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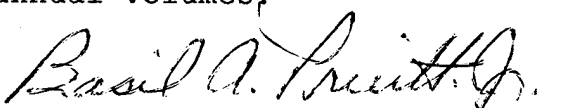
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FOREWORD

Military mission relevancy and cogency of this Institute's clinical, teaching, and research activities are a source of reassurance in the face of the anticipated decrease and redistribution of military funding. In the past four years, the Institute's clinical studies have led to modification of fluid management and revolutionary changes in the treatment of inhalation injury. Laboratory research projects have identified previously undescribed postburn changes in cellular and organ function and defined the similarities of such responses to those following other forms of trauma. The burn patient is an universal trauma model. Those developments and a general improvement in clinical care management have resulted in continuing improvement in outcome to an unprecedented level of survival in an ever increasing number of admissions.

The Institute has, in the past five years, discharged its teaching mission by providing undergraduate, graduate, and postgraduate experience for physicians from all three United States military services and civilian medical students, residents, and fellows as well as physician members of foreign medical services. Since 1987, burn-specific educational experience has been provided to 23 military physicians at the PGY-1 level, 108 military residents, and 54 civilian residents. De facto burn fellowships have been provided to five United States military surgeons, 8 United States civilian surgeons, and three individuals in NRC fellowship status. Twenty-two military physicians and 14 civilian physicians from foreign countries made educational visits to the Institute ranging in duration from one day to 12 or more months.

The Institute has also demonstrated repeatedly its readiness to meet all exigencies, whether they be disasters in other counties, military combat operations, or peacetime domestic and intercontinental aeromedical transfer of critically ill and injured patients. The ability of the staff to meet military requirements, provide humanitarian care, deliver multilevel medical teaching while conducting both clinical and laboratory research, and still set the standard of burn care in the United States, makes them and this Institute unique in the United States military medical services as documented by these annual volumes.



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

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

ACCESSION: DAOG6380

SUMMARY DATE: 921001 **SUMMARY KIND:** D **PREV DATE:** 911001 **DISTRIBUTION:** CX

PROGRAM #: 62787A **PROJ #:** 30162787A874 **TASK AREA:** EF **WORK UNIT:** 164

TITLE: Clinical Operation, Center for Treatment of Burned Patients

SUBJ1: 060500 - Medicine and Medical Research
SUBJ2: 061300 - Microbiology
SUBJ3: 061500 - Pharmacology

START DATE: 5007 **END DATE:** 9909 **PERFORMANCE METHOD:** C

CONTRACT/GRANT NO:

CONT TOTAL: $
CUM TOTAL: $
TOTAL LAB FUNDS: $

RESOURCES ESTIMATE

FY	WORK YRS	$(Thousands)
91	47.2	$4,393
92	49.8	$4,168
93	49.8	$5,489

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ASSOC1:

ASSOC2:

FOREIGN INTELLIGENCE CONSIDERED: Y **CIVILIAN APPLICABILITY:** M

KEYWORDS: RA II; Volunteers; Adults; Children; Burns (Injuries); Diagnosis (Medicine); Healing; Health Care Facilities

OBJECTIVE: A DTIC literature search was not conducted since the objectives of this work are broad-based to provide specialized care for thermally injured patients, investigate diagnostic and therapeutic technics to improve the survival and function of thermally injured patients, and to promulgate scientific medical information to health professionals.

APPROACH: Thermally injured patients from the Continental United States and throughout the world are transported to this Institute for intensive, specialized treatment. Carefully controlled evaluations of new treatment technics are conducted by professional staff.

PROGRESS: 9110-9209. During calendar year 1991, 218 seriously burned patients were admitted and treated at this Institute. Clinical activities included studies of host resistance, endocrine changes after injury, nutritional support regimens, skin substitutes, and postinjury infection control. For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report* for fiscal years 1950 through 1992.

ABSTRACT

PROJECT NUMBER: 3M162787A874-164, Applied Research and Exploratory Development

PROJECT TITLE: Clinical Operation, Center for Treatment of Burned Patients

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

PERIOD COVERED IN THIS REPORT: 1 January 1991 - 31 December 1991

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Two hundred and eighteen patients were admitted to this Institute during calendar year 1991. Principal activities included care of severely burned patients, research to improve survival and function of such patients, and education and training of health care professionals and paraprofessionals. Areas of research included an ongoing study of 5% aqueous mafenide acetate soaks for the topical treatment of burn wounds following grafting, studies of neuroendocrine abnormalities in burn injuries, evaluation of in vitro cultivated keratinocytes used for the closure of burn wounds, evaluation of clotrimazole for the prevention of fungal colonization in thermally injured patients, a study of the efficacy of a polyetherurethane membrane dressing in the treatment of skin graft donor sites, evaluation of the effect of sucralfate on prevention of stress ulcers and nosocomial pneumonia in thermally injured patients, a study of salt and water balance in the

thermally injured patient, assessment by flow cytometry of peripheral blood cells, a study of the effect of recombinant human insulin-like growth factor treatment on the rate of healing of burn patients who require skin grafting, evaluation of the effect of nutritional support on immune function in patients with thermal injury, a comprehensive analysis of the perceived needs of families of critically injured burned patients, a project to characterize certain biochemical indicators of infection in the thermally injured, a study to determine vecuronium bromide requirements of thermally injured patients, a study of the efficacy of low-dose dopamine therapy in thermally injured patients, evaluation of the effects of heparinized versus nonheparinized flush solutions on the patency of arterial pressure monitoring lines, a study of the efficacy of silicone gel in the prevention of hypertrophic burn scar formation, a study of the effects of weak direct current on the healing of donor sites, determination of intestinal permeability after burn injury, an assessment of the short- and long-term effects of Dermagraft™ dermal replacement, measurement of plasma amino acid and pyridoxal-5'-phosphate levels, and determination of protein turnover, amino acid flux, and nitrogen balance in thermally injured patients.

CLINICAL OPERATION
CENTER FOR TREATMENT OF BURNED PATIENTS

The Clinical Division of this Institute admitted 218 soldiers and other authorized patients with thermal, chemical, or electric injury during calendar year 1991. Aeromedical teams from the Institute conducted 51 missions to transfer 55 (25.5%) of the admitted patients. All missions were within the continental United States. Eighteen missions (35.3%) were carried out by rotary-wing aircraft and 33 (64.7%) by fixed-wing aircraft. One hundred and twenty-six patients (57.8%) were admitted within 24 h of injury and 134 (61.5%) were admitted within 48 h of injury. One hundred and eighty patients (82.6%) were male and 31 (17.4%) were female.

The ages of these patients ranged from 3 months to 91 yr, with an average age of 28.7 yr. The extent of burn averaged 18.7% of the total body surface area, with an average full-thickness component of 9.9%. Fifty-three patients (24.3%) were in the pediatric age group (< 15 yr), with an average age of 4.5 yr and an average burn size of 12.8% of the total body surface area. There were 12 patients (5.5%) with high voltage electric injury and 8 patients (3.7%) with chemical injury. The average hospital stay of all patients, excluding convalescent leave for active duty military patients, was 28.2 days. The sources of admission are identified in Table 1 and the causes of burn injury are detailed in Table 2.

Inhalation injury was identified in 33 patients (15.1%). Forty-four patients (20.2%) had some associated injury (includes 33 patients with inhalation injury) which included fractures or dislocations in 10 patients, lacerations in 6 patients, and head injuries in 3 patients.

During calendar year 1991, 409 operative procedures were performed on 148 patients, an average of 2.8 operative procedures per patient.

Morbidity and Mortality. Seventeen patients (7.8%) died during calendar year 1991. Autopsies were performed in 7 (41.2%) of these hospital deaths. The average burn size of patients who died was 62.2% of the total body surface area and the full-thickness burn averaged 47.6% of the total body surface area. Age ranged from 2 to 91 yr. Ten of these patients (58.8%) had inhalation injury as a primary or contributing cause of death. Fifteen patients (82.2%) had burn injuries ≥ 30% of the total body surface area, 14 (88.2%) exceeding 40%, and 12 (70.6%) exceeding 50% of the total body surface area. Two (11.8%) of the 17 deaths occurred in pediatric patients.

Infection was once again the most common complication following thermal injury, with pneumonia occurring in 24 patients (11%). The most common organism isolated in patients with bacterial pneumonia

TABLE 1. Sources of Admission (1991)

AREA	A	AD	AF	AFD	N/MC	ND	VAB	OTHER	TOTAL
First Army	2	1	–	–	–	–	–	–	**3**
Third Army	1	3	1	1	3	2	1	–	**12**
Fifth Army	7	18	2	5	4	3	20	93	**152**
Sixth Army	2	–	–	–	1	–	2	–	**5**
Alaska	–	–	–	–	–	–	1	–	**1**
Germany	2	1	–	–	–	–	–	–	**3**
Hawaii	1	–	–	–	–	–	–	–	**1**
Honduras	1	–	–	–	–	–	–	–	**1**
Japan	–	–	–	–	1	–	–	–	**1**
Korea	1	–	–	–	–	–	–	–	**1**
Mexico	–	–	–	–	–	–	–	1	**1**
Panama	1	–	–	–	–	–	–	–	**1**
Saudi Arabia	31	–	1	–	3	–	–	–	**35**
TOTAL	**50**	**23**	**4**	**6**	**12**	**5**	**24**	**94**	**218**

A indicates United States Army; AF, United States Air Force; N, United States Navy; M, United States Marine Corps; D, dependent; VAB, Veterans Administration beneficiary; and OTHER, civilian emergency, US Public Health Service beneficiary, and Bureau of Employees Compensation beneficiary.

TABLE 2. Burn Etiology (1991)

Causes	Number of Patients	Percentage of Admissions	Deaths	Mortality (%)
Hot liquids	59	27.1	1	5.9
Gasoline, diesel, and kerosene	56	25.7	2	11.8
Structural fires	18	8.3	7	41.2
Bomb, shell, simulator grenade, and gunpowder explosions	16	7.3	-	-
Contact	12	5.5	-	-
Electrical	12	5.5	-	-
Open flames	11	5.0	1	-
Butane, propane, or natural/sewer gas explosions	9	4.1	1	5.9
Chemical	8	3.7	-	-
Self-inflicted	7	3.2	3	17.6
Aircraft, helicopter	3	1.4	-	5.9
Motor vehicle accidents	3	1.4	-	-
Other	3	1.4	7	-
Smoking, clothes ignition	1	0.5	1	5.9
TOTAL	**218**	**100.0**	**17**	**7.8**

was *Staphylococcus aureus* in 4 patients. Gram-negative organisms were responsible for pneumonia in the remaining 20 patients. No patients had bacterial invasion of the burn wound; however, 3 had burn wound invasion by fungi or yeast. Aspergillus was the organism identified in viable tissue in 2 of these patients and Mucor species in 1. One patient had suppurative thrombophlebitis during this reporting period.

Table 3 lists the effect of age and extent of injury on survival and Table 4 lists mortality rates associated with increments of 10% of the total body surface area for the years 1987 through 1991. Table 5 summarizes the survival of patients with extensive burns from 1963 through 1991. Table 6 compares mortality before and after the use of topical chemotherapy on the burn wound. Table 7 lists the causes of death for calendar year 1991.

Educational Activities. During calendar year 1991, the professional staff of the Clinical Division continued to provide education to professional and paraprofessional groups at the local, national, and international levels. A total of 31 resident physicians were attached for periods of 1 to 2 months, including 7 from Wilford Hall USAF Medical Center, 6 each from Brooke Army Medical Center and the University of Texas Health Science Center (San Antonio TX), 4 from the Pensacola Naval Air Station, 2 from Travis Air Force Base, and 1 each from Bethesda Naval Medical Center, Fitzsimons Army Medical Center, Metropolitan Medical Center (St. Louis MO), Providence Hospital (Southfield MI), the University of Iowa, and the University of Puerto Rico. Five interns from Brooke Army Medical Center had 1-month rotations at the Institute. A total of 5 medical students spent time at the Institute, including 1 each from Creighton University (Omaha NE), Louisiana State University, Mercer University Medical Center (Macon GA), Morehouse College (Atlanta GA), the Uniformed Services University of the Health Sciences, and the University of Texas Medical Branch (Galveston TX). A total of 15 physicians visited from foreign countries for periods ranging from 1 day to 1 month, including 3 from Germany, 2 each from Hungary, Thailand, and Uruguay, and 1 each from Argentina, Honduras, Poland, Northern Ireland, and the United Kingdom. One foreign medical student from Taiwan visited the Institute for a period of 3 months and 1 travelling fellow from the United Kingdom visited the Institute for a period of 1 week. The Respiratory Therapy Branch had 80 trainees, the Physical Therapy Branch had 1 trainee, and the Occupational Therapy Branch had 2 trainees. Thirty-five scientific publications appeared in refereed medical journals and 188 scientific presentations were conducted for military and civilian audiences. Numerous scientific presentations were made at the Academy of Health Sciences and various military installations throughout the continental United States, to include support of the Combat Casualty Care Courses for the United States Army. In addition, weekly professional staff conferences were conducted for and by Institute personnel.

TABLE 3. Age, Body Surface Involvement, and Mortality (1991)

Age (Yr)	Total Body Surface Area Burn (%)										Cases	Deaths	Mortality (%)
	0-9	10-19	20-29	30-39	40-49	50-59	60-69	70-79	80-89	90-100			
0	5	1	–	–	–	–	–	–	–	–	6	–	–
1	9	1	2	–	1	–	–	–	–	–	13	1	7.7
2	5	–	1	3	–	–	–	–	–	–	9	–	–
3	1	1	1	–	1	–	–	–	–	–	4	–	–
4	2	1	1	–	–	–	–	–	–	–	2	–	–
5 – 9	7	2	2	–	1	1	–	–	–	–	13	1	7.7
10 – 14	5	1	–	–	–	–	–	–	–	–	6	–	–
15 – 19	3	1	1	1	–	–	–	–	–	–	6	–	–
20 – 29	32	15	9	3	1	3	–	2	3	–	68	2	2.9
30 – 39	12	13	5	5	2	–	1	–	–	–	38	2	5.3
40 – 49	6	3	3	–	1	2	–	1	1	2	19	5	26.3
50 – 59	5	3	2	–	1	1	–	–	–	–	12	1	8.3
60 – 69	9	1	1	2	4	–	–	–	1	–	18	4	22.2
70 – 79	2	–	–	–	–	–	–	–	–	–	2	–	–
80 – 89	1	–	–	–	–	–	–	–	–	–	1	–	–
90 – 100	–	–	–	–	–	–	–	–	–	–	1	1	100.0
Total Cases	104	42	27	14	12	7	1	3	5	3	218		
Total Deaths	–	1	1	1	2	4	1	1	4	2		17	
Mortality (%)	–	2.4	3.7	7.1	16.7	57.1	100.0	33.3	80.0	66.7			7.8

TABLE 4. Total Body Surface Area Burn Involvement (%) and Mortality (1987–91)

	0-9	10-19	20-29	30-39	40-49	50-59	60-69	70-79	80-89	91-100	Total
1991											
Number of Patients	104	42	27	14	12	7	1	3	5	3	218
Number of Deaths	-	1	1	1	2	4	1	1	4	2	17
Mortality (%)	-	2.4	3.7	7.1	16.7	57.1	100.0	33.3	80.0	66.7	7.8
1990											
Number of Patients	75	43	36	19	13	6	7	7	8	2	216
Number of Deaths	-	2	2	3	1	-	1	1	5	2	17
Mortality (%)	-	4.7	5.6	15.8	7.7	-	14.3	14.3	62.5	100.0	7.9
1989											
Number of Patients	66	52	26	22	22	11	2	4	6	-	211
Number of Deaths	-	3	-	3	4	7	0	1	4	-	22
Mortality (%)	-	5.8	-	13.6	18.2	63.6	-	25.0	66.7	-	10.4
1988											
Number of Patients	80	55	21	21	16	8	6	6	5	2	220
Number of Deaths	-	2	1	2	2	2	3	1	3	2	18
Mortality (%)	-	3.6	4.8	9.5	12.5	25.0	50.0	16.7	60.0	100.00	8.2
1987											
Number of Patients	67	52	36	20	19	12	10	-	2	3	221
Number of Deaths	-	-	1	2	3	4	6	-	2	3	21
Mortality (%)	-	-	2.8	10.0	15.8	33.3	60.0	-	100.0	100.00	9.5

TABLE 5. Survival and Nonsurvival by Year for Patients with Burns ≥ 30% of the Total Body Surface Area (1963–91)

Year	SURVIVORS			NONSURVIVORS		
	Number of Cases	Average Burn (%) Total	3°	Number of Cases	Average Burn (%) Total	3°
1991	30	46.9	28.9	15	68.0	53.5
1990	49	50.5	24.5	13	67.7	48.2
1989	48	44.5	24.5	19	56.7	40.2
1988	56	40.9	20.6	15	66.2	53.3
1987	46	43.7	17.2	20	63.2	45.1
1986	178	21.8	7.3	24	68.3	47.9
1985	48	43.6	21.7	32	65.6	45.7
1984	43	46.4	24.8	32	59.5	41.4
1983	37	43.5	17.5	31	61.7	49.9
1982	53	43.7	24.8	46	59.5	43.5
1981	54	42.7	17.5	40	64.9	41.8
1980	62	42.7	15.1	63	65.5	43.1
1979	61	45.4	13.4	72	66.8	38.0
1978	67	45.7	14.8	59	60.0	36.6
1977	66	42.2	14.4	64	60.0	31.1
1976	69	45.5	15.0	74	67.3	34.5
1975	80	46.1	14.7	89	63.5	33.6
1974	55	43.9	12.2	93	62.2	37.0
1973	47	43.7	19.6	107	62.2	38.0
1972	62	42.0	17.2	93	50.7	38.8
1971	63	41.9	14.0	66	62.0	39.1
1970	92	39.4	10.7	58	57.7	37.4
1969	113	43.2	11.1	68	59.9	27.1
1968	143	44.2	12.6	35	56.8	25.9
1967	103	42.7	13.3	48	61.7	32.8
1966	68	41.5	14.9	59	59.9	31.3
1965	47	43.8	21.0	33	66.0	33.4
1964	40	41.8	14.8	35	67.7	44.8
1963	28	45.8	19.6	53	58.6	42.6

TABLE 6. Comparison of Burn Mortality Rates (1962-3 and 1964-90)

	TOTAL BODY SURFACE AREA BURN (%)														
	0-29			30-39			40-49			50-59			60-100		
YEARS	Number of Patients	Number of Deaths	Mortality (%)	Number of Patients	Number of Deaths	Mortality (%)	Number of Patients	Number of Deaths	Mortality (%)	Number of Patients	Number of Deaths	Mortality (%)	Number of Patients	Number of Deaths	Mortality (%)
1962-3	140	6	4.3	36	16	44.4	35	22	61.1	23	18	78.3	55	49	89.1
1964-90	3,774	135	3.6	951	167	17.6	747	221	29.6	521	234	44.9	964	769	79.8
1991	173	2	1.2	14	1	7.1	12	2	16.7	7	4	57.1	12	8	66.7

TABLE 7. Causes of Death (1991)

Patient	Age	Sex	BURN SIZE (%) Total	3°	Postburn Day	Cause of Death
1	41	M	96	92	1	96% total body surface area burn with acute myocardial infarction and severe inhalation injury.
2	91	F	90	90	2	90% total body surface area burn with acute coronary insufficiency.
3	67	M	90	83	16	90% total body surface area burn with fungal burn wound infection and cardiomyopathy.
4	29	M	90	83	32	90% total body surface area burn with inhalation injury and pneumonia.
5	40	M	84	80	3	*84% total body surface area burn.
6	21	F	82	74	2	*82% total body surface area burn with resuscitation failure.
7	41	M	75	57	1	*75% total body surface area burn with inhalation injury.
8	31	M	67	15	9	*67% total body surface area burn with acute aspiration of gastric contents.
9	6	F	60	60	17	*60% total body surface area burn with inhalation injury.
10	45	M	59	45	28	*59% total body surface area burn with inhalation injury and acute myocardial infarction.

TABLE 7 (Continued)

Patient	Age	Sex	BURN SIZE (%) Total	3°	Postburn Day	Cause of Death
11	41	M	57	18	2	*57% total body surface area burn with acute airway obstruction.
12	56	M	54	51	8	*54% total body surface area burn with inhalation injury and pneumonia.
13	69	M	45	40	0	45% total body surface area burn with inhalation injury.
14	67	M	40	21	7	40% total body surface area burn with inhalation injury and pneumonia.
15	62	M	38	-	42	*38% total body surface area burn with acute bacterial pneumonia.
16	1	F	20	-	1	*20% total body surface area burn with inhalation injury.
17	30	M	10	-	6	*10% total body surface area burn with inhalation injury.

*Autopsy not performed.

PRESENTATIONS

Cioffi WG Jr: Early burn care. Presented at the 97th Army General Hospital, Frankfurt, Germany, 14 January 1991.

Driscoll DM: Burn wound management. Presented to the Nursing Service Branch, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 15 January 1991.

Duncan DJ: Initial management of burn injuries. Presented to the Nursing Service Branch, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 15 January 1991.

Duncan DJ: Standards of care for the large burn victim during the initial 48 hours. Presented to the Nursing Service Branch, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 15 January 1991.

Milner EA: Nutrition and the thermally injured patient. Presented to the Nursing Service Branch, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 15 January 1991.

Black CI: Perioperative care of the burn victim. Presented to the Nursing Service Branch, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 16 January 1991.

Duncan DJ: Burn wound management. Presented to the Nursing Service Branch, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 16 January 1991.

Hollan E: Infection control in the burn unit. Presented to the Nursing Service Branch, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 16 January 1991.

Matta CB: Aeromedical evacuation of the burn patient. Presented to the Nursing Service Branch, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 16 January 1991.

Molter NC: Pain management. Presented to the Nursing Service Branch, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 16 January 1991.

Newsome DM: Acid base balance. Presented to the Nursing Service Branch, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 16 January 1991.

Summers TM: Psychological aspects of burn care. Presented to the Nursing Service Branch, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 16 January 1991.

Cioffi WG Jr: Early burn care. Presented at the Nuremberg Army General Hospital, Nuremberg, Germany, 17 January 1991.

Summers TM: Nursing care of the burn patient. Presented to the Burn Family Support Group, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 22 January 1991.

Summers TM: Crisis and families. Presented as part of the US Army Intensive Care Nursing Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 23 January 1991.

Black CI: Perioperative care of the burn victim. Presented as part of the US Army Operative Room Nursing Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 23 January 1991.

Molter NC: Experiences in constructing an instrument: needs of the families of critically ill patients. Presented at the Research Colloquium, University of Texas School of Nursing, Austin, Texas, 24 January 1991.

Summers TM: Stress and crisis management in the intensive care unit. Presented as part of the US Army Intensive Care Nursing Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 25 January 1991.

Pruitt BA Jr: Recent progress in burn care. Presented at the 19th Conference of the Medical Committee of the American Armies, San Antonio, Texas, 28 January 1991.

McManus WF: Advances in burn care. Presented to the National Safety Council, San Antonio, Texas, 28 January 1991.

Cioffi WG Jr: Pulmonary injury in burns. Presented as part of the OT/PT Management of Burns Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 4 February 1991.

McManus WF: Aeromedical transportation of the burned patient. Presented as part of the OT/PT Management of Burns Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 4 February 1991.

McManus WF: Burn wound management. Presented as part of the OT/PT Management of Burns Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 4 February 1991.

Pruitt BA Jr: Introduction to burn trauma and burn injury pathophysiology. Presented as part of the OT/PT Management of Burns Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 4 February 1991.

Rue LW 3d: Special injuries: chemical, electrical, and toxic epidermal necrolysis. Presented as part of the OT/PT Management of Burns Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 4 February 1991.

Allen CD: Scar control/management. Presented as part of the OT/PT management of Burns Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 5 February 1991.

Basham FD: Respiratory therapy. Presented as part of the OT/PT Management of Burns Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 5 February 1991.

Burgess MC: Orientation to the intensive care environment. Presented as part of the OT/PT Management of Burns Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 5 February 1991.

Harden NG: OT/PT management of burns. Presented as part of the OT/PT Management of Burns Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 5 February 1991.

Hollan E: Infection control. Presented as part of the OT/PT Management of Burns Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 5 February 1991.

Milner EA: Nutritional management. Presented as part of the OT/PT Management of Burns Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 5 February 1991.

Mingrone MM: Scar control/management. Presented as part of the OT/PT Management of Burns Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 5 February 1991.

Stetz CK: Minor burn wound management. Presented as part of the OT/PT Management of Burns Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 5 February 1991.

Summers TM: Psychological evaluation and considerations. Presented as part of the OT/PT Management of Burns Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 5 February 1991.

Duncan DJ: Initial management of the burn victim. Presented at the Baptist Memorial Hospital School of Nursing, San Antonio, Texas, 6 February 1991.

Duncan DJ: Standards of care for the large burn victim in the initial 48 hours. Presented at the Baptist Memorial Hospital School of Nursing, San Antonio, Texas, 6 February 1991.

Molter NC: The gift of a shared vision. Presented at the Army Nurses' Corp Birthday Celebration, William Beaumont Army Medical Center, Fort Bliss, El Paso, Texas, 10 February 1991.

Matta CB: Initial management of the burn victim in the theater of operation. Presented as part of the United States Air Force Battlefield Nursing Course, Brooks Air Force Base, San Antonio, Texas, 12 February 1991.

Molter NC: Pain management. Presented as part of the OT/PT Management of Burns Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 13 February 1991.

Duncan DJ: Aeromedical transport of the burn victim. Presented as part of the US Army Intensive Care Nursing Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 14 February 1991.

Duncan DJ: Initial management of the burn victim. Presented as part of the US Army Intensive Care Nursing Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 14 February 1991.

Black CI: Perioperative care of the burn patient. Presented as part of the US Army Perioperative Nursing Course, Fort Sam Houston, San Antonio, Texas, 20 February 1991.

Black CI: Surgical care of the burn patient. Presented to the families of burn victims, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 22 February 1991.

Pruitt BA Jr: Diagnosis and treatment of surgical infections. Presented to the Department of General Surgery, Wilford Hall USAF Medical Center, Lackland Air Force Base, San Antonio, Texas, 23 February 1991.

Loresch DC: Thermal and environmental injuries. Presented as part of the Emergency Medical Technician Course, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 25 February 1991.

Duncan DJ: Burn wound management. Presented as part of the US Army Intensive Care Nursing Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 27 February 1991.

Pruitt BA Jr: Importance of burn centers in burn care and burn research. Presented at the 10th Anniversary Symposium, North Carolina Jaycee Burn Center, Chapel Hill, North Carolina, 2 March 1991.

Black CI: Perioperative care of the burn patient. Presented to the operating room staff, Brooke Army Medical Center, Fort Sam Houston, San Antonio, Texas, 5 March 1991.

Pruitt BA Jr: Early treatment of the burn patient. Presented at the Trauma/Critical Care 1991 Symposium, Las Vegas, Nevada, 17 March 1991.

Pruitt BA Jr: Late treatment of burn patients. Presented at the Trauma/Critical Care 1991 Symposium, Las Vegas, Nevada, 19 March 1991.

Pruitt BA Jr: Role of colloids in resuscitation from burn injury. Presented at the FDA/NIH Workshop for the Assessment of Plasma Volume Expanders, Bethesda, Maryland, 26 March 1991.

Latendresse LM: Prevention and management of thermal injuries. Presented at the Health Careers High School, San Antonio, Texas, 2 April 1991.

Newsome DM: Prevention and management of thermal injuries. Presented at the Health Careers High School, San Antonio, Texas, 2 April 1991.

Pruitt BA Jr: Ventilatory management of inhalation injury. Presented at the Mini-Beffa Conference on Inhalation Injury, Baltimore, Maryland, 2 April 1991.

Driscoll DM: Intragastric pH monitoring. Presented at the 23rd Annual Meeting of the American Burn Association, Baltimore, Maryland, 5 April 1991.

Duncan DJ: Burn management overview. Presented as part of the Recruiting Command Student Tour, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 8 April 1991.

LeVoyer T: Intestinal permeability following thermal injury. Presented at the 11th Annual Meeting of the Surgical Infection Society, Fort Lauderdale, Florida, 8 April 1991.

Matta CB: Initial management of the burn victim in the theater of operation. Presented as part of the USAF Battlefield Nursing Course, Brooks Air Force Base, San Antonio, Texas, 9 April 1991.

Cioffi WG Jr: Dissociation of blood volume and flow in regulation of salt and water balance in thermally injured patients. Presented at the 111th Annual Meeting of the American Surgical Society, Boca Raton, Florida, 12 April 1991.

Black CI: Perioperative care of burn patients. Presented as part of the US Army Perioperative Nursing Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 16 April 1991.

Pinkston GD: Thermal and environmental injuries. Presented as part of the Emergency Medical Technicians Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 16 April 1991.

Milner EA: Nutritional management of burn patients. Presented as part of the AMSC Postgraduate Nutrition Short Course, Walter Reed Army Institute of Research, Washington, DC, 17 April 1991.

Pruitt BA Jr: Current treatment of burn wounds. Presented at the Veterans Administration Medical Center, Mountain Home, Tennessee, 20 April 1991.

McManus WF: Advances in burn care. Presented at the Gary P. Wrattan Surgical Symposium, San Francisco, California, 25 April 1991.

Cioffi WG Jr: Ventilatory support in patients with inhalation injury. Presented at the Mini Beffa Conference, Baltimore, Maryland, 28 April 1991.

Pruitt BA Jr: Presentation, evaluation, and management of lung injury. Presented at the Conference on Toxic Smoke Inhalation and Lung Injury, Naval Medical Research Institute, Gaithersburg, Maryland, 29 April 1991.

Pruitt BA Jr: Pathophysiology and treatment of inhalation injury. Presented at the Conference on Toxic Smoke Inhalation and Lung Injury, Naval Medical Research Institute, Gaithersburg, Maryland, 1 May 1991.

Duncan DJ: Burn care – family concerns. Presented to families of burn victims, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 7 May 1991.

Driscoll DM: Battlefield nursing. Presented to the American Association of Critical Care Nurses' National Training Institute, Boston, Massachusetts, 13 May 1991.

Driscoll DM: Compassion knows no boundaries: the 1989 train disaster in the USSR. Presented at the American Association of Critical Care Nurses' National Training Institute, Boston, Massachusetts, 15 May 1991.

Driscoll DM: Initial management of burn trauma. Presented at the American Association of Critical Care Nurses' National Training Institute, Boston, Massachusetts, 16 May 1991.

Duncan DJ: Burn nursing at the US Army Institute of Surgical Research. Presented as part of the AMEDD Officer Basic Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 17 May 1991.

Pinkston GD: Thermal and environmental injuries. Presented as part of the Emergency Medical Technician Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 20 May 1991.

Matta CB: Initial management of burn victims in the theater of operations. Presented as part of the United States Air Force Battlefield Nursing Course, Brooks Air Force Base, San Antonio, Texas, 21 May 1991.

Milner EA: Nutritional support of thermally injured patient. Presented as part of the Nutritional Assessment Postgraduate Short Course, Brooke Army Medical Center, Fort Sam Houston, San Antonio, Texas, 21 May 1991.

Newsome DM: Acid base balance. Presented as part of the Intensive Care Unit Course, Fort Sam Houston, San Antonio, Texas, 22 May 1991.

Pruitt BA Jr: Planning and provision of burn care during Operation Desert Shield/Desert Storm. Presented to the Rhode Chapter of the American College of Surgeons/Providence Surgical Society, Providence, Rhode Island, 22 May 1991.

Driscoll DM: Compassion knows no boundaries: the 1989 train disaster in the USSR. Presented as part of the AMEDD Army Officer Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 23 May 1991.

Duncan DJ: Management of the burn trauma patient. Presented as part of the US Army Emergency Nursing Care Course, Fort Sam Houston, San Antonio, Texas, 23 May 1991.

Black CI: Perioperative care of the burn patient. Presented to perioperative nursing residents, Brooke Army Medical Center, Fort Sam Houston, San Antonio, Texas, 29 May 1991.

Pruitt BA Jr: Infection as a comorbid factor in burn patients. Presented as the 18th Preston A. Wade Trauma Lecturer at the New York Hospital-Cornell Medical Center, New York, New York, 29 May 1991.

Summers TM: Crisis and families. Presented as part of the Intensive Care Unit Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 29 May 1991.

Duncan DJ: Burn management at the US Army Institute of Surgical Research. Presented as part of the Educator's Tour, Junior Officer Panel, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 30 May 1991.

Pruitt BA Jr: Resuscitation of the thermally injured patient with and without inhalation injury. Presented at the 2nd

International Conference on Shock and Symposium/Workshop on Pathology and Therapy of Burn Injury, Vienna, Austria, 3 June 1991.

Becker WK: Hypertonic saline-dextran and the recovery of hepatic blood flow and high-energy phosphate content following hemorrhage. Presented at the 2nd International Conference on Shock, 5th Annual Meeting of the European Shock Society, 14th Annual Meeting of the Shock Society (USA), and 3rd Annual Vienna Shock Forum, Vienna, Austria, 4 June 1991.

Summers TM: Stress and crisis management in the Intensive Care Unit. Presented as part of the Intensive Care Nursing Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 7 June 1991.

Becker WK: Burns: mass casualty management and planning. Presented to the Israeli Burn Association, Jerusalem, Israel, 9 June 1991.

Milner EA: Nutritional management of thermally injured patients. Presented to nursing students at the University of Texas Health Sciences Center, San Antonio, Texas, 10 June 1991.

Cioffi WG Jr: The effect of GM-CSF function following thermal injury. Presented at the 10th Annual Meeting of the Surgical Infection Society, Cincinnati, Ohio, 15 June 1991.

McManus WF: Management of burns. Presented to the 8th Medical Brigade, New York, New York, 15 June 1991.

Pruitt BA Jr: Urgent treatment of extensive burns. Presented at the 10th International Congress of Emergency Surgery, Lisbon, Portugal, 18 June 1991.

Vaughan GM: Syrian hamster pineal isoproterenol responsiveness extends into the early light phase. Presented at the annual meeting of the Society of Uniformed Endocrinologists, Washington, DC, 20 June 1991.

Driscoll DM: Initial management of the burn victim. Presented as part of the Intensive Care Nursing Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas 21 June 1991.

Pruitt BA Jr: Diagnosis and treatment of inhalation injury. Presented as part of the 55th Annual Continuing Education Course in Surgery, Minneapolis, Minnesota, 21 June 1991.

Pruitt BA Jr: Diagnosis and treatment of opportunistic infections in burn patients. Presented as part of the 55th Annual Continuing Education Course in Surgery, Minneapolis, Minnesota, 22 June 1991.

Duncan DJ: Burn wound management. Presented as part of the Intensive Care Nursing Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 28 June 1991.

Milner EA: Nutrition and the burn patient. Presented to families of burn victims, US Army Institute of Surgical Research, Fort Sam Houston, 9 July 1991.

Cioffi WG Jr: Advanced trauma life support. Presented at the University of Texas Health Science Center, San Antonio, Texas, 10 July 1991.

Black CI: Perioperative care of the burn patient. Presented to families of burn patients, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 16 July 1991.

Driscoll DM: Contingency planning and provision of burn care in Operation Desert Storm. Presented at Nursing Research Forum, US Army Medical Research and Development Command, Bethesda Naval Hospital, Bethesda, Maryland, 17 July 1991.

Pruitt BA Jr: Fluid resuscitation of burn patients. Presented as part of the US Army Institute of Surgical Research Lecture Series, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 17 July 1991.

Pruitt BA Jr: Shock and fluid resuscitation - pediatric patients. Presented as part of the Advanced Burn Life Support Course, Lincoln, Nebraska, 19 July 1991.

Molter NC: Overview of Nursing at the US Army Institute of Surgical Research. Presented as part of the Educator's Tour, Reserve Officer Training Corps, Recruiting Command, Fort Sam Houston, San Antonio, Texas, 22 July 1991.

Burgess MC: Pediatric thermal injuries. Presented as part of the Advanced Burn Life Support Course, Portsmouth, Virginia, 23 July 1991.

Pruitt BA Jr: Diagnosis and treatment of burn wound infection. Presented as part of the US Army Institute of Surgical Research Lecture Series, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 24 July 1991.

Pruitt BA Jr: Excision and closure of the burn wound. Presented as part of the US Army Institute of Surgical Research Lecture Series, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 27 July 1991.

Kelly KM: Initial management of the burn victim in the theater of operation. Presented at the United States Air Force Battlefield

Nursing Course, Brooks Air Force Base, San Antonio, Texas, 30 July 1991.

Pruitt BA Jr: Current techniques of burn care. Presented at the Surgical Grand Rounds, Wilford Hall USAF Medical Center, Lackland Air Force Base, San Antonio, Texas, 3 August 1991.

Driscoll DM: Nursing care and wound care of the burn patient. Presented to the families of burn victim, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 6 August 1991.

Milner EA: Nutrition and the burn patient. Presented to the families of burn victims, US Army Institute of Surgical Research, Fort Sam Houston, 13 August 1991.

Wiegum LM: Psychosocial care of the burn victim. Presented to the families of burn victim, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 20 August 1991.

Pruitt BA Jr: End points in resuscitation. Presented at the End Points in Resuscitation Symposium, Uniformed Services University of Health Care and Veterans Administration Medical Center, Washington, DC, 23 August 1991.

Becker WK: Urea-cycle amino acids and growth in rats. Presented at the 34th World Congress of Surgery of the ISS/SIC and 12th World Congress of CICD, Stockholm, Sweden, 25 August 1991.

Pruitt BA Jr: Resuscitation of the thermally injured patient with and without inhalation injury. Presented at the International Surgical Week, Stockholm, Sweden, 26 August 1991.

Rue LW 3d: Thromboembolic complications in thermally injured patients. Presented at the International Surgical Week, Stockholm, Sweden, 26 August 1991.

Burgess MC: Initial management of the burn victim. Presented to Nursing Service Branch, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 27 August 1991.

Driscoll DM: Burn wound management. Presented to Nursing Service Branch, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 27 August 1991.

Hollan E: Infection control. Presented to the Nursing Service Branch, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 27 August 1991.

Morales MS: Toxic epidermal necrolysis. Presented to the Nursing Service Branch, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 27 August 1991.

Mozingo DW: Pulmonary amino acid flux in critically ill patients. Presented at the International Surgical Week, Stockholm, Sweden, 27 August 1991.

Black CI: Perioperative care of the burn patient. Presented to Nursing Service Branch, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 28 August 1991.

Newsome D: Aeromedical transport. Presented to the Nursing Service Branch, US Army Institute of Surgical Research, Fort Sam Service Branch, Fort Sam Houston, San Antonio, Texas, 28 August 1991.

Pruitt BA Jr: The changing epidemiology of infection in burn patients. Presented at the International Surgical Week, Stockholm, Sweden, 28 August 1991.

Pruitt BA Jr: The metabolic effects of burn injury. Presented at the International Surgical Week, Stockholm, Sweden, 28 August 1991.

Sanford P: Pediatric burns. Presented to the Nursing Service Branch, US Army Institute of Surgical Research, Fort Sam Houston, Branch, US Army Texas, 28 August 1991.

Wiegum L: Psychosocial aspects of burn care. Presented to Nursing Service Branch, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 28 August 1991.

Driscoll DM: Pain management. Presented to the Nursing Service Branch, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 29 August 1991.

Becker WK: Small animal models of smoke exposure and inhalation injury. Presented at the 34th World Congress of Surgery of the ISS/SIC and 12th World Congress of CICD, Stockholm, Sweden, 30 August 1991.

Pruitt BA Jr: Planning and preparation of burn care for peacetime disasters and warfare casualties. Presented to the International Surgical Group, Malmö, Sweden, 2 September 1991.

Driscoll DM: Management of the burn patient. Presented at the Baptist Memorial Hospital School of Nursing, San Antonio, Texas, 3 September 1991.

Pruitt BA Jr: The organization and delivery of burn care for Operation Desert Storm. Presented at the annual meeting of the Halsted Society, High Hampton, Virginia, 4 September 1991.

Becker WK: Burn care in Saudi Arabia. Presented at the Kirov Traumatology Hospital, Kirov, USSR, 6 September 1991.

Becker WK: Burn care in Saudi Arabia. Presented at the Kirov Medical Institute, Kirov, USSR, 6 September 1991.

Stetz CJ: Burn care in the 90s. Presented to the Texas Association of Home Health Agencies, San Antonio, Texas, 11 September 1991.

Molter NC: Overview of nursing at US Army Institute of Surgical Research. Presented as part of the AMEDD Officer Basic Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 24 September 1991.

Barrett J: Emergency burn care and hazardous materials. Presented as part of the Emergency Medical Technician Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 4 October 1991.

Pruitt BA Jr: The care and closure of the burn wound. Presented to the Department of Surgery, Duke University Medical Center, Durham, North Carolina, 8 October 1991.

Pruitt BA Jr: Transfer of mass burn casualties. Presented at the International Congress on the Management of Mass Burn Casualties, Antwerp, Belgium, 13 October 1991.

Milner EA: Nutrition and the burn patient. Presented to burn victims families, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 15 October 1991.

Pruitt BA Jr: Personal view on the management of mass burn casualties. Presented at the International Congress on the Management of Mass Burn Casualties, Antwerp, Belgium, 15 October 1991.

Stetz CJ: Burn wound care. Presented at the Nursing Student Recruiting Session, Lewis University, Romeoville, Illinois, 22 October 1991.

Stetz CJ: Initial management of the burn trauma patient. Presented at the Nursing Student Recruiting Session, Lewis University, Romeoville, Illinois, 22 October 1991.

Black CI: Perioperative care of the burn victim. Presented as part of the families of burn victims, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 23 October 1991.

Driscoll DM: Compassion knows no boundaries – USSR burn mission). Presented at the University of San Francisco, San Francisco, California, 29 October 1991.

Driscoll DM: Latest trends in burn care. Presented at the University of San Francisco, San Francisco, California, 29 October 1991.

Driscoll DM: Nursing burn care: humanitarian mission to Russia. Presented at San Jose State University, San Francisco, California, 29 October 1991.

Driscoll DM: Fluid resuscitation in burn wound management. Presented to the University of California at San Francisco, San Francisco, California, 30 October 1991.

Driscoll DM: Latest trends in burn care – humanitarian mission to Russia. Presented at the Samuel Merritt School of Nursing, San Francisco, California, 31 October 1991.

Driscoll DM: Nursing burn care – humanitarian mission to Russia. Presented at California State University at Hayward, San Francisco, California, 31 October 1991.

Driscoll DM: Overview of burn care infection control issues. Presented to California State University at Sacramento, San Francisco, California, 31 October 1991.

Driscoll DM: Initial management of the burn victim. Presented as part of the Intensive Care Unit Nursing Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 1 November 1991.

Driscoll DM: Nursing burn care. Presented at California State University at Sacramento, San Francisco, California, 1 November 1991.

Pruitt BA Jr: Fluid resuscitation of injured man. Presented at the 39th Annual Detroit Trauma Symposium, Department of Surgery, Wayne State University, Detroit, Michigan, 1 November 1991.

Pruitt BA Jr: Opportunistic infections in injured man. Presented at the 39th Annual Detroit Trauma Symposium, Department of Surgery, Wayne State University, Detroit, Michigan, 2 November 1991.

Driscoll DM: Learn not to burn. Presented at LaSoya Elementary School, San Antonio, Texas, 5 November 1991.

McManus WF: Resuscitation and metabolic support of the burn patient. Presented at the Trauma Symposium, William Beaumont Army Medical Center, Fort Bliss, El Paso, Texas, 14 November 1991.

Driscoll DM: Burn care in Russia: an observation. Presented at the 10th Annual Trauma Symposium, El Paso, Texas, 15 November 1991.

Kelly KM: Aeromedical evacuation of the burn patient. Presented to the 57th Aeromedical Evacuation Squadron, Scott Air Force Base, Illinois, 20 November 1991.

Kelly KM: Burn wound management. Presented to the 57th Aeromedical Evacuation Squadron, Scott Air Force Base, Illinois, 20 November 1991.

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ABSTRACT

PROJECT NUMBER: 3M162787A874-164, Applied Research and Exploratory Development

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

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

PERIOD COVERED IN THIS REPORT: 1 January 1991 - 31 December 1991

INVESTIGATORS: John G. Thomas, MD, Major, MC
William F. McManus, MD, Colonel, MC
Basil A. Pruitt, Jr., MD, Colonel, MC

During the period of this report, 148 patients were anesthetized a total of 409 times, an average of 2.8 times per patient. The most commonly used anesthetic agent was narcotics (95%) followed by nitrous oxide (75%) and isoflurane (66%).

ANESTHESIOLOGY

PREOPERATIVE PROCEDURES

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

Preparation. All patients are placed on NPO status after 2400 h the day prior to surgery with the exception of children, who may receive clear liquids up to 5 h prior to surgery, and infants, who may receive clear liquids up to 3 h prior to surgery. Any patient with an enteral feeding tube, the proximal end of which is shown to be beyond the ligament of Treitz, may have tube feedings continued perioperatively.

Premedication. Routine medications, such as cimetidine, sucralfate, or cardiovascular medications, are continued up to the time of surgery. Benzodiazepines, such as diazepam, are routinely given as premedicants for patients on a PO diet. Morphine sulfate or midazolam hydrochloride is often given as a premedicant for ICU patients. Atropine (20 µg/kg IV) is given routinely to pediatric patients under the age of 1 yr immediately prior to induction of anesthesia. Glycopyrrolate, from 0.005 mg/kg to a maximum dose of 0.2 mg/kg, is given intravenously immediately prior to induction with ketamine.

Fluids. All fluids, except hyperalimentation solutions, are changed to lactated Ringer's or lactated Ringer's with 5% glucose upon arrival in the operating room. Plasmalyte® is used as a packed RBC diluent; however, its use is kept to a minimum to avoid sodium loading.

TYPES OF ANESTHESIA

At this Institute, narcotics, including fentanyl citrate and sufentanil citrate, are the most frequently used anesthetic agents, most often in combination with nitrous oxide and isoflurane. Halothane and ketamine are also used, but to a lesser extent (see Table 1). Enflurane was not used.

TABLE 1. Pattern of Anesthesia Administration (1988-91)

Agent	1988		1989		1990		1991	
	Number	%	Number	%	Number	%	Number	%
Enflurane	23	4.9	3	0.6	-	-	-	-
Halothane	7	1.5	12	2.4	12	2.8	35	8.6
Isoflurane	278	59.5	167	33.9	80	18.3	271	66.3
Ketamine hydrochloride	35	7.5	36	7.3	21	4.8	37	9.1
Local	9	1.9	6	1.2	9	2.1	17	4.2
Narcotics	114	24.5	269	54.7	387	88.8	388	94.9
Nitrous oxide	1	0.2	1	0.2	94	21.6	308	75.3
Regional	-	-	-	-	-	-	14	3.4
TOTAL	**467**	**100.0**	**493**	**100.0**	*	*	*	-

*Two separate methods were used to enter data into the data base. The previous anesthesiologist entered only the primary agent used for a particular case whereas MAJ Thomas entered the combination of main agents used. This accounts for the dramatic increase in nitrous oxide use from 1989 to 1991.

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

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

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

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

Halothane. Halothane is an halogenated alkane that has been relatively little used during recent years. Biotransformation can account for as much as 25% of an inhaled dose. Halothane hepatitis, although rare, fortunately has not been reported in burned patients. Since the successful introduction of enflurane and isoflurane, few indications for halothane's use exist in this patient population that may be predisposed to hepatitis from

multiple transfusions with blood products. Halothane is much less pungent and causes a more rapid anesthetic induction than enflurane or isoflurane. As a result, its use is indicated primarily in the burned pediatric patient who requires that his airway be secured by an endotracheal tube following a smooth, rapid induction of anesthesia.

Regional Anesthesia. Although regional anesthesia is generally considered one of the safest methods available, its use in the thermally injured patient is limited for 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.

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

Muscle Relaxants. Succinylcholine was not used during this reporting period. The risk of hyperkalemia following succinylcholine contraindicates it use in the great majority of thermally injured patients. On the other hand, nondepolarizing muscle relaxants (vecuronium bromide, pancuronium bromide, and atracurium besylate) were used in 94% of the operative cases over the past year.

MONITORING TECHNIQUES

Adequate Oxygenation. Monitoring included inspired and expired oxygen concentration (in-circuit oxygen analyzer and gas sampling oxygen analyzer), arterial hemoglobin oxygen saturation (pulse oximeter), and patient's color.

Adequate Ventilation. Monitoring included respiratory rate, chest excursions, auscultation of breath sounds (esophageal and precordial stethoscopes), end-tidal carbon dioxide concentration (continuous capnometer and mass spectrometer which was replaced by a more modern infrared gas analyzer), pulmonary function parameters (Siemens™ ventilator), and arterial blood gases (if indicated).

The noninvasive measurement of end-tidal carbon dioxide, arterial hemoglobin oxygen saturation by pulse oximetry, and pulmonary function parameters, e.g., tidal volume and peak inspiratory pressure, all represent no risk to the patient, are easily obtainable, and are accurate. These monitors have become standard in our anesthetic care of the burned patient.

Hemodynamic Stability. Monitoring included continuous EKG, auscultation of heart sounds (precordial and esophageal stethoscopes), peripheral pulse, arterial blood pressure, central venous and wedge pressures (if indicated), cardiac output (if indicated), systemic vascular resistance (if indicated), serial hematocrits, and urine output.

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

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

Body Temperature. Skin, rectal, nasopharyngeal, or esophageal temperatures are continually monitored. Because of the greatly increased evaporative losses in burned patients, hypothermia can be a serious problem. Several methods were employed to maintain body temperature during anesthesia. Ambient temperatures were maintained between 85°F and 95°F. Anesthetic gases were heated and humidified and radiant heat lamps were used when necessary. Scrub solutions, intravenous fluids, and blood products were all warmed prior to use.

RESULTS

Complications. There were no anesthesia complications noted during this reporting period.

Patient Data. Tables 2 and 3 provide overall anesthetic patient data.

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

PRESENTATIONS/PUBLICATIONS

None.

TABLE 2. Use of Selected Intraoperative Monitors* (1989–91)

Monitor/Parameter	1989		1990		1991	
	Number	%	Number	%	Number	%
Inspired oxygen concentration	493	100.0	430	98.6	397	97.1
Temperature	485	98.4	429	98.4	399	97.6
Pulse oximeter (hemoglobin saturation)	488	99.0	435	99.8	409	100.0
End-tidal carbon dioxide	473	95.9	415	95.2	397	97.1
Pulmonary function	461	93.5	403	92.4	391	96.8
Arterial line	124	25.1	147	33.7	162	39.6
Swan-Ganz catheter	42	8.5	51	11.7	40	9.8
Central venous pressure	21	4.3	54	12.4	45	11.0

*Blood pressure and heart rate and rhythm are monitored intraoperatively for every patient. In some patients with toxic epidermal necrolysis, the heart rate and rhythm are ascertained from the blood pressure trace from a sterile arterial line.

TABLE 3. Overall Anesthetic Patient Data (1971-91)

Year	Number of Patients	Number of Patients Anesthetized	% of All Patients	Number of Times Anesthetized	Number of Times Anesthetized Per Patient
1991	218	148	67.9	409	2.8
1990	216	159	71.6	436	2.7
1989	218	172	78.9	493	2.9
1988	223	161	72.2	467	2.9
1987	221	179	81.0	463	2.6
1986	207	143	69.1	410	2.9
1985	197	133	67.5	388	2.9
1984	190	139	73.2	461	3.3
1983	179	98	54.8	291	3.0
1982	231	151	65.4	532	3.5
1981	208	127	61.1	404	3.2
1980	243	148	60.9	531	3.6
1979	267	161	60.3	554	3.4
1978	268	151	56.3	435	2.9
1977	242	129	53.3	344	2.7
1976	277	139	50.2	476	3.4
1975	254	142	55.9	490	3.5
1974	226	123	54.4	380	3.1
1973	273	141	51.7	377	2.7
1972	301	183	60.8	575	3.1
1971	301	179	59.5	475	2.7

TABLE 4. Recent Trends in Operative Procedures (1987-91)

Procedure	1987		1988		1989		1990		1991	
	Number	%	Number	%	Number	%	Number	%	Number	%
Autograft	389	43.8	395	42.7	424	43.6	385	45.3	342	43.7
Chondrectomy	5	0.6	2	0.2	6	0.6	5	0.5	-	-
Excision	397	44.7	421	45.6	453	46.6	398	46.8	376	48.1
Eye and lid	9	1.0	11	1.2	1	0.1	1	0.1	17	2.2
Intra-abdominal	7	0.8	6	0.6	17	1.7	5	0.5	5	0.6
Orthopedic	27	3.0	36	3.9	25	2.6	23	2.7	16	2.0
Plastic	5	0.6	12	1.3	11	1.1	21	2.5	5	0.6
Other	50	5.6	41	4.4	36	3.7	12	1.4	21	2.7
TOTAL	**889**	**100.0**	**924**	**100.0**	**973**	**100.0**	**850**	**100.0**	**782**	**100.0**

RESEARCH AND TECHNOLOGY WORK SUMMARY

ACCESSION: DA335682

SUMMARY DATE: 921001 **SUMMARY KIND:** D **PREV DATE:** 911001 **DISTRIBUTION:** CX

PROGRAM #: 62787A **PROJ #:** 30162787A874 **TASK AREA:** BN **WORK UNIT:** 161

TITLE: Effect of Nutritional Support on Immune Function in Patients with Thermal Injury - A Component Study

SUBJ1: 060500 - Medicine and Medical Research
SUBJ2: 060800 - Food, Food Service, and Nutrition

START DATE: 9010 **END DATE:** 9309 **PERFORMANCE METHOD:** C

CONTRACT/GRANT NO:

CONT TOTAL: $
CUM TOTAL: $
TOTAL LAB FUNDS: $

RESOURCES ESTIMATE

FY	WORK YRS	$(Thousands)
91	0.5	$27
92	0.5	$36
93	0.5	$38

RESPONSIBLE DOD ORGANIZATION: 404885
MRDC INSTITUTE OF SURGICAL RESEARCH USAISR
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FOREIGN INTELLIGENCE CONSIDERED: Y **CIVILIAN APPLICABILITY:** M

KEYWORDS: RAII; Volunteers; Adults; Burns (Injuries); Nutrients; Nutrition; Hypermetabolism; Immunosuppression; Therapy

OBJECTIVE: A DTIC literature search was conducted under DTIC request numbers W6R09K/W6R10L dated 9 October 1990. The objective of this work is to evaluate the effect of variations in amino acid structure and omega-3 and omega-6 lipid content in the diet of nutritional indices as well as cell structure and immune function in thermally injured patients. Improved nutritional support will enhance the outcome and decrease the morbidity of such patients.

APPROACH: This prospective, double-blind study will randomize patients to receive one of two solutions as their enteral support product. Blood and urine samples will be collected on days 0 (preinfusion), 5, 10, 15, and 21 to determine plasma amino acid levels and polyunsaturated fatty acids and urinary polyamines and orotic acid. The patient's weight, nitrogen balance (Waxman's formula), VO_2, VCO_2, resting energy expenditure, CBC, platelet count, electrolytes, Ca, Mg, and phosphorus data will be recorded from the clinical records. Patients with inhalation injury will be analyzed separately. Differences between the treatment groups will be determined by ANOVA. The end point of a positive study outcome will be a statistically significant increase in lymphocyte function, i.e., mitogen stimulation of lymphocye activity. It is not expected that significant differences in patient outcome will occur; however, these variables will be monitored in case such a difference should occur.

PROGRESS: 9110-9209. The order of availability of products for tube feedings changed. An addendum reflecting this change was

submitted and approved during this reporting period. The initial tube feeding product was recently received from the manufacturer and patient enrollment will begin shortly. For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report* for fiscal years 1991 and 1992.

ABSTRACT

PROJECT NUMBER: 3M162787A874-161, Applied Research and Exploratory Development

PROJECT TITLE: Effect of Nutritional Support on Immune Function in Patients with Thermal Injury - A Component Study

INSTITUTION: US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas 78234-5012,[1] and Walter Reed Army Institute of Research, Washington, DC 20307[2]

PERIOD COVERED IN THIS REPORT: 15 October 1991 - 30 September 1992

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Basil A. Pruitt, Jr, MD, Colonel, MC_1

Patients with thermal injury are known to have defects in immune function. Infection is the leading cause of death in this patient population. Nutritional support, most often by the enteral route, is a standard management tool in burn patients. If it can be demonstrated that components of enteral nutrition products augment immune function in burn patients, it may be possible to reduce the high incidence of infectious complications in this group of patients.

The objective of this study is to compare the efficacy of various nutritional components specifically designed to promote immune function in hypermetabolic patients.

EFFECT OF NUTRITIONAL SUPPORT ON IMMUNE FUNCTION IN PATIENTS WITH THERMAL INJURY - A COMPONENT STUDY

Nutrition support (support beyond spontaneous oral intake) is a frequent treatment modality in burn patients. The increased energy expenditure associated with cutaneous thermal injury, along with increased protein catabolism and nitrogen excretion, are well documented (1). Nutrition support in the burn patient is generally accepted as beneficial; however, strong evidence to document a salutary effect associated with nutrition support is lacking.

Infectious complications are frequent in burn patients and are the leading cause of death (2). Cutaneous thermal injury is also associated with defects in immune function, predominantly cellular immunity (3). It has been suggested that the composition of the nutritional prescription may be important in supporting cellular immunity. Based on this suggestion, new nutrition products which purport to have a beneficial effect on immune function when compared to standard nutrition support products have been developed. Specific nutrients which have been demonstrated to affect lymphocyte function include arginine, polyunsaturated fatty acids, and purines/pyrimidines (4).

Enteral nutrition products containing these nutrients have, in preliminary experimental and clinical trials, been found to achieve some of the goals they were designed to meet (5). It is still unclear whether they truly have a measurable beneficial clinical effect in hypermetabolic patients.

The objective of this study is to compare the efficacy of various nutritional components specifically designed to promote immune function in hypermetabolic patients.

MATERIALS AND METHODS

Study Design. This clinical protocol will be a randomized, prospective, double-blind trial of enteral nutrition products containing components thought to promote immune function in burn patients requiring enteral nutritional support. Entry will be controlled for burn size, predicted mortality, and for the presence of inhalation injury. Patients eligible to enter this trial will be randomized to receive one of two solutions as their enteral support product. Once entered into the trial, the research dietician will randomly pull a card from a file containing an equal distribution of the two products. The research dietician will be the only member of the research team aware of this result and will also be responsible for preparation and distribution of the product to the nursing personnel responsible for the patient. The tube feeding products will be colored by the addition of a food coloring dye so that they are indistinguishable from each other. The use of such dyes is standard practice at this Institute. The compositions

of the tube feeding products in grams per 1,500 ml are listed below. The solutions are isocaloric and isonitrogenous. This study will initially evaluate Impact® and Solution D (see Table 1). If warranted, additional studies based on the other solutions listed will be proposed. Solutions will be obtained from Sandoz Nutrition (Minneapolis MN).

Table 1. Composition of Solutions (g/1,500 ml)

		Solution			
	Impact®	A	B	C	D*
Intact protein	65	65	65	65	65
Arginine	18.75	18.75	18.75	–	–
Glycine	–	–	–	32.32	32.32
Fish oil	16.65	16.65	–	16.65	–
Structured lipid	25	25	–	25.0	–
Corn oil	–	–	34	–	34
MCT oil	–	–	7.65	–	7.65
RNA	2.256	–	2.25	–	–
CHO (maltodextran)	198	198	198	184.43	184.43

*Isocaloric/isonitrogenous control solution.

Description of Procedures. All feeding will be performed through enteral feeding tubes placed fluoroscopically in the distal duodenum or proximal jejunum. Feeding will begin between 48 and 96 h postburn. Tube feeding will begin at 25 cc/h full strength and will be advanced to the amount and strength necessary to meet 100% of predicted energy requirements using the Institute's standard formula. Advancement will be performed at a rate of 25 cc/h every 12 h. Tube feeding will not be discontinued for operative procedures. The clinician responsible for the patient may decrease or temporarily discontinue for the following reasons:

1. Diarrhea, defined as ≥ 5 liquid stools or a total stool volume of > 1,200 g/day.

2. Evidence of tube dysfunction or malposition.

3. Evidence of reflux of tube feeding into stomach or documented aspiration of tube feeding solution.

4. Abdominal distension associated with abdominal pain (cramps).

5. Any intraabdominal condition that contraindicates use of the gut as the route of nutritional support.

6. Glucose intolerance not responsive to standard insulin therapy. Tube feeding will not be increased beyond the calculated maximal rate to provide 100% support. If it is the opinion of the clinician that support at level of > 100% of estimated requirements is necessary, the patient will be withdrawn from the study.

With the exception of the research dietician, personnel involved in this study and in the patient's care will be unaware of which product is being used. Data concerning the total volume intake of product (hourly and daily), carbohydrate infusion rate (mg/kg/min), and percent of estimated caloric requirements (daily) will be available to the clinician, as it is considered essential to manage the patient safely. It will not be possible from this information alone to break the blinded nature of this study.

The following data will be collected and recorded on days 0 (preinfusion), 5, 10, 15, and 20:

1. Weight.

2. Nitrogen balance (Waxman's Formula).

3. VO_2, VCO_2, and REE by indirect calorimetry.

4. CBC and platelet count.

5. Electrolytes, Ca, Mg, and phosphorus.

6. BUN.

7. Creatinine.

8. Glucose, albumin, bilirubin, SGOT, and alkaline phosphatase.

*9. Plasma amino acid levels and polyunsaturated fatty acids and urinary polyamines and orotic acid.

*10. Prealbumin.

*11. T-cell surface markers.

*12. WBC, differentiated.

*13. EA rosetting (to be performed by COL Wright) and granulocyte surface antigens CR1 and CR3.

*14. MOA63108, CD 11/18, and ICAM by FACS (PMNs).

*Indicates experimental procedures. The remainder are routine care items.

In addition to the these studies, the following data will be collected. Importance will be assessed to insure comparability of study groups.

1. Preburn weight.
2. Age.
3. Sex.
4. Total body surface area burn size.
5. Presence or absence of inhalation injury.
6. Daily weight, unless clinically contraindicated.
7. Weekly nitrogen balance.
8. Weight at discharge.
9. Weekly prealbumin and results of liver function tests.
10. Percent estimated requirements met.

During the initial 21-day study period, no other exogenous nutritional support will be given, with the exception of D5W or D5W electrolyte solutions needed to maintain fluid balance. If during this period it is felt that other exogenous support is necessary, the patient will be withdrawn from the study. After 21 days, other types of support can be added at the discretion of the responsible clinician. During this time period, the patient will continue to receive only the study product as an enteral supplement if a combination of oral and tube feedings is felt necessary. The patient's active participation in the study will end when the patient is felt to no longer require tube feedings and the tube is removed.

Infectious complications will be recorded and classified as follows:

1. Septicemia - Documented only by positive blood cultures.

2. Tracheobronchitis - Defined as > 25 WBC/hpf and < 5 epithelial cells per high power field on tracheal aspirate and the presence of a predominant organism on culture.

3. Pneumonia - Defined as localized infiltrate on chest x-ray, fever > 102.5°F, and sputum leukocytosis.

4. Wound infection - Histopathologically documented burn wound invasion.

5. Urinary tract infection - > 10^5 organisms/ml on catheterized or clean-catch specimen.

6. Miscellaneous infections - Sinusitis, meningitis, phlebitis, etc.

The incidence of infection will be as described in the monthly infection report as prepared by the US Army Institute of Surgical Research Infection Control Committee. Cause of death and autopsy results and cultures, if applicable, will also be recorded.

Patient Criteria. Sixty patients admitted to the US Army Institute of Surgical Research will be enrolled in this study. Properly signed and witnessed DA Forms 5303-R, Volunteer Agreement Affidavit, will be obtained from each patient prior to beginning the study.

Patient Inclusion. Patients meeting the following criteria may be enrolled in the study upon giving written informed consent:

1. Male or female patients $\geq$ 18 yr. Female patients must have been surgically sterilized, be postmenopausal (> 45 yr and the lack of menstrual periods for > 1 yr), or have a negative pregnancy test prior to initiation into the study.

2. Patients with burn sizes > 20% of the total body surface area. The presence of an inhalation injury will not be exclusionary but those patients with inhalation injury will be separately randomized from those without such injury. Inhalation injury will be diagnosed by either a positive bronchoscopy or ^{133}Xenon lung scan.

Patient Exclusion. Patients meeting any of the following criteria will be excluded from participation in this study:

1. Patients < 18 yr.

2. Patients who are pregnant or nursing.

3. Patients with an injury as a result of an electrical burn or toxic epidermal necrolysis.

Determination of Number of Subjects Required. Using an expected 50% difference in the results of PBMC stimulation between the two treatment groups as an index and a power of 60% with a 0.05 significance level, it is estimated that 15 patients per group are

required. There will be 4 groups (2 = no inhalation injury, 2 = inhalation injury) for a total of 60 patients.

Data Analysis Plan. Inhalation and noninhalation groups will be analyzed separately. Differences between treatment groups will be determined by ANOVA. The endpoint to determine a positive study outcome will be a statistically significant increase in lymphocyte function, i.e., ConA stimulation of MLR activity. It is not expected that significant differences in patient outcome variables will occur; however, these variables will be monitored in case such a difference should occur.

RESULTS

The order of availability of products for the tube feedings changed. Therefore, an addendum reflecting this change was submitted and approved during this reporting period. The initial tube feeding product was recently received from the manufacturer and patient enrollment will begin shortly.

DISCUSSION

When the projected total of 60 patients have completed the study, the data will be analyzed as to the efficacy of various nutritional components specifically designed to promote immune function in hypermetabolic patients.

PRESENTATIONS/PUBLICATIONS

None.

REFERENCES

1. Wilmore DW, Long JM, Mason AD Jr, et al: Catecholamines: mediator of the hypermetabolic response to thermal injury. *Ann Surg* 180:653–69, 1974.

2. Pruitt BA Jr: The diagnosis and treatment of infection in the burn patient. *Burns Incl Therm Inj* 11:79–91, 1984.

3. Kupper TS, Green DR, Durum SK, Baker CC: Defective antigen presentation to a cloned T helper cell by macrophages from burned mice can be isolated by interleukin-1. *Surgery* 98:199–206, 1985.

4. Rudolph FB, Kulkarni AD, Fanslow WC, et al: Role of RNA as a dietary source of pyrimidines and purines in immune function. *Nutrition* 6:45–52, 1990.

5. Bower RH: A unique enteral formula as adjunctive therapy for septic and critically ill patients. Multicenter study – design and rationale. *Nutrition* 6:92–5, 1990.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

ACCESSION: DAOG6971

SUMMARY DATE: 921001 **SUMMARY KIND:** D **PREV DATE:** 911001 **DISTRIBUTION:** CX

PROGRAM #: 62787A **PROJ #:** 30162787A874 **TASK AREA:** EA **WORK UNIT:** 162

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

SUBJ1: 060500 - Medicine and Medical Research
SUBJ2: 061300 - Microbiology
SUBJ3: 061500 - Pharmacology

START DATE: 7610 **END DATE:** 9909 **PERFORMANCE METHOD:** C

CONTRACT/GRANT NO:

CONT TOTAL: $
CUM TOTAL: $
TOTAL LAB FUNDS: $

RESOURCES ESTIMATE

FY	WORK YRS	$(Thousands)
91	0.5	$185
92	0.5	$ 50
93	0.5	$195

RESPONSIBLE DOD ORGANIZATION: 404885
MRDC INSTITUTE OF SURGICAL RESEARCH USAISR
FT SAM HOUSTON, TX 78234
PRUITT, B A
210-221-2720

PERFORMING ORGANIZATION: 404885
MRDC INSTITUTE OF SURGICAL RESEARCH USAISR
FT SAM HOUSTON, TX 78234
FITZPATRICK, J C
210-221-8440

ASSOC1:

ASSOC2:

FOREIGN INTELLIGENCE CONSIDERED: Y **CIVILIAN APPLICABILITY:** M

KEYWORDS: RA II; Volunteers; Adults; Children; Burns (Injuries); Wounds and Injuries; Skin Grafts; Therapy

OBJECTIVE: A DTIC literature search was conducted under DTIC request numbers W6R17I/W6B23L dated 19 October 1989. The objectives of this work are to evaluate the efficacy of 5% aqueous mafenide acetate-soaked dressings, employed either for final debridement of burn wounds or following application of meshed cutaneous autograft to prevent infection and desiccation of the tissue exposed in the interstices of such grafts.

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

PROGRESS: 9110-9209. One hundred and fifty-eight patients were treated with 5% aqueous mafenide acetate soaks. Eleven of these patients exhibited mild cutaneous atopy. This low incidence of mild side effects of 5% aqueous mafenide acetate and its continued clinical effectiveness speak for the continued use of this valuable therapeutic agent. For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report* for fiscal years 1977 through 1992.

ABSTRACT

PROJECT NUMBER: 3M162787A874-162, Applied Research and Exploratory Development

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

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

PERIOD COVERED IN THIS REPORT: 1 October 1991 - 30 September 1992

INVESTIGATORS: John C. Fitzpatrick, MD, Major, MC
Basil A. Pruitt, Jr, MD, Colonel, MC

During this reporting period, 5% aqueous mafenide acetate dressings have continued to be efficacious in the care of the burn wound. One hundred and fifty-eight patients were treated with 5% aqueous mafenide acetate dressings, employed either for final debridement of a wound or following application of meshed cutaneous autografts to prevent desiccation of tissue exposed in the interstices of such grafts. A 6.9% incidence of skin rash (atopy) was noted as the only adverse reaction. The clinical results achieved by the use of 5% aqueous mafenide acetate solution strongly support its continued use.

5% AQUEOUS SULFAMYLON® SOAKS USED IN TOPICAL TREATMENT OF BURNED PATIENTS

During this reporting period, the evaluation of 5% aqueous mafenide acetate solution for topical treatment of the burn wound has continued at this Institute where it was used for 158 of 237 patients (66.7%). The 5% aqueous mafenide acetate-soaked dressings are used as wet-to-dry dressings to debride nonviable tissue elements in preparation for split-thickness autograft procedures or as continuous wet dressings to protect freshly excised wounds that are not autografted. In addition, when meshed cutaneous autografts are applied, dressings are soaked with 5% aqueous mafenide acetate solution to decrease the rate of bacterial growth and to prevent desiccation of tissue exposed in the interstices of such grafts.

Eleven patients (6.9%) demonstrated allergic reactions (atopy) with the use of 5% aqueous mafenide acetate solution and these patients demonstrated rapid resolution of the atopic reaction following administration of an antihistamine and/or discontinuation of the 5% aqueous mafenide acetate-soaked dressings. Saline or other aqueous topical antimicrobial agents were substituted when 5% aqueous mafenide acetate-soaked dressings were discontinued and no other adverse reactions were noted in this group of patients.

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

PRESENTATIONS/PUBLICATIONS

None.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

ACCESSION: DAOG6970

SUMMARY DATE: 921001 **SUMMARY KIND:** D **PREV DATE:** 911001 **DISTRIBUTION:** CX

PROGRAM #: 62787A **PROJ #:** 30162787A874 **TASK AREA:** BG **WORK UNIT:** 163

TITLE: Neuroendocrine Assessment of Burned Patients

SUBJ1: 060500 - Medicine and Medical Research
SUBJ2: 061500 - Pharmacology

START DATE: 7910 **END DATE:** 9909 **PERFORMANCE METHOD:** C

CONTRACT/GRANT NO:

CONT TOTAL: $
CUM TOTAL: $
TOTAL LAB FUNDS: $

RESOURCES ESTIMATE

FY	WORK YRS	$(Thousands)
91	0.5	$194
92	0.5	$ 36
93	0.5	$ 30

RESPONSIBLE DOD ORGANIZATION: 404885
MRDC INSTITUTE OF SURGICAL RESEARCH USAISR
FT SAM HOUSTON, TX 78234
PRUITT, B A
210-221-2720

PERFORMING ORGANIZATION: 404885
MRDC INSTITUTE OF SURGICAL RESEARCH USAISR
FT SAM HOUSTON, TX 78234
VAUGHAN, G M
210-221-5416

ASSOC1:

ASSOC2:

FOREIGN INTELLIGENCE CONSIDERED: Y **CIVILIAN APPLICABILITY:** M

KEYWORDS: RA II; Volunteers; Adults; Lab Animals; Hamsters; Rats; Burns (Injuries); Pineal Gland; Catecholamines; Indoles

OBJECTIVE: A DTIC literature search was conducted under DTIC request numbers W6R11I/W6R13L dated 19 October 1989. The objective of this work is to characterize alterations of neuroendocrine function in burned patients in order to improve survival.

APPROACH: Photic control of the melatonin rhythm will be studied and the findings related to the sympathetic unresponsiveness of terminal critical injury.

PROGRESS: 9110-9209. In order to investigate the pineal model of sympathetic responsiveness, it will be necessary to assess circulating levels of melatonin (MEL), both during the day and night. Previous MEL assays, all "first generation", are still unable to assess the low daytime serum MEL. Presently, we have developed the first "second generation" assay using the G280 antibody of Kennaway. With iodo-MEL tracer and a precipitating antibody-carrier system, we have lowered the sample volume to 0.2 ml, the analytic least detectable to 0.25 pg/ml, and the functional least detectable to 0.6 pg/ml. In spite of chloroform extraction, assay recovery is 100%. All values in a patient with no pineal gland are < 0.25 pg/ml and propranolol blocked the nocturnal surge of serum MEL in hamsters. In hamsters, isoproternol (ISO, single injection) responsiveness of serum MEL extends into the early light phase and exhibits the same pattern of response seen in pineal MEL content. Morning responsiveness deteriorates greatly by 4 h into light, though not as an acute result of the presence of light, suggesting entrainment (not gating) of beta-adrenergic responsiveness into the dark and early

light part of the cycle. The normal fall of the endogenous sympathetic-drive nocturnal serum MEL surge to daytime MEL levels is also acutely independent of the onset of light, but occurs just before the expected onset of light. The mechanisms for failure of sympathetic responsiveness can now be addressed with this assay. For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report* for fiscal years 1980 through 1992.

ABSTRACT

PROJECT NUMBER: 3M162787A874-163, Applied Research and Exploratory Development

PROJECT TITLE: NEUROENDOCRINE ASSESSMENT OF THE BURNED PATIENT: Validation of a New Sensitive Serum Melatonin Radioimmunoassay (RIA) Employing the Kennaway G280 Antibody - Syrian Hamster Morning Adrenergic Response

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

PERIOD COVERED IN THIS REPORT: 1 October 1991 - 30 September 1992

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

A new procedure with the G280 antibody of Kennaway provides an assay for circulating melatonin (aMT) with a sample volume (200 µl), an analytic (0.33 pg/ml) and functional (0.62-0.80 pg/ml) detectability, a 50% displacement dose (6.4 pg/ml), a K_d (0.657 pM), and measured circulating daytime levels lower than reported for previous procedures, and 100% assay recovery. The normal daytime range in adult human and Syrian hamster serum was 0.4 to 4 pg/ml. The pattern of fall of the nocturnal surge of Syrian hamster serum aMT near the time of lights-on was unaltered by extended darkness. Isoproterenol (ISO) injection 1 h after lights-on, when aMT had reached daytime levels, raised serum and pineal aMT dramatically 2 h postinjection. The same dose of ISO injected 4 h into light produced only a small detectable increase. Novel extension of nocturnal darkness did not affect the responses to ISO. Thus, when they are allowed to occur at the usual time on a 10-h dark schedule, both the fall from the nocturnal aMT surge and the subsequent loss of pineal beta-adrenergic responsiveness in this species occur endogenously (probably entrained) rather than from gating by acute effects of morning light. Changes in daytime serum aMT consistent with concomitant changes in the pineal can be measured with a sufficiently sensitive RIA.

VALIDATION OF A NEW SENSITIVE SERUM MELATONIN RADIOIMMUNOASSAY (RIA) EMPLOYING THE KENNAWAY G280 ANTIBODY - SYRIAN HAMSTER MORNING ADRENERGIC RESPONSE

Lack of generalized catecholamine responsiveness characterizes the preterminal condition of nonsurviving burned and other critically ill patients with multiorgan failure. We have been interested in characterizing melatonin (acetylmethoxytryptamine) secretion in an animal model (Syrian hamster) which exhibits a normal daytime fall of catecholamine responsiveness in the pineal gland similar to that in normal humans (1,2). In both species, beta-adrenergic activity stimulates melatonin (aMT) secretion at night.

Acute exposure of Syrian hamsters in vivo or of their pineals in vitro to either the transmitter norepinephrine (NE) in the presence of a blocker of protective reuptake or to the beta-agonist isoproterenol (ISO), which does not require prevention of uptake, raised pineal aMT content (in vivo) or aMT secretion from pineals into the medium (in vitro) when the agonist was applied one time during the second half of the 10-h dark phase, but not during the light phase (3-5). We have reported (4) that a single intraperitoneal injection of 1 µg/g NE (after reuptake blockade) in this species failed to stimulate pineal aMT content when given 2 h into the light phase. However, pineals taken 3/4 h into the light phase responded to NE in the incubation medium. NE-induced aMT secretion into the medium was markedly diminished in pineals taken 2-3/4 h into light (3).

In order to assess the Syrian hamster pineal beta-adrenergic response in the early morning, 1 µg/g ISO, which is effective at night (5) and does not require the complication of reuptake blockade, was injected subcutaneously. Because we wanted to observe daytime circulating aMT, which is too low to be assessed with current aMT assays, we developed a new RIA procedure, using the previously reported G280 antibody of Kennaway, to achieve sufficient sensitivity.

MATERIALS AND METHODS

Assay of Serum aMT. G280 antibody was supplied by D. Kennaway (Department of Obstetrics and Gynecology, University of Adelaide, Adelaide, South Australia). Its specificity profile and use for RIA of circulating aMT have been reported (6,7). That procedure involved a sample volume of 0.5 ml extracted with dichloromethane/hexane in the presence of borate buffer, an assay buffer containing bovine albumin and globulin at pH 7.4, ^{3}H-aMT tracer (specific activity 87 Ci/mmol), final G280 antibody dilution of 1:480,000, final reaction volume of 0.8 ml, incubation for 15 min at 37°C, immersion in an ice bath for 1 h or more, and charcoal separation of the bound and free tracer. Instead (see Table 1), we

TABLE 1. G280 Assay Procedure

1.	Extract 200 μl melatonin standards (triplicates from < 1 to 100 pg/ml in buffer*) and samples (duplicates) with 2 ml $CHCl_3$ in 12 x 75-mm glass tubes (VCA).
2.	Wash $CHCl_3$ with 200 μl 0.1 N NaOH (VCA).
3.	Wash $CHCl_3$ with 200 μl H_2O (VCA).
4.	Add 200 μl H_2O (only centrifuge and aspirate).
5.	Evaporate $CHCl_3$ at room temperature in a vacuum centrifuge (1-2 h), replenish with N_2.
6.	Elute overnight with 500 μl buffer at 4°C after vortexing.
7.	Wash eluate with 2 ml petroleum ether - vortex, let settle at 4°C 1 h, aspirate organic phase.
8.	Vortex eluate, let organic traces evaporate 1 h at 4°C.
9.	Transfer 400 μl eluate to assay tubes (we use glass tubes), place tubes in ice bath.
10.	Add 50 μl sheep gamma globulin (Sigma 15131) from a solution containing 0.05 mg/ml in buffer.
11.	Add 50 μl G280 (a 1:240,000 dilution in buffer). This will give a final incubation dilution of 1:2,880,000.
12.	Add 50 μl ^{125}I-melatonin (New England Nuclear, 2200 Ci/mmole, approximately 4000 cpm) in buffer.
13.	Add 50 μl donkey anti-sheep-gamma globulin (Fitzgerald 40-DS40) after prior dilution 1:10 in buffer (adjust dilution for maximum tracer precipitation).
14.	Vortex, incubate at 4°C for 20 h.
15.	With tubes at 4°C, add 2 ml of cold (4°C) 17.5 g/dl ammonium sulfate in H_2O (keep tubes at 4°C until centrifuged).
16.	Incubate 30 min in 4°C bath.
17.	Centrifuge 30 min at 4°C, 2000 g.
18.	Decant supernatant.
19.	Count gamma scintillations from precipitate radioactivity to 10,000 counts/tube or 10 min, express this as cpm.

*Buffer - 0.01 M (sodium) phosphate, 0.9% NaCl, 0.1% gelatin, 100 mg/L thimerosal, pH 7.0. VCA indicates vortex, centrifuge, aspirate aqueous layer.

use a sample volume of 0.2 ml extracted with chloroform, alkaline and neutral H_2O washes, a pH 7.0 buffer with gelatin, ^{125}I-aMT tracer (2-iodo-aMT, specific activity 2200 Ci/mmol), final G280 antibody dilution of 1:2,880,000, final reaction volume of 0.6 ml, incubation for 20 h at 4°C, and separation of the bound tracer using a precipitating antibody (and sheep gamma globulin carrier) present during the G280 antibody incubation, and final precipitation with ammonium sulfate.

Though the G280 is caprine, omission of the donkey anti-sheep antibody severely reduces precipitating aMT tracer. With unextracted buffer reaction medium, second antibody and carrier quantities were optimized for tracer precipitation (up to 94% of tracer counts with larger nonroutine amounts of G280 present). Binding characteristics of the G280 antibody in unextracted buffer were explored by varying the concentrations of G280 and tracer. Bound (precipitated) counts were corrected for nonspecific binding, which was less than 2% of the free.

With use of the reagent concentrations in Table 1, procedural recovery of aMT after extraction was assessed by comparison of results with those from standard curves not extracted with chloroform and not exposed to petroleum ether (beginning at step 10 after volume adjustment), or extracted and the eluate frozen on dry ice for decanting the petroleum ether and thawed and retained in the same tubes for continuation of the procedures. The latter sequence (extraction without transfer to different assay tubes) was termed "extracted" and the previous sequence was termed "unextracted". The routine procedure (Table 1) used for this recovery comparison and at other times for assessment of assay performance and determination of unknowns was termed "extracted and transferred", and was applied to standards and all samples when present.

Serum samples used in assessment of assay performance were pools from Sprague-Dawley rats, Syrian hamsters, or humans, or were from individual healthy adult humans or from one man following successful irradiation of a germinoma that had previously been present at two sites (pineal and anterior hypothalamus).

The analytic least detectable value (ALD) in buffer was the aMT concentration in a given assay obtained at two standard deviations below the mean cpm of 6 or 9 zero standard replicates. The functional least detectable (FLD) was the aMT concentration below which the between-assay coefficient of variation was more than 20%, determined from different human, hamster, and rat serum samples, each measured in one duplicate pair of tubes over several assays. The FLD provides a more conservative index of the lower end of the useful range of the assay in its practical performance with serum samples (8,9).

Experimental Design. Young adult male Syrian (*Mesocricetus auratus*) hamsters were obtained from Harlan Sprague-Dawley (Indianapolis IN) and maintained (3-5 per clear plastic cage) in the experimental light/dark cycle of 14/10 h (lights off, 2000; lights on, 0600) for at least two weeks prior to the study, with access to tap water and laboratory chow ad libitum. Injections were given subcutaneously in a volume of 0.15 ml. Groups of 8 to 10 hamsters were sampled by guillotine at 2000 (just before lights out), 2200, 2400, 0400, 0550, 0615, 0620, 0700, 0900, 1000, and 1200 h. Sampling in the dark was done with the aid of a 25-watt lamp behind a Kodak 1A safe light red filter. The animals sampled at 0900 h had been injected with physiologic saline (0.9% NaCl) at 0700, and those sampled at 1200 h had received NaCl at 1000 h. Other groups were sampled at 0400 h (after 1 µg/g propranolol injected at 0200 h), at 0900 h (after 1 µg/g ISO injected at 0700), and at 1100, 1200, and 1300 h (after ISO injected at 1000 h). On another day, the portion of the above protocol from 0550 h onward was repeated with the exceptions that at 0600 h, on only the morning of this part of the study, the lights were not turned on (i.e., novel morning darkness extended from the night through all sampling), and that the first sampling after 0600 h was at 0620 h. Trunk serum was saved at −60°C. Pineal glands from animals sampled at 0900 h and beyond were saved at −60°C, using the Rollag antibody as described previously (10), with an ALD of 5 pg per pineal.

Analyses. Assay results were calculated from a four-parameter logistic regression of precipitated standard cpm (including zero aMT) with preextraction aMT expressed in pg/ml. The relevant formulas for regression (predicted cpm) and calculations are shown in the legend of Table 2. Calculations (P1D), regressions (PAR, P6D), ANOVA (P7D) and covariance (P1V), and t tests with the Bonferroni correction for multiple nonindependent comparisons where applicable (P7D), were performed with the indicated programs of the BMDP statistical package (11) on a VAX-4000 computer system.

RESULTS

G280 Assay Performance. The high affinity of the G280 antibody obtained under these conditions suggests that use of constant concentrations of tracer and antibody in a low range (fig 1, Table 3) should provide an assay with high sensitivity. Rearrangement of the elements of the mass law equation (total tracer and total nontracer ligand expressed separately and equation elements combined into the tracer binding proportions (B_0, B/B_0, see Table 2)) allows prediction of the aMT concentration that might be required for a given B/B_0, with the routine concentrations of tracer and antibody (the latter indexed by the B_0), as a function of the K_d (dissociation constant) applying to both tracer and nontracer ligand (molar in the reaction mixture):

TABLE 2. Assay Parameters, Calculations, and Characteristics*

	Mean	SE	Comment
Total cpm	4129	205	Total per tube
Logistic parameters			
a	2151	77	Fitted max cpm (zero aMT)
b	70	6	Fitted min cpm (@ infinite aMT)
c	6.44	0.11	Fitted ED_{50} (pg/ml)
d	1.0332	0.0109	Slope factor
Nonspecific binding			
NSB_o (%)	1.90	0.11	cpm/total (without antibody)
NSB_b (%)	1.74	0.14	b/total (for infinite aMT)
ALD (pg/ml)	0.33	0.03	For zero-aMT cpm mean - 2 SD
FLD (pg/ml)	[0.62 - 0.8]		aMT limit for BACV = 20%
B/B_o (%)	95.6	0.4	For aMT = 0.33 pg/ml (ALD)
B/B_o (%)	91.8	0.3	For aMT = 0.62 pg/ml (FLD)
B/B_o (%)	89.6	0.3	For aMT = 0.8 pg/ml (FLD)
ED_5 (pg/ml)	0.38	0.01	For B/B_o = 95%
ED_{10} (pg/ml)	0.77	0.02	For B/B_o = 90%
ED_{20} (pg/ml)	1.69	0.04	For B/B_o = 80%
ED_{50} (pg/ml)	6.44	0.11	For B/B_o = 50%
ED_{80} (pg/ml)	24.75	0.57	For B/B_o = 20%
ED_{90} (pg/ml)	54.53	1.71	For B/B_o = 10%
ED_{95} (pg/ml)	113.11	4.58	For B/B_o = 5%

*20 assays, standard and sample volume - 0.2 ml, standards ranging from < 1 to 100 pg/ml. cpm indicates counts per minute in precipitate, unless specified as total; aMT, melatonin in pg/ml before extraction; ALD, analytic least detectable; FLD, functional least detectable; BACV, between-assay coefficient of variation; and ED, effective dose.

$$\text{Predicted cpm} = \frac{a - b}{1 + \left(\frac{aMT}{c}\right)^d} + b. \qquad B = \frac{cpm - b}{\text{total cpm}}. \qquad B_o = \frac{a - b}{\text{total cpm}}.$$

$$\text{Predicted aMT} = c\left(\frac{1}{B/B_o} - 1\right)^{1/d}. \qquad \text{Predicted } B/B_o = \frac{1}{1 + \left(\frac{aMT}{c}\right)^d}.$$

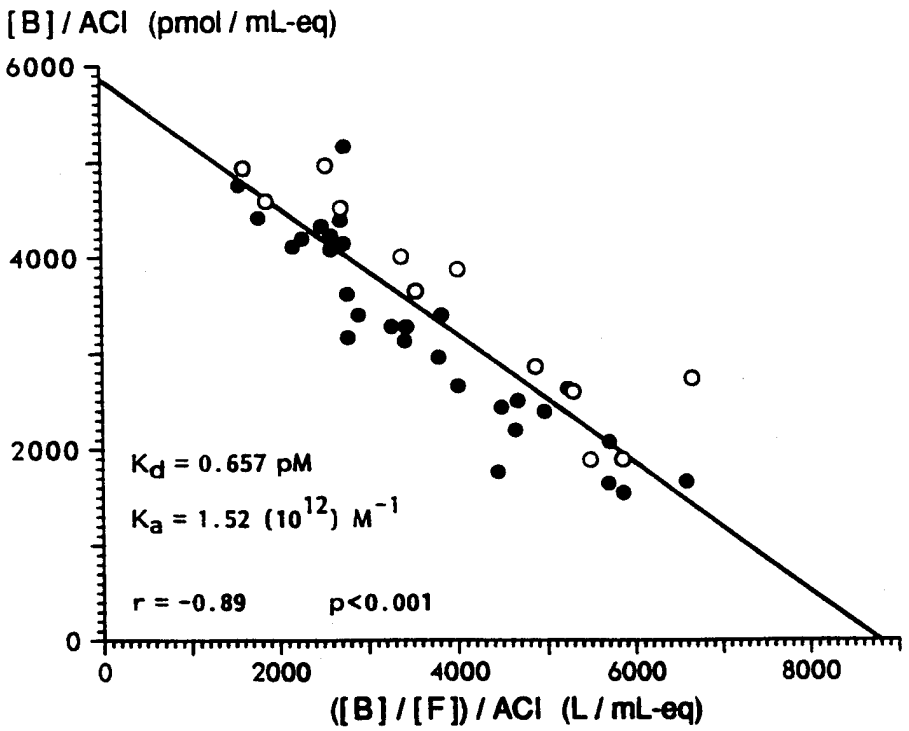


FIGURE 1. Scatchard analysis of G280 antibody. Data are means of duplicates or triplicates. [B] (concentration of bound ligand or bound antibody binding sites) and [F] (concentration of free ligand) are in pM of reaction mixture. ACI (antibody concentration index) is in milliliter equivalents of undiluted G280 goat serum (ml-eq) per liter of reaction mixture. The K_d (dissociation constant = -slope) = $1/K_a$ (association constant). The Bmax (y intercept) is 5800 pmol/ml-eq. Closed circles represent tracer ^{125}I-aMT as the only ligand present, and open circles, nonradioactive aMT as 18% to 91% of total ligand (90% to 91% for 8/12 samples). For the open circles, [B] was taken as bound tracer augmented by the amount of aMT in the fraction (of total nonradioactive aMT present) equivalent to the fraction of tracer that was bound, allowing a different affinity for aMT to be manifested as an altered slope. Analysis of covariance detected no difference between slopes with and without aMT. The slope from the pooled data was used for the plot and the K_d. Ranges in the data: total ligand, 0.80-5.4 pM; total antibody binding sites, 0.40-6.03 pM; total ligand/antibody sites ratio, 0.33-5.70; ligand bound, 14% to 86%; and antibody sites bound, 28% to 82%.

TABLE 3. Tracer and aMT Concentrations Before Extraction and in Assay Reaction Mixture (with 2.0 pM Antibody Binding Sites)

Identification	Concentration in Standard or Sample Before Extraction pg/ml	pM	Amount Per Assay Tube pg	pmol	Concentration in Reaction Mixture (pM)
Total cpm (e.g., 4129)	-	-	0.265*	0.00115*	1.91*
aMT at ALD	0.33	1.42	0.0528	0.000228	0.379
aMT at FLD	0.62	2.67	0.0992	0.000428	0.713
aMT at FLD	0.80	3.45	0.128	0.000552	0.920
aMT of typical lowest standard	0.50	2.16	0.0800	0.000345	0.575
aMT at ED50	6.44	27.8	1.03	0.00444	7.40
aMT of highest standard	100	431	16.0	0.0690	115

*With observed total tracer activity (4129 cpm) to be corrected to 94% as ^{125}I-aMT, the logistically projected amount precipitated at infinite G280 concentration applied in determination of the binding constants in Figure 1, but not in the use of the assay; the gamma counter efficiency for the glass tubes used was 70%; pg tracer given as aMT equivalent; tracer quantity not included for aMT (the other entries) below amount per tube or in reaction mixture; this amount of tracer would be 1.66 pg/ml or 7.16 pM if present as aMT equivalent in an unextracted sample.

$$[aMT] = \left(\frac{1}{B/B_o} - 1\right)\left[^{125}I\text{-}aMT\right] + \frac{K_d}{(1-B_o)(B/B_o)} - \frac{K_d}{1 - B_o(B/B_o)}$$

With use of aMT-free buffer, B_o averages about 51% for the amount of tracer (e.g., about 4129 cpm) and of antibody to be used in the assay. The standard deviation of the B_o replicates (as B/B_o, i.e., each divided by the mean) is usually about 2.5%, giving a mean (100%) minus two standard deviations as 95% B/B_o. The above formula with the K_d thus gives a theoretical ALD prediction of about 0.24 pM in the reaction mixture or about 0.21 pg/ml in an unextracted sample, without use of standard curve observations. The ALD, found to be in this general range (Table 2) from actual standard curves, lends credence to the antibody affinity determination and the high sensitivity of the assay.

Incorporation of (nonradioactive) aMT did not alter the binding slope (fig 1) or the binding profile with the higher ligand concentrations (fig 2) needed for an assay, suggesting that G280 binds ^{125}I-aMT and aMT with virtually identical characteristics throughout the concentration range of their use in the assay. Figure 3 shows the typical distribution of tracer quantity expressed as observed total cpm per tube among assays (constant in a given assay) and the resulting B_o. The negative relationship of B_o with varying total cpm, the latter mainly a function of variable time for radioactive decay following preparation in buffer, is consistent with the supplier's assertion that ^{125}I-aMT undergoes catastrophic decay, leaving nonradioactive products without apparent immunoactivity in this system. The proportion (about 94%) of cpm precipitated in the presence of nonroutine excess amounts of G280 does not appear to change during the time of use, indicating stable attachment of the ^{125}I atom to the aMT prior to decay.

Table 2 gives routine assay logistic parameters and characteristic relationships between aMT concentration and tracer binding inhibition. Standards from less than 1 to 100 pg/ml produce a range of displacement from about 5% to 95%, with 50% displacement at 6.44 pg/ml (the ED_{50}). The analytic (ALD) and functional (FLD) least detectable concentrations produce displacements of about 5% and 10%, respectively. An assay tracer binding profile (fig 4) shows essentially parallel binding of dilutions of serum.

Figure 5 shows that omission of chloroform extraction of a human day serum sample results in a false high value of 17 pg/ml and nonparallel dilution, compared to 2.2 pg/ml with parallel dilution after extraction. (Nonspecific crossreactants are reflected by nonparallelism only if the former happen to have different antibody binding characteristics from those of aMT over changing concentrations in the relevant ranges.) Of importance, there is no detectable loss of aMT itself in the extraction, washing, and transfer procedures (fig 6). Thus, standards and

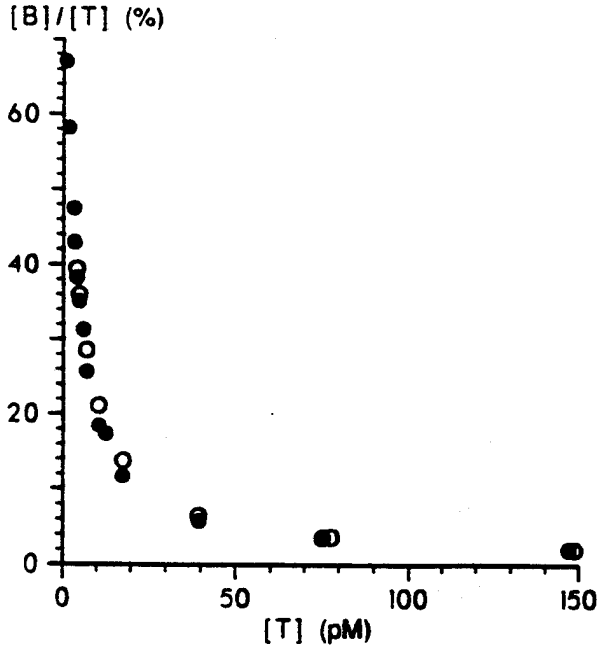


FIGURE 2. Proportion of ^{125}I-aMT tracer bound as a function of total ligand concentration in the reaction mixture over a range wider than that in a typical assay standard curve with the constant G280 antibody concentration used in the assay. Open circles represent constant tracer concentration typical of an assay, with added nonradioactive aMT including the range used in the assay and closed circles, variable tracer concentration as the only ligand.

samples are routinely extracted to minimize nonspecific serum crossreactivity, provide a common procedural base for any factors potentially affecting variation in results, and allow assay recovery from serum that is not significantly different from 100% after dilution or after addition of known amounts of aMT (fig 7). For human serum samples ranging 1 to 80 pg/ml aMT, there were whole blood aliquots collected simultaneously in heparin (7 samples, 20–80 pg/ml aMT) or potassium EDTA (12 samples, 1–46 pg/ml aMT) for comparison of plasma values (data not shown). Analysis of covariance showed no difference in either series of plasma values from those of serum.

Figure 8 shows the assay coefficients of variation. The serum between-assay coefficient of variation is less than 20% for aMT above 0.62 to 0.80 pg/ml and less than 10% for aMT above 2 pg/ml. The FLD is in the range of 0.62 to 0.80 pg/ml, in comparison with the ALD of 0.33 pg/ml.

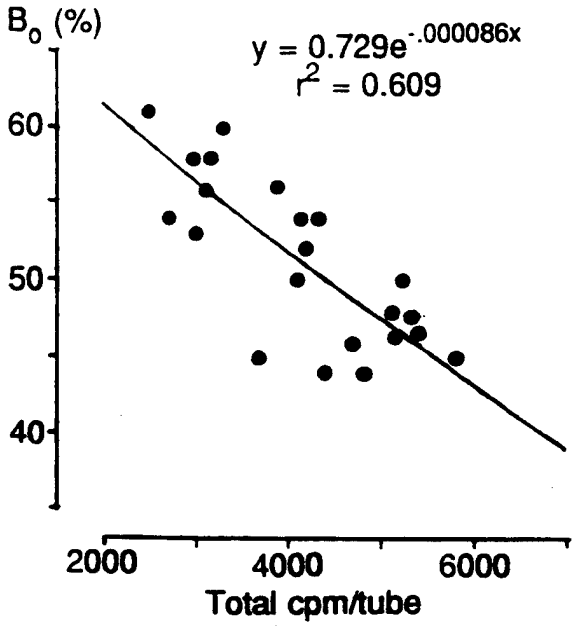


FIGURE 3. Proportion of tracer cpm bound by the small constant assay quantity of G280 antibody in the absence of aMT, as a function of total tracer cpm. The data include the mean zero-standard binding in the assays summarized in Table 2. Variation in the total cpm was due largely to physical decay over variable time intervals after preparation of the tracer for use.

The assay can detect the human nocturnal aMT surge (fig 9) and appears to detect normal daytime values which range 0.4 to 4.0 pg/ml (fig 10). The latter were all above the ALD and most were above the FLD. In contrast, all (including nocturnal) values from the patient with postirradiation pineal and hypothalamic atrophy (and hence likely reduced or absent pineal function) were below the ALD (fig 10).

Syrian Hamster Study. Subcutaneous ISO injection (1 μg/g) after 1 h of morning light (when serum aMT has already reached the daytime level) is capable of elevating mean circulating aMT by at least fivefold above the daytime mean of just less than 2 pg/ml. Delaying the injection until 4 h into the light phase produced a markedly smaller, though still significant response (fig 11). Assay of the injectate did not detect aMT crossreactivity. Neither the baseline aMT values nor the postinjection responses to ISO were altered by extension of nocturnal darkness into the morning experimental time. The lower limit (0.7 pg/ml) of the mean ± 2 SD control range (daytime values after 0800 h) is essentially the same as the FLD (fig 11).

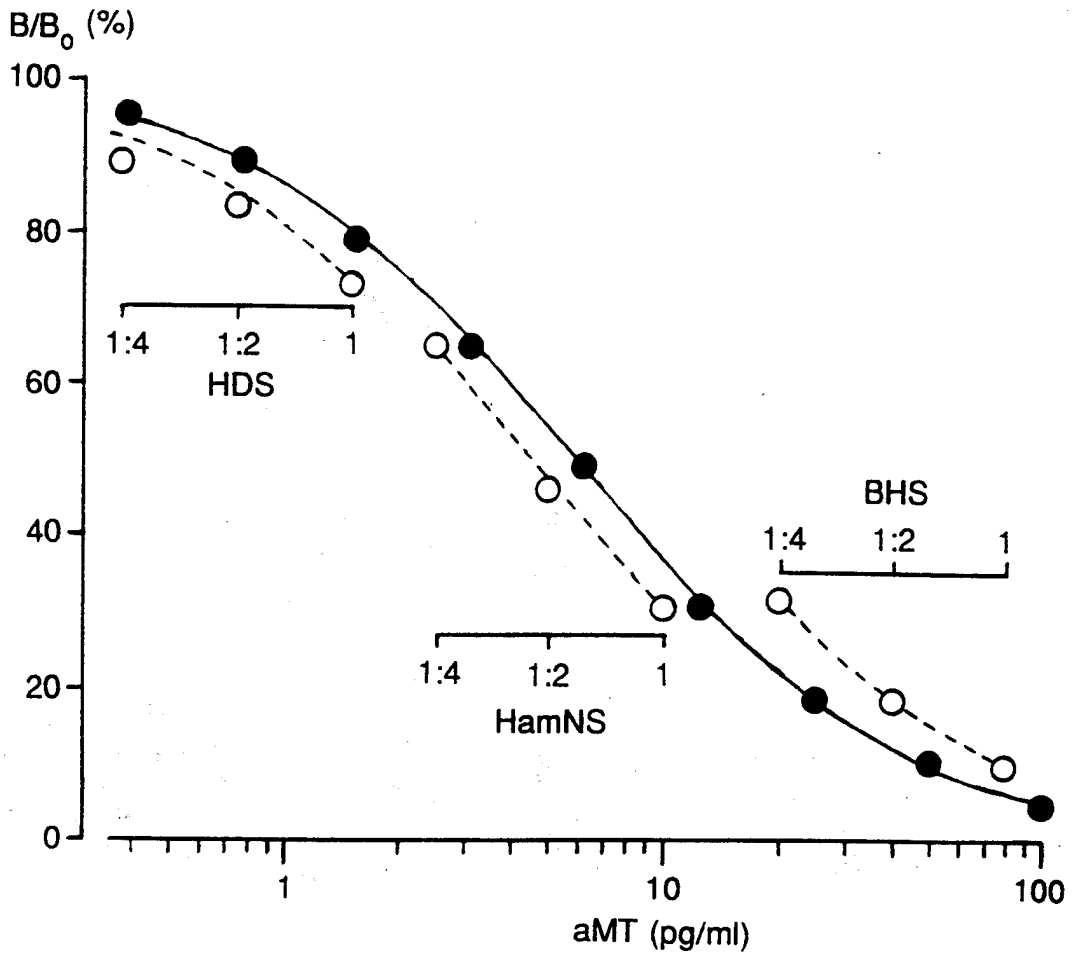


FIGURE 4. Binding profile of standards in an assay run. Closed circles represent observed means of standard triplicates; open circles, dilutions of serum (means of duplicates) displaced uniformly to the right or left of the standard curve; and dashed lines, projected from undiluted serum are parallel to the fitted standard curve. HDS indicates pooled human day serum; HamNS, pooled Syrian hamster night serum; and BHS, pooled burn human serum collected from burn patients at about 0500 h. Abscissa, aMT prior to extraction.

Figure 12 shows that in these animals on this 10-h dark schedule, a large part of the fall of the nocturnal serum aMT surge occurred near the expected time of lights-on, even if the lights did not come on. An injection of propranolol prevented or inhibited most of the rise otherwise seen at 0400 h.

Pineal aMT content, available in the present experiment from the animals sampled after 0800 h, showed a large rise from ISO injected after 1 h of morning light and a smaller (but still

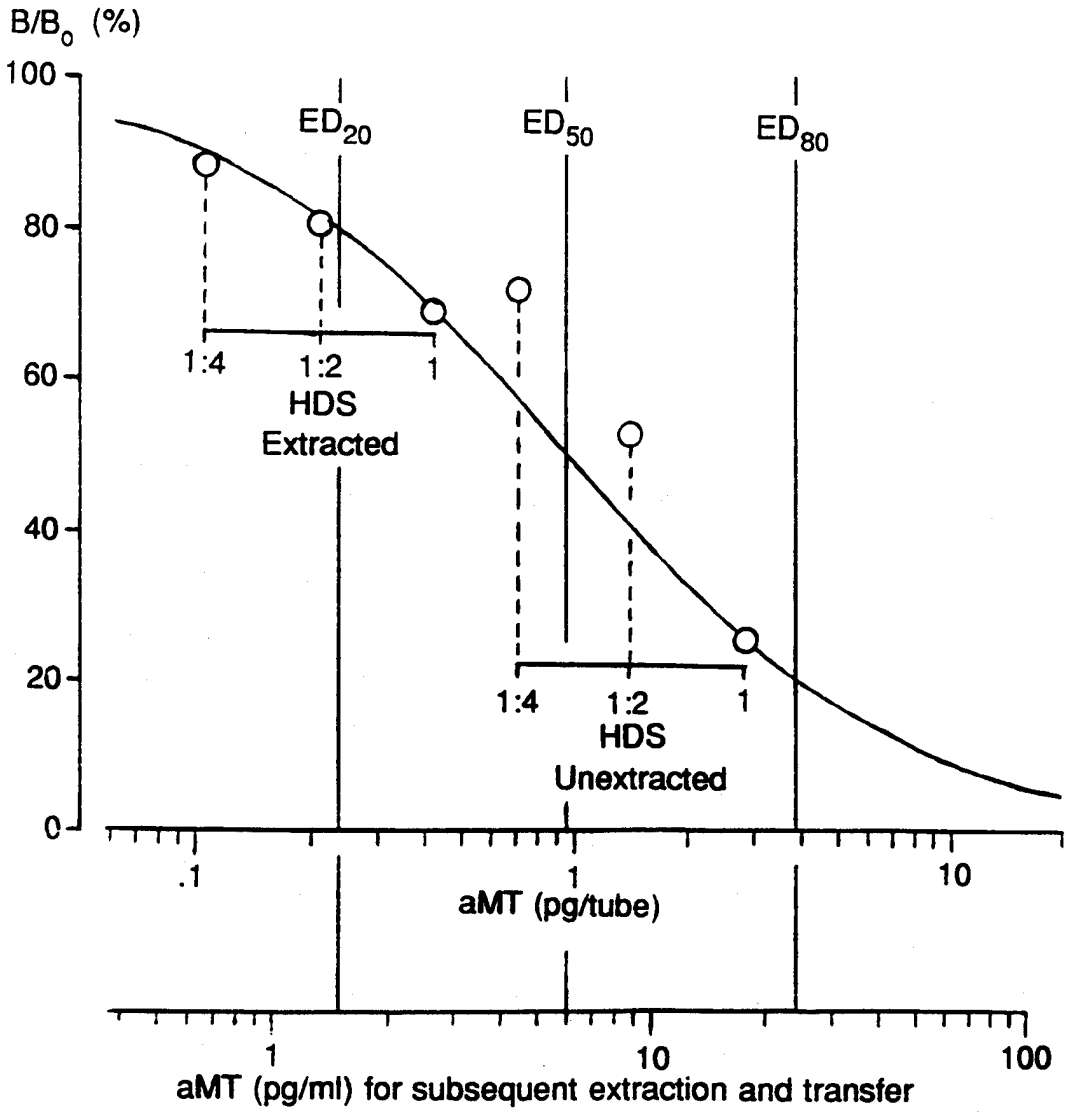


FIGURE 5. Effect of omitting sample extraction. In this (extracted) assay, one set of pooled human day serum (HDS) was left unextracted: 200 µl serum placed in tubes with 300 µl buffer beginning at step 6 of Table 1, omitting steps 7 and 8. The other set of HDS aliquots was prepared in the regular fashion (Table 1). Standards are represented only by the logistically fitted line, and undiluted serum sample coordinates are placed on the fitted standard curves with serum dilutions as shown. In this case, two abscissas show aMT both as concentration and amount per tube.

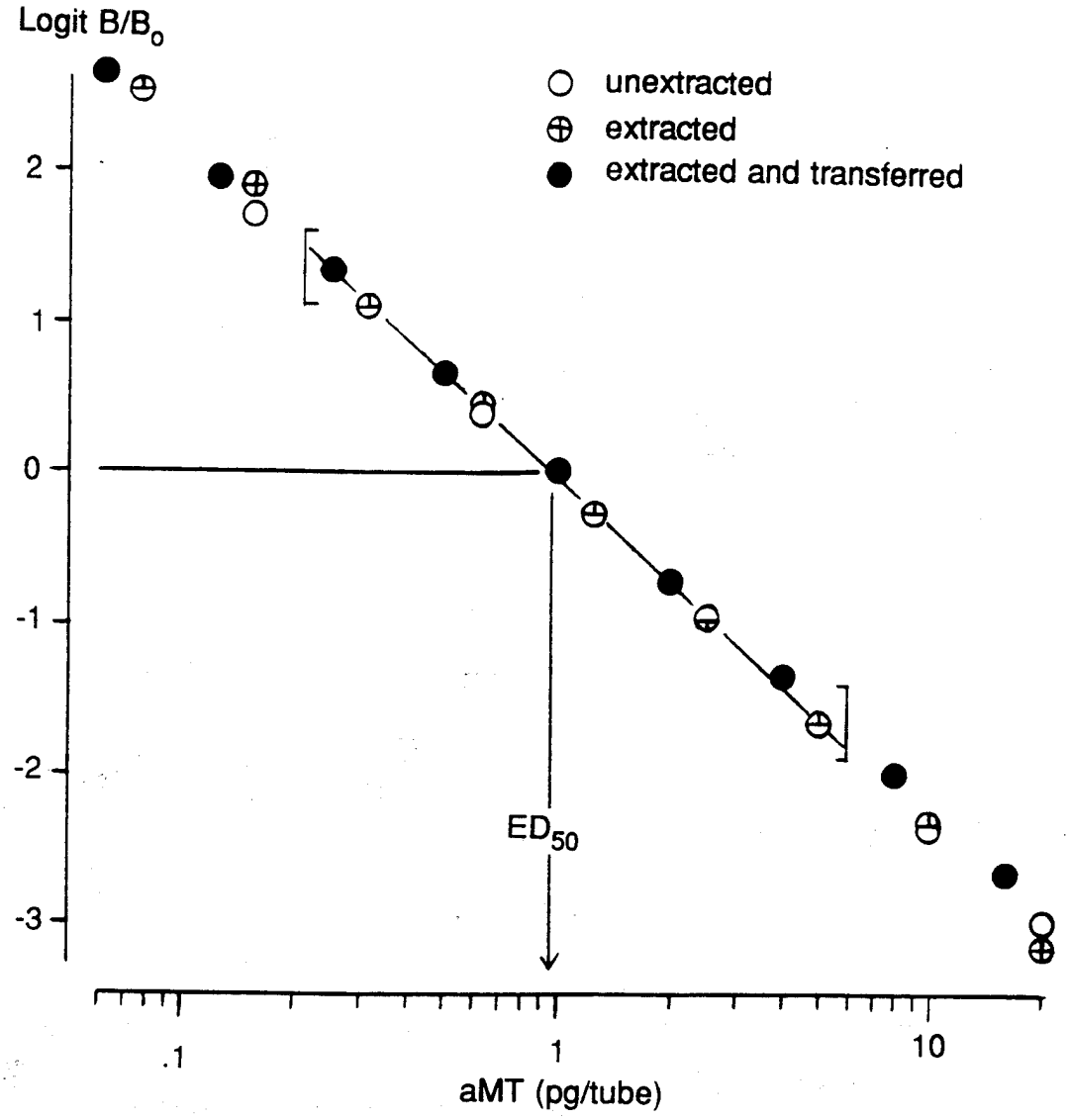


FIGURE 6. Procedural recovery of standards. Symbols are means of 6 to 9 replicates. Values surrounded by brackets were subjected to analysis of covariance showing no difference in slope or position among the three procedural groups.

significant) rise from injection delayed until 4 h into the light phase (fig 13). This pattern and the lack of detectable influence from novel extension of darkness resemble the findings in serum inthe same animals. The responses of pineal aMT 2 h after 1 µg/g ISO injected either after brief exposure to light at night (5) or at 1 h into the expected time of morning light are similar (fig 14), but contrast with the small response after 4 h of light and the absent response by the end of the light phase (5).

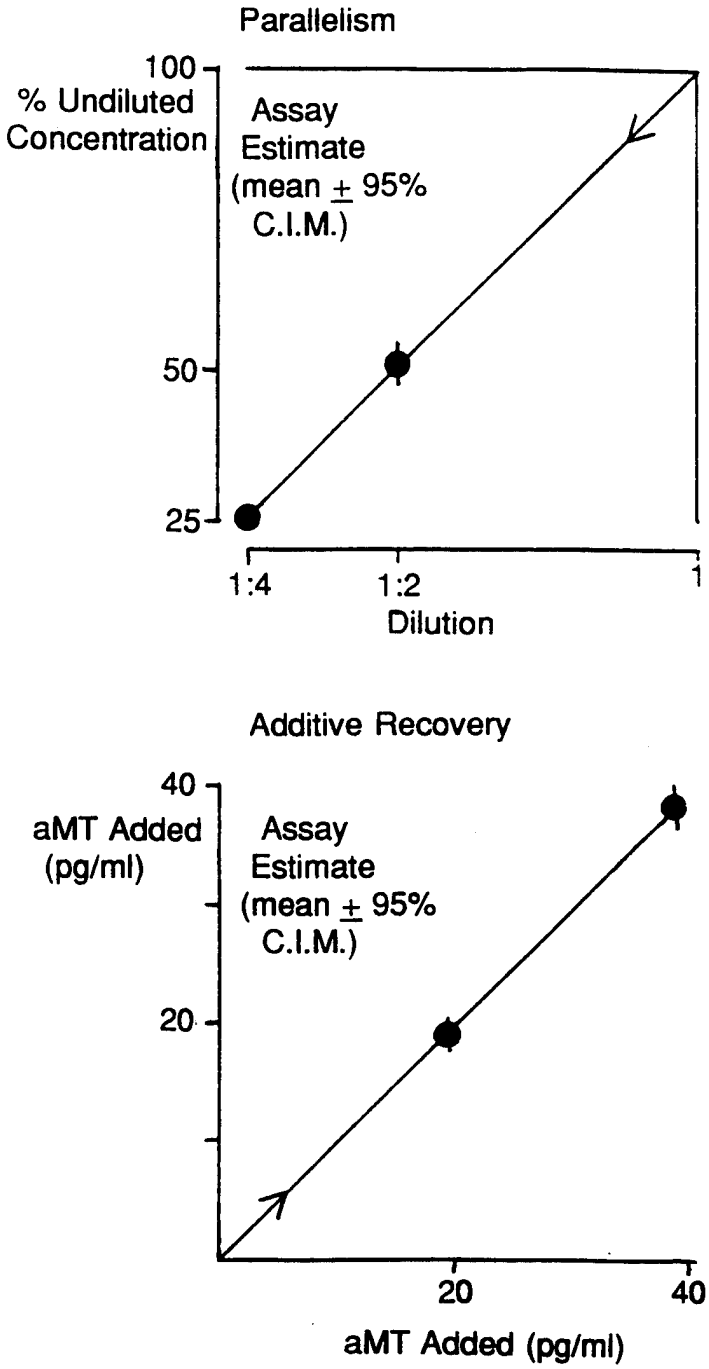


FIGURE 7. Dilutional (A) and additive assay (B) recovery in serum, measured over eight assays. C.I.M. indicates confidence interval of the mean. Diagonal lines are the lines of identity. In the top panel, starting samples were pooled hamster night serum (14 pg/ml, 5 assays) and pooled human burn serum (54 pg/ml, 3 assays). In the lower panel, starting samples were pooled hamster night serum (14 pg/ml, 5 assays) and day serum from an individual human (0.9 pg/ml, 3 assays).

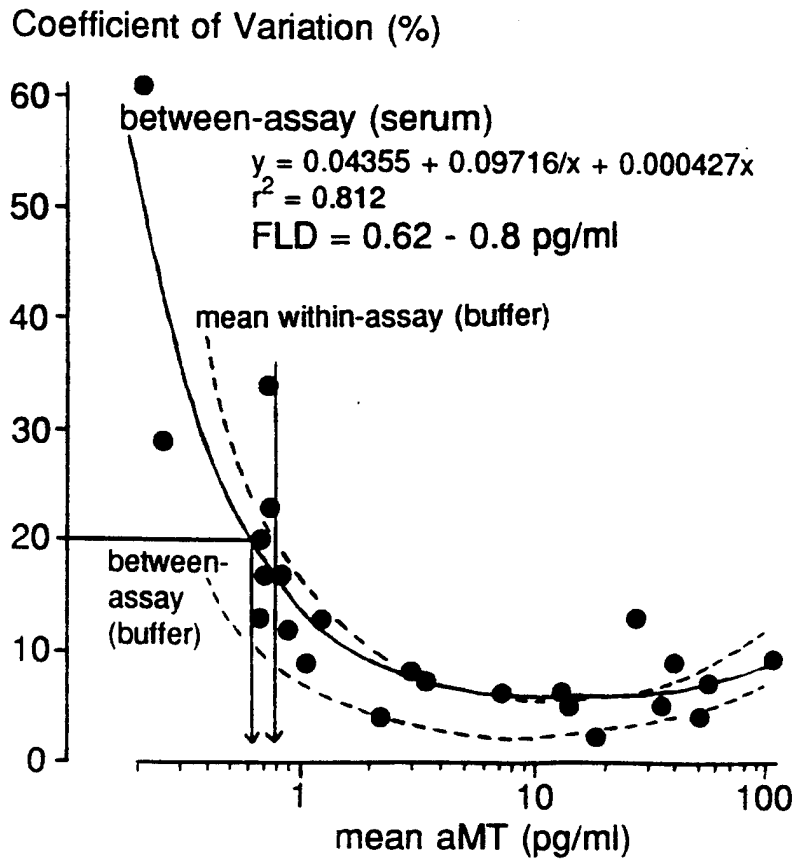


FIGURE 8. Assay variation in the assays of Table 1. The functional least detectable value is obtained from serum between-assay variation as a function of the pre-extraction concentration. Serum samples were measured in duplicate in each of three to nine assays and plotted (closed circles). The uninterrupted fitted line gives 0.62 pg/ml at 20% between-assay coefficient for serum, and 0.8 pg/ml is taken from the concentration above which all observed serum variation between assays was less than 20%. The buffer results are given only as their fitted curves (dashed lines) and are derived from the standards present as triplicates in each assay and whose cpm were treated as unknowns to calculate predicted aMT.

DISCUSSION

The magnitude of circulating daytime aMT levels has so far been uncertain, because values have usually been below the assay detection limits or have varied widely among assays even when above the quoted least detectable. RIA sensitivity can be indexed by several parameters including the ALD, the ED_{50} (effective dose at 50% tracer displacement), and the K_d or K_a (association constant)

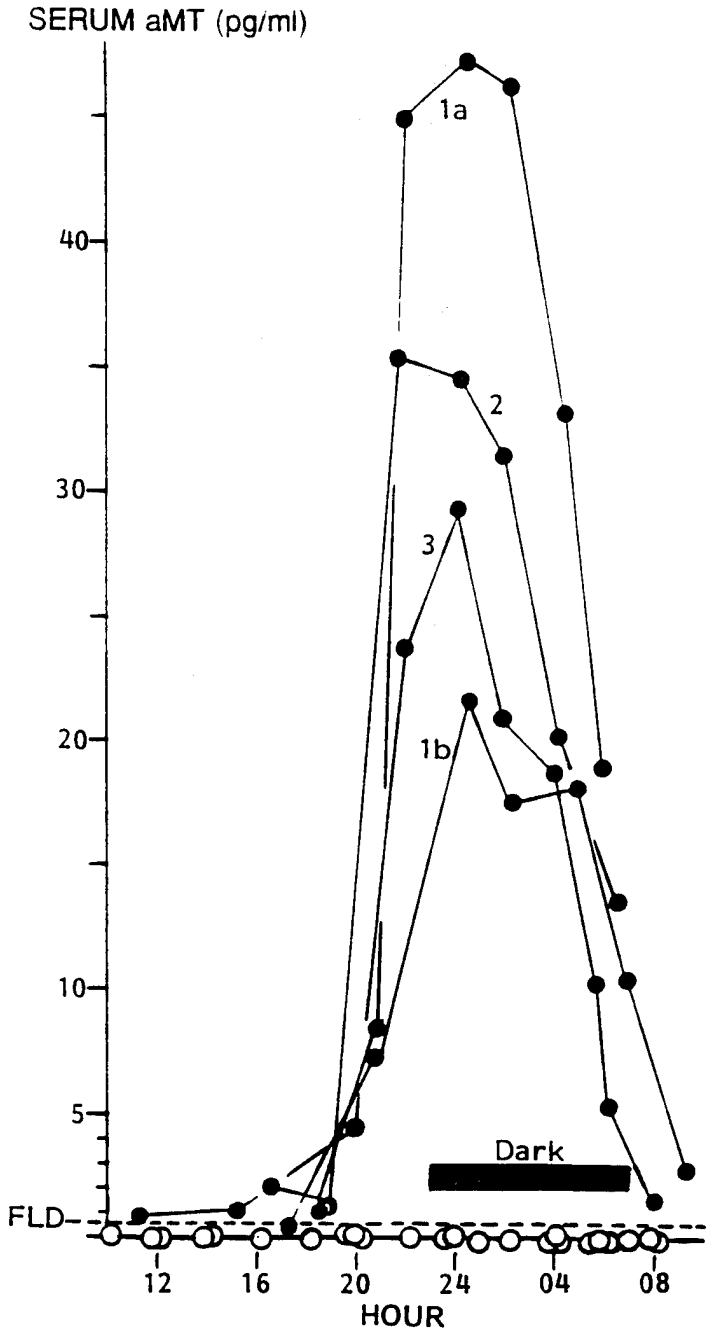


FIGURE 9. Serum aMT in three healthy adult humans [subject 1 at age 40 (1a) and 47 (1b), subject 2 at age 41, and subject 3 at age 48] as closed circles. In comparison, note the values (open circles) for one patient with anterior hypothalamic and pineal atrophy sampled on several occasions 2 to 17 yr after successful irradiation of germinoma at those two sites at age 32. FLD indicates functional least detectable taken as 0.62 pg/ml.

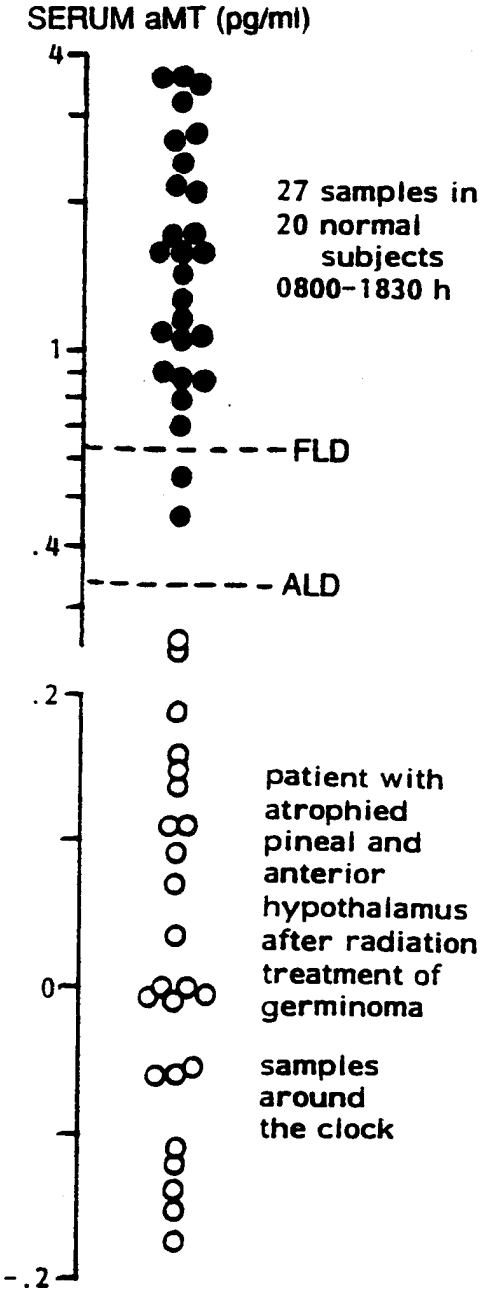


FIGURE 10. Daytime samples from healthy subjects 22 to 60 yr old (closed circles) compared with the around-the-clock samples of the pineal atrophy patient (open circles) from Figure 9. The ordinate scale is expanded to show what is equivalent to the bottom of the previous figure. The logistic standard curve fit was projected to negative aMT values for cpm above the fitted zero-standard cpm. The pineal patient's samples were distributed very similar to the zero standards (not shown). ALD indicates analytic least detectable; FLD, functional least detectable taken as 0.62 pg/ml.

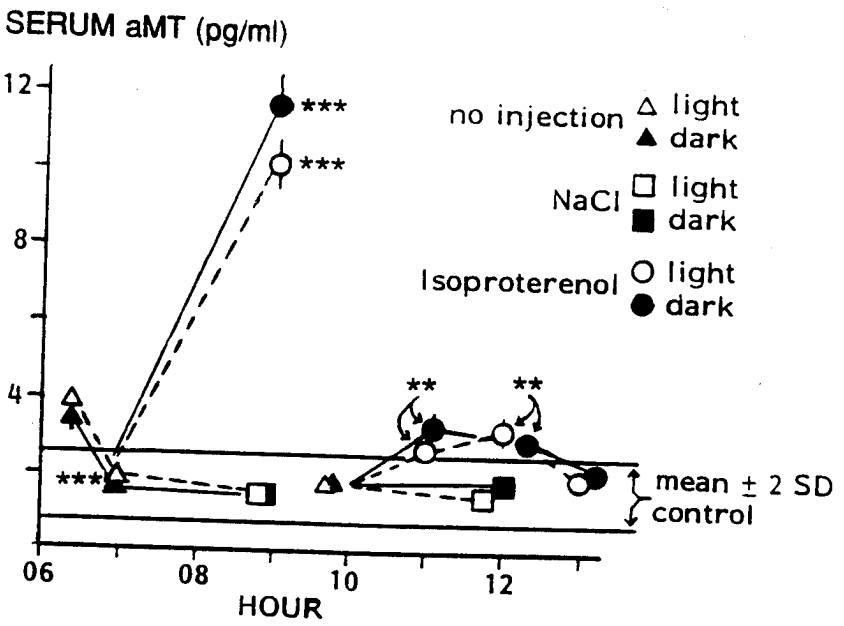


FIGURE 11. Effect of a single 1 µg/g subcutaneous injection of isoproterenol (ISO) on Syrian hamster serum aMT in the early light phase. Injection was at either 0700 or at 1000 h (not both). Open circles and dashed lines indicate sampling after the usual lights-on (0600 h) had occurred; closed circles and continuous lines indicate sampling in novel morning darkness (lights-on at 0600 h had not occurred). The control range shown is based on all samples from hamsters (not injected with ISO) taken after 0800 h. Within either light or novel dark: ***$P < 0.001$ (0620 vs 0700 h, 0900 ISO vs NaCl); **$P < 0.01$ (1100 and 1200 h ISO vs 1200 NaCl). There were no significant differences between light and novel dark.

of the antibody and melatonin under the conditions of the assay. In reports that define the cited estimates of "sensitivity" or "detectability", this has usually been the amount of curve-fitted aMT at tracer displacement of two standard deviations below the mean of zero-standard replicates, or occasionally the amount at 5% displacement of tracer, which may provide a similar estimate. In any case, even if the precise determinant was not given, the estimates reported are herein termed the ALD. They represent what was perceived, within a given assay run and its analytic constraints, as the minimum aMT required to give a consistent analytic response in buffer or special (sometimes stripped) serum matrix. Such media for standards are constant and/or lack many components that may vary among samples of native serum or plasma. The ALD and ED_{50} are herein expressed per milliliter of sample rather than per assay tube, to assess procedures as they are

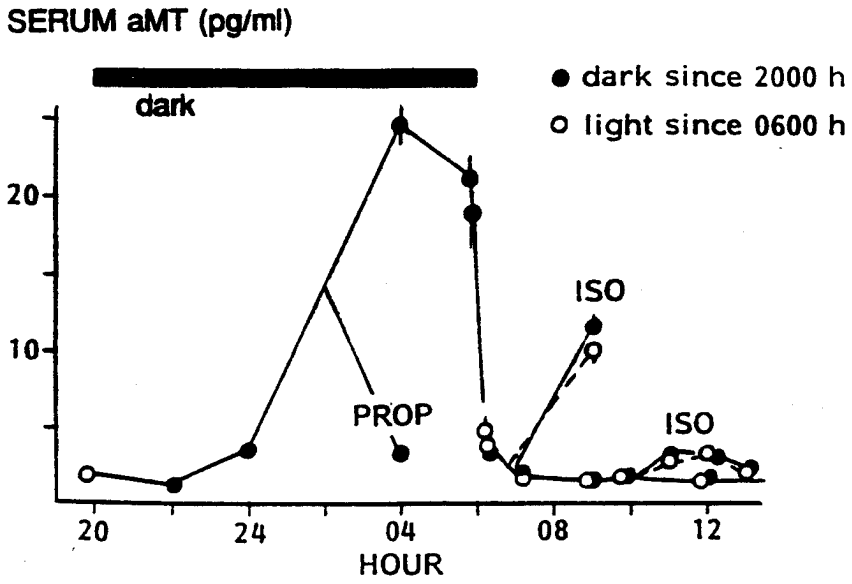


FIGURE 12. Nocturnal serum aMT plotted with the data from the last figure. PROP indicates 1 µg/g propranolol given subcutaneously at 0200 h.

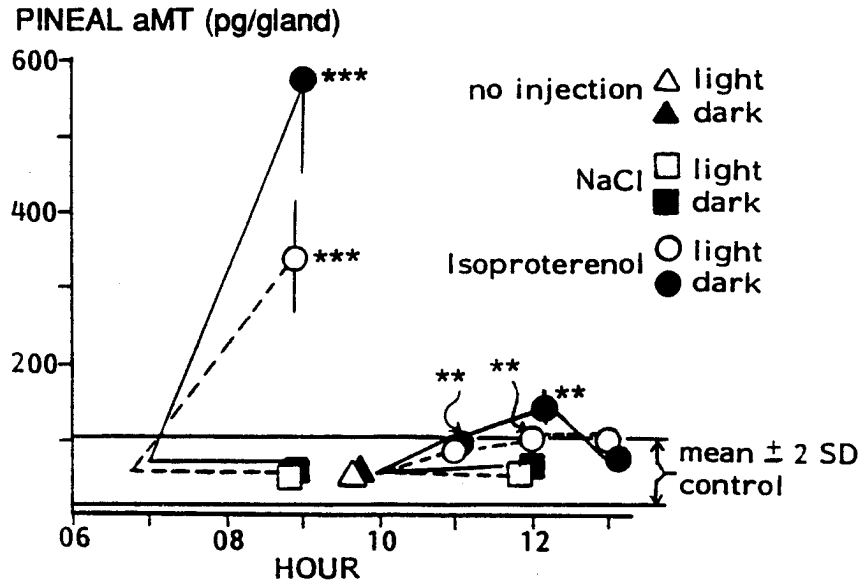


FIGURE 13. Effect of isoproterenol (ISO) on pineal aMT content for the animals of Figure 11 in which pineal aMT was determined. ***P < 0.001, **P < 0.01, ISO vs respective NaCl. No differences were detected between light and novel dark. Control range based on animals not receiving ISO and sampled after 0800 h.

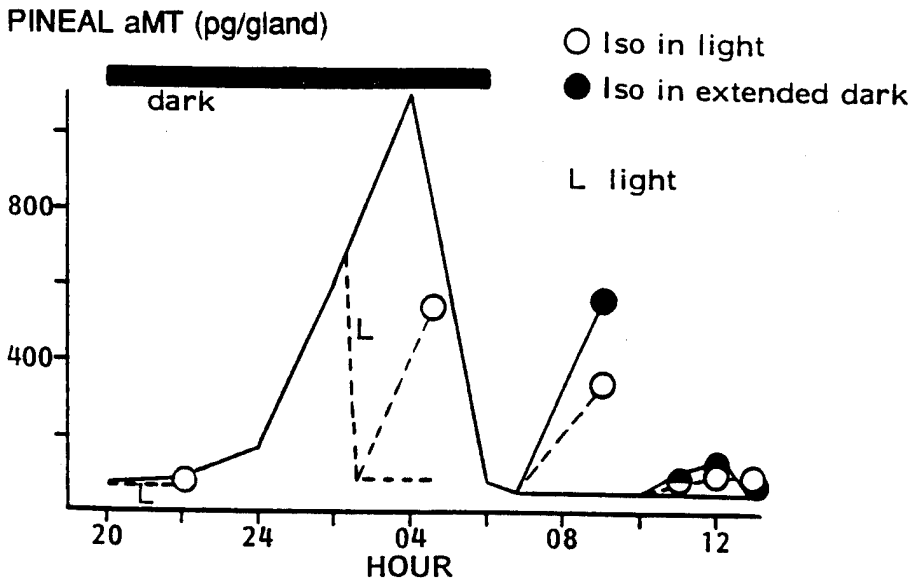


FIGURE 14. Nocturnal Syrian hamster pineal aMT content from previously published data (5,10) plotted along with the morning data of the present study from the previous figure. ISO indicates isoproterenol (1 μg/g) injected subcutaneously in the morning or during short light exposure (L) at night. The nocturnal pineal data are from hamsters other than those providing the nocturnal serum data of the present study (fig 12).

actually used. Expressed this way, parameters of sensitivity and detectability are worsened with smaller sample volume under conditions otherwise the same. Higher sample volumes to enhance these parameters may also increase nonspecific serum or plasma factors in the assay and interact with sample collection limitations.

Since the projected aMT value in a range near the limit of detection varies greatly for any small error in assay signal (e.g., B/B_0 where the slope is less steep), aMT values in this range, even if above the ALD, are liable to larger relative errors than values above this range. The sources of such error, usually conceived to be either systematic (nonspecific serum factors) or random, operate variably within and between given assay runs. If the between-assay (between-run) coefficient of variation (BACV) for a control serum or plasma sample in several assays is determined by using within-assay means of the same number of replicates (e.g., a single pair of duplicates) as used for determination of unknown samples, then across-assay variation for this control sample results from the composite of within- and between-assay error forces that probably also affect the unknown serum or plasma determinations. The error in any given determination includes a contribution from being in a given assay run as opposed to another. This part of the

error is not disclosed by within-assay parameters. The effects of nonspecific factors peculiar to native samples together with those of the random error forces become magnified (relative to the mean analyte value) in the low analyte range, and the BACV thus includes some of these influences. This allows one to determine a lower limit of analyte in serum or plasma (the FLD) below which the BACV rises above a given level (e.g., 20%) (8,9), indicating the sizeable risk of measurement error, but at a more conservative threshold than indicated by the ALD with only buffer or standard matrix influences operating within assay runs. Values above the FLD (determined in serum) may more likely be taken to represent the output of the method.

Values above the FLD are not necessarily free of nonspecific error influences but the role of these influences exerted more powerfully (relative to the analyte content) at the lower range of measurement may be minimized above the FLD. Less assay specificity allows such serum influences to be exerted into higher ranges of analyte, and this might be indexed mainly or only by higher apparent analyte levels, particularly when they should be low. Thus, particularly for samples with low expected analyte content, if assay recovery for a given procedure is at least comparable to that of others, lower values usually indicate greater assay specificity. Such comparisons for daytime aMT values, which should be low, may be informative. For some of the RIA kits mentioned below, extraction of samples, but not standards, may be employed, and a difference in intra-assay precision and/or recovery between standards and samples risks an overstatement of sensitivity.

The present antibody (G280) was previously used in a procedure (7) with an ED_{50} of 93 pg/ml and an ALD of 15 pg/ml on 500-µl samples, compared with 6.4 and 0.33 pg/ml on 200-µl samples in the present procedure. Large reductions in the amount of tracer (greater specific activity of ^{125}I vs ^{3}H label) and of antibody were possible because of the high antibody affinity and likely are mostly responsible for the improvement. Another procedure used previously in our laboratory employed the Rollag antibody and ^{125}I-aMT analogue tracer and was modified to enhance sensitivity (less antibody and tracer than in the original Rollag method) and specificity (petroleum ether wash)(10). With a sample volume of 500 µl, the ED_{50} was 47 pg/ml and ALD 5 pg/ml. Daytime normal adult human serum samples registered a mean of about 14 pg/ml. In contrast, the present assay with a smaller sample volume gives a normal range for human day serum of 0.4 to 4 pg/ml and a FLD (0.6-0.8 pg/ml) well below the previous ALD, suggesting previous nonspecific serum crossreactivity in daytime samples.

A similar contrast can be made with most of the other commonly used aMT RIA's. Unless specified differently, methods are RIA and the tracer ^{3}H-aMT. The procedure of Pang et al (12,13) utilizes samples of 500 µl with an ED_{50} of 500 pg/ml, a reported ALD of 10 pg/ml, and human day serum values of 5 to 20 pg/ml. The widely

used kit assay of WHB (Bromma, Sweden) (14-18) extracts 1 ml of serum and utilizes 20% to 80% of the buffer reconstitution so that there is potential variation in the pre-extraction concentration-based parameters. The ED_{50} appears to be 58 to 232 pg/ml, and ALD, 11 to 45 pg/ml, though ALDs of 3, 8, and 16 pg/ml have been mentioned. Mean human day serum values have ranged 5 to 27 pg/ml. The K_d (from the reported K_a) was 3.3 (10^{-10}) M in comparison with our 6.57 (10^{-13}) M.

The widely used kit procedure of CIDtech (Hamilton, Ontario, Canada) (19-21), with extraction of 1 ml serum, has involved an ED_{50} of 50 to 60 pg/ml, an ALD of 5 to 14 pg/ml, and human day serum results centering around 5 to 26 pg/ml. In one report (21), three BACV values were given (from 16% to 26%) in a pattern suggesting a FLD of 34 pg/ml. Higher numbers of within-assay replicates of the BACV control samples might tend to lower the BACV by reducing the within-assay contribution to the BACV. If such replication is greater than for the unknown samples, then the BACV (and the FLD) might be underestimated for the purpose of assessing potential error in unknown samples. Though the within-assay replication for BACV samples was not given, the large number of assays (apparently 32) suggests some fidelity in the above tentative FLD estimate as a lower limit. Conservatively, it would appear higher than the present 0.62 to 0.80 pg/ml from within-assay duplicates.

The widely used Stockgrand Ltd. kit assay (Guildford, Surrey, UK) (22-29) has the advantage that it does not require sample extraction. The sample volume is 500 µl and the ED_{50} originally (22) was about 120 pg/ml, though it may be less with use of the lower amount of antibody recommended subsequently. The ALD has been in the range of 5 to 10 pg/ml. Human day serum is often stated to be below the ALD, though means of up to about 15 pg/ml have been reported. In one report (24) BACV (10% to 21%) was given for three aMT levels in a pattern compatible with a FLD of about 25 pg/ml. Without knowledge of the extent of within- and between-assay replication, and with only three BACV-aMT coordinates, only a tentative interpretation is warranted. A comparison (27) of plasma aMT estimates in 43 samples between this RIA and the GC-MS assay of Lewy and Markey revealed a correlation that was almost entirely due to the approximately 30% of the values above 7 pg/ml on GC-MS (all of these being above about 18 pg/ml on RIA). The other samples ranged from 1 to 7 pg/ml on GC-MS but 10 to 39 pg/ml on the RIA. About five of these values may have been below the RIA ALD and assigned the ALD value for the RIA (10 pg/ml). RIA values between 24 and 30 pg/ml (13 values on the plot) were associated with GC-MS values of 2 to 27 (2 to 12 in all but two) pg/ml. If the results from the procedure with the lower values (GC-MS) can be taken as the comparison standard, then the data are compatible with an RIA FLD of about 23 pg/ml, below which RIA variation from the regression line at a given GC-MS value appeared increased. However, the functional error profile for

GC-MS at a spectrum of aMT levels is also not known and may have contributed to the scatter in the data, presumably more so below some aMT level. That study interpreted results of daytime levels (for a small effect of exercise) but without assessing whether the results were below a FLD in either assay.

A modification of this RIA has been used in which the sample volume (250-300 µl), amount of antibody, and reaction volume are reduced, and the reaction occurs in the scintillation vials (30-32). An ED_{50} of about 55 pg/ml and ALDs of 2, 10, and 6.5 pg/ml have been reported. Human day serum was about 16 pg/ml in one report or usually less than the ALD in another. The K_d for the antibody under these conditions was given as 17.6 pM. We have employed this (Stockgrand) antibody in our laboratory (33) by extracting 250-µl samples and standards (the latter in buffer) using a procedure including all the steps in Table 1, except that the initial antibody dilution was 1:3000 (50 µl, one-fourth that usually used for this antibody in the original procedure), ^{125}I-aMT (instead of ^{3}H-aMT) was the tracer, the buffer was the originally described tricine but at pH 7.0 for optimal binding, and the carrier-second-antibody system was in higher concentration (optimized). The ED_{50} was 28 pg/ml and the ALD 2.8 pg/ml (at zero-standard B/B_o minus 2 SD, giving a B/B_o of 95%). Further reduction in amount of antibody (presumably to increase sensitivity) was not possible, because the B_o was already 28%. There were 17 daytime human samples (0.4 to 3.7 pg/ml in the currently reported G280 assay) available for comparison with this modified Stockgrand antibody procedure. There was 100% assay recovery in both procedures. All values were higher with the latter procedure, in which 9 registered above 4 pg/ml, up to 9.6 pg/ml (data not shown). We did not determine the FLD. We found a K_d of 6.0 pM for ^{125}I-aMT.

The procedure of Claustrat and colleagues (34,35) utilizes diethylether extraction of 300-µl samples and a radioiodinated aMT analogue as tracer. The ED_{50} appears to be 100 pg/ml, and the ALD is reported to be 5 pg/ml. Human day serum values appeared to be 5 to 10 pg/ml. The procedure of Tiefenauer and Andres (36) was originally described in buffer aMT standards without extraction and also utilizes an iodinated aMT analogue as tracer. The ED_{50} was approximately 400 pg/ml aMT and the K_d (from the reported K_a) was 1.6 (10^{-9}) M for the analogue and 8.3 (10^{-10}) M for ^{3}H-aMT. The method sold as kits (Tecova AG, Wohlen, Switzerland; Euro-Diagnostics, Apeldoorn, Holland) calls for extracton of 500 µl samples in diethylether or chloroform. Reports citing the original description and/or a kit (37-43) have involved extraction and ALDs of 5 or 10 pg/ml. Human day serum has ranged from less than 5 pg/ml to means of 7 to 14 pg/ml or 25 pg/ml.

The assay of Vakkuri and colleagues (44-46) utilizes 2-^{125}I-aMT as tracer (as does the presently reported G280 assay), but extracts 1 ml serum in chloroform for an ED_{50} of about 45 pg/ml. The ALD

was reported to be 4 pg/ml and human day serum ranged from 4 to 15 pg/ml and had a mean value at 1100 h of 9.7 pg/ml in one report (46) In the latter report, three BACV values (10% to 24%) were given over a range of aMT compatible with a FLD of about 10 pg/ml.

An assay kit marketed by Nichols Institute (San Juan Capistrano CA) involves diethylether extraction of 500 µl serum or plasma for an ED_{50} of 25 pg/ml and an ALD of 3 pg/ml. The tracer (aMT or analogue not specified) is radioiodinated. Of 21 human day serum samples, one registered 1.7 and the others 5 to 15 pg/ml (telephonic communication with Nichols in-house representative). An ELISA kit (ALPCO, Windham, NH) is available in which an aMT analogue containing a tracer enzyme competes with 50 µl unextracted sample for an antibody attracted to an immobilized second antibody. The ALD is stated to be 2.6 pg/ml and the ED_{50} about 148 pg/ml, and human day serum apparently ranged from undetectable to 15 pg/ml. Independent evaluation of these kits is needed for assessment of their performance.

Another kit (Elias USA, Osceola, WI) utilizes a radioiodinated aMT analogue in an RIA directly with 200 µl unextracted EDTA plasma for an ED_{50} of 28 pg/ml and an ALD of 1.5 pg/ml. A comparison (assay brochure) of human plasmas with the Lewy and Markey GC-MS method shows a correlation, but this appears entirely due to samples above 22 pg/ml RIA (above 15 pg/ml GC-MS). Of the 45 other plotted points, those from daytime were not identified. At RIA 2.5 to 4 pg/ml (well above the ALD), the 10 plotted values for GC-MS ranged from 0 to 14 pg/ml, and samples with RIA at 15 to 18 pg/ml (5 points) ranged from 2.5 to 17 on GC-MS. At 12.5 to 15 pg/ml GC-MS, the 7 points ranged from 3 to 21 on RIA, and samples with GC-MS at 2.4 pg/ml (18 points) ranged from 2.5 to 18 pg/ml on RIA. It is not possible to determine whether the highly variable results below about 20 pg/ml stemmed from one or both (in combination) comparison procedures, though this extreme variation occurred at 10- to 15-fold above the ALD of both procedures. Method-related sources for this variation would only be identified if samples with the same aMT content at the levels of interest were assayed in multiple assays with both procedures to see if repeated results on the same sample show the same large variation in either procedure. This would also allow a precision-based FLD to determine below what level either procedure gives a result not interpretable as representative of the procedure.

The GC-MS procedure of Lewy and Markey (47-53) has the lowest ALD reported so far, though the sample volume is 1 ml. The within-assay analytic parameters used to define threshold criteria for the ALD were not explicit, though it is mentioned (47) that a judgment of the presence of the more intense ion peak above background noise may be aided by specificity parameters such as the presence of the less intense ion peak, peak symmetry, and absence of shoulders. Thus, the original concentration required to satisfy threshold criteria might be ascertained for a given recovery. For

samples with less recovery, a greater pre-extraction concentration (i.e., a greater ALD) would be required for detection. Because of the extensive washing, extraction, and derivatization procedures, a deuterated aMT internal standard is added to the plasma sample (15-40 pg/ml) in sufficient quantity to provide another ion peak to determine recovery. This procedural recovery was said to vary as much as fivefold (47), which would mean that the pre-extraction ALD (reported as 1 pg/ml at an unspecified recovery) might vary greatly among plasma samples. From the description given, it also seems likely that a small signal above a valid ALD (the signal itself being accepted as qualitatively representative of some aMT from its specificity earmarks) might give a quantitative value containing a component of noise or error from random, procedural, or nonspecific serum factors, any of which may vary among samples. Then, at higher concentrations, the physico-chemical parameters begin to provide specificity presumably for almost all of what is quantitated as aMT, providing the powerful asset of this important procedure. Though a functional assessment of these intervening levels (e.g., with a FLD threshold) is not given, nadir daytime human plasmas have registered variously 1.5 to 4.9, 2 to 10, and a mean of about 7 pg/ml taken from a plot.

Use of a FLD (precision based detectability) would not be restricted to RIA methodology (as much as are the ED_{50} and the B/B_0-determined ALD) and might provide a common reference frame for the lower limit above which aMT values can be taken to represent reliably the output of a given method. Comparisons between methods would be meaningful only if this assessment is available, because otherwise, the perturbingly large scatter from the regression line projected into the lower daytime range cannot be partitioned according to method in the comparison. In the higher ranges, for methods capable of detecting the nocturnal surge, their outputs are expected to be correlated anyway. The FLD has not been applied intentionally to aMT assays previously. However, when BACV values were given for RIA's in a way to afford a tentative FLD estimate, they have been a little above twice the conservative estimate of the ALD.

The present G280 assay utilizes a smaller sample volume than almost all other assays and gives a FLD (0.62-0.80 pg/ml) about twice the ALD (0.33 pg/ml), the former well below even the ALD of other RIAs. Normal human daytime samples in our assay so far have been above the ALD and most above the FLD, so far not extending above 4 pg/ml. In other RIAs, human day values have either been quite higher and/or not in the usable range of the assay. Our least detectable (either as ALD or FLD) and daytime nadir seem to register lower than reported for the GC-MS method. Some of this smaller difference in daytime nadir might stem partly from use of different subjects in different locations.

Daytime young adult Syrian hamster samples have averaged about 25, 17, or 30 pg/ml in our modified Rollag antibody assay (54,55);

5 to 20 or 18 to 20 pg/ml in the Pang assay (56-59); 10 to 100 in the procedure of Brown et al (60) and 22 pg/ml with the Stockgrand antibody (61). These values are all several fold above those assays' ALDs. A modified Claustrat procedure (62), perhaps with a different antibody, gave mean values ranging 20 to 60 pg/ml in Syrian hamsters during the day. On the other hand, our present results show mean daytime normal Syrian hamster serum aMT of about 1.8 pg/ml, with a range for individuals similar to that in normal humans.

The fall of Syrian hamster serum aMT near the end of the dark phase appears entrained, not gated, since it was not different if darkness was suddenly extended past the end of the night. Even after serum aMT (present results) and pineal aMT (10) fall to daytime levels, the ability of subcutaneously injected ISO to stimulate pineal and serum aMT persists in SH for several hours. Unlike the nocturnal surge itself, this response to a beta-adrenergic stimulus is not blocked by the acute presence of light. By 4 h into the light phase, this response is already markedly attenuated. Thus, the fall of the nocturnal surge and the fall of beta-adrenergic responsiveness are controlled endogenously but are several h out of phase. The persistence of a beta-adrenergic aMT response in the early light phase suggests that the preceding fall-off of the nocturnal aMT surge may be produced by endogenous withdrawal of the adrenergic drive to the pineal that is present in the second half of darkness and that can also be withdrawn artificially by propranolol administration.

The aMT response to either the endogenous neurotransmitter (producing the normal nocturnal aMT surge) or injected ISO (inducing a secondary surge at night after acute inhibition by light) requires new protein (N-acetyltransferase, NAT) synthesis from stimulation of RNA production in the SH (63). However, a dose of actinomycin D (ACTD) given in close proximity before late dark phase ISO injection after acute light exposure (but not ACTD given earlier) apparently failed to inhibit the pineal aMT response to ISO. Cycloheximide was inhibitory at either time. Thus, one must consider that ISO injected at night after acute light might exert its effect at a post-transcriptional but still pre-translational site, if recent (e.g., nocturnal) adrenergic tone has already stimulated transcription (64). Such an action of ISO might explain the presently observed morning ISO response by its re-engendering the effectiveness of mRNA left over from the nocturnal surge. Thus, later daytime unresponsiveness to relatively brief adrenergic stimuli to the pineal in SH might reflect nonpersistence of the stimulus past a long lag period required to replenish NAT mRNA after it is depleted from waning of the previous (more prolonged) nocturnal surge of pineal sympathetic tone (63). However, the available data do not yet exclude possible adrenergic responsiveness also of pineal transcription varying around the clock in this species, becoming minimal after a few hours of light and rising before the nocturnal surge of NAT/aMT, shortly before

the ineffective dose of ACTD in the previous studies (63) could have become effective. Whether morning aMT responsiveness wanes from falling NAT mRNA stores and/or from falling responsiveness of new mRNA synthesis, this does not occur from an acute effect of morning light and is thus endogenously determined.

The close parallel between pineal and serum changes in aMT, even seen when aMT is low and the response to ISO is minimal, indicates that alterations of daytime circulating aMT can be measured in this assay with a demonstrated basis of reliability. This improvement (enabling use with daytime levels) may tentatively classify it as a second-generation assay. However, it is still possible that normal daytime levels may be shown to be even lower should there be developed a third-generation assay with greater sensitivity and specificity in the lower range manifested in part by a substantially reduced FLD.

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PRESENTATIONS/PUBLICATIONS

Vaughan GM: Syrian hamster morning melatonin dynamics revealed by serum assay. Presented at the International Symposium on Melatonin and the Pineal Gland, Paris, France, 6 September 1992.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

ACCESSION: DA335693

SUMMARY DATE: 920910 **SUMMARY KIND:** H **PREV DATE:** 911001 **DISTRIBUTION:** CX

PROGRAM #: 62787A **PROJ #:** 30162787A874 **TASK AREA:** CH **WORK UNIT:** 167

TITLE: Effect of Clotrimazole on the Prevention of Fungal Colonization and Infection in Thermally Injured Patients

SUBJ1: 060500 - Medicine and Medical Research

START DATE: 9011 **END DATE:** 9209 **PERFORMANCE METHOD:** C

CONTRACT/GRANT NO:

CONT TOTAL: $
CUM TOTAL: $
TOTAL LAB FUNDS: $

RESOURCES ESTIMATE

FY	WORK YRS	$(Thousands)
91	0.4	$19
92	0.1	$ 4
93	0.0	$ 0

RESPONSIBLE DOD ORGANIZATION: 404885
MRDC INSTITUTE OF SURGICAL RESEARCH USAISR
FT SAM HOUSTON, TX 78234
PRUITT, B A
210-221-2720

PERFORMING ORGANIZATION: 404885
MRDC INSTITUTE OF SURGICAL RESEARCH USAISR
FT SAM HOUSTON, TX 78234
CIOFFI, W G
210-221-8440

ASSOC1: MC MANUS, A T

ASSOC2: JORDAN, B S

FOREIGN INTELLIGENCE CONSIDERED: Y **CIVILIAN APPLICABILITY:** M

KEYWORDS: RA II; Volunteers; Adults; Burns (Injuries); Fungi; Therapy; Immunosuppression

OBJECTIVE: A DTIC literature search was conducted under DTIC request numbers W6045B/W6046D dated 6 November 1990. The objective of this work is to assess the effectiveness of clotrimazole in preventing fungal colonization and infection in patients with thermal injury.

APPROACH: Patients were randomized in a pair-wise fashion to receive either standard wound care of alternating mafenide acetate and silver sulfadiazine or the standard wound care with the addition of clotrimazole cream. Routine microbiologic surveys were performed and, if clinically indicated by the appearance of the wound, biopsies were obtained for histologic evaluation.

PROGRESS: 9110-9209. This study was approved by the USAISR Research Council and US Army Institute of Surgical Research Human Use Committee during the first quarter of Fiscal Year 1991. Twenty-five patients were enrolled in the study. Analyses of the data revealed no benefit of clotrimazole in preventing colonization of the burn wound with fungus. For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report* for fiscal years 1991 and 1992.

ABSTRACT

PROJECT NUMBER: 3M162787A874-167, Applied Research and Exploratory Development

PROJECT TITLE: Effect of Clotrimazole on the Prevention of Fungal Colonization and Infection in Thermally Injured Patients

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

PERIOD COVERED IN THIS REPORT: 19 November 1991 - 30 September 1992

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Twenty-five patients were enrolled in this study, 13 receiving clotrimazole therapy and 12 serving as controls. The overall colonization rate was 52% for all patients enrolled in this study. For those patients who survived the first postburn week, the colonization rate was 59%. Clotrimazole did not appear to affect the rate of colonization. This occurred despite the similar age and burn size of the two patient groups.

EFFECT OF CLOTRIMAZOLE ON THE PREVENTION OF FUNGAL COLONIZATION AND INFECTION IN THE THERMALLY INJURED PATIENT

Opportunistic fungal infections have been well documented as complications of uncontrolled diabetes, neoplasia, and diseases associated with a depressed immune system (1). Enhanced growth of fungi and yeasts has been associated with decreased host resistance, metabolic disturbances such as metabolic acidosis, impaired phagocytosis, ecologic disturbances of flora such as those due to the use of antibiotics, and local tissue defects, which provide a portal of entry for these organisms (2). Although there has been an overall decrease in the incidence of bacterial burn wound infection as a consequence of topical antimicrobial agents, opportunistic infections by fungi and yeast have proved to be a problem facing the thermally injured patient (3).

Nash et al (4), in a review of the experience at this Institute, demonstrated that since the introduction of topical mafenide acetate, the incidence of infection caused by the broad hyphae fungi, such as Aspergillus and Fusarium, increased 10-fold (4). Spebar and Lindberg (5), in a review of 1,245 patients at this Institute, noted a 35% colonization rate with fungi or yeast. Of those patients, 7% with candidal colonization developed invasive infection, whereas 47% of patients colonized with fungi developed invasive infections. It appeared to those authors that patients at greatest risk for mycotic infection were those with extensive thermal injuries (> 55% of the total body surface area), and those receiving intravenous antibiotics for bacterial sepsis. A more recent review of 2,141 patients over a 10-yr period at this Institute revealed 54 patients with invasive fungal infection as compared with 68 patients with bacterial burn wound invasion. Patients sustaining fungal burn wound infections had an average burn size of 62% of the total body surface area, associated inhalation injury in 47% of the cases, and an overall mortality of 75%. The infecting organism was Aspergillus or Fusarium in 68% of cases and Candida in 16%. The annual incidence for invasive fungal burn wound infection was 6%. It was clinically observed that 33% of patients with a wound biopsy histologic classification of 1C or greater had evidence of split-thickness skin graft loss as a consequence of the fungal proliferation.

The majority of the fungi colonizing, and potentially invading, the burn wound probably originate from the environment. Stone et al (6) reported that most of the cases at their institution originated from contaminated air conditioning ducts. Consequently, efforts to protect the wound from fungal colonization is an attractive approach to the problem. This might include specific filters designed to decrease patient exposure to fungi, added precautions with linen which might potentially harbor fungal spores, and finally, the use of topical antifungal agents in a prophylactic fashion.

One percent clotrimazole cream is a synthetic antifungal agent used topically for dermal infections (7). It is a member of the imidazole family of antifungal agents which presumably acts by promoting intracellular leakage of phosphorus compounds, resulting in a breakdown of cellular nucleic acids. This preparation has been heralded as a potential agent for the prevention of fungal wound colonization.

The objective of this study was to determine whether or not topically applied clotrimazole cream diminishes the incidence of fungal burn wound colonization and infection in thermally injured patients.

MATERIALS AND METHODS

Study Design. Twenty-five patients were randomized in a pair-wise fashion to receive either standard wound care of alternating mafenide acetate and silver sulfadiazine or the standard wound care with the addition of clotrimazole cream (Lotrimin® Cream 1%, Schering Corporation, Kenilworth, NJ). Routine microbiologic surveys were performed and, when clinically indicated by the appearance of the wound, biopsies were obtained for histologic evaluation.

Description of Procedures. Twenty-five patients were randomized to receive either the standard wound care, i.e., morning application of 10% mafenide cream at a thickness of approximately 1/16th of an inch and a similar evening application of 1% silver sulfadiazine cream, or the standard wound care with the addition of 1% clotrimazole cream applied prior to each application of the topical antimicrobial agents. Burn wounds were examined by a physician on a daily basis for any evidence of burn wound infection. Routine microbiologic cultures were obtained every Monday, Wednesday, and Friday, and as clinically indicated. Isolates from each source were typed and compared and the timing of colonization for each source was also recorded. Wounds having a clinical appearance suggestive of invasive fungal infection underwent a full-thickness lenticular biopsy for histologic evaluation. Evidence of invasive fungal burn wound infection was considered an indication for surgical excision of the wound. Serial liver function tests, WBC counts, BUNs, and creatinines were monitored to rule out any toxic effect of the medication.

Patient Criteria. Twenty-five patients were enrolled in this study. Properly signed and witnessed DA Forms 5303-R, Volunteer Agreement Affidavit, were obtained from each patient prior to beginning the study.

Patient Inclusion. Patients meeting the following criteria were eligible for enrollment in this study:

1. Male or female patients $\geq$ 18 yr. Female patients must have been surgically sterilized, be postmenopausal (> 45 yr and lack of menstrual periods for at least 1 yr), or have a negative pregnancy test prior to initiation into the study.

2. Patients admitted to the US Army Institute of Surgical Research within 48 h postburn.

3. Patients with burns > 30% of the total body surface area (the presence of an inhalation injury not being exclusionary).

Patient Exclusion. Patients meeting any of the following criteria were excluded from participation in this study:

1. Patients < 18 yr.

2. Patients not admitted to the US Army Institute of Surgical Research within 48 h postburn.

3. Patients with burns < 30% of the total body surface area.

4. Patients who were pregnant or nursing.

Data Collection. Microbiologic survey data of blood, sputum, urine, and topical cultures were recorded for all patients. Wounds were examined on a daily basis and any suggestion of fungal colonization or fungal burn wound invasion was recorded and documented as indicated.

RESULTS

Twenty-five patients were enrolled in this study, 13 receiving clotrimazole therapy and 12 serving as controls. Three patients were subsequently found to be colonized with fungus on the day of injury and were excluded from further analysis, leaving 22 patients for evaluation (see Table 1). Five controls and three treated patients were colonized with true fungus, while two controls and three treated patients were colonized with Candida species.

DISCUSSION

The overall colonization rate was 52% for all patients enrolled in this study. For those patients who survived the first postburn week, the colonization rate was 59%. Clotrimazole did not appear to affect the rate of colonization. This occurred despite the similar age and burn size of the two patient groups.

PRESENTATIONS/PUBLICATIONS

None.

TABLE 1. Results from a Study of the Use of Clotrimazole to Prevent Fungal Colonization of Burn Wounds (Mean ± SEM)

Group	n	Age (Yr)	Total Body Surface Area Burn Size (%)	Number of Patients Colonized (%)	Percentage of Patients Colonized	Postburn Day of Colonization
Treatment	11	35.2 ± 3.4	59.1 ± 5.6	6	54.5	16 ± 2.6
Control	11	44.3 ± 6.5	66.2 ± 7.1	7	63.6	19 ± 2.6

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

ACCESSION: DA335814

SUMMARY DATE: 921001 **SUMMARY KIND:** D **PREV DATE:** 911001 **DISTRIBUTION:** CX

PROGRAM #: 62787A **PROJ #:** 30162787A874 **TASK AREA:** EF **WORK UNIT:** 168

TITLE: Determination of Vecuronium Bromide Requirements in the Thermally Injured Patient

SUBJ1: 060500 - Medicine and Medical Research
SUBJ2: 061500 - Pharmacology

START DATE: 9101 **END DATE:** 9309 **PERFORMANCE METHOD:** C

CONTRACT/GRANT NO:

CONT TOTAL: $
CUM TOTAL: $
TOTAL LAB FUNDS: $

RESOURCES ESTIMATE

FY	WORK YRS	$(Thousands)
91	0.4	$19
92	0.5	$20
93	0.5	$21

RESPONSIBLE DOD ORGANIZATION: 404885
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PERFORMING ORGANIZATION: 404885
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ASSOC1: MONGAN, P D

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FOREIGN INTELLIGENCE CONSIDERED: Y **CIVILIAN APPLICABILITY:** M

KEYWORDS: RA II; Volunteers; Adults; Burns (Injuries); Anesthesia; Muscle Relaxants; Dosage; Pharmacokinetics

OBJECTIVE: A DTIC literature search was conducted under DTIC request numbers W6025N/W6029N dated 29 January 1990. The objectives of this work are to determine the ED_{95} of vecuronium bromide for train-of-four twitch height depression in thermally injured patients and compare these data with historical data previously determined for nonthermally injured patients.

APPROACH: Forty patients will be enrolled in this study. Two 25-ga needles will be inserted subcutaneously at the elbow to stimulate the ulnar nerve. Once the train-of-four twitch height depression is stable for 3 min, 100 µg/kg of vecuronium bromide will be administered intravenously. Maximal twitch height depression will be recorded and plotted. Because of parallel slopes of individual response curves, an incremental dose required to achieve 95% twitch height depression will be calculated and administered intravenously. The maximal effect of this second dose will also be recorded and plotted. These two measurements will be utilized to construct an individual dose-response curve for each patient. Individual data will be analyzed to determine an average ED_{95} for the entire cohort. Data will then be analyzed for standard deviation and a Pearson R will be used to ascertain if statistically significant difference ($P < 0.01$) exists in the ED_{95} between thermally injured and nonthermally injured patients.

PROGRESS: 9110-9209. Fifteen patients have been enrolled in this study to date, 13 during this reporting period. Upon completion of enrollment, data will be analyzed as indicated. For technical

reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report* for fiscal years 1991 and 1992.

ABSTRACT

PROJECT NUMBER: 3M162787A874-168, Applied Research and Exploratory Development

PROJECT TITLE: Determination of Vecuronium Bromide Requirements in the Thermally Injured Patient

INSTITUTIONS: US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas 78234-5012[1]; Department of Surgery, Brooke Army Medical Center, Fort Sam Houston, San Antonio, Texas 78234-6200;[2] and Wilford Hall United States Air Force Medical Center, Lackland Air Force Base, San Antonio, Texas 78236[3]

PERIOD COVERED IN THIS REPORT: 1 October 1991 - 30 September 1992

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The primary application of this study is to determine the ED_{95} dose of vecuronium bromide for train-of-four twitch height depression in the thermally injured patient. This has previously not been done by any investigator. Other muscle relaxants, including d-tubocurarine, pancuronium bromide, metocurine iodide, and atracurium besylate have been studied in the past in relatively small cohorts of burned patients. It has been determined in these studies, and also clinically, that the burn patient is resistant to the effects of nondepolarizing muscle relaxants. Numerous theories exist as to the etiology of this phenomenon, but none have been substantiated. Regardless of the pharmacokinetic and pharmacodynamic reasons for this resistance, the knowledge of the dose-effect relationship is useful in the clinical practice of anesthetizing burned patients.

DETERMINATION OF VECURONIUM BROMIDE REQUIREMENTS IN THE THERMALLY INJURED PATIENT

The US Army Institute of Surgical Research provides surgical and anesthetic management to hundreds of military members, dependents, and civilian emergencies yearly. As has been evident in recent military conflicts, a large number of casualties involve thermally injured patients. In order to provide optimum treatment of injured personnel, to serve the present and future needs of military medicine, and to further the body of knowledge pertaining to anesthetic management of burned patients, we must actively research and answer questions pertaining to the clinical rationale for management of the thermally injured patient.

Martyn et al (1) at the Anesthesia Services of the Massachusetts General Hospital and the Department of Anesthesiology, Harvard Medical School (Boston MA) have provided the bulk of knowledge concerning clinical pharmacology of nondepolarizing muscle relaxants in patients with burns. His group demonstrated persistent resistance to neuromuscular-blocking effect of metocurine iodide in the burned patient (2,3). His group also demonstrated a shift in the dose-response curve of pancuronium bromide (4) as well as d-tubocurarine (5,6) in the burned patient. More recently, a group from the University of Washington School of Medicine and the Harborview Medical Center (Seattle WA) investigated and published data concerning the clinical response of the burned patient to atracurium besylate and the relationship of that response to the percent of total body surface area burned and the number of days postburn (7). To date, no study has been published concerning the dose-response curve for vecuronium bromide in the burned patient.

Vecuronium bromide, one of the newer, intermediate-acting, nondepolarizing muscle relaxants has the advantage of having the highest therapeutic ratio of any of the currently available nondepolarizing muscle relaxants. Because of the lack of deleterious side effects and its intermediate half-life which adds to its versatility, vecuronium bromide has become one of the relaxants of choice at this institution and others for aiding in rapid control of the airway and for maintenance of relaxation during operative procedures. Accurate knowledge of the ED_{95} of vecuronium bromide in the burned patient would help to make the practice of anesthesia in the thermally injured patient more precise and safe.

The objectives of this study are to determine the ED_{95} of vecuronium bromide for train-of-four twitch height depression in the thermally injured patient and compare the ED_{95} determined for these patients to that previously determined for nonthermally injured patients.

MATERIALS AND METHODS

Study Design. Patients are premedicated at the discretion of the anesthesiologist. After placement of monitors and preoxygenation, patients are induced with sufentanil citrate and thiopental sodium or ketamine as indicated by the patient's condition. The patients are intubated and controlled ventilation is instituted with nitrous oxide and oxygen in a 2:1 ratio. Normothermia is maintained and no volatile anesthetics are utilized during the study portion of the anesthetic. Two 25-ga needles are inserted subcutaneously at the elbow to stimulate the ulnar nerve. Stimulation is a train-of-four supramaximal square wave pulse administered over 2 sec by a Grass Nerve Stimulator (Grass Instruments, Inc., Boston, MA). This stimulation is repeated every 12 sec and the evoked tension of thenar adduction is measured by a Grass FT10 Force Transducer (Grass Instruments) and recorded by a Grass 7 Polygraph (Grass Instruments). Once the train-of-four twitch height is stable for 3 min, 100 μg/kg of vecuronium bromide is administered intravenously. Maximal twitch height depression is recorded and plotted on 6.5 probit/log paper. Because of parallel slopes of individual response curves, an incremental dose required to achieve 95% twitch height depression is calculated and administered intravenously. The maximal effect of this second dose is recorded and plotted. These two measurements are utilized to construct an individual dose-response curve for each patient. The individual data are analyzed to determine an average ED_{95} for the entire cohort. The data are analyzed for standard deviation and a Pearson R is used to correlate ED_{95} with the percent total body surface area burn size. A student t test will be used to ascertain if a statistically significant difference ($P < 0.01$) exists in the ED_{95} between thermally injured and nonthermally injured patients. This two-dose method for determining individual dose-response curves and ultimately ED_{95} values has been shown to be a valid one by Meretoja and Wirtavuori (8).

Description of Procedures. Patients undergo study after induction of general anesthesia as described above. Two 25-ga needles are inserted at the elbow to stimulate the ulnar nerve. The forearm, fingers, and thumb are positioned in the force transducer so that the initial resting tension of the adductor pollicis brevis muscle is at least 200 g. The ulnar nerve is stimulated supramaximally with train-of-four square wave pulse over 2 sec. This stimulation is repeated every 12 sec and the evoked tension of thenar adduction is measured and recorded. Once the train-of-four twitch height is stable for 3 min, 100 μg/kg vecuronium bromide is administered intravenously. Maximal twitch height depression is recorded on 6.5 probit/log paper. Because of parallel slopes of individual response curves, an incremental dose required to achieve 95% twitch height depression is calculated and given intravenously. The maximal effect of this second dose is also recorded and plotted. The patients then undergo their operative procedure as planned. The time required for these

measurements has been approximately 25–30 min. Additional surgeries for each patient, if necessary, are performed using the specific dose determined in this study.

Patient Criteria. Forty patients admitted to the US Army Institute of Surgical Research will be enrolled in this study. Properly signed and witnessed DA Forms 5303-R, Volunteer Agreement Affidavit, are obtained from each patient prior to beginning the study.

Patient Inclusion. Patients meeting the following criteria are enrolled in the study upon giving written informed consent:

1. Male or female patients ≥ 18 yr scheduled by their attending surgeon for excision and grafting of their burns.

2. Patients > 1 week postburn.

3. Patients with burns > 33% of the total body surface area (the presence of an inhalation injury not being exclusionary).

Patient Exclusion. Patients meeting the following criteria are excluded from participation in the study:

1. Patients < 18 yr.

2. Patients < 1 week postburn.

3. Patients with burns < 33% of the total body surface area.

4. Patients with toxic epidermal necrolysis.

5. Patients with any condition known to alter response to neuromuscular blocking drugs, i.e., myotonia, myasthenia gravis, myasthenic syndrome, Von Recklinghausen's disease, systemic lupus erythematosus, polymyositis, dermatomyositis, polyarteritis nodosa, lower motor neuron disorders, and sepsis.

6. Patients receiving any drug known to alter response to neuromuscular blockers, i.e., aminoglycosides, polymyxin B, tetracyclines, colistin, sodium colistimethate, phenytoin sodium, and carbamazepine. If a patient is to receive aminoglycoside antibiotics perioperatively, the dose is administered after data collection has been completed, prior to surgical incision.

Determination of Number of Subjects Required. Forty patients will be required for this study. Though we do not have reasonable estimates of variance in burned patients, previous work indicates a reasonable requirement for 40 patients to establish response parameters in burned patients with enough precision for comparison with published control data.

Data Collection. Data pertaining to the patients' burn size is derived from the clinical record. Data collected from patients is in the form of evoked polygraph recordings taken in the operating room.

Data Analysis Plan. The individual dose-response curve data will be analyzed to determine an average ED_{95} and variance parameters. Also, multivariate correlation will be sought for ED_{95} with percent total body surface area burn size, postburn day, and sepsis, if present. Student t test will be used to ascertain if a statistically significant difference exists between the ED_{95} for normal patients (published widely in the literature) and for thermally injured patients.

RESULTS

Fifteen patients have been enrolled in this study to date, 13 during this reporting period. There were no side effects or adverse reactions.

DISCUSSION

The small number of patients limits the validity of statistical manipulation of the data at this point. When the projected total of 40 patients have completed the study, the data will be analyzed as to the clinical pharmacology of vecuronium bromide in patients with burns.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

ACCESSION: DA335813

SUMMARY DATE: 921001 **SUMMARY KIND:** D **PREV DATE:** 911001 **DISTRIBUTION:** CX

PROGRAM #: 62787A **PROJ #:** 30162787A874 **TASK AREA:** BN **WORK UNIT:** 169

TITLE: Short-Term Anabolic Effects of Recombinant Human Insulin-Like Growth Factor I in Thermally Injured Patients

SUBJ1: 060500 - Medicine and Medical Research
SUBJ2: 061500 - Pharmacology

START DATE: 9102 **END DATE:** 9309 **PERFORMANCE METHOD:** C

CONTRACT/GRANT NO:

CONT TOTAL: $
CUM TOTAL: $
TOTAL LAB FUNDS: $

RESOURCES ESTIMATE

FY	WORK YRS	$(Thousands)
91	0.3	$16
92	0.5	$20
93	0.5	$21

RESPONSIBLE DOD ORGANIZATION: 404885
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PERFORMING ORGANIZATION: 404885
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ASSOC1: GRAVES, T A

ASSOC2: BECKER, W K

FOREIGN INTELLIGENCE CONSIDERED: Y **CIVILIAN APPLICABILITY:** M

KEYWORDS: RA II; Volunteers; Adults; Burns (Injuries); Hormones; Protein Metabolism; Therapy; Recombinant Proteins

OBJECTIVE: A DTIC literature search was conducted under DTIC request numbers W6P15L/W6P18N dated 3 January 1991. The objectives of this work are to assess the biochemical and physiologic efficacy of recombinant human insulin-like growth factor I in thermally injured patients.

APPROACH: Fifteen patients with thermal injury will be studied between postburn days 7 and 15. Recombinant human insulin-like growth factor I will be administered as a continuous intravenous infusion at a rate of 20 µg/kg/h for a period of 3 days. Differences among pretreatment, during treatment, and posttreatment indices will be valued using ANOVA.

PROGRESS: 9110-9209. Six patients have been enrolled in this study to date, 2 during this reporting period. Four of six patients demonstrated a significant insulin-like effect of recombinant human insulin-like growth factor I. These four patients also had a 30% decrease in protein oxidation and a 15% decrease in protein breakdown, effects which are desirable in catabolic burn patients. For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report* for fiscal years 1990 through 1992.

ABSTRACT

PROJECT NUMBER: 3M162787A874-169, Applied Research and Exploratory Development

PROJECT TITLE: Short-Term Anabolic Effects of Recombinant Human Insulin-Like Growth Factor I in Thermally Injured Patients

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

PERIOD COVERED IN THIS REPORT: 1 October 1991 - 30 September 1992

INVESTIGATORS: William G. Cioffi, Jr, MD, Major, MC
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Bryan S. Jordan, RN, MSN
Avery A. Johnson, BS
George M. Vaughan, MD, Colonel, MC
William F. McManus, MD, Colonel, MC
Basil A. Pruitt, Jr, MD, Colonel, MC

This trial was designed to determine the anabolic effects of recombinant human insulin-like growth factor I (rhIGF-1) in thermally injured patients. rhIGF-1 is administered for 3 days by continuous intravenous infusion. Patients are evaluated at baseline (before drug administration), after 3 days of drug administration, and 3 days after cessation of drug administration. Indices to be measured will allow one to determine if administration of rhIGF-1 results in a decrease in protein catabolism and hypermetabolism which are associated with thermal injury.

Eight patients have been enrolled in this study to date, including two patients enrolled since 30 September 1992. No untoward effects of the rhIGF-1 infusion have been noted. Insulin and C peptide levels decreased significantly in all patients during rhIGF-1 infusion as compared to preinfusion values. No significant trend in 3-methylhistidine excretion was noted. Stable isotope data have been collected and are currently being analyzed. Resting energy expenditure did not appear to change with rhIGF-1 infusion.

Data from the intravenous glucose tolerance test showed that serum glucose curves were similar both preinfusion and during and after rhIGF-1 infusion for all patients. However, serum insulin levels were significantly less and did not show an increase during the glucose infusion while the patients were receiving rhIGF-1.

When 15 patients have completed the study, all data will be analyzed so that the effect of rhIGF-1 infusion on protein kinetics can be ascertained.

SHORT-TERM ANABOLIC EFFECTS OF RECOMBINANT HUMAN INSULIN-LIKE GROWTH FACTOR I IN THERMALLY INJURED PATIENTS

No therapeutic modalities exist which decrease the catabolic state which occurs during the hypermetabolism characteristic of thermal injury. The current nutritional treatment of thermally injured patients using high caloric enteral and/or parenteral feeding does little to decrease the erosion of lean body mass. Attempts to reverse catabolism by experimental treatment with growth hormone have been promising under certain conditions (1,2). The administration of pharmacologic doses of growth hormone to healthy adults results in a protein-sparing effect, i.e, nitrogen balance becomes positive. Serum IGF-1 levels increase in parallel. Clinical trials using growth hormone in a variety of catabolic patients demonstrated that growth hormone was somewhat effective in conserving body proteins. However, the most severely ill patients did not improve their nitrogen balance, and significant increases in endogenous serum IGF-1 levels were not found. Long-term studies have yet to be published demonstrating favorable effects of growth hormones on clinical outcome (3). However, growth hormone is an insulin antagonist, and therefore, may contribute to stress-induced insulin resistance in critically ill patients.

Data confirming the role of insulin-like growth factors in the regulation of growth, metabolism, and differentiation have expanded remarkably during the past decade. The two major components of this family of hormones, IGF-1 and IGF-2, have both anabolic and insulin-like properties. Both growth factors share a structural homology with pro-insulin. Human IGF-1 consists of 70 amino acids with a molecular weight of 7,649. Circulating IGF-1 is normally bound to specific carrier proteins, and less than 20% of circulating IGF-1 is in the unbound state. The development of a specific RIA for this growth factor and the biosynthesis of recombinant IGF-1 have provided the opportunity to study its biologic effects and therapeutic potential in humans.

In 1971, Daughaday et al (4), proposed that growth hormone regulates the synthesis and release of IGF-1 by the liver. IGF-1 is considered to be one of the potential mediators of the anabolic effects of growth hormone. Serum concentration of IGF-1 is low in infants and rises during childhood to reach peak levels during adolescence. In the normal adult, IGF-1 is present in the serum in concentrations comparable to the preadolescent child. IGF-1 levels are low in growth hormone-deficient individuals and levels rise in response to administration of exogenous growth hormone.

To date, there is no evidence of a protein-sparing effect of recombinant human IGF-1 in healthy patients. Studies in a burned rodent model suggest that recombinant human IGF-1 was able to reduce the hypermetabolism which contributes to a loss of lean body protein and body fat (5). Other reports in animals demonstrate

that recombinant human IGF-1 administered to starving rats reduces protein loss by reducing proteolysis (6). Furthermore, numerous reports show that IGF-1 is a growth-promoting agent in different models of impaired growth; hence, IGF-1 has many of the properties of an anabolic hormone.

Human trials have been performed showing the safety of intravenous administration of recombinant human IGF-1. The in vivo pharmacologic effects of this compound on healthy subjects include a dose-dependent hypoglycemia which can be ameliorated if the patients are fed a normal diet. Serum levels of insulin and C peptide are reduced substantially following rhIGF-1 administration. IGF-1 administration also lowered serum levels of triglycerides and total cholesterol. Additionally, GFR and renal plasma flow increased by approximately 25% in normal healthy controls. Tubular reabsorption of fluid and sodium increases in a similar manner, although no significant total body weight gain or edema were noted. Finally, IGF-1 has a positive cardiac chronotropic effect. No untoward effects of IGF-1 were noted in these trials.

The purpose of this trial is to determine the effects of a continuous intravenous infusion of recombinant human IGF-1 on the catabolic response to thermal injury.

MATERIALS AND METHODS

Study Design. This is a pilot study to assess the biochemical and physiologic efficacy of rhIGF-1 in up to 15 clinically stable burn patients in an open label-designed trial. Patients are enrolled in the study after completion of fluid resuscitation. Patients are studied between postburn days 7 and 15. The duration of treatment with rhIGF-1 is 3 days and the drug is administered by continuous intravenous infusion at a rate of 20 μg/kg/h.

Patient Criteria. Up to 15 patients will be enrolled in this study. Properly signed and witnessed DA Forms 5303-R, Volunteer Agreement Affidavits, are obtained from each patient prior to beginning the study.

Patient Inclusion. Patients meeting the following criteria are eligible for enrollment in the study:

1. Male or female patients > 18 yr. Female patients must have been surgically sterilized, be postmenopausal (≥ 45 yr and lack of menstrual periods for > 1 yr), or have a negative serum pregnancy test prior to initiation into the study.

2. Patients admitted to the US Army Institute of Surgical Research within 48 h postburn.

3. Patients with burns > 25% and < 85% of the total body surface area.

4. Patients who are hemodynamically stable and have successfully completed resuscitation.

Patient Exclusion. Patients meeting any of the following criteria are excluded from participation in the study:

1. Patients < 18 yr.

2. Patients not admitted to the US Army Institute of Surgical Research within 48 h postburn.

3. Patients with burns < 25% or > 85% of the total body surface area.

4. Patients who are pregnant or nursing.

5. Patients with high voltage electric injury.

6. Patients with a history of diabetes or requirement of insulin treatment during the course of the study.

7. Patients with evidence of significant preexisting cardiovascular disease.

8. Patients with a history of cancer, except those patients who have undergone curative surgical resection.

9. Patients with a history of known or suspected hypersensitivity to insulin or insulin-like growth factor.

10. Patients receiving any investigational drugs within the previous 30 days, except for 5% aqueous mafenide acetate.

11. Patients with blood glucose levels > 200 mg/dl at time of enrollment.

12. Patients with serum creatinine levels > 2.5 mg/dl at time of enrollment.

13. Patients with a body weight > 125 kg.

14. Patients with gross malnutrition.

15. Patients with an inability to tolerate an enteral diet.

16. Patients with abnormal liver enzymes.

Description of Procedures. Up to 15 patients will be enrolled in the study. After obtaining written informed consent, a baseline examination is undertaken, to include a medical history and physical examination, nutritional assessment (indirect calorimetry

to assess resting energy expenditure), EKG, chest roentgenogram, measurement of body weight, analysis of serum IGF-1 and glucose levels, evaluation of a 24-h urine sample for 3-methylhistidine excretion, intravenous glucose tolerance test (IVGTT) (during which time serum insulin and glucose levels are measured), and a stable isotope study using N-15 lysine to obtain a measurement of whole body protein synthesis and degradation.

After completion of the baseline studies, the patient begins receiving an intravenous infusion of rhIGF-1 (20 μg/kg/h) for a period of 3 days. During the infusion, daily physical examinations, nutritional assessment, and analyses of serum glucose levels are obtained (as well as serum glucose at 6 and 12 h after beginning the infusion). After 3 days of drug administration, the patient has an assessment of resting energy expenditure performed by indirect calorimetry, an evaluation of a 24-h urine sample for 3-methylhistidine excretion, IVGTT, and a stable isotope study using N-15 lysine. Upon completion of these studies, the drug is stopped. Three days later, all studies are repeated. On the day of each study, blood is drawn at 0700 h for thyroid function tests and serum cortisol levels.

Prior to beginning the study, patients are started on enteral nutrition which is continued at the same rate, protein content, carbohydrate content, and fat content for the duration of the study. Caloric requirements are estimated from the Institute's formula and patients receive at least 80% but not greater than 100% of that amount. No oral feedings are allowed during the study period. The calorie to nitrogen ratio is 150:1. The patients are not scheduled for surgery during the 7-day study period, but can have surgery prior to enrollment in the study. Serum BUN and creatinine as well as SGOT, SGPT, alkaline phosphatase, and bilirubin are measured every 3 days during the study period.

Intravenous Glucose Tolerance Test (IVGTT). Glucose is administered as a 50% aqueous solution in approximately a 50-ml bolus delivered intravenously over 90 sec in a dose of 0.5 g/kg. Blood samples (4-5 ml) are taken before the infusion and at 10, 20, 30, 40, 60, 90, 120, and 150 min afterwards. A monoexponential regression of serum glucose and insulin concentrations with time gives the best fit maximum and minimum values and the disappearance constant for each patient. These indices allow assessment of insulin secretion and resistance and glucose handling. Enteric glucose is infused at a constant rate throughout the 7-day course of the study. The rate of intravenous infusion of 5% glucose is determined by the patient's fluid needs, but 2 h before each IVGTT, the infusate is changed to a solution without glucose. The IVGTT is performed three times during the course of the study, i.e., a baseline study, a study during the infusion of rhIGF-1, and a study after stopping the administration of the hormone. This part of the study occurs after performing the N-15 lysine protocol.

N-15 Lysine Test. In order to determine whole body protein synthesis and degradation, the alpha N-15 lysine technique is used as described by Wolfe et al (7). This technique uses a stable, nonradioactive N-15 lysine isotope. This is injected as a constant infusion of N-15 lysine at a dose of 0.08 µmoles/kg/min. A priming dose of N-15 lysine of 6.8 µmoles/kg is administered prior to starting the infusion. In addition, the urea pool is primed by infusing N-15,N-14 urea at a dose of 3.2 µmoles/kg as a bolus dose. Sterile, nonpyrogenic amino acid is dissolved in sterile saline and the solution infused at a rate not to exceed 25 ml/h. After 1 h of the infusion, hourly plasma samples are obtained for 3 h. A 3-h urine collection is performed during this time. The percent enrichment of urine and plasma with N-15 lysine and N-15 urea is determined using GC-MS.

$$\text{Synthesis (g/kg/day)} = \frac{\text{Lysine Flux} - \text{Lysine Breakdown}}{\text{3.4 mM Lysine/g N}} \times 6.25 \text{ g P/g N} \times 24 \text{ h}$$

$$\text{Lysine Breakdown (µmoles/kg/min)} = \frac{\text{Urea Production (µmole/kg/min)} \times \frac{\text{2 µmole N}}{\text{µmole urea}} \times \text{Urea N (APE) Enrichment}}{\text{Plasma Lysine Enrichment (APE)}}$$

$$\text{Lysine Flux} = \frac{\text{Rate of Urinary N Excretion}}{\text{Rate of Excretion of N-15 Urea}} \times \text{Rate of Infusion of N-15 Lysine}$$

Data Collection. A medical history, measurement of body weight, physical examination, electrocardiogram, and chest roentgenogram are obtained. Data from analyses of serum IGF-1, creatinine, alkaline phosphatase, bilirubin, and glucose levels, resting energy expenditure assessments by indirect calorimetry, 24-h urine sample evaluations for 3-methylhistidine excretion, thyroid function, cortisol, SGOT, and SGPT tests, IVGTT, and N-15 studies are collected for each study patient. A USAMRDC Form 60-R, Volunteer Registry Data Sheet, containing demographics is also completed for each study patient.

Data Analysis Plan. Glucose tolerance test data were analyzed by calculation of the area under the glucose and insulin curves for the test time period. A ratio of the area under the glucose curve divided by the area under the insulin curve was then calculated. All before and during IGF-1 infusion data were compared for each patient using a paired student t test.

RESULTS

Eight patients have been enrolled in this study to date, including two enrolled since 30 September 1992. The mean age of these patients was 41.6 yr and mean total body surface area burn size was 56.2%. All patients completed the study and no untoward effects of IGF-1 were noted. Of note, hypoglycemia did not occur in any of the patients. All patients demonstrated a significant rise in serum IGF-1 levels while on therapy. Similarly, IGF-1 binding protein 2 and 3 levels increased during therapy (see Table 1). Although the negative nitrogen balance tended to decrease while on therapy, this difference was not statistically significant. Resting energy expenditure was not different during the two time periods, 2,844 versus 2,805 kcal/day, respectively. The results from the glucose tolerance testing did not reveal a significant difference in the area under the glucose curves before or during IGF-1 infusion. However, the area under the insulin curve decreased significantly during IGF-1 therapy (see Table 2). This resulted in an increase in the glucose area/insulin area ratio while on therapy from 7.09 to 11.99 ($P = 0.06$). Two of the 8 patients failed to demonstrate a significant insulin-like effect of the IGF-1 despite documented elevated serum levels of IGF-1. While all other patients demonstrated a blunted insulin response during the glucose tolerance testing, these 2 patients demonstrated a similar insulin response before and during IGF-1 therapy (see figs 1-4).

Analysis of the N-15 lysine data revealed a significant decrease in lysine oxidation during IGF-1 therapy ($P = 0.038$) and a near significant reduction in endogenous lysine ($P = 0.059$), an index of lysine breakdown (see Table 3).

DISCUSSION

The data from this study indicate that the continuous infusion of IGF-1 into severely catabolic, hypermetabolic, thermally injured patients can be performed safely without untoward effects. The infused IGF-1 had a significant insulin-like effect in six of the eight patients. Despite this insulin-like effect, no hypoglycemic episodes were noted in any of the patients during the IGF-1 infusion. The reason for lack of an insulin-like effect in two of the patients is unclear. Both patients had significant elevations of serum IGF-1 levels, indicating that they received the drug. Neither of the patients had clinical indications of infection during their trial, although one patient did require an insulin infusion to maintain normoglycemia after the trial was completed.

TABLE 1. IGF-1 and Binding Protein Levels, Nitrogen Balance, and Resting Energy Expenditure (Mean ± SEM)

Treatment Period	IGF-1 (ng/ml)	IGF-1 Binding Protein 1 (mg/ml)	IGF-1 Binding Protein 2 (mg/ml)	Nitrogen Balance (g/day)	Resting Energy Expenditure (kcal/day)
Before	83.4 ± 13.4	1274 ± 302	494 ± 36	−10.6 ± 3.3	2884 ± 198
During	675 ± 42.6*	1738 ± 313*	723 ± 56*	−7.8 ± 3.8	2805 ± 219

*P < 0.05 vs before treatment.

TABLE 2. Results of Glucose Tolerance Testing (Mean ± SEM)

Treatment Period	Glucose Curve Area	Insulin Curve Area	Glucose Curve Area/ Insulin Curve Area Ratio
Before	26197 ± 3016	4628 ± 868	7.09 ± 1.7
During	24582 ± 4177	2943 ± 853*	11.99 ± 2.9**

*P < 0.05, **P = 0.06 vs before treatment.

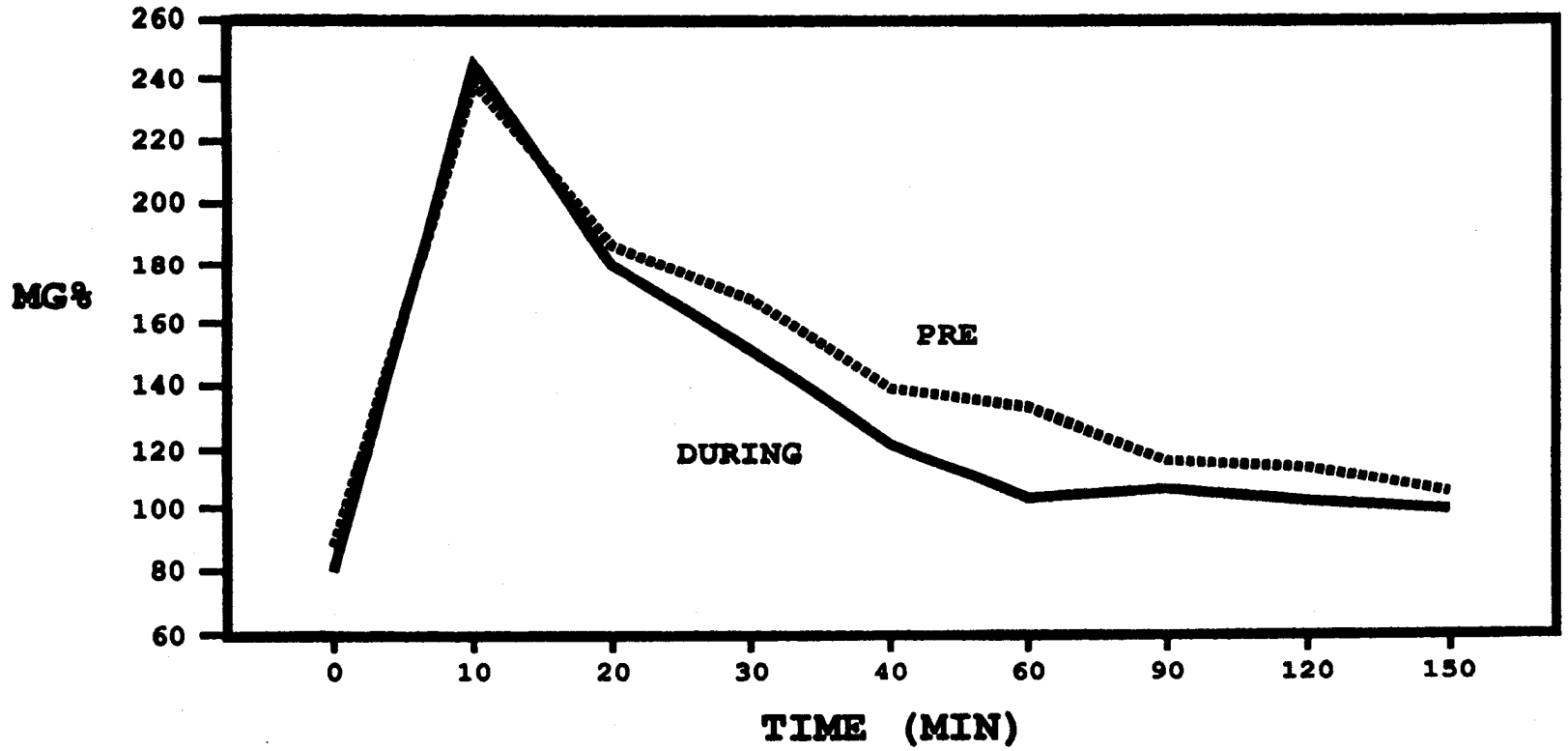


FIGURE 1. Peripheral blood glucose levels during the glucose tolerance test before and during IGF-1 infusion are depicted for patient #2.

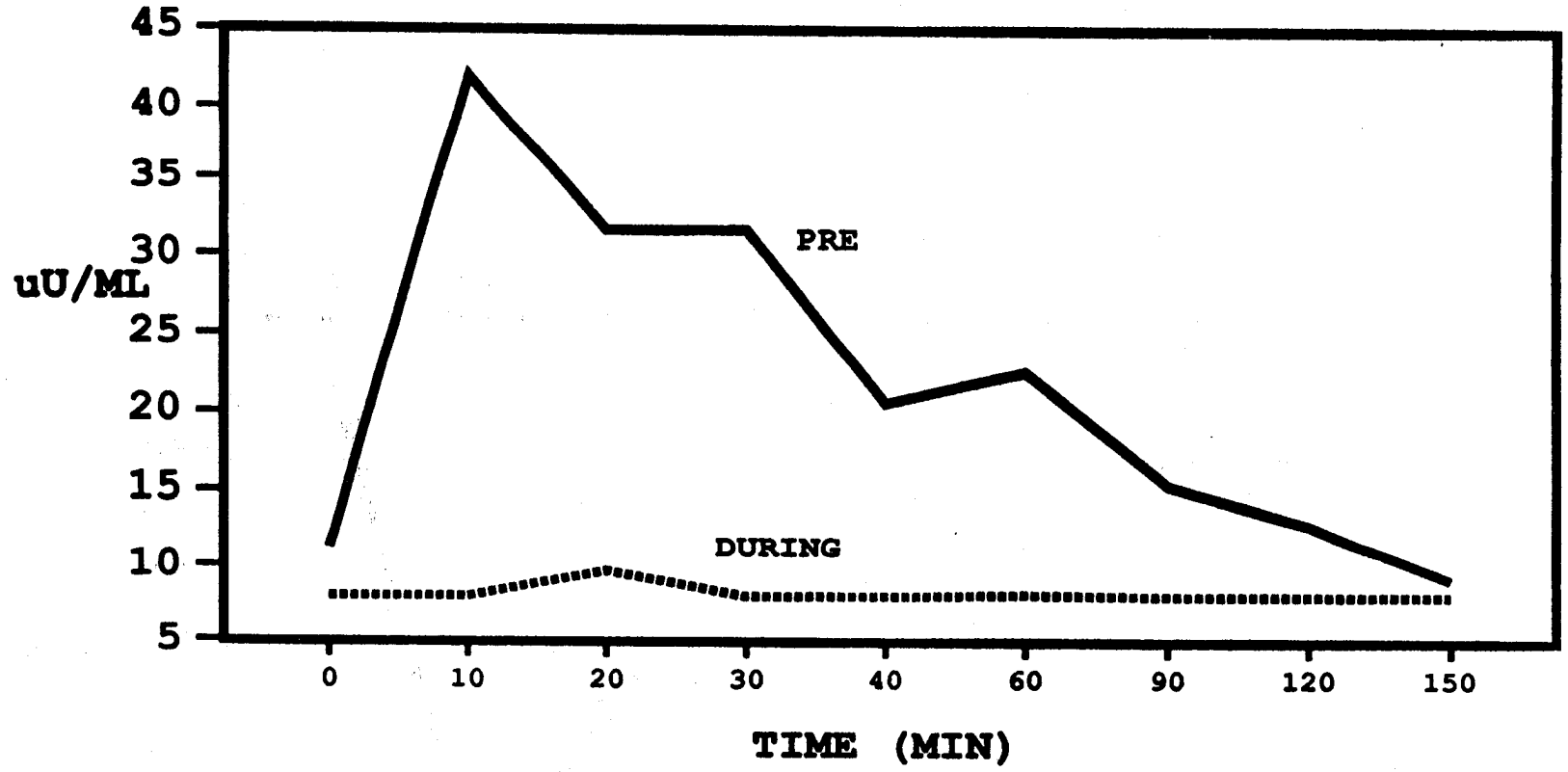


FIGURE 2. Insulin levels during the glucose tolerance test before and during IGF-1 infusion are depicted for patient #2. Note the sustained blunted insulin response during IGF-1 infusion.

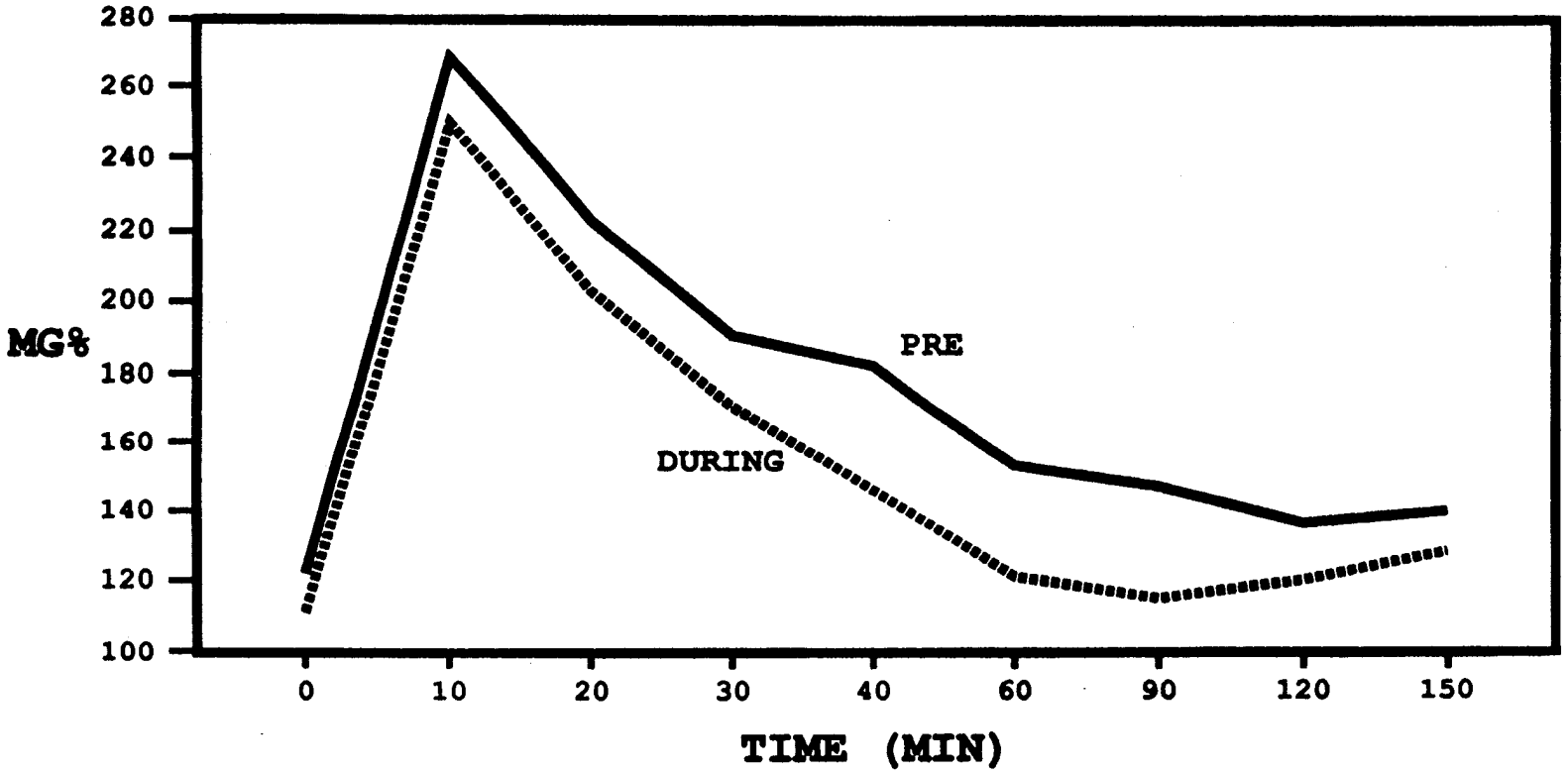


FIGURE 3. Glucose levels during the glucose tolerance test before and during IGF-1 infusion are depicted for patient #4.

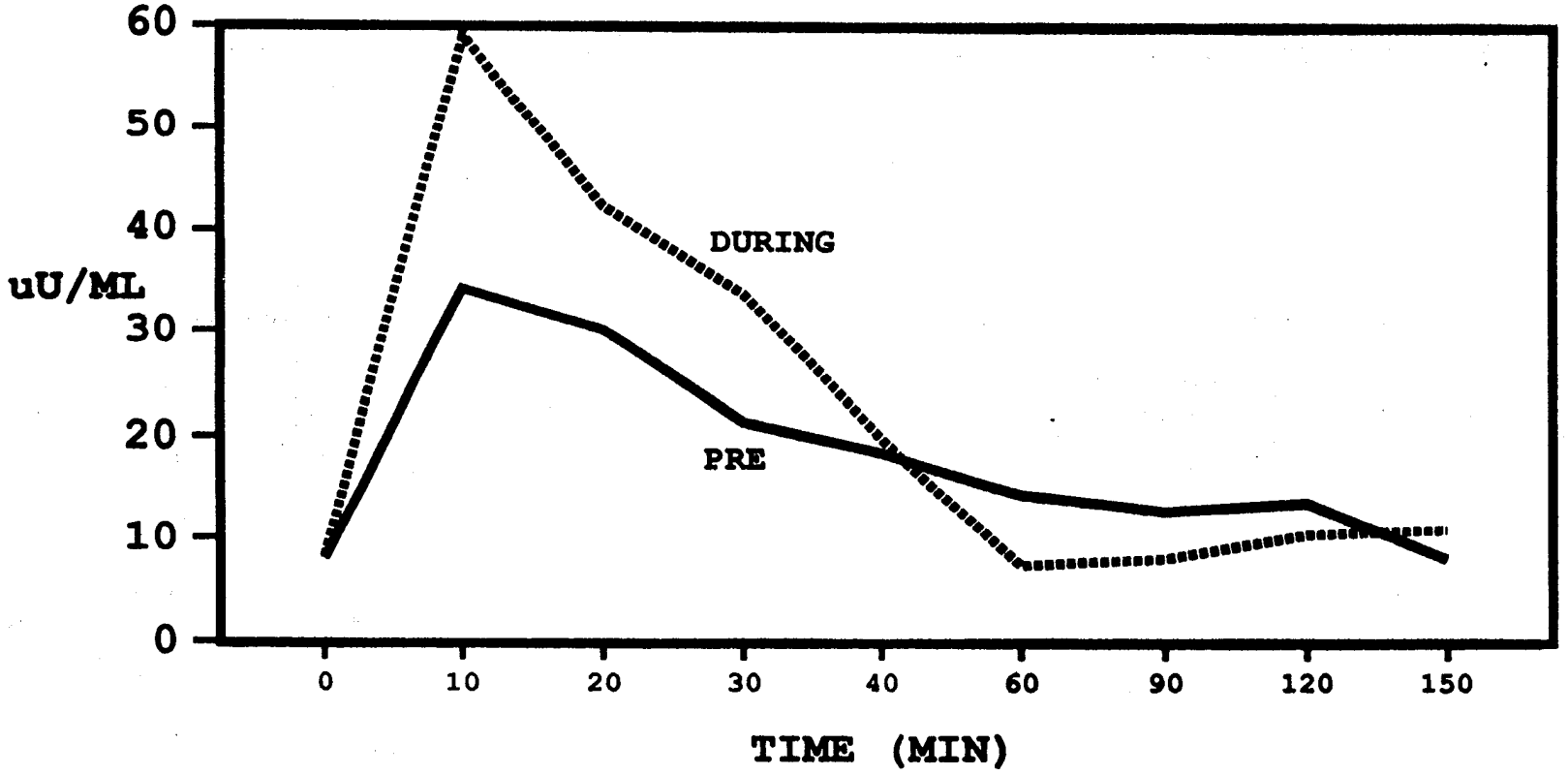


FIGURE 4. Insulin levels during the glucose tolerance test before and during IGF-1 infusion are depicted for patient #4. Note the lack of a blunted insulin response during the IGF-1 infusion.

TABLE 3. N-15 Lysine Results (Mean ± SEM)

Treatment Period	Percent Enrichment of Plasma with N-15 Lysine	Percent Enrichment of Plasma with N-15 Urea	Lysine Oxidation (µm/kg/min)	Rate of Appearance of Endogenous Lysine in Plasma (µm/kg/min)
Before	3.08 ± 0.19	0.02245 ± 0.004	0.04470 ± 0.011	3.092 ± 0.242
During	4.32 ± 0.52	0.02225 ± 0.0035	0.03303 ± 0.008*	2.523 ± 0.383

*P < 0.05 vs before treatment.

In this small group of patients, continuous infusion of IGF-1 resulted in a significant decrease in lysine oxidation. If one assumes lysine oxidation to be representative of the oxidation rate of all amino acids, this decrease would equate to a 26% decrease in protein oxidation (0.118 vs 0.087 g/kg/day). The rate of appearance of endogenous lysine decreased from 3.09 to 2.52 μm/kg/min (P = 0.0594). One of the patients who did not demonstrate an insulin-like effect of the IGF-1 was the only patient in whom lysine did not decrease while on therapy. This near significant decrease in protein breakdown suggests that IGF-1 therapy may conserve lean body mass in thermally injured patients.

The lack of adverse effects and the suggestion of a protein-sparing effect with IGF-1 infusion in this small group of catabolic, thermally injured patients suggest that future studies are warranted to determine the role of IGF-1 therapy in the treatment of thermally injured patients. Cross-limb studies to identify the site of IGF-1 action, as well as extension of the studies to other stable isotopes of amino acids and glucose are necessary to document the utility of this drug regimen. In addition, wound and muscle biopsies before and during infusion will be necessary to identify the effects of IGF-1 on protein synthesis.

PRESENTATIONS/PUBLICATIONS

None.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

ACCESSION: DA315359

SUMMARY DATE: 921001 **SUMMARY KIND:** D **PREV DATE:** 911001 **DISTRIBUTION:** CX

PROGRAM #: 62787A **PROJ #:** 30162787A874 **TASK AREA:** EB **WORK UNIT:** 170

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

SUBJ1: 060500 - Medicine and Medical Research

START DATE: 8610 **END DATE:** 9309 **PERFORMANCE METHOD:** C

CONTRACT/GRANT NO:

CONT TOTAL:	$
CUM TOTAL:	$
TOTAL LAB FUNDS:	$

RESOURCES ESTIMATE

FY	WORK YRS	$(Thousands)
91	1.5	$520
92	1.5	$366
93	1.5	$700

RESPONSIBLE DOD ORGANIZATION: 404885
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PRUITT, B A
210-221-2720

PERFORMING ORGANIZATION: 404885
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FITZPATRICK, J C
210-221-8440

ASSOC1: CIOFFI, W G

ASSOC2: DRISCOLL, D M

FOREIGN INTELLIGENCE CONSIDERED: Y **CIVILIAN APPLICABILITY:** M

KEYWORDS: RA II; Volunteers; Adults; Burns (Injuries); Skin Grafts; Healing; Keratinocytes; Cell Cultures; Biopsy

OBJECTIVE: A DTIC literature search was conducted under DTIC request numbers W6J05C/W6K07C dated 20 October 1989. The objectives of this work are to evaluate cultured keratinocytes as grafts for epithelial closure of burn wounds and identify technical and immunological requirements to establish banks of frozen histocompatible keratinocytes for wound coverage of burned soldiers. Use of cultured keratinocytes to hasten wound coverage of extensive thermal injuries will translate into improved survival of patients with thermal injuries.

APPROACH: The utility of cultured keratinocytes will be assessed initially with cultured autologous keratinocytes. Keratinocytes will be cultured from biopsies taken early after admission of patients with large burns and limited unburned donor sites for standard partial-thickness autografts. If such grafts are deemed clinically useful, efforts will expand into investigations of allogeneic skin cultures. In accordance with an addendum, reasons for delayed graft loss will be evaluated.

PROGRESS: 9110-9209. Three patients with burns greater than 85% of the total body surface area were enrolled in this study during this reporting period. Twenty-two sites were covered in nine procedures. Initial graft take on postburn day 10 averaged 36%. Final graft take averaged 4%. Delayed loss of previously intact epithelial autografts appears to result from infection and possibly the lack of a dermal analogue. One application of cultivated keratinocytes over dermabraded allograft did not show any improvement in take over placement on fascia, but further

attempts will be necessary to fully evaluate this technique. For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report* for fiscal years 1986 through 1992.

ABSTRACT

PROJECT NUMBER: 3M162787A874-177, Applied Research and Exploratory Development

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

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

PERIOD COVERED IN THIS REPORT: 1 October 1990 - 30 September 1991

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There are more than two million burn injuries annually in the United States. More than 10,000 deaths each year result from serious thermal injury. The ultimate outcome of burn patients is determined by wound coverage, and therefore, the ultimate goal in burn wound care is to achieve timely, permanent closure of the open wound. The objective of this study is to determine the suitability of cultured autologous epithelium for the closure of burn wounds compared to similar wounds covered with fresh autograft. As well, methods to optimize wound take and preoperative wound bed preparation are to be investigated.

Three patients with burns greater than 85% of the total body surface area were enrolled in this study during this reporting period but were unable to complete all parts of the study due to poor graft take. Twenty-two sites were covered in nine operative procedures. Keratinocyte grafts were applied to a mean of 9.45% of the body surface area per patient. Net total body surface area burn wound covered by keratinocyte grafts was 0.33% (range 0.11% to 0.55%).

Future investigation into this wound care technology should focus upon means by which engraftment success can be optimized. Investigation of the causes for delayed graft loss will be pursued by evaluation of the impact of microbial density and the possible role of autoimmune phenomenon; the utility of allograft dermis to increase the definitive total body surface area healed with cultivated keratinocytes will also be examined.

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

There are more than two million burn injuries annually in the United States. More than 10,000 deaths each year result from serious thermal injury. The ultimate outcome of burn patients is strongly influenced by timely wound coverage, and therefore, the ultimate goal in burn wound care is to achieve timely, permanent closure of the open wound. Currently, the only adequate permanent coverage is split-thickness autograft, as all other biologic membranes are temporary wound covers and artificial skin substitutes require ultimate thin split-thickness autografting. Often the surface area and depth of burn are so extensive that the patient's available donor sites are insufficient to provide adequate wound coverage. Consequently, a new source of autograft would be most desirable.

Human keratinocytes can now be cultured in vitro to produce confluent epithelial sheets (1). These cells can be grown from relatively small initial samples of the patient's unburned skin and can be expanded over a period of weeks to months to a size sufficient to cover the entire body surface area. The use of cultured autologous epithelium in burn patients has been reported by several institutions and is becoming a well-recognized therapeutic modality for the extensively burned patient (2-5), but has been hampered by problems with both immediate and delayed graft loss which may be related to infection, lack of a dermal surface for adherence of the graft the wound bed, rejection, or a combination of these factors.

The objective of this study is to determine the suitability of cultured autologous epithelium for the closure of burn wounds compared to fresh autograft applied to similar wounds. Methods to optimize wound take and preoperative wound bed preparation are to be investigated.

MATERIALS AND METHODS

Patient Criteria. Thirty patients will be enrolled in this study. Properly signed and witnessed DA Forms 5303-R, Volunteer Agreement Affidavit, will be obtained from each patient prior to beginning the study.

Patient Inclusion. Patients meeting the following criteria are eligible for enrollment in this study:

1. Patients hospitalized for burn injury.

2. Male or female patients $\geq$ 18 and $\leq$ 65 yr. Female patients must be previously surgically sterilized, be

postmenopausal (> 45 yr and lacked menstrual periods for > 1 yr), or have a negative pregnancy test.

3. Patients with burn wounds > 40% of the total body surface area.

Patient Exclusion. Patients meeting any of the following criteria are excluded from participation in this study:

1. Patients < 18 or > 65 yr.

2. Patients who are pregnant or nursing.

3. Patients with burns of ≤ 40% of the total body surface area.

Procedures. Within 48 h of admission to the Institute and after obtaining informed consent, skin samples consisting of epithelium and partial-thickness dermis are harvested under local anesthesia after alcohol skin preparation. A surface area of 10 cm^2 is harvested. The skin is then placed in a transport medium and transported to the tissue culture facilities where the epidermis is separated from the dermal elements. The epidermis is then trypsinized and the keratinocytes inoculated in tissue culture flasks containing a defined medium which does not require a feeder layer of lethally irradiated 3T3 cells (5). The cells are grown to sufficient numbers of confluent cell sheets to allow the grafting of between 20% and 40% of the patient's total body surface area. This requires approximately three weeks. During this time, the patient proceeds to the operating room and conventional therapy consisting of harvesting available donor sites and subsequent autografting are undertaken. Additional trips to the operating room for debridement and placement of various types of temporary biological dressings may be required in order to prepare the patient's other burns for grafting. Approximately three weeks postburn, the patient is returned to the operating room after preparation of cultured epithelium of sufficient area to cover the still open burn wound. At this time, the available donor sites are harvested and autograft placed on the burn wound. The remainder of the burn wound is covered with the cultured autologous epithelium mounted on the backing recommended by the supplier. This backing is also used to cover the autograft applied during the same operation. All grafted areas are recorded by location. The nature of the grafts applied and the nature of the recipient bed, i.e., freshly excised deep dermis, freshly excised fat, early granulation tissue (> 7 days old), chronic granulation tissue, and fresh fascial excision, are specified. Surface and tissue cultures are sent from random recipient bed sites.

The fresh autografts are treated with standard dressings and postoperative care. They are inspected on postoperative day 3 or 4. Perioperative antibiotics are used as customary within the

Institute. The cultured epithelial grafts, covered by the backing recommended by the supplier, are left covered by this adherent gauze for 7 to 10 days. Inspection of the gauze is undertaken on a daily basis to determine the presence of any large bullae which might elevate the graft off the recipient bed. These areas are aspirated through the overlying tissue. Any drainage is cultured. If the patient develops signs of infection, i.e., fever, leukocytosis, erythema, or other systemic signs, the dressings are inspected and, if they appear suspect, the gauze is changed and the grafted wound examined. Cultures are taken if indicated and the need to alter the wound care or begin antibiotic therapy is determined at that time.

After the initial 7- to 10-day period, the wounds grafted with the cultured epithelium are inspected and a decision made whether to leave them exposed or to reapply a protective dressing. Areas of graft take and loss are recorded and compared to areas grafted with fresh autograft. Additional grafting procedures are undertaken as needed to close the patient's burn wounds. Cultured epithelium is used at these later graftings if the patient's own donor sites are insufficient to cover the recipient bed. Healed cultured grafts are examined with regard to fragility. Punch biopsies from areas of adherent cultured epithelium are taken once the patient's burn wounds are fully healed. Patients enrolled in this study are followed after discharge from the hospital to determine any incidence of late sequelae such as contractures and breakdown of the grafted wounds.

Photographs are obtained of the burn wound prior to grafting, at the first dressing change, and weekly thereafter during the period of hospitalization. Additional photographs are obtained at any follow-up outpatient visits which take place. At these times, the graft viability is evaluated by quantitatively estimating the percentage of the grafted area which is covered by viable graft and by qualitatively evaluating the durability of the grafts and their tendency to ulcerate. The quantitative scale ranges from 0 to 10, corresponding to 0% and 100%, respectively. The first qualitative scale grades the durability of the graft into three categories, A = stability to minor trauma equal to that found in a typical unmeshed autograft, B = fragile graft, but adequate wound coverage, and C = very fragile coverage, at high risk for graft loss. The second qualitative scale also grades the grafts into three categories, A = no tendency to ulcerate, B = scattered small ulcerated areas, and C = large ulcerations involving at least 25% of the area grafted with cultured epithelium. All grafted areas are evaluated independently by the primary investigator and the Chief, Clinical Division. Punch biopsies from the areas of healed cultured epithelium are obtained under local anesthesia prior to discharge from the hospital and again at the follow-up visit 1 yr following discharge. These will be examined for evidence of surviving dermal elements.

Sterile Techniques. The skin biopsy is harvested using sterile technique following standard operative preparation and is transported to the tissue culture laboratory in sterile media. Manipulations of the keratinocytes are done under a laminar flow hood using sterile technique. The holding media is sterile and contains penicillin, streptomycin, and fungizone. The keratinocytes are then transported back to the Institute's operating room in sterile containers, which are only opened when they are to be placed on the surgical field.

RESULTS

Three patients with burns greater than 85% of the total body surface area were enrolled in this study during this reporting period but were unable to complete all parts of the study due to poor graft take. Twenty-two sites were covered in nine operative procedures. The mean age of the treated patients was 26 yr (range 25-27) with a mean burn size of 86.9% of the total body surface area (range 84.3% to 89%). All patients had documented inhalation injury requiring mechanical ventilatory support. The mean length of hospitalization was 254.66 days (195+, 208, and 361), with one patient still hospitalized. No mortalities occurred. Insufficient graft was present on any of the patients to allow 5-mm biopsies to be obtained for assessment of reasons for delayed graft loss.

Keratinocyte grafts were applied to a mean of 9.45% of the body surface area (range 4.5% to 15.3%) per patient. Mean graft take on postoperative day 10 was 37% (range 0% to 80%); final graft take on postoperative day 28 was 5% (range 0% to 10%). Net total body surface area burn wound covered by keratinocyte grafts was 0.33% (range 0.11% to 0.55%). Complete data on grafting procedures in these three patients is found at Table 1. Since the ultimate goal of this technology is definitive body surface area wound coverage, the percent of the total body surface area burn wound covered is the most objective means of assessing the impact of cultivated keratinocytes.

To further delineate the experience with this wound care approach, the success of engraftment was assessed with respect to the level of wound excision and the extent of burn. Table 2 summarizes our experience during the preceding fiscal year with respect to the excisional wound bed. Despite application of cultivated epithelial autografts to a larger proportion of the total body surface area (20.9% vs 12%), patients undergoing fascial excisions had a smaller area of definitive wound coverage (2.8% vs 6.1%) than patients undergoing dermal wound excisions. All patients enrolled in the study during the present year required fascial excision for all graft sites; due to poor graft take during the year and to new reports in the literature describing improved graft take with the technique of keratinocyte placement on dermabraded allograft, a protocol addendum was developed to evaluate this method of dermal substitution. At the time of this

TABLE 1. Individual Patient Data on Grafting Procedures

	TBSA Grafted (%)	Graft Site	Take		TBSA Covered (%)
			Initial (%)	Final (%)	
Patient 1	19.77	Fascia	40.00	–	–
	6.38	Fascia	33.00	–	–
	19.77	Fascia	10.00	5.00	1.00
Subtotal	15.31		27.67	1.67	0.33
Patient 2	7.00	Fascia	–	–	–
	6.38	Allograft	40.00	10.00	0.21
Subtotal	4.54		20.00	5.00	0.11
Patient 3	10.82	Fascia	70.00	10.00	1.10
	8.12	Fascia	–	–	–
	1.93	Allograft	80.00	10.00	0.20
	9.15	Fascia	60.00	10.00	0.91
Subtotal	7.51		52.50	7.50	0.55
TOTAL	9.45		37.00	5.00	0.33

TBSA indicates total body surface area.

TABLE 2. Wound Bed Data from Fiscal Year 1991

Excision Type	n=	Mean Age (Yr)	Mean Burn Size (% TBSA)	Keratinocyte Application (% TBSA)	Definitive Coverage (% TBSA)
Fascial	7	31.2 (18–48)	71.8 (44–85)	20.9 (4–59)	2.8 (0–6.3)
Dermal	9	28.5 (10–56)	65.5 (42–82)	12 (5.4–24.5)	6.1 (0–18.6)

( ) indicates range; TBSA, total body surface area.

report, two engraftments have been made using this procedure on small areas; no difference in results has yet been noted.

DISCUSSION

An increasing number of extensively burned patients are being successfully resuscitated and supported during the early postinjury period. Though modern intensive care management has contributed significantly to improved survival, ultimate outcome is still dependent upon definitive closure of the burn wound. The disparity between available donor sites and burn areas requiring coverage has stimulated interest in alternative means of wound coverage. Rheinwald and Green's report (6) of the ability to cultivate human keratinocytes in vitro has encouraged many investigators to explore the potential application of this technology for burn wound closure. Multiple reports exist in the literature which promote the use of cultured autologous epithelium in the closure of burn wounds (2-5).

An objective means of assessing the impact of this technology is to determine the actual total body surface area of wound closure. In this cohort of extensively burned patients (the target population for this technology), only 0.33% (range 0.11% to 0.55%) of the total body surface area was definitively covered with cultured epithelial autografts despite application to an average of 9.45% of the body surface area. Future investigation into this wound care technology should focus upon means by which engraftment success can be optimized. Investigation of the causes for delayed graft loss will be pursued by evaluation of the impact of microbial density and the possible role of an autoimmune phenomenon; the utility of allograft dermis to increase the clinical effectiveness of cultivated keratinocytes will also be examined.

PRESENTATIONS/PUBLICATIONS

None.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

ACCESSION: DA346158

SUMMARY DATE: 921001 **SUMMARY KIND:** K **PREV DATE:** 911001 **DISTRIBUTION:** CX

PROGRAM #: 62787A **PROJ #:** 30162787A874 **TASK AREA:** EA **WORK UNIT:** 171

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

SUBJ1: 060500 - Medicine and Medical Research
SUBJ2: 061200 - Medical Facilities, Equipment, and Supplies

START DATE: 9003 **END DATE:** 9207 **PERFORMANCE METHOD:** C

CONTRACT/GRANT NO:

CONT TOTAL: $
CUM TOTAL: $
TOTAL LAB FUNDS: $

RESOURCES ESTIMATE

FY	WORK YRS	$(Thousands)
91	0.5	$23
92	0.1	$ 4
93	0.0	$ 0

RESPONSIBLE DOD ORGANIZATION: 404885
MRDC INSTITUTE OF SURGICAL RESEARCH USAISR
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PRUITT, B A
210-221-2720

PERFORMING ORGANIZATION: 404885
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FT SAM HOUSTON, TX 78234
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210-221-8440

ASSOC1: CIOFFI, W G

ASSOC2:

FOREIGN INTELLIGENCE CONSIDERED: Y **CIVILIAN APPLICABILITY:** M

KEYWORDS: RA II; Volunteers; Adults; Burns (Injuries); Dressings; Skin Grafts; Excision

OBJECTIVE: A DTIC literature search was conducted under DTIC request numbers W6010C/W6007E dated 6 March 1990. The objectives of this work are to evaluate the efficacy of Eurothane® in the treatment of skin graft donor sites in comparison with fine-mesh gauze. Methods to hasten donor site healing and consequently burn wound closure will have a positive effect on the care of patients with thermal injury.

APPROACH: Patients underwent split-thickness skin graft harvesting from an anterior thigh. One-half of this donor site was treated with Eurothane® and the other half was covered with fine-mesh gauze. Records were maintained comparing the time of complete epithelization and the time at which reharvesting of the donor site was felt to be appropriate. Data analyses were performed using the paired t test.

PROGRESS: 9003-9207. This study was approved by the USAISR Research Council and US Army Institute of Surgical Research Human Use Committee during the second quarter of Fiscal Year 1990. Seventeen patients were enrolled in the study, with 13 patients completing the study. While there were no apparent risk with the use of Eurothane®, the only benefit appeared to be a decrease in postoperative pain. There did not appear to be any improvement in wound healing. For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report* for fiscal years 1990 through 1992.

ABSTRACT

PROJECT NUMBER: 3M162787A874-171, Applied Research and Exploratory Development

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

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

PERIOD COVERED IN THIS REPORT: 1 October 1991 - 1 July 1992

INVESTIGATORS: Robert L. Waguespack, MD, Captain, MC
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Loring W. Rue, III, MD, Major, MC
William F. McManus, MD, Colonel, MC
Basil A. Pruitt, Jr, MD, Colonel, MC

Eurothane® (BEAM Tech Ltd, Cheshire, United Kingdom) is manufactured from a polyetherurethane fabricated into a membrane structure designed to provide an ideal environment for natural healing by control of the factors which influence tissue repair. The objective of this study was to evaluate the efficacy of Eurothane® in the treatment of skin graft donor sites in comparison with fine-mesh gauze.

Seventeen patients were enrolled in this study, with 13 completing the study. While there were no apparent risks with the use of Eurothane®, the only benefit appeared to be a decrease in postoperative pain. There did not appear to be any improvement in wound healing.

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

Eurothane® (BEAM Tech Ltd, Cheshire, United Kingdom) is manufactured from a polyetherurethane fabricated into a membrane structure. The chemistry combines hard and soft blocks of urethane, producing a soft, flexible, elastic, asymmetric membrane 0.5 mm thick, having open pores on the wound contact surface and an ultraporous skin forming the outer surface.

Eurothane® is designed to provide an ideal environment for natural healing by control of the factors which influence tissue repair. The self-adhesive membrane properties of Eurothane® provide for thermal insulation and create an environment for optimal wound healing by controlling the exudate uptake and moisture vapor transmission. Maximum exudate absorption is four times the original weight of the dressing. Water removal from the wound is achieved by absorption into the open pores of the dressing with subsequent moisture vapor transmission at the surface. The dressing will not support the growth of microorganisms and the outer surface composition seems to prevent ingress of bacteria.

Availability of skin graft donor sites is a limitation in definitive burn wound closure. Methods to hasten donor site healing and consequently burn wound closure would have a positive effect on the care of thermally injured patients. The objective of this study was to evaluate the efficacy of Eurothane® in the treatment of skin graft donor sites in comparison with fine-mesh gauze.

MATERIALS AND METHODS

Study Design. Forty-two patients with burn injuries less than 70% of the total body surface area undergoing an initial split-thickness skin graft harvest from an anterior thigh were authorized for enrollment in this study. One surgeon harvested the skin grafts utilizing the same dermatome at the same thickness (0.010 in). One-half of the donor site was treated with Eurothane® after obtaining hemostasis with warm saline laparotomy pads. The dressing extended at least 1 cm beyond the wound margin for optimal adherence. The other half of the donor site was covered with fine-mesh gauze and hemostasis was achieved with warm saline laparotomy pads. The fine-mesh gauze was applied to the wound edges without overlap. The Eurothane®-treated donor site areas were inspected after removal of the dressing on the seventh postoperative day or at the time of spontaneous separation of the dressing. If the wound was completely reepithelialized, it was exposed to air. If spontaneous separation from an incompletely healed wound occurred before the seventh postoperative day, and

provided no contraindications existed, additional Eurothane® was applied.

Criteria for Admission to the Study. Patients admitted to the US Army Institute of Surgical Research were offered the opportunity to participate in this study. Properly signed and witnessed DA Forms 5303-R, Volunteer Agreement Affidavit, were obtained from each patient prior to initiation of the study.

Patient Inclusion. Patients meeting the following criteria were enrolled in the study upon giving written informed consent:

1. Male or female patients $\geq$ 18 yr. Female patients must have been surgically sterilized, postmenopausal (> 45 yr and the lack of menstrual periods for > 1 yr), or have had a negative pregnancy test before initiation into the study.

2. Patients with burn sizes < 70% of the total body surface area.

Patient Exclusion. Patients meeting any of the following criteria were excluded from participation in the study:

1. Patients < 18 yr.

2. Patients who were pregnant or nursing.

3. Patients with an injury as a result of an electrical burn or toxic epidermal necrolysis.

4. Patients with burn sizes $\geq$ 70% of the total body surface area.

Data Collection. Photographs were taken immediately after harvest, after removal of the Eurothane®, and at the time of separation of fine-mesh gauze as a comparison. Donor sites were examined daily for signs of infection or adverse reaction to the dressing. Donor sites from which the dressing prematurely separates were examined for signs of infection or tissue reaction. Adverse reactions and premature separations of the dressing were recorded. A record was maintained comparing the time of complete donor site epithelialization and the time at which reharvesting of the donor site was felt to be possible.

Data Analysis Plan. Data analysis was performed using the paired t test. Each patient served as his/her own control.

RESULTS

Seventeen patients were enrolled in this study, with 13 patients completing the study. While there were no apparent risks with the use of Eurothane®, the only benefit appeared to be a

decrease in postoperative pain. There did not appear to be any improvement in wound healing.

DISCUSSION

This study demonstrated that while Eurothane® decreases postoperative pain, it does not improve wound healing.

PRESENTATIONS/PUBLICATIONS

None.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

ACCESSION: DA318017

SUMMARY DATE: 921001 **SUMMARY KIND:** H **PREV DATE:** 911001 **DISTRIBUTION:** CX

PROGRAM #: 62787A **PROJ #:** 30162787A874 **TASK AREA:** FA **WORK UNIT:** 172

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

SUBJ1: 060500 - Medicine and Medical Research

START DATE: 8902 **END DATE:** 9202 **PERFORMANCE METHOD:** C

CONTRACT/GRANT NO:

CONT TOTAL: $
CUM TOTAL: $
TOTAL LAB FUNDS: $

RESOURCES ESTIMATE

FY	WORK YRS	$(Thousands)
91	0.5	$25
92	0.0	$ 0
93	0.0	$ 0

RESPONSIBLE DOD ORGANIZATION: 404885
MRDC INSTITUTE OF SURGICAL RESEARCH USAISR
FT SAM HOUSTON, TX 78234
PRUITT, B A
210-221-2720

PERFORMING ORGANIZATION: 404885
MRDC INSTITUTE OF SURGICAL RESEARCH USAISR
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MC MANUS, A T
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ASSOC1: BURLESON, D G

ASSOC2: MC MANUS, W F

FOREIGN INTELLIGENCE CONSIDERED: Y **CIVILIAN APPLICABILITY:** M

KEYWORDS: RA II; Volunteers; Adults; Burns (Injuries); Immunosuppression; Blood

OBJECTIVE: A DTIC literature search was conducted under DTIC request numbers W6K33C/W6K34C dated 20 October 1989. The objectives of this work are to determine the significance of TNF levels in burn patients and whether elevations in these levels predict impending infection or recovery from infection. There are currently no reliable blood tests for identifying the early stages of burn wound infection. Such tests would be of prognostic usefulness and might serve as a guide to therapy

APPROACH: A 5-ml sample of whole blood was drawn on a twice weekly basis from consecutive burn patients with burns exceeding 20% of the total body surface area. Patients were monitored on a prospective basis for the development of infections.

PROGRESS: 8902-9202. Twenty-three patients were enrolled in the study and serial plasma samples were drawn for analysis by ELISA. This study was terminated at the request of the primary investigator. For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report* for fiscal years 1989 through 1992.

ABSTRACT

PROJECT NUMBER: 3M162787A874-172, Applied Research and Exploratory Development

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

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

PERIOD COVERED IN THIS REPORT: 1 October 1991 - 30 September 1992

INVESTIGATORS: Albert T. McManus, PhD
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William F. McManus, MD, Colonel, MC
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Burn patients have a significant infection diathesis due in part to the loss of the normal epithelial skin barrier and to postburn immunosuppression. Burn patient wounds are therefore frequently contaminated with various microorganisms. Control of such contamination is attempted through the use of topical antimicrobial agents. Despite topical wound care, invasive burn wound infection occurs in certain patients with major thermal injuries. Currently, the diagnosis of a developing infection depends upon the clinical observation of the patient's wounds and a review of the patient's vital signs and laboratory test data. When such evidence indicates that an invasive burn wound infection could be developing, appropriate burn wound biopsies are obtained and sent for histopathological examination to check for the presence of invasive infection. Blood cultures are also obtained. There are currently no reliable blood tests for detecting burn wound infections early in the course of development. The development of positive blood cultures indicates that the infection has progressed to a very significant degree and is life-threatening to the patient. It would therefore be desirable to have a blood test available which would indicate early burn wound invasion before the patient has become critically and possibly irreversibly septic.

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

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

Twenty-three patients were enrolled in this study and serial plasma samples were drawn for analysis by ELISA. This study has been terminated.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

ACCESSION: DA346159

SUMMARY DATE: 921001 **SUMMARY KIND:** K **PREV DATE:** 911001 **DISTRIBUTION:** CX

PROGRAM #: 62787A **PROJ #:** 30162787A874 **TASK AREA:** DA **WORK UNIT:** 173

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

SUBJ1: 060500 - Medicine and Medical Research

START DATE: 9003 **END DATE:** 9209 **PERFORMANCE METHOD:** C

CONTRACT/GRANT NO:

CONT TOTAL: $
CUM TOTAL: $
TOTAL LAB FUNDS: $

RESOURCES ESTIMATE

FY	WORK YRS	$(Thousands)
91	0.5	$23
92	0.2	$ 2
93	0.0	$ 0

RESPONSIBLE DOD ORGANIZATION: 404885
MRDC INSTITUTE OF SURGICAL RESEARCH USAISR
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ASSOC1: SUMMERS, T M

ASSOC2: MC MANUS, W F

FOREIGN INTELLIGENCE CONSIDERED: Y **CIVILIAN APPLICABILITY:** M

KEYWORDS: RA II; Volunteers; Adults; Burns (Injuries); Hospitalization; Medical Services; Health Care Facilities

OBJECTIVE: A DTIC literature search was conducted under DTIC request numbers W6R49L/W6R44M dated 9 January 1990. The objectives of this work are to describe the needs of family members of critically injured burn patients as they perceive them across a span of time (six weeks) and to compare perceptions of needs by individual family members with those of nurses who interact with them in the burn unit. When anxiety is reduced and healthy coping mechanisms are promoted to facilitate dealing with the crisis of a severe burn injury, the family will be better able to provide the crucial support necessary for the patient to cope. Understanding families' perceptions of their needs will better enable nursing personnel to intervene in the most appropriate manner.

APPROACH: This descriptive/comparative multisite study established a comprehensive data base related to the perceived needs of families of critically burned patients.

PROGRESS: 9110-9209. Thirteen burn units, including this Institute, were enrolled in this study. Eight units completed data collection. Five other units voluntarily disenrolled from the study. Analyses of data collected at this Institute indicate that nurses are only moderately accurate in rating the importance of the needs perceived by family members. Data were analyzed from questionnaires received from one primary family visitor for each patient enrolled in the study. Responses were compared to those on a questionnaire completed by the nurse caring for the patient at the time that the family member completed the questionnaire. Some differences in needs were noted across the time span, but

many needs continued to be significantly important to families for the entire six-week period. For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report* for fiscal years 1990 through 1992.

ABSTRACT

PROJECT NUMBER: 3M162787A874-173, Applied Research and Exploratory Development

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

INSTITUTION: US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas 78234-5012,[1] and the School of Nursing, University of Wisconsin, Milwaukee, Wisconsin 53201[2]

PERIOD COVERED IN THIS REPORT: 1 October 1991 - 30 September 1992

INVESTIGATORS: Nancy C. Molter, CCRN, Colonel, AN[1]
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Jane Leske, RN, MSN, PhD[2]
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Basil A. Pruitt, Jr., MD, Colonel, MC[1]

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

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

An initial six-month data collection pilot was completed at this Institute, resulting in minor revisions of the protocol hypotheses and refinement of data collection methodology. Twelve

patients were enrolled in the study conducted at this Institute, with 32 family responses and 22 staff responses.

After approval of the revised protocol, requests for participation in the study were sent to 154 burn units listed in the directory of the American Burn Association. Thirteen burn units completed the human use review process and enrolled patients in the study.

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

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

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

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

When the needs of families of burn-injured patients are described, specific interventions will need to be evaluated for effectiveness. Once the psychometric properties of the Critical

Care Family Needs Inventory (CCFNI) are determined in the burn patient population, selected dimensions of the tool may be used more effectively to study relationships between specific interventions and observed outcomes.

Several exploratory descriptive studies have been done using the CCFNI, or a variation of it, to identify family needs of critically ill patients. These studies have been conducted in different types of critical care units, with different types of patient diagnoses, and in different geographic locations. Five excellent published reviews in the literature analyzed 34 published and 23 nonpublished studies related to family needs of critically ill patients (14-18). Four of the reviews are qualitative (14-17) and Leske's (18) is quantitative.

Leske (18) integrated original data from 27 investigators in 15 states over a period of 10 yr. Thirty-eight different critical care units were represented. The sample included 905 family members of 668 critically ill patients. The mean level of importance for all needs on the CCFNI are reported with the needs categorized into five categories, i.e., assurance, proximity, information, comfort, and support needs. In comparing her findings with those from the reviews by Simpson (14) and Hickey (15), there appeared to be no substantive difference between the qualitative and quantitative reviews. All described basically the same top needs.

Only one study evaluated the change in level of importance of needs across a time span. Bouman (19) had 34 family members rate their needs using the CCFNI within the first 36 h after patient admission and then again at 96 h postadmission. As a group, the cognitive needs, i.e., need to know specific facts about treatment and progress, expected outcome, honest answers to questions, and clear explanations, decreased in importance at the second time frame.

Four studies reported that families perceived that their needs were met the majority of the time (20-23) However, it is not enough to focus on the level of importance or the level of satisfaction or dissatisfaction without relating the two (24). Lynn-McHale and Bellinger (25) concluded that nurses must be accurate in assessing the importance of the need to the family member before planning interventions to meet the need.

A review of the literature over the past 15 years related to family needs of burn patients indicates that most studies/case reports are concerned with informational needs, usually at the time of discharge (26-29). A retrospective survey of 68 family members (30) did evaluate family needs related to receiving information

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

Six studies compared nurses perceptions of the families needs with those of the families (23-25,31-33). All reported that nurses were only moderately successful at rating the needs at a similar level of importance as the family members. Forrester et al (24) collected data from 92 confederate pairs of responses from a modified CCFNI (30 items) obtained from 92 family members of adult patients in a variety of clinical care units and 49 nurses providing direct care for those patients. Statistically significant differences in ratings were found for 15 (50%) of the needs. Johnston (33) compared the perceptions of 25 spouses of patients in a coronary care unit with the perceptions of 17 nurses who cared for the patients. The majority of nurses identified the needs in a different priority than did the spouses. These two studies were the only ones that used the confederated methodology and thus eliminated a significant limitation found in other comparison studies.

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

The major objectives of the study are to describe the needs of family members of critically injured burned patients as they perceive them across a span of time, compare perceptions of needs by individual family members with those of nurses who interact with them in the burn unit, and describe the psychometric properties of the CCFNI when used with the burn unit population.

Hypotheses. The following hypotheses were tested:

1. The needs of families change across a time span from 72 h postadmission until six weeks postadmission and/or transfer to the acute care ward. The family needs in the burn population will be different than those in the general critical care population.

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

3. The majority of needs perceived by the family are being met.

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

A significance level of $P \leq 0.05$ was used to accept or reject the hypotheses.

MATERIALS AND METHODS

Participant Criteria. A convenience sample was used of burn patient family members who were ≥ 18 yr, able to read English, had a family relationship or were a common law spouse, housemate, legal guardian, or close friend, designated by the patient if there was no family relative. There was no limitation to the number of family members per patient who could enroll in the study to complete the CCFNI at the first time interval. However, one family member was designated by the family present at the beginning of the study as the primary visitor for the patient during the hospitalization. The primary visitor was not necessarily the legal next-of-kin, but rather the person the family felt was able to visit the most consistently. This individual completed subsequent versions of the CCFNI across the appropriate time frames. A nurse caring for the patient was asked to complete the CCFNI within 48 h of having contact with the primary visitor after he/she completed the CCFNI. The nurses' CCFNIs reflected the nurses' perceptions of the needs of the primary visitor only.

Study Design. This was a descriptive/comparative study to establish a comprehensive data base related to the perceived needs of family of critically burned patients. A letter inviting participation in this study was sent to all burn units within the United States and one in Canada listed in the directory of the American Burn Association.

Eight units participated, representing 6 of 12 regions of the American Burn Association. The number of patients per unit ranged from 1 to 38. Participating units had from 6 to 40 beds, with a range of 5 to 16 ICU beds. The average census for 1991 for the units was 149 (range 48-229). Registered nursing staff ranged from 14 to 35 per unit, with licensed vocational nursing staff ranging from 3 to 55. Table 1 contains a summary of burn unit profile data.

TABLE 1. Burn Unit Profile

	Unit							
Variable	1	2	3	4	5	6	7	8
Burn region*	12	5	4	6	5	1	3	5
Beds	40	12	20	32	12	10	18	6
ICU beds	16	7	8	5	6	5	6	6
1991 admissions	216	229	48	220	122	116	110	120
RNs	35	21	25	14	21	28	30	15
LVNs	55	8	3	3	4	-	-	-
Study participants	38	6	3	3	2	1	11	17

*As defined by the American Burn Association.

Data were collected for a minimum of six months up to one year. Data collection ended for all units by May 1992. For confidentiality, the unit data collection coordinators coded all data forms and maintained the key to the data at the facility. Only coded forms were forwarded to the Institute for analyses. The principal investigators at the Institute had the specific unit code key for participating units and each unit received their unit code number, study instruments, and specific data collection instructions upon enrollment in the study.

Description of Procedures. After the family member visited the patient at least once within the first 72 h postadmission to the critical care burn unit, and after verbal/written informed consent was obtained, the unit data collection coordinator had the family member complete the Family Member Data Form and the CCFNI. Any

number of family members for a specific patient could participate in completing the first version of the CCFNI. One member of the family was then designated by the family as the "primary visitor". The family member designated as the primary visitor was asked to complete the CCFNI again at 2, 4, and 6 weeks as appropriate and at the time of patient transfer from the ICU (see Table 2).

TABLE 2. CCFNI Administration Template

Code	Time Period
1	Within 72 h of admission, after first visit
2	2 weeks postadmission
3	4 weeks postadmission
4	6 weeks postadmission
5	Transfer from unit (did not include death)

The data collection coordinator contacted a nurse who had interacted with the primary visitor within 48 h of the primary visitor's completion of the CCFNI and asked the nurse to complete a staff CCFNI and Burn Unit Personnel Data Form. Nurses could participate more than once in the study with the same primary visitor or with different primary visitors for different patients. However, individual nurses completed only one Burn Unit Personnel Data Form.

The data collection coordinator collected the data about the unit once using the Unit Demographic Data Form and data about each study patient using the Patient Data Form. Once the family members completed the Family Member Data Form, the Hollingshead Index of Socioeconomic Status (unpublished manuscript) was used to calculate the class level which was, in turn, recorded on the form. All data were coded at the collection site and only coded data were forwarded to the Institute.

Reliability and Validity of the Critical Care Family Needs Inventory. The internal psychometric properties of the CCFNI were reported by Leske (34). Family response data on 677 subjects collected by 21 investigators were used as the aggregate data base. High reliability was indicated by the internal alpha coefficient of

0.92 for the total CCFNI for the general critical care population. The alpha coefficient for the family study population CCFNIs was 0.88 and 0.93 for the staff version.

Leske used principal factor analysis with varimax rotation to report construct validity. Analysis resulted in a five factor solution. She labeled the five dimensions of the CCFNI as needs for support, comfort, information, proximity, and assurance. Principal components factor analysis with varimax rotation resulted in a six-factor solution for the burn population data base. Each factor has from three to nine variables per group. Twelve needs did not load on any factor and three needs loaded on two different factors. A comparison of results with Leske's (Table 3) shows many similarities but Leske had a larger data base. Therefore, Leske's five domains of needs are used when discussing findings and implications of this study.

Macey and Bouman (35) reported the reading level of the CCFNI to be at the ninth grade level using the Gunning Fog Index (36).

Sample. A convenience sample of 109 family members of 76 patients in eight critical care burn units was included. The range was between one to four family members per patient. However, one family member was designated by the family present at the beginning of the study as the primary visitor who would complete subsequent versions of the CCFNI across the time frames. Most family members were high school graduates or had some college education (58%) and were working as managers in small businesses or were professionals in narrow or limited fields. The rest of the sample were skilled laborers with a partial high school education as classified by the Hollingshead Two-Factor Index of Socioeconomic Status (unpublished manuscript). The mean age was 32.5 yr (range 16-73). The majority of the sample were spouses (25%), parents (39%), or siblings or children of the patient (26%). Women composed 78% of the sample. Religion was important to 53% of the group and they equally were divided into those who had experience with a family member in an ICU and those who had not. Ninety-four percent of the sample had no previous experience with a family member hospitalized for burn injury. The demographic profile of the family member sample is summarized in Table 4.

The patient profile for the study is displayed in Table 5. The age range was 1 to 93 years with a mean of 37 years. Patients had from 3% to 86% total body surface area burn sizes with an average of 36%. The mean third-degree injury was 19.1% of the total body surface area. Inhalation injury was present in 44.7% of the sample; 86.8% had no other injuries. Only 26% of the patients had preexisting medical conditions.

TABLE 3. Comparison of Factor Analysis of Family Needs Between Burn Population Study and General Critical Care Population Study

Burn Study Factors	General Critical Care Study Categories
FACTOR 1 (Family + staff responses = 329 cases, alpha coefficient = 0.86; family responses only = 184 cases, alpha coefficient = 0.71)	
To talk to the doctor every day	Information
To have questions answered honestly	Assurance
To know why things were done for the patient	Information
To know exactly what is being done for the patient	Information
To be called at home about changes in the patient's condition	Proximity
To receive information about the patient at least once a day	Proximity
To feel that hospital personnel care about the patient	Assurance
To know specific facts concerning the patient's progress	Assurance
To see the patient frequently	Proximity
FACTOR 2 (Family + staff responses = 77 cases, alpha coefficient = 0.79; family responses only = 173 cases, alpha coefficient = 0.78)	
To have good food available in the hospital	Comfort
To have comfortable furniture near the waiting room	Comfort
To have a phone near the waiting room	Comfort
To talk about the possibility of the patient's death	Support
To have the bathroom near the waiting room	Comfort
To have the waiting room near the patient	Proximity

TABLE 3 (Continued)

Burn Study Factors	General Critical Care Study Categories
FACTOR 3 (Family + staff responses = 316 cases, alpha coefficient = 0.79; family responses only = 176 cases, alpha coefficient = 0.72)	
To talk to the doctor every day	Information
To have a specific person to call at the hospital when unable to visit	Information
To have visiting hours changed for specific conditions	Proximity
To visit at any time	Proximity
To know which staff members could give what type of information	Information
To have someone help with financial problems	Support
To have another person with me when visiting the critical care unit	Support
FACTOR 4 (Family + staff responses = 331 cases, alpha coefficient = 0.81; family responses only = 185 cases, alpha coefficient = 0.81)	
To know the expected outcome	Assurance
To have explanations of the environment before going into the critical care unit for the first time	Support
To now why things were done for the patient	Information
To feel hope	Assurance
To know how the patient is being treated medically	Information
To know exactly what is being done for the patient	Information

TABLE 3 (Continued)

Burn Study Factors	General Critical Care Study Categories
FACTOR 5 (Family + staff responses = 328 cases, alpha coefficient = 0.74; family response only = 183 cases, alpha coefficient = 0.74)	
To have someone be concerned with my health	Support
To talk to the same nurse every day	Proximity
To feel it is alright to cry if I want to	Support
To be told about other people that could help with problems	Support
To be told about someone to help with family problems	Support
FACTOR 6 (Family + staff responses = 324 cases, alpha coefficient = 0.62; family response only = 181 cases, alpha coefficient = 0.62)	
To have directions as to what to do at the bedside	Support
To help with the patient's physical care	Information
To be told about transfer plans while they are being made	Proximity

TABLE 3 (Continued)

Burn Study Factors	General Critical Care Study Categories
NEEDS MISSING IN FACTORS 1 THROUGH 6	
To talk about feelings about what has happened	Support
To have friends nearby for support	Support
To know the types of staff members taking care of the patient	Information
To be assured that the best care possible is being given the patient	Assurance
To have a place to be alone while in the hospital	Support
To feel accepted by hospital staff	Comfort
To have a pastor visit	Support
To be assured it is alright to leave the hospital	Comfort
To be alone whenever I want	Support
TO have explanations given that are understandable	Assurance
To have visiting hours start on time	Proximity
To be told about chaplain services	Support

TABLE 4. Demographic Profile of Family Members

Variable	Unit 1	2	3	4	5	6	7	8	Total
Family members	56	7	7	3	2	1	15	18	109
Socioeconomic class*									95
2	-	-	-	-	1	-	-	1	2
3	12	1	2	-	-	-	3	5	23
4	16	-	3	2	1	-	3	5	30
5	24	6	2	1	-	1	9	7	50
Relationship									
Spouse	9	5	2	1	1	1	1	7	27
Parent	30	2	2	-	2	-	3	5	42
Sibling	8	-	-	1	-	-	2	2	15
Child	7	-	1	1	-	-	2	2	13
Significant other	-	-	1	-	-	-	1	1	3
Other	2	-	1	-	-	-	6	1	10
Age (range, 16-73 yr)									108
16-30	15	-	4	-	-	-	5	3	27
31-45	18	6	-	-	1	1	5	7	38
46-59	16	1	3	2	1	-	2	6	31
> 60	7	-	-	1	-	-	3	1	12
Mean	24.4	37.6	34.7	55	41.5	33	41.7	42.5	32.5
Sex									
Male (22%)	15	-	2	1	-	-	4	2	24
Female (78%)	41	7	5	2	2	1	11	16	85

TABLE 4 (Continued)

Variable	Unit 1	2	3	4	5	6	7	8	Total
Religious	52		6						104
Yes	37	5	6	1	-	-	4	2	55
No	15	2	-	2	2	1	11	16	49
Previous ICU experience	55		6						106
Yes	29	1	2	2	2	1	11	10	58
No	26	6	4	1	-	10	4	7	58
Previous burn experience	52		6						
Yes	-	-	1	1	-	-	-	4	6
No	52	7	5	2	2	1	15	14	98

*2 indicates college graduate, business manager, professional; 3, partial college, administrative personnel, small businessmen; 4, high school graduate, clerical workers, technicians; and 5, partial high school, skilled manual laborers.

TABLE 5. Patient Profile

Variable	Unit 1	2	3	4	5	6	7	8	Total
Patients (n)	38	6	3	3	2	1	6	17	76
Age (yr)									
Range	1-93	5-41	26-64	48-86	26-34	36	3-60	18-78	1-93
Mean	26	30	39	66	30	36	30	39	37
Total burn size (% TBSA)									
Range	7-85	20-70	32-35	11-31	20-33	65	24-86	3-70	3-86
Mean	39	40	33	21	26.5	65	53	23	36
3° burn size (% TBSA)									
Range	0-85	0-35	7-22	0-10	3-25	35	4-86	0-50	0-86
Mean	23	12.5	12	4.6	14	35	47	13.6	19.1
Inhalation injury (yes/no)	18/20	0/6	2/1	0/3	1/1	0/1	6/0	7/10	34/42
Other injuries (yes/no)	4/34	0/6	0/3	1/2	0/2	0/1	2/4	3/14	10/66
Existing medical problems (yes/no)	8/30	0/6	0/3	3/0	0/2	0/1	3/3	6/11	20/56

*TBSA indicates total body surface area.

The sample of burn nursing personnel included 73 nurses, with 71.2% being women. Table 6 summarizes other demographic information, including age (mean 33 yr), highest level of education (42.5% with a Bachelor's or higher degree), highest level of nursing education (38.3% with a BSN or higher degree), number of years of nursing experience (mean 8.7 yr), number of years of burn experience (mean 3.8 yr), and position in the unit (93.1% staff RN or LVN).

RESULTS/DISCUSSION

HYPOTHESIS - The needs of families change across a time span from 72 h until six weeks postadmission and/or transfer to the acute care ward. The family needs in the burn population will be different than those in the general critical care population. This hypothesis is rejected. Of the top needs ranked by family members in this study (Table 7), only the need "to see the patient frequently" changed in level of importance over the time frames. The longer the patient remained hospitalized, the less important the need was ranked ($P = 0.038$). "To feel accepted by the hospital staff" was the only other of the 45 needs that statistically changed in level of importance over the time frames. This need became increasingly more important as length of hospitalization increased.

All of the needs ranked most highly by the family members are contained in three of the five categories described by Leske (18) and parallel the importance ranked by the 677 subjects in her analysis. Assurance needs were ranked highest by most family members (mean 3.89-3.97) and included the need to have questions answered honestly, to feel that the hospital personnel care about the patient, to know specific facts concerning the patient's progress, to know the expected outcome, to feel there is hope, to be assured that the best care possible is being given the patient, and to receive understandable explanations.

Three proximity needs, i.e, to be called at home about changes in the patient's condition, to receive information about the patient at least once a day, and to see the patient frequently, were cited as very important in most every other family need study. Although the following five lowest ranking needs were ranked lower in the burn population than in the general critical care population analyzed by Leske (18), they were all ranked in the lower third of her study:

1. To be assured it is alright to leave the hospital (mean 1.15).

TABLE 6. Burn Unit Personnel Profile

Variable	n	Percent
Sex	73	
Male	21	28.8
Female	52	71.2
Age (yr)	70	
23-30	23	32.8
31-40	38	54.2
41-52	9	12.9
Range 23-52, mean 33		
Highest level of education	73	
High school/GED	11	15.1
Diploma in nursing	18	24.7
Associate's degree	13	17.8
Bachelor's degree	28	38.4
Master's degree	3	4.1
Highest level of nursing education	73	
LVN	19	26.0
RN (diploma)	16	21.9
Associate's degree in nursing	10	13.7
BSN	25	34.2
MSN or MN	3	4.1
Nursing experience	69	
0-2	12	17.4
3-5	17	24.6
6-10	22	31.9
> 10	18	26.1
Range 0-35, mean 8.7		
Burn experience (yr)	69	
0-2	37	53.6
3-5	14	20.3
6-10	11	15.9
> 10	7	10.1
Range 0-19, mean 3.8		
Position in unit	72	
Staff nurse/LVN	67	93.1
Clinical nurse specialist	4	5.6
Manager	1	1.4

TABLE 7. Most Important Needs as Ranked by Family Members*

Need	Mean Level of Importance*
To have questions answered honestly	3.97
To be called at home about changes in the patient's condition	3.93
To feel that hospital personnel care about the patient	3.93
To know specific facts concerning the patient's progress	3.93
To know the expected outcome	3.92
To feel there is hope	3.92
To be assured that the best care possible is being given the patient	3.91
To know how the patient is being treated medically	3.89
To have explanations given that are understandable	3.89
To receive information about the patient at least once a day	3.86
To see the patient frequently	3.86
To know why things were done for the patient	3.85
To know exactly what is being done for the patient	3.85

*Scale from 1 (not important) through 4 (very important).

2. To have someone be concerned about the family member's health (mean 2.58).

3. To have a place to be alone while in the hospital (mean 2.70).

4. To be told about chaplain services (mean 2.72).

5. To be alone whenever they want (mean 2.75).

From the data, it is concluded that the majority of family needs of critically burned patients do not change in level of importance as length of stay increases. It is clear that the needs of these families are very similar to those of families in the general critical care population.

HYPOTHESIS - Burn nurses have a different perception of importance of family needs than do family members of burn patients. This hypothesis was accepted for all but nine needs (overall mean for each need):

1. To be assured that the best care possible is being given to the patient (mean 3.89).

2. To have another person with me when visiting the critical care unit (mean 2.77).

3. To have someone be concerned with my health (mean 2.54).

4. To talk to the same nurse every day (mean 3.18).

5. To feel it is alright to cry when I want to (mean 2.95).

6. To be alone whenever I want (mean 2.69).

7. To be told about someone to help with family problems (mean 3.10).

8. To have visiting hours start on time (mean 3.23).

9. To be told about chaplain services (mean 2.67).

Only one of these needs, the need for assurance about the quality of care being given, was in the list of top needs ranked by the family members of burned patients. Two of the needs were ranked significantly different across the five time frames:

1. To feel accepted by the staff - decreasing in importance with length of hospitalization ($F = 2.7$, $df = 4,332$, $P = 0.028$).

2. To see the patient frequently - also decreasing in importance over the five time frames ($F = 2.5$, $df = 4,330$, $P = 0.038$).

Table 8 summarizes the comparison of means between the family members' and nurses' ranking of needs for all the needs. The needs are grouped in the five domains identified by Leske's factor analysis (34).

Comparing these results with those of Forrester et al (24), there were major differences. Using a modified version of the CCFNI consisting of 30 items, 15 of the needs were perceived to be of similar importance by the nurse-family confederate pairs (n=92) in their study. None of those needs were perceived by family members in their sample as being most important. Three of the needs in this group, i.e., to know why things were being done for the patient, to feel there was hope, and to receive information about the patient once a day, were perceived as most important by the burn population family members.

In comparing the needs identified by Forrester et al (24) and this study that were perceived as statistically different in level of importance between family members and nurses, only one need, i.e., to be assured that the best possible care is being given to the patient, was identified by both studies. It was ranked high in importance by both study populations.

Johnston (33) used factor analysis to group needs into three factors, i.e., spouse, nurse, and shared factors. Several themes emerged in her comparison, spouses rated comfort needs more highly than did nurses, spouses thought it more important to be involved in the patient's care than did nurses, and nurses put more priority on providing support than merited by the ranking of these needs by the spouses.

The data from this investigation support the conclusion that nurses are not very accurate in estimating the level of importance of needs for family members of critically burned patients. Therefore, each family member requires individualized assessment before planning interventions.

HYPOTHESIS - The majority of needs perceived by the family are being met. This hypothesis is accepted. Ten needs were met < 75% of the time (Table 9) with only one (to be called at home about

TABLE 8. Family Versus Staff in Burn Population Across Five Time Frames (72 h through 6 Weeks Postadmission)*

Needs by Domain	Family (Mean)	Staff (Mean)	F	df	P
ASSURANCE					
To know the expected outcome	3.91	3.67	18.9	1331	0.0001
To have questions answer honestly	3.97	3.79	23.8	1330	0.0001
To feel there is hope	3.92	3.69	21.4	1327	0.0001
To be assured that the best care possible is being given to the patient	3.91	3.85	2.1	1330	ns
To have explanations given that are understandable	3.89	3.59	27.1	1334	0.0001
To feel that hospital personnel care about the patient	3.93	3.67	28.9	1332	0.0001
To know specific facts concerning the patient's progress	3.93	3.50	58.9	1331	0.0001
INFORMATION					
To talk to the same doctor every day	3.67	3.19	32.9	1330	0.0001
To know which staff members could give what type of information	3.65	3.03	68.4	1330	0.0001

TABLE 8 (Continued)

Needs by Domain	Family (Mean)	Staff (Mean)	F	df	P
To have a specific person to call at the hospital when unable to visit	3.53	2.92	39.8	1330	0.0001
To know why things were done for the patient	3.85	3.48	39.4	1329	0.0001
To know about the types of staff members taking care of the patient	3.50	2.85	60.3	1329	0.0001
To know how the patient is being treated medically	3.89	3.40	69.0	1330	0.0001
To know exactly what is being done for the patient	3.85	3.41	48.4	1330	0.0001
To help with the patient's physical care	3.37	2.67	45.5	1326	0.0001
SUPPORT					
To have explanations of the environment before going into the critical care unit for the first time	3.60	3.43	5.0	1329	0.025
To talk about feelings about what has happened	3.48	3.15	15.0	1328	0.0001
To have directions as to what to do at the bedside	3.56	3.25	12.6	1330	0.0001

TABLE 8 (Continued)

Needs by Domain	Family (Mean)	Staff (Mean)	F	df	P
To have friends nearby for support	3.51	3.19	15.4	1327	0.0001
To have a place to be alone while in the hospital	2.70	2.38	7.1	1325	0.008
To have someone help with financial problems	3.41	3.11	9.1	1322	0.003
To have a pastor visit	3.03	2.65	11.2	1322	0.001
To talk about the possibility of the patient's death	3.01	2.58	10.7	1318	0.001
To have another person with me when visiting the critical care unit	2.85	2.66	2.1	1330	ns
To have someone be concerned with my health	2.58	2.48	0.9	1330	ns
To feel it is alright to cry when I want to	3.01	2.87	1.5	1327	ns
To be told about other people who could help with problems	3.34	3.15	5.2	1327	0.023
To be told about chaplain services	2.72	2.60	0.8	1326	ns
To be alone whenever I want	2.75	2.60	2.1	1324	ns
To be told about someone to help with family problems	3.13	3.05	0.8	1325	ns

TABLE 8 (Continued)

Needs by Domain	Family (Mean)	Staff (Mean)	F	df	P
PROXIMITY					
To have visiting hours changed for special conditions	3.61	3.06	40.6	1330	0.0001
To visit at any time	3.39	2.92	20.9	1325	0.0001
To talk to the same nurse every day	3.23	3.12	1.4	1332	ns
To have visiting hours start on time	3.31	3.12	3.7	1329	ns
To be told about transfer plans while they are being made	3.80	3.38	29.7	1324	0.0001
To be called at home about changes in the patient's condition	3.93	3.61	31.6	1330	0.0001
To receive information about the patient at least once a day	3.86	3.63	15.1	1330	0.0001
To see the patient frequently	3.86	3.22	76.3	1330	0.0001
To have the waiting room near the patient	3.37	3.00	13.2	1294	0.0001
COMFORT					
To have good food available in the hospital	2.79	2.37	15.5	1324	0.0001

TABLE 8 (Continued)

Needs by Domain	Family (Mean)	Staff (Mean)	F	df	P
To have comfortable furniture in the waiting room	2.78	2.25	28.7	1330	0.0001
To feel accepted by hospital staff	3.47	3.01	35.4	1332	0.0001
To have a telephone near the waiting room	3.17	2.60	29.9	1328	0.0001
To be assured it is alright to leave the hospital for awhile	1.15	1.49	21.1	1324	0.0001
To have a bathroom near the waiting room	2.94	2.45	19.6	1327	0.0001

*ANOVA of Critical Care Family Needs Inventory. ( ) indicates not significant. Information needs concerned knowing why things were done for the patient as well as specifics of what is being done related to the medical therapies.

TABLE 9. Relationship of Family Versus Staff Responses to Perceived Family Needs Met < 75% of the Time*

Need	Family % Met	Staff % Met	Relationship: Level of Importance Chi-Square Pearson Value	df	P =	Relationship: Need Met Chi-Square Pearson Value	df	P =
To have visiting hours changed for special conditions	71.2 (131/184)	46.6 (69/148)	41.4	3	0.000001	49.4	2	0.000001
To visit at any time	52.5 (95/181)	18.5 (27/146)	28.9	3	0.000001	61.6	2	0.000001
To have comfortable furniture in the waiting room	68.4 (128/187)	28.1 (41/146)	35.4	3	0.000001	91.6	2	0.000001
To talk about the possibility of the patient's death	65.5 (110/168)	41.0 (59/144)	13.2	3	0.004	71.9	2	0.000001
To talk to the same nurse each day	65.6 (118/180)	25.3 (37/146)	7.6	3	0.056**	80.6	2	0.000001
To have a bathroom near the waiting room	70.7 (133/188)	42.4 (59/139)	29.6	3	0.00001	62.3	2	0.000001
To help with the patient's physical care	67.6 (123/182)	50.0 (73/146)	44.4	3	0.000001	51.6	2	0.000001
To be told about transfer plans while they are being made	72.9 (129/177)	40.6 (58/143)	27.7	3	0.000001	74.8	2	0.000001
To be called at home about changes in the patient's condition	70.6 (127/180)	61.5 (88/143)	38.1	3	0.000001	33.8	2	0.000001

( ) indicates n. *Staff were allowed the option of selecting "unknown" while family were not allowed. **Not significant.

changes in the patient's condition) ranked in the priority group of needs. The relationship between the percentage of family versus staff responses was significant for the level of importance of the need for all but one need (to talk to the same nurse each day). The relationship between the percentage of family versus staff responses was significant for the issue of whether the need was met.

In comparing the unmet needs of the current study with those from previous studies (20-23), 16 needs were identified by at least one study (Table 10). All but two (to know about the types of staff members taking care of the patient and to know specific facts about the patient's progress) were identified in at least two studies. Three needs were ranked among the most important needs in the majority of the studies, i.e., to talk to the physician once a day, to know specific facts about the patient's progress, and to be called at home about changes in the patient's condition. The need to talk to the doctor every day was ranked in the top 10 to 15 needs of every study (#14 in this study). Only in this investigation was the need found to be met the majority of the time.

It can be concluded that the majority of family needs are perceived as being met. Certainly the team approach to burn care facilitates this result.

HYPOTHESIS - Family needs differ with respect to percent burn, age, sex, relationship of family member to the patient, perception of severity of injury, socioeconomic status, and self-reported importance of religion in their lives. The majority of needs did not differ with respect to the aforementioned variables. However, for each variable, there were significant differences for some needs when all time frames were considered. There were also some differences among the five time frames for selected variables. Comparisons of the burn family population are made with the general critical care population studied by Leske (37). In her analysis, data about needs from 905 family members of 668 critically ill patients were related to the variables of age, gender, relationship to patient, previous ICU experience, and patient diagnosis.

Differences in Level of Importance of Needs Based on Selected Variables.

Percent of Total Body Surface Area Burned (1%-50% vs 51%-100%). Only three needs were significantly affected by this variable when classified into two categories of 1% to 50% total body surface area burns and 51% to 100% total body surface area

TABLE 10. Comparison of Unmet Family Needs as Reported by Molter (20), Rodgers (21), Spatt et al (22), Prowse (23), and This Study

Unmet Need	Molter (1979)	Rodgers	Spatt et al	Prowse	Molter (1993)
To talk to the physician once a day*	X	X	X	X	
To be told about chaplain services	X				
To have a place to be alone while in the hospital	X	X		X	
To have someone help with financial problems	X				
To be alone whenever I want to be	X				
To have a bathroom near the waiting room	X				X
To help with the patient's physical care		X			X
To know about the types of staff members taking care of the patient			X		
To know specific facts about the patient's progress*			X		
To be called at home about changes in the patient's condition*			X		X
To have visiting hours changed for special conditions			X		X

TABLE 10 (Continued)

Unmet Need	Molter (1979)	Rodgers	Spatt et al	Prowse	Molter (1993)
To visit at any time			X		X
To have comfortable furniture in the waiting room					X
To talk about the possibility of the patient's death					X
To talk to the same nurse each day			X		X
To be told about transfer plans while they are being made					X

*Needs cited as among 10 most important needs in at least one study.

burns (data were collapsed over all time frames and analyzed by ANOVA), i.e., to have visiting hours changed for special conditions ($F = 4.2$, $df = 1,164$, $P = 0.04$), to feel accepted by the hospital staff ($F = 5.2$, $df = 1,168$, $P = 0.02$), and to have someone to help with financial problems ($F = 4.0$, $df = 1,161$, $P = 0.05$). The importance of two needs appeared to change significantly in relation to percent total body surface area burned over the five time frames, i.e., to be assured it is alright to leave the hospital for awhile ($F = 2,8$, $df = 4,162$, $P = 0.03$) and to help with the patient's physical care ($F = 3.2$, $df = 4,162$, $P = 0.01$). In both cases, importance decreased over time. None of these needs were ranked in the top third of the priority rating by this sample.

Age. Table 11 contains a summary of the needs that differed in importance based on the age of the family members. Four age categories were delineated for the analysis, i.e., 16 to 30, 31 to 45, 46 to 59, and over 59 yr. The greatest number of family members were in the age group of 31 to 45 yr. Of the seven needs identified that differed with respect to age (to know the expected outcome, to know exactly what is being done for the patient, to have a pastor visit, to feel it is alright to cry when I want to, to be told about transfer plans while they are being made, to receive information about the patient once a day, and to see the patient frequently), four of them (the first and last two) were considered most important needs for the entire sample. These needs represent the domains of assurance, support, and proximity. Leske found no differences among age groups except for older family members rating comfort needs more highly. Two needs decreased in level of importance significantly as hospital stay lengthened, i.e., to be assured it is alright to leave the hospital for awhile ($F = 2.9$, $df = 4,167$, $P = 0.023$) and to help with the patient's physical care ($F = 3.2$, $df = 4,168$, $P = 0.015$).

Gender of Family Member. When data were collapsed across all time frames, five needs (see Table 12) differed in importance based on the gender of the family member (78% female, n=109). These needs represent those from the domains of assurance and support needs. Leske found significant differences in all domains but assurance. Spatt et al (22) found that men tended to rate the level of importance of most needs lower than women. This was also true in this study where every need was rated lower in importance by men than by women.

Only the need "to be assured it is alright to leave the hospital for awhile" varied significantly across time frames ($F = 3.0$, $df = 4,178$, $P = 0.021$), decreasing in importance with length of hospitalization. The statistics related to this variable are contained in Table 12.

TABLE 11. Family Needs Differing in Importance Based on Age of Family Member*

Need	Grand Mean	Mean Per Age Group 16-30 (42-43)	31-45 (68-71)	46-59 (56-58)	> 59 (20-22)	F	df	P =
To know the expected outcome	3.92	3.94	3.96	3.96	3.64	7.4	3172	0.0001
To know exactly what is being done for the patient	3.86	3.90	3.85	3.92	3.67	2.8	3171	0.039
To have a pastor visit	3.03	2.97	2.78	3.32	3.18	2.8	3171	0.039
To feel it is alright to cry when I want to	3.02	2.90	3.25	3.03	2.46	3.8	3170	0.012
To be told about transfer plans while they are being made	3.81	3.93	3.75	3.88	3.55	3.1	3169	0.028
To receive information about the patient at least once a day	3.88	3.95	3.85	3.95	3.66	4.3	3172	0.006
To see the patient frequently	3.88	3.97	3.88	3.88	3.70	3.3	3170	0.021

( ) indicates n. *Data were collapsed across all time frames.

TABLE 12. Family Needs Differing in Importance Based on Gender of Family Member*

Need	Grand Mean	Mean: Male (37-38)	Mean: Female (153-155)	F	df	P =
To know the expected outcome	3.92	3.71	3.97	23.0	1182	0.0001
To have questions answered honestly	3.98	3.92	4.00	8.9	1181	0.003
To talk about feelings about that has happened	3.48	3.22	3.55	6.6	1181	0.011
To know why things were done for the patient	3.86	3.75	3.89	4.3	1182	0.039
To feel it is alright to cry when I want to	3.02	2.64	3.11	7.3	1181	0.008

( ) indicates n. *Data from family members were collapsed from all time frames.

Family Members' Relationship to Patient. Collapsing data across all time frames, data for four categories of relationships were analyzed, i.e., spouse, parent, child, and significant other. The majority of the family members were either spouses or parents of the burned patient. Thirteen needs differed in importance based on the relationship (see Table 13). Eight of the needs were in the support domain with two in each of the comfort and information domains and one from the proximity domain. Leske found no significant differences in ratings among spouses, parents, and adult children of the patients in her analysis. None of these needs were rated by the total sample of the current study in the top priority ranking.

Perception of Severity of Injury. Of the fourteen needs (see Table 14) that significantly differed in level of importance based on the family members' perception of severity of injury, two were rated as among the most important needs for the total sample (to feel that the hospital personnel care about the patient and to have explanations given that are understandable). Four needs differed significantly within the family members and the study coordinators groups (to talk to the doctor every day, to have a specific person to call at the hospital when unable to visit, to feel accepted by the hospital staff, and to have a bathroom near the waiting room). The family members differed on nine additional needs while the coordinators only differed on one additional need. Prowse (23) found that spouses' perception of severity of illness did not affect the ordering of their needs.

Socioeconomic Status. Twelve needs differed significantly in level of importance based on aggregate data from four socioeconomic classes defined with the Hollingshead Two-Factor Index of Social Position (see Table 15). Class 2 represents college graduates representative of professional or business administration roles. Individuals classified into the third class have partial college education working as administrative personnel or are small business owners. Class 4 represents high school graduates working in clerical roles or as technicians. Individuals in Class 5 have a partial high school education and generally are manual laborers or unskilled workers or homemakers.

Six of the needs that differed significantly were rated in the top priority ranking by the entire sample (to have questions answered honestly, to know why things were done for the patient, to know how the patient is being treated medically, to know exactly what is being done for the patient, to have explanations given that are understandable, and to be called at home about changes in the patient's conditions). Only one need (to help with the patient's

TABLE 13. Family Needs Differing in Importance Based on Family Members' Relationship to Patient*

Need	Grand Mean	Spouse (52)	Parent (84)	Child (22)	SO (4)	F	df	P =
To talk about feelings about what has happened	3.45	3.38	3.55	3.13	4.00	2.8	3144	0.039
To have good food available in the hospital	2.81	2.78	2.87	2.44	3.94	2.8	3142	0.043
To have directions as to what to do at the bedside	3.58	3.31	3.77	3.50	3.73	4.7	3145	0.003
To know which staff members could give what type of information	3.66	3.50	3.77	3.55	4.00	3.4	3143	0.018
To have friends nearby for support	3.55	3.46	3.66	3.25	3.99	3.0	3143	0.033
To know about the types of staff members taking care of the patient	3.49	3.27	3.67	3.26	3.80	4.8	3143	0.003
To have a pastor visit	2.97	2.62	3.27	2.62	2.92	5.2	3143	0.002
To have another person with me when visiting the critical care unit	2.79	3.38	3.15	2.78	2.98	6.7	3146	0.000

TABLE 13 (Continued)

Need	Grand Mean	Spouse (52)	Parent (84)	Child (22)	SO (4)	F	df	P =
To be told about other people who could help with problems	3.32	3.14	3.49	3.01	3.76	3.9	3141	0.010
To have a bathroom near the waiting room	2.91	3.10	2.71	3.10	3.68	2.7	3146	0.048
To be alone whenever I want	2.76	3.00	2.75	2.30	2.43	2.9	3144	0.037
To have visiting hours start on time	3.32	2.99	3.59	3.00	4.00	5.7	3142	0.001
To be told about chaplain services	2.67	2.14	3.11	2.24	2.66	12.0	3141	0.000

( ) indicates n; SO, significant other. *Data from family members were collapsed from all time frames.

TABLE 14. Comparison of Family Needs Differing in Importance Based on Family Members' and Staff Coordinators' Perceptions of Severity of Injury*

Need	Grand Mean	Mean: Critical (59-61)	Mean: Very Ill (68-76)	Mean: Stable (44-54)	F	df	P =
To talk to the doctor every day							
Family members	3.66	3.82	3.58	3.57	3.8	2171	0.030
Staff Coordinators	3.68	3.87	3.64	3.47	6.0	2160	0.003
To have a specific person to call at the hospital when unable to visit							
Family members	3.52	3.75	3.44	3.37	3.4	2172	0.036
Staff Coordinators	3.53	3.77	3.58	3.11	8.6	2161	0.0
To have directions as to what to do at the bedside							
Family members	3.56	3.77	3.48	3.43	4.0	2172	0.019
To have a place to be alone while in the hospital							
Family members	2.71	2.58	3.00	2.46	4.5	2171	0.013
To feel accepted by hospital staff							
Family members	3.46	3.63	3.43	3.31	3.3	2175	0.038
Staff Coordinators	3.48	3.66	3.33	3.47	4.1	2164	0.019
To have someone help with financial problems							
Family members	3.40	3.67	3.49	2.93	9.2	2169	0.000

TABLE 14 (Continued)

Need	Grand Mean	Mean: Critical (59-61)	Mean: Very Ill (68-76)	Mean: Stable (44-54)	F	df	P =
To have a pastor visit							
Family members	3.01	3.14	3.18	2.63	5.2	2172	0.006
To talk about the possibility of the patient's death							
Family members	3.02	3.09	3.23	2.62	5.0	2165	0.008
To have another person with me when visiting the critical care unit							
Family members	2.84	3.31	2.48	2.82	8.1	2175	0.000
To talk to the same nurse every day							
Family members	3.23	3.56	3.12	3.12	3.1	2175	0.049
To have a bathroom near the waiting room							
Family members	2.94	2.64	2.91	3.32	6.4	2175	0.002
Staff Coordinators	2.93	2.66	2.85	3.41	7.5	2163	0.001
To feel that hospital personnel care about the patient							
Family members	3.94	4.00	3.87	3.97	4.7	1173	0.01
To have explanations given that are understandable							
Family members	3.89	3.90	3.82	3.99	3.5	2175	0.032

TABLE 14 (Continued)

Need	Grand Mean	Mean: Critical (59-61)	Mean: Very Ill (68-76)	Mean: Stable (44-54)	F	df	P =
To have comfortable furniture in the waiting room							
Staff Coordinators	2.80	2.89	2.55	3.15	5.5	2163	0.005

( ) indicates n. *Data from family members were collapsed across all time frames.

TABLE 15. Family Needs Differing in Importance Based on Socioeconomic Status of Family Member*

Need	Grand Mean	Mean Class 2 (5)	Class 3 (40-41)	Class 4 (54-57)	Class 5 (79-83)	F	df	P =
To have questions answered honestly	3.98	3.81	4.00	3.96	3.99	2.9	3165	0.036
To talk about feelings about what has happened	3.48	2.60	3.50	3.45	3.54	2.8	3165	0.039
To have friends nearby for support	3.50	3.16	3.64	3.26	3.63	3.7	3165	0.012
To know why things were done for the patient	3.86	3.43	3.97	3.82	3.86	3.6	3166	0.014
To know about the types of staff members taking care of the patient	3.50	2.60	3.46	3.51	3.56	3.2	3165	0.026
To know how the patient is being treated medically	3.89	2.79	3.98	3.91	3.90	23.1	3166	0.0001
To know exactly what is being done for the patient	3.86	3.43	3.93	3.83	3.87	3.1	3166	0.028
To have a pastor visit	3.05	1.43	3.12	3.27	2.97	5.3	3166	0.002
To have another person with me when visiting the critical care unit	2.84	2.37	3.12	2.49	2.96	2.8	3169	0.042
To have explanations given that are understandable	3.89	3.20	3.92	3.88	3.92	7.3	3169	0.0001
To be told about transfer plans while they are being made	3.81	3.18	3.94	3.73	3.84	3.8	3164	0.011

TABLE 15 (Continued)

Need	Grand Mean	Mean Class 2 (5)	Class 3 (40-41)	Class 4 (54-57)	Class 5 (79-83)	F	df	P =
To be called at home about changes in the patient's condition	3.94	3.39	3.98	3.92	3.97	7.3	3167	0.0001

( ) indicates n. *Data were collapsed across all time frames. Socioeconomic class 2 is defined as college graduate, business manager, professional; class 3, partial college, administrative personnel, small businessmen; class 4, high school graduate, clerical workers, technicians; and class 5, partial high school, skilled manual laborers.

care) differed significantly among time frames based on this variable, decreasing in importance as time progressed.

Self-Reported Importance of Religion in Life. Thirteen needs significantly differed in importance based on whether religion was viewed as an important factor in the family member's life (see Table 16). All decreased in importance if religion was not perceived as important. Four of the needs (to know the expected outcome, to have questions answered honestly, to know why things were done for the patient, to be assured that the best care possible is being given the patient, and to know exactly what is being done for the patient) were rated by the entire sample in the top priority ranking.

Summary. None of the most important needs changed level of importance with all variables. No important needs changed based on the relationship of the family member to the patient or the percent total body surface area burned. Table 17 contains a summary of the most important needs that changed level of importance based on at least one of the family member variables.

CONCLUSIONS

Family members of critically ill burned patients ranked their most important needs at essentially the same level of importance as family members of patients in the general critical care population. When evaluated across a time span of from 72 h postadmission to six weeks postadmission, only two needs did not change significantly in level of importance in the aggregate sample (to feel accepted by the staff and to see the patient frequently). The need to feel accepted by the staff increased in importance over time and the need to see the patient frequently decreased.

When paired with the family responses of the patient they were caring for, nurses perceived the importance of family needs at a lower level than the family member. There were only nine needs where there was statistically significant agreement between the nurse and family members. However, only one of these needs was in the group of needs of priority ranking for the sample. As indicated in previous studies, nurses are not very accurate at estimating how important needs are to individual family members. Therefore, each family member should be assessed. It is reasonable, however, to develop strategies that address the needs ranked as most important in priority.

For this sample, only one need in the group of priority needs was not met at least 75% of the time (to be called at home about changes in the patient's condition). The units involved had a

TABLE 16. Family Needs Differing in Importance Based on Whether Religion is Important in Family Member's Life*

Need	Grand Mean	Mean: Yes (125-130)	Mean: No (56-57)	F	df	P =
To know the expected outcome	3.91	3.94	3.83	4.7	1175	0.032
To have questions answered honestly	3.98	3.99	3.95	3.9	1174	0.049
To talk about feelings about what has happened	3.47	3.55	3.29	4.8	1174	0.029
To have directions as to what to do at the bedside	3.57	3.65	3.39	5.3	1175	0.022
To know why things were done for the patient	3.85	3.89	3.77	4.2	1174	0.042
To know about the types of staff members taking care of the patient	3.50	3.57	3.33	4.9	1173	0.028
To be assured that the best care possible is being given to the patient	3.91	3.95	3.82	6.9	1174	0.009
To have a place to be alone while in the hospital	2.67	2.85	2.26	11.5	1173	0.001
To know exactly what is being done for the patient	3.86	3.92	3.72	13.5	1174	0.0001
To have a pastor visit	3.02	3.23	2.54	18.5	1174	0.0001

TABLE 16 (Continued)

Need	Grand Mean	Mean: Yes (125-130)	Mean: No (56-57)	F	df	P =
To talk to the same nurse every day	3.22	3.31	3.02	4.0	1177	0.048
To be alone whenever I want	2.74	2.84	2.52	3.9	1176	0.05
To be told about chaplain services	2.70	2.89	2.29	12.9	1172	0.0001

( ) indicates n. *Data were collapsed across all time frames.

TABLE 17. Summary of Variables that Significantly Changed the Reported Level of Importance of Needs of Family Members of Critically Burned Patients

Most Important Family Needs	Age	Sex	Severity of Injury	Socioeconomic Status	Importance of Religion
To know the expected outcome	X	X		X	X
To know exactly what is being done for the patient	X			X	X
To receive information about the patient once a day	X				
To see the patient frequently	X				
To have questions answered honestly		X		X	X
To know why things were done for the patient		X		X	X
To feel that hospital personnel care for the patient			X		
To have explanations that are understandable			X	X	
To know how the patient is being treated medically				X	
To be called at home about changes in the patient's condition				X	

TABLE 17 (Continued)

Most Important Family Needs	Age	Sex	Severity of Injury	Socioeconomic Status	Importance of Religion
To be assured that the best care possible is being given to the patient					X

variety of support services that facilitated meeting family needs, information and support groups (75% and 38%, respectively), clinical nurse specialists or psychiatric support personnel (57%), dedicated social services support (75%), written and/or video information material (75% and 50%, respectively), and primary nursing (50%).

Although importance of family needs varied with respect to many variables, none of the important needs changed with all variables. No needs significantly changed in level of importance based on the variables of percent total body surface area burn the patient sustained and the relationship of the family member to the patient.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

ACCESSION: DA346179

SUMMARY DATE: 921001 **SUMMARY KIND:** D **PREV DATE:** 911001 **DISTRIBUTION:** CX

PROGRAM #: 62787A **PROJ #:** 30162787A874 **TASK AREA:** EA **WORK UNIT:** 174

TITLE: Effect of Sucralfate on Prevention of Stress Ulcers and Nosocomial Pneumonia in Thermally Injured Patients

SUBJ1: 060500 - Medicine and Medical Research

START DATE: 9001 **END DATE:** 9509 **PERFORMANCE METHOD:** C

CONTRACT/GRANT NO:

CONT TOTAL: $
CUM TOTAL: $
TOTAL LAB FUNDS: $

RESOURCES ESTIMATE

FY	WORK YRS	$(Thousands)
91	0.5	$25
92	0.1	$26
93	0.1	$28

RESPONSIBLE DOD ORGANIZATION: 404885
MRDC INSTITUTE OF SURGICAL RESEARCH USAISR
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PERFORMING ORGANIZATION: 404885
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ASSOC1: MCMANUS, A T

ASSOC2: MCMANUS, W F

FOREIGN INTELLIGENCE CONSIDERED: Y **CIVILIAN APPLICABILITY:** M

KEYWORDS: RA II; Volunteers; Adults; Burns (Injuries); Ulcers; Pneumonia, Therapy

OBJECTIVE: A DTIC literature search was conducted under DTIC request numbers W6N47E/W6N40C dated 30 May 1990. The objective of this work is to verify that sucralfate is effective in the prevention of stress ulcer-induced gastrointestinal bleeding and reduction of the incidence of nosocomial pneumonia.

APPROACH: Three hundred burn patients will be randomized to receive either standard prophylaxis (cimetidine and antacids) or sucralfate. Differences between treatment groups in the rate of occurrence of pneumonia and clinically evident gastrointestinal bleeding will be evaluated for significance using the Chi square technique.

PROGRESS: 9110-9209. Eighty-eight patients have been enrolled in this study, 28 during this reporting period. Upon completion of enrollment, the data will be analyzed as indicated. For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report* for fiscal years 1990 through 1992.

ABSTRACT

PROJECT NUMBER: 3M162787A874-174, Applied Research and Exploratory Development

PROJECT TITLE: Effect of Sucralfate on Prevention of Stress Ulcers and Nosocomial Pneumonia in Thermally Injured Patients

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

PERIOD COVERED IN THIS REPORT: 1 October 1991 - 30 September 1992

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Support for investigating alternative methods for stress ulcer prophylaxis resides in the fact that pneumonia is now the most significant nosocomial infection in thermally injured patients and adds significantly to their morbidity and mortality. Any therapy which could decrease this incidence would be of obvious benefit. Additionally, it might be of benefit if a nonsystemic agent could replace one which is systemic in nature and has known adverse effects on organ function. Therefore, the objective of this study is to verify that sucralfate is effective in the prevention of stress ulcer-induced gastrointestinal bleeding and in the reduction of nosocomial pneumonia.

Sixty-three patients have been enrolled in this study to date, 43 during this reporting period. One patient receiving sucralfate had a clinically significant gastrointestinal bleed which necessitated performance of a subtotal gastrectomy. There have been no other episodes of significant gastrointestinal bleeding in either patient population.

When the projected total of 300 patients have completed the study, the data will be analyzed as to the efficacy of sucralfate in the prevention of stress ulcer-induced gastrointestinal bleeding and reduction of nosocomial pneumonia.

EFFECT OF SUCRALFATE ON PREVENTION OF STRESS ULCERS AND NOSOCOMIAL PNEUMONIA IN THERMALLY INJURED PATIENTS

Prior to adoption of measures to neutralize gastric acid, gastrointestinal bleeding was a relatively frequent lethal complication in thermally injured patients. Endoscopically documented mucosal ulcerations could be identified in almost all seriously injured patients (1). The current therapy, aimed at maintaining gastric pH above 4.5, has virtually eliminated this complication (2).

In 1978, Atherton and White (3) proposed that the stomach might serve as a reservoir for bacteria that then colonized the respiratory tract of mechanically ventilated patients. It is now accepted that when the pH of the gastric contents rises above 4, the stomach becomes rapidly colonized with bacteria. Some authors have suggested that in patients with gram-negative nosocomial pneumonia, the bacteria appear to be of gastric origin. Since the incidence of pulmonary aspiration of gastric contents may be as high as 74% in mechanically ventilated patients (4), a method of stress ulcer prophylaxis which does not allow bacterial colonization of the stomach may be beneficial.

A recently reported prospective randomized trial documented that sucralfate was as effective as antacids or H_2 blockers in preventing stress-induced bleeding. The incidence of nosocomial pneumonia was lower in patients receiving sucralfate than those receiving antacids or H_2 blockers (5). However, the lowest incidence of pneumonia was in the group receiving H_2 blockers alone.

Sucralfate, a chemical complex of sucrose octasulfate and aluminum hydroxide, appears to protect against stress ulcer bleeding through pepsin absorption, mucosal protein binding, and cytoprotection without significantly altering gastric pH (6). It has been suggested recently that sucralfate may also have intrinsic antibacterial activity (7).

Previous trials investigating the use of sucralfate as prophylaxis for stress ulcer-induced bleeding have all suffered from the same flaw. The population of patients studied was usually not at significant risk for the development of stress ulcers. Patients with significant thermal injury represent a population at significant risk for the development of stress ulcers. With preliminary work suggesting that sucralfate is adequate for prophylaxis in populations at less risk, the next logical step is to attempt its use in patients at higher risk.

Therefore, the purpose of this study is twofold. First, the efficacy of sucralfate in the prevention of stress ulcer-induced gastrointestinal bleeding will be investigated. Second, the

incidence of pneumonia in patients randomized to receive standard therapy with antacids and H_2 blockers will be compared to that in patients who receive sucralfate.

MATERIALS AND METHODS

Study Design. Three hundred patients will be randomized to receive either standard prophylaxis or sucralfate. The gastric pH of all patients will be checked and recorded every hour. The incidence of gastrointestinal bleeding, pneumonia, and tracheobronchitis will be recorded for each patient. Any patient demonstrating clinically evident bleeding will undergo upper gastrointestinal endoscopy to verify the source of the bleeding. Sputum Gram stain and cultures and gastric aspirate cultures will be obtained every Monday, Wednesday, and Friday and as clinically indicated. Isolates from each source will be typed and compared. The timing of colonization for each source will be recorded. Differences between treatment groups in the rate of occurrence of pneumonia and clinically evident gastrointestinal bleeding will be evaluated, with the patients stratified for the presence of inhalation injury.

Criteria for Admission. Three hundred patients will be enrolled in this study. Properly signed and witnessed DA Forms 5303-R, Volunteer Agreement Affidavit, are obtained from each patient prior to initiation of the study.

Patient Inclusion. Patients meeting the following criteria are eligible for participation in this study after giving written informed consent:

1. Male or female patients ≥ 18 yr. Female patients must have been surgically sterilized, be postmenopausal (> 45 yr and lack of menstrual periods for > 1 yr), or have a negative pregnancy test immediately prior to entry into the study.

2. Patients admitted to the US Army Institute of Surgical Research within 48 h postburn.

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

Patient Exclusion. Patients meeting any of the following criteria are excluded from participation in this study:

1. Patients under < 18 yr.

2. Patients who are pregnant or nursing.

3. Patients admitted to the US Army Institute of Surgical Research more than 48 h postburn.

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

5. Patients with a previous history of peptic ulcer disease.

6. Patients who are presently receiving H_2 antagonists.

7. Patients with a diagnosis of pneumonia at the time of admission to the US Army Institute of Surgical Research.

Description of Procedures. Patients meeting entry criteria are randomized to receive either standard prophylaxis or sucralfate. Standard anti-ulcer prophylaxis consists of the administration of cimetidine and antacids. Cimetidine (300 mg IV) is administered every 6 h. The dose of cimetidine is adjusted depending upon the patient's renal function and gastric pH. Antacids are administered as a 30-cc bolus via the nasogastric tube every 2 h. Any evidence of clinically significant upper gastrointestinal bleeding results in withdrawal of the patient from the study. Withdrawal from the study for any reason results in the classification of the patient as a treatment failure. Gastric pH is checked every hour and if the pH is < 4.5, the dose of antacids is doubled and administered on an hourly basis until the pH is ≥ 4.5. Sucralfate (1 g suspended in 20 cc water) is administered via the nasogastric tube every 6 h. The tube is clamped for 1 h following administration. The gastric pH of these patients is checked and recorded every hour. The incidence of clinically evident gastrointestinal bleeding is recorded. Any patient who demonstrates clinically evident bleeding undergoes upper gastrointestinal endoscopy to verify the source of the bleeding. The diagnosis of pneumonia is based upon roentgenographic findings consistent with pneumonia, sputum leukocytes > 20 WBC/hpf, and growth of a predominant organism on sputum culture. A diagnosis of tracheobronchitis is made based upon an elevated sputum culture leukocytosis (> 20 WBC/hpf) and a predominant organism in the sputum culture. The incidence of pneumonia and tracheobronchitis is recorded for each patient. Sputum Gram stain and cultures and gastric aspirate cultures are obtained every Monday, Wednesday, and Friday and as clinically indicated. Isolates from each source are typed and compared. The timing of colonization for each source is recorded.

Determination of Number of Subjects Required. Between 1983 and May 1985, there were 220 patients who developed pneumonia out of a total of 1,300 admissions for an incidence of 17%. For patients with burns exceeding 20% of the total body surface area, this incidence is estimated to be 25% to 30%. Assuming an incidence of 25% and a 50% reduction in the incidence of pneumonia in sucralfate-treated patients as suggested in the literature, 150 patients per arm will be required to prove the hypothesis with a type I error < 0.05 and a type II error < 0.2.

Data Collection. The gastric pH of all patients is checked and recorded every hour. The incidence of clinically evident gastrointestinal bleeding, pneumonia, and tracheobronchitis is recorded for each patient and the timing of colonization for each source is recorded.

Data Analysis Plan. Differences between treatment groups in the rate of occurrence of pneumonia and clinically evident gastrointestinal bleeding is evaluated for significance using the Chi-square technique.

RESULTS

Ninety-one patients have been enrolled in this study to date, 28 during this reporting period. One patient receiving sucralfate had a clinically significant gastrointestinal bleed which necessitated performance of a subtotal gastrectomy. There have been no other episodes of significant gastrointestinal bleeding in either patient population.

DISCUSSION

When the projected total of 300 patients have completed the study, the data will be analyzed as to the efficacy of sucralfate in the prevention of stress ulcer-induced gastrointestinal bleeding and reduction of nosocomial pneumonia.

PRESENTATIONS/PUBLICATIONS

Cioffi WG Jr: The efficacy of sucralfate in the prevention of stress ulcers and nosocomial pneumonia in thermall injury patients. Presented at the 12th Annual Meeting of the Surgical Infection Society, Los Angeles, California, 9 April 1992.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

ACCESSION: DA346178

SUMMARY DATE: 920121 **SUMMARY KIND:** H **PREV DATE:** 911001 **DISTRIBUTION:** CX

PROGRAM #: 62787A **PROJ #:** 30162787A874 **TASK AREA:** CA **WORK UNIT:** 175

TITLE: Study of Effects of Intermittent vs. Continuous Administration of EXOSURF® in Patients with ARDS Induced by Thermal Injury

SUBJ1: 060500 - Medicine and Medical Research

START DATE: 9001 **END DATE:** 9201 **PERFORMANCE METHOD:** C

CONTRACT/GRANT NO:

CONT TOTAL: $
CUM TOTAL: $
TOTAL LAB FUNDS: $

RESOURCES ESTIMATE

FY	WORK YRS	$(Thousands)
91	0.5	$25
92	0.0	$0
93	0.0	$0

RESPONSIBLE DOD ORGANIZATION: 404885
MRDC INSTITUTE OF SURGICAL RESEARCH USAISR
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PRUITT, B A
210-221-2720

PERFORMING ORGANIZATION: 404885
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CIOFFI, W G
210-221-8440

ASSOC1: RUE, L W

ASSOC2:

FOREIGN INTELLIGENCE CONSIDERED: Y **CIVILIAN APPLICABILITY:** M

KEYWORDS: RA II; Volunteers; Adults; Burns (Injuries); Morbidity; Inhalation; Therapy

OBJECTIVE: A DTIC literature search was conducted under DTIC request numbers W6N55B/W6N57E dated 30 May 1990. The objectives of this work are to determine whether a twofold increment in EXOSURF® dose alters the effects of aerosolized EXOSURF® on shunt fraction and other physiologic indices of pulmonary function and to assess patient tolerance to continuous aerosolized EXOSURF® for 5 days.

APPROACH: Twenty-four patients with early onset ARDS secondary to thermal and smoke inhalation injury will be randomized to one of four treatment groups. Patients in each group will receive continuous aerosol administration of the appropriate agent for 5 days. A variety of ventilatory, hemodynamic, and blood gas data will be collected.

PROGRESS: 9001-9201. This study has been terminated due to problems encountered with the aerosol generator. No patients were enrolled in the study. For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report* for fiscal years 1990 through 1992.

ABSTRACT

PROJECT NUMBER: 3M162787A874-175, Applied Research and Exploratory Development

PROJECT TITLE: A Multicenter Double-Blind, Randomized, Controlled Pilot Study of the Effect of Intermittent Versus Continuous Administration of EXOSURF® in Patients with Adult Respiratory Distress Syndrome Induced by Thermal and Smoke Inhalation Injury

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

PERIOD COVERED IN THIS REPORT: 1 October 1991 - 30 September 1992

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This investigation is a multicenter, double-blind, randomized, parallel, placebo-controlled, pilot study of the effect of intermittent versus continuous administration of EXOSURF® aerosol on pulmonary function in patients with early adult respiratory distress syndrome due to cutaneous thermal injury and smoke inhalation. Twenty-four patients will be assigned randomly to four treatment groups.

The objectives of this study are to determine whether a twofold increment in EXOSURF® dose alters the effects of aerosolized EXOSURF® on shunt fraction and other physiologic measurements of pulmonary function and to assess patient tolerance to continuous aerosolized EXOSURF® for 5 days. Improvement in shunt fraction will be an indication that EXOSURF® actually is being deposited in the alveolar regions of the lung and is improving gas exchange, compliance, and/or functional residual capacity. Secondary measures of efficacy will be hypoxemia ratio, respiratory system compliance, days on mechanical ventilation, and survival. Safety will be determined by studying the effect of aerosolized EXOSURF® on blood chemistries, hematologic indices, urinalyses, ECGs, and chest roentgenographs.

This study was terminated due to problems encountered with the aerosol generator. No patients were enrolled in the study.

A MULTICENTER DOUBLE-BLIND, RANDOMIZED, CONTROLLED PILOT STUDY OF THE EFFECT OF INTERMITTENT VERSUS CONTINUOUS ADMINISTRATION OF EXOSURF® IN PATIENTS WITH ADULT RESPIRATORY DISTRESS SYNDROME INDUCED BY THERMAL AND SMOKE INHALATION INJURY

Acute, severe pulmonary insufficiency afflicts patients of all ages, ranging from the newborn to geriatric age groups. A common, often fatal form of acute pulmonary insufficiency in adults is termed adult respiratory distress syndrome (ARDS) (1,2).

ARDS develops as a result of various and diverse etiologies. ARDS may occur after direct lung injuries, such as chest contusion, aspiration, near drowning, or inhalation of smoke or irritant gases. ARDS may also develop after various nonpulmonary disorders, including sepsis, trauma, shock, burns, fractures, transfusions, and pancreatitis (1,3-7).

Inhalation injury is common in patients with thermal injury. An estimated 10-40% of thermal injury patients have a concurrent inhalation injury, which frequently causes pulmonary complications (8,9). Mortality rates of individuals with smoke inhalation injury can increase significantly when compared to similar burn patients without injury (8). The presence of smoke inhalation injury may increase mortality in every age group and burn size (8). The number of burn patients with inhalation injury who develop respiratory compromise each year remains uncertain. However, pulmonary complications, including ARDS, are now some of the leading causes of mortality in burn patients with inhalation injury.

ARDS, often rapid in onset and requiring mechanically assisted ventilation, results in a mortality which exceeds 65% (1-5). The clinical picture is one of increased pulmonary endothelial and epithelial permeability, resulting in exudation of protein-rich fluid in interstitial and alveolar spaces, severe hypoxia secondary to right-to-left shunting of blood, microatelectasis, and a decreased amount and/or inactivation of surfactant (1-7,10-15).

Surfactant is a lipid protein complex which lines the alveolar surface of the lung (16). Surfactant reduces surface tension at the air-liquid interface, lowers end-expiratory volumes of the lung, increases lung compliance, and aids in keeping the small alveoli as dry as possible (17,18). Surfactant is a mixture of several phospholipids and numerous proteins (16); however, the main constituent is dipalmitoyl-phosphatidylcholine (DPPC), comprising approximately 50% of the complex. DPPC is the component responsible for the surface tension-lowering properties of the complex. Another component of surfactant is phosphatidylglycerol (PG). While as surface active in DPPC, the role of PG in surfactant is unclear since PG may be unnecessary for good surfactant function (19,20). However, PG is a good marker for

mature lung and surfactant in the neonate (21). Most of the proteins in the surfactant are of serum origin, although there are several surfactant-specific proteins with molecular weights in the ranges of 34-36 and 5-14 kD (16,22-25). The surfactant-specific proteins aid in the spreading of surfactant on the alveolar surface. The lung lavage fluid content of the lower molecular weight protein is decreased in several animal models of lung injury (26). Analyses of bronchoalveolar lavage from ARDS patients and from animal models of acute lung injury demonstrate that the alveolar phospholipids have a reduced content of DPPC and PG and a decreased lecithin-sphingomyelin ratio similar to the situation in the neonate (13,15). The ARDS patient has a decreased pulmonary compliance, which is one of the physiologic correlates of increased alveolar surface tension (1,2). It is believed that the initial lung injury precipitating ARDS is a breakdown of the alveolar endothelial-epithelial permeability barrier. As a consequence of the alveolar injury, there is a marked disturbance of the surfactant system (10-15). The etiology of ARDS is complex and numerous substances are released into the lung which may cause cellular damage and surfactant inactivation (6).

The present treatment for patients with ARDS secondary to smoke inhalation consists of mechanical ventilation with positive end-expiratory pressure, supplemental oxygen, and vigorous pulmonary toilet, all of which are supportive attempts to maintain arterial oxygenation rather than specific disease treatment. In fact, high inspired oxygen concentrations when administered to patients on mechanical ventilation may in and of themselves cause further lung damage. Such supportive therapeutic approaches are often unsuccessful and may result in significant morbidity.

The administration of exogenous surfactant along with mechanical ventilation and positive end-expiratory pressure significantly improves the survival of rabbits in a lavage surfactant-depleted model of ARDS and in mice infected with influenza (27-30). In an oxygen toxicity model of ARDS, the administration of a natural surfactant to oxygen-exposed rabbits improved lung compliance, decreased lung edema, and appeared to mitigate the lung injury (31). Similarly, administration of exogenous surfactant to ARDS patients should improve compliance of the lung and increase functional residual capacity, allowing the patient to be ventilated with lower peak pressures and lower inspired oxygen concentrations. By virtue of its surface tension-lowering and antiedema properties, exogenous surfactant may prevent or help reverse alveolar edema (32). Thus, exogenous surfactant administration early in the course of ARDS may help stabilize the lungs, thereby decreasing the need for mechanical ventilation.

Preliminary studies in patients in whom exogenous surfactant was administered within 72 h of onset of severe ARDS showed transient improvement in gas exchange. Four grams of

porcine-derived surfactant were administered as a 50-ml intratracheal bolus. This large dose of surfactant was well tolerated (33). Natural surfactant equivalent to 110 ml/kg of DPPC has also been tracheally instilled in the terminally ill child with ARDS. Within 4 h, the arterial oxygen tension rose from 19 to 200 mmHg and there was significant clearing of pulmonary infiltrates on the chest film. Thus, the initial experiences suggest that surfactant administration may be useful in the treatment of ARDS.

Natural surfactant, a combination of lipids and proteins, exhibits not only surface tension-reducing properties but also rapid spreading and absorption. Although DPPC by itself markedly reduces surface tension, alone it is ineffective in ARDS because DPPC spreads and absorbs poorly (34-35). The rapid spreading and absorption necessary for normal natural surfactant function is conferred by the apoproteins. The compound to be used in this study, EXOSURF® (Burroughs Wellcome Company, Research Triangle Park, NC), is a totally synthetic surfactant patented in 1982. Since alcohols spread rapidly on the surface of water, it was postulated that adding alcohol to DPPC would create an effective synthetic surfactant. In this sense, the alcohol constituent of EXOSURF® serves the same function as the apoprotein moieties of natural surfactant. EXOSURF is a 13.5:1.5:5.8:1 mixture of DPPC, hexadecanol(cetyl alcohol), sodium chloride, and tyloxapol.

Toxicology studies of EXOSURF® administered intratracheally as a liquid bolus have been completed. Fifty-two newborn rabbit pups who were dosed on the first day of life and subsequently sacrificed at 14 days received doses of EXOSURF® at 1, 2, and 3 times the recommended neonatal dose of this single intratracheal bolus. Several pups in both the control- and EXOSURF®-treated groups died acutely. Postmortem examination showed no significant changes attributable to EXOSURF®. A second study using 215 rabbit pups who received EXOSURF® 4 times a day in doses ranging from 1 to 3 times the recommended dose were sacrificed 2 weeks later. Again, no significant pathology attributable to EXOSURF® was noted (Documents TTEP/85/0003 and TTEP/85/0004, Burroughs Wellcome Company).

To date, four toxicity studies of aerosolized EXOSURF® in adult animals have been performed. Pilot studies with rats and monkeys have been performed for 5 days (Documents TTEP/87/0005 and TTEP/87/0006, Burroughs Wellcome Company). Aerosolized EXOSURF® resulted in no gross or microscopic signs of toxicity. Two larger studies (Documents TTEP/87/0019 and TTEP/87/0020, Burroughs Wellcome Company) using larger groups of rats and monkeys once again demonstrated no toxicity secondary to aerosolized EXOSURF® administration.

To date, there have been 15 clinical trials with EXOSURF® used in the pediatric age group. All studies were randomized, paralleled, placebo-controlled trials of liquid bolus administration of EXOSURF®. Preliminary analysis of these studies

indicate significant reductions in deaths in EXOSURF®-treated groups with no difference in the incidence of bronchopulmonary dysplasia or intraventricular hemorrhages between the treated and control subjects.

Pilot studies utilizing aerosolized EXOSURF® in adult patients with ARDS have been initiated. Thirteen patients have been treated with aerosolized EXOSURF® without adverse reactions. Patients responded with a decreased oxygen requirement, increased PaO_2/FIO_2 ratio, and decreased shunt fraction.

Pulmonary complications are now the major determinant of mortality in patients with significant thermal injury and smoke inhalation (37). Data suggest that surfactant depletion and inactivation may be partially responsible for pulmonary dysfunction following smoke inhalation.

The purpose of this study is to determine whether administration of exogenous surfactant will result in an improvement in pulmonary functions in patients with thermal- and inhalation injury-induced ARDS. Additionally, this study will attempt to ascertain whether continuous or intermittent administration of aerosolized EXOSURF® are equally effective.

MATERIALS AND METHODS

Study Design. This study is designed as a multicenter, double-blind, randomized, parallel design, pilot investigation of the effect of EXOSURF® or saline insufflation on pulmonary function in patients with early pulmonary insufficiency secondary to smoke inhalation injury. Patients will be randomized to one of 4 groups. Group A will be administered EXOSURF® for 12 h/day, Group B will be administered saline for 12 h/day, Group C will be administered EXOSURF® for 24 h/day, and Group D will be administered saline for 24 h/day. Patients in each treatment group will receive continuous aerosol administration of the appropriate agent for the designated time of 5 days. Randomization will be such that twice as many patients will be enrolled in the treatment groups as the control groups.

Drug Preparation. EXOSURF® will be formulated, packaged, and labelled by the Burroughs Wellcome Company. EXOSURF® will be packaged in individual 50-ml glass vials which will be identified by a self-adhesive label containing the code number, dosage instructions, and storage instructions. EXOSURF® is formulated as a lyophilized powder in sealed glass vials and is stable at room temperature for prolonged periods. The drug will be prepared in the Pharmacy within 4 h before use and will be refrigerated until delivery to the Institute. EXOSURF® or saline will then be administered as an aerosol generated by an air-driven nebulizer with a large drug reservoir.

Description of Procedures. Twenty-four patients who develop early evidence of acute lung injury as the result of thermal and smoke inhalation injury will be offered the opportunity to participate in this study. After written informed consent is obtained, the patient will be screened as indicated in Table 1. The patient will then be transferred to the ventilator adapted for this study, i.e., containing the necessary output to control the nebulizer. After the patient is stabilized, baseline measurements of respiratory system compliance, airway resistance, temperature, hemodynamics, cardiac output, ventilatory data, and blood gases will be recorded (see Table 1). From these data, the hypoxemia ratio, lung injury score, alveolar-arterial PO_2 gradient, arterial to alveolar ratio, systemic oxygen transport, and shunt fraction will be calculated. A baseline blood and tracheal fluid sample will be obtained, processed, and forwarded on dry ice to the Burroughs Wellcome Company for assay of EXOSURF® constituents.

The pharmacist will be asked to contact the Burroughs Wellcome Company to determine whether the patient is to receive EXOSURF® or saline. Five vials of EXOSURF® or 175 ml of 0.1N NaCl will be prepared and placed in an opaque canister for each 4-h administration segment. The canister will be attached to the nebulizer and airflow adjusted to one-half the volume the patient is receiving from the ventilator. When the nebulizer output is stabilized, the output will be connected to the endotracheal input at the same time as the ventilator output is reduced by 50%, such that the minute ventilation the patient receives during treatment is the same as before treatment.

On each day, canisters will be replaced at 4 and 8 h for patients in Groups A and B and at 4, 8, 12, 16, and 20 h for patients in Groups C and D. At each canister replacement, the used canister of EXOSURF® will be removed from the ventilator circuit without being opened and replaced with a new canister.

Treatment and monitoring will continue for 5 days, unless clinical signs contraindicate continued aerosol therapy. A daily chest roentgenograph will be made each morning. If sepsis develops during the 5 days of the study, the site of infection and identification of the organism will be recorded. A 7-ml arterial blood sample will be obtained at 0, 12, 24, 108, 120, and 144 h and stored for possible determination of DPPC, hexadecanol, and tyloxapol. A 10-ml urine sample will be retained from 24-h urine collections obtained on day 1 (0-24 h) and day 5 (96-120 h) and stored for possible determination of DPPC, hexadecanol, and tyloxapol. In addition, tracheal suctioning will be performed at 0, 24, 120, and 144 h and the fluid suctioned from the airways will be retained for analysis.

After 5 days of treatment, or if the decision is made to discontinue treatment at any time prior to 5 days, the patient will be monitored for an additional 24 h, if possible. The data

TABLE 1. Study Plan Flow Chart

	Screening	Treatment Period	Posttreatment[a]	Follow Up
Adverse experiences		X	X	X
APACHE II score	X		X	
Arterial catheter	X	X		
Body temperature	X	X		X
Blood gas measurements	X	X		X
Burn surface area	X			
Cardiac output		X		
Chest roentgenograph	X	X	X	X
EXOSURF® pharmacokinetic blood sample		X	X	
EXOSURF® pharmacokinetic urine sample		X		
Hemodynamic measurements		X		
Hematology, blood chemistry	X	X	X	X
Medical history	X		X	X
Physical examination	X			X
Respiratory system compliance		X		X[b]
Shunt fraction		X		
Swan-Ganz catheter	X	X		
Tracheal secretion sample		X	X	
Urinalysis	X		X	X
Ventilatory data		X		X[b]
12-lead ECG	X		X	X

[a]Performed 24 h after the final EXOSURF® administration.
[b]Pulmonary function testing will be substituted if patient is not on mechanical ventilation.

collected during this 24-h postdosing interval will be appropriate to the clinical state of the patient at that time. Ventilatory data will be recorded at each change of settings during the 24-h postdosing interval. Blood gases, shunt fraction, hypoxemia ratio

and score, temperature, alveolar-arterial PO_2 gradient, arterial to alveolar ratio, and hemodynamics will be collected or calculated every 4 h during the 24-h postdosing interval if the patient is on mechanical ventilation and catheters are in place. Compliance, airway resistance, and cardiac output will be measured every 8 h during the 24-h postdosing interval. If the patient is taken off the ventilator or catheters are removed during the 24-h postdosing interval, other available data will continue to be collected at the specified times. A chest roentgenograph will be obtained each morning.

Treatment may be restarted after this 24-h postdosing interval if the clinical status of the patient appears to deteriorate significantly. Treatment for > 5 days may be permitted if the patient appears to be improving during treatment, but deteriorates during the 24-h postdosing interval. However, treatment beyond 5 days (in increments of up to 5 days) will be instituted only upon joint approval of the primary investigator and the Burroughs Wellcome Company.

The patient will be evaluated at approximately 30 days after the start of the EXOSURF® administration. At that time, information will be recorded as indicated in Table 1. If the patient is on mechanical ventilation at day 30, then compliance and airway resistance will be measured instead of pulmonary function testing. Pulmonary function testing will be substituted if the patient is not on mechanical ventilation; however, this test will be omitted if the patient's health status does not permit performing the testing by day 40. If the patient dies during this one-month interval, the last available data and cause of death will be recorded. In addition, if an autopsy is performed, a copy of the report will be forwarded to the Burroughs Wellcome Company.

Patient Criteria. Twenty-four patients admitted to the US Army Institute of Surgical Research will be enrolled in this study. Properly signed and witnessed DA Forms 5303-R, Volunteer Agreement Affidavit, will be obtained from each patient prior to initiation the study.

Patient Inclusion. Patients meeting all of the following criteria will be eligible for enrollment in the study:

1. Male or female patients ≥ 18 yr. Female patients must have been surgically sterilized, be postmenopausal (> 45 yr and lack of menstrual periods for > 1 yr), or have a negative pregnancy test prior to initiation into the study.

2. Patients with a total body surface area burn < 85%.

3. Patients with documented inhalation injury, i.e., history of smoke exposure, bronchoscopic evidence of inhalation

injury, including carbonaceous deposits at or below the trachea carina and tracheal and/or bronchial erythema/edema.

4. Patients who are intubated and on mechanical ventilation between 48 and 120 h, inclusive, after initial thermal and inhalation injury.

5. Patients with clinical evidence of established ARDS as evidenced by hypoxemia ratio (PaO_2/FIO_2 (FIO_2 expressed as a decimal between 0.21 and 1.0)) between 50 and 299, inclusive, and diffuse pulmonary infiltrates by chest roentgenograph in one or more quadrants.

6. Patients with a Swan-Ganz catheter.

Patient Exclusion. Patients meeting any of the following criteria will be excluded from participation in the study:

1. Male or female patients < 18 yr.

2. Patients who are pregnant or nursing.

3. Patients with a total body surface area burn ≥ 85%.

4. Patients without documented inhalation injury, i.e., history of smoke exposure, bronchoscopic evidence of inhalation injury, including carbonaceous deposits at or below the trachea carina and tracheal and/or bronchial erythema/edema.

5. Patients who do not require mechanical ventilation between 48 and 120 h, inclusive, after initial thermal and inhalation injury or a Swan-Ganz catheter.

6. Patients without clinical evidence of established ARDS as evidenced by hypoxemia ratio (PaO_2/FIO_2 (FIO_2 expressed as a decimal between 0.21 and 1.0)) between 50 and 299, inclusive, and diffuse pulmonary infiltrates by chest roentgenograph in one or more quadrants.

7. Patients with ECG evidence of acute infarction or coronary artery ischemia or wedge pressure > 22 mmHg by Swan-Ganz catheter.

8. Patients with evidence of bacterial pneumonia, *Pneumocystis carinii*, or other opportunistic pulmonary infections.

9. Patients with evidence of renal failure, as defined by sustained oliguria with urine output < 30 ml/h.

10. Patients with evidence of hepatic failure as defined by bilirubin > 5 or SGOT or SGPT > 5 times the upper limit of the normal range.

11. Patients known to have AIDS.

12. Patients with a clinical diagnosis of septic syndrome at time of screening.

13. Patients with head injuries with a Glasgow coma score < 6.

14. Patients receiving chronic medications for COPD, asthma, or emphysema.

15. Patients with the presence of any physiological or psychological condition other than ARDS which contraindicates the administration of EXOSURF®.

Data Analysis. Data collection forms for this study will be forwarded to the Clinical Monitor at the Burroughs Wellcome Company. The primary measure of efficacy will be improvement in shunt fraction, as shunt fraction is a sensitive indicator of lung function and has been shown to correlate with disease severity. Secondary measures will be the hypoxemia ratio, respiratory system compliance, and days on mechanical ventilation. A lung injury score will be used a means to quantify the degree of respiratory failure and/or ARDS. Survival at 30 days will also be assessed.

Efficacy will be assessed by analyzing each EXOSURF® treatment arm relative to its placebo group and to both placebo groups combined. For each physiologic parameter, changes from baseline will be calculated in each treatment group and compared to one another. Data analyses will focus upon differences in mean values at specified times, mean differences from baseline, and areas under the curves for respective values with time. In these critically ill patients, individual values for shunt fraction are expected to vary widely within and between patients. Thus, an area under the curve analysis for shunt fraction throughout the treatment period will be the preferred analysis. Rank order between the two treatment groups is expected rather than a statistically significant improvement in any efficacy parameter because of the small sample size. However, the data from this pilot study should give an estimate of the patient variance in shunt fraction for this subtype of ARDS and permit informed power calculations for the design of subsequent pivotal evaluations of EXOSURF® efficacy and safety in thermal and inhalation injury patients with ARDS.

Safety will be analyzed primarily for signs of intolerance to the dosing regimens by comparing the number of adverse experiences and dosing interventions in each treatment group.

Thermal and inhalation injury patients with sepsis at the time of screening will be excluded from the study in order to achieve a more homogeneous patient group. The time course, survival rate, and changes in circulating mediators suggest major differences in

sepsis-induced ARDS and in thermal and inhalation injury-induced ARDS complicated by sepsis. However, in this small group of patients, data will not be excluded from analysis for patients who develop sepsis after entering the study.

RESULTS

No patients were enrolled in this study. Laboratory experience with the VISAN® nebulizer, which was to be used for the delivery of the aerosolized surfactant, uncovered multiple inherent defects.

DISCUSSION

This study was terminated due to problems encountered with the aerosol generator in an animal model.

PRESENTATIONS/PUBLICATIONS

None.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

ACCESSION: DA346201

SUMMARY DATE: 921001 **SUMMARY KIND:** D **PREV DATE:** 911001 **DISTRIBUTION:** CX

PROGRAM #: 62787A **PROJ #:** 30162787A874 **TASK AREA:** ED **WORK UNIT:** 176

TITLE: A Clinical Study of the Efficacy of Topical Silicone Gel (Silastic™ Gel Sheeting) in the Prevention of Hypertrophic Burn Scar Formation

SUBJ1: 060500 - Medicine and Medical Research

START DATE: 9006 **END DATE:** 9309 **PERFORMANCE METHOD:** C

CONTRACT/GRANT NO:

CONT TOTAL: $
CUM TOTAL: $
TOTAL LAB FUNDS: $

RESOURCES ESTIMATE

FY	WORK YRS	$(Thousands)
91	0.5	$23
92	0.3	$24
93	0.3	$26

RESPONSIBLE DOD ORGANIZATION: 404885
MRDC INSTITUTE OF SURGICAL RESEARCH USAISR
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PRUITT, B A
210-221-2720

PERFORMING ORGANIZATION: 404885
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HARVEY, K D
210-221-8957

ASSOC1: CIOFFI, W G

ASSOC2:

FOREIGN INTELLIGENCE CONSIDERED: Y **CIVILIAN APPLICABILITY:** M

KEYWORDS: RA II; Volunteers; Adults; Burns (Injuries); Skin Grafts; Scars; Dressings

OBJECTIVE: A DTIC literature search was conducted under DTIC request numbers W6007D/W6008D dated 30 May 1990. The objective of this work is to evaluate the efficacy of silicone gel in the prevention of hypertrophic burn scar formation. Improved management of hypertrophic scar formation will benefit military survivors of burn injury.

APPROACH: Forty-two patients who have undergone meshed, split-thickness skin grafting of at least one upper extremity will be enrolled in this study. When the graft has healed and the wound is closed, topical silicone gel will be applied to the grafted area. Data from treated and untreated sites of each patient will be subjected to a one-way, within subjects ANOVA. Data groups will be formed for treated and untreated sites and will undergo a one-way, between patients ANOVA.

PROGRESS: 9110-9209. Twenty-nine patients have been enrolled in this study to date, 18 during this reporting period. Three patients were withdrawn from the study due to early discharge from the Institute prior to completion of the study. Immediate visual smoothening and softening of the treatment sites have been noted. Risks have been minimal with no resultant problems. Upon completion of enrollment, the data will be analyzed as indicated. For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report* for fiscal years 1990 through 1992.

ABSTRACT

PROJECT NUMBER: 3M162787A874-176, Applied Research and Exploratory Development

PROJECT TITLE: A Clinical Study of the Efficacy of Topical Silicone Gel (Silastic™ Gel Sheeting) in the Prevention of Hypertrophic Burn Scar Formation

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

PERIOD COVERED IN THIS REPORT: 1 October 1991 - 30 September 1992

INVESTIGATORS: Karoline D. Harvey, OTR, Captain, SP
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Basil A. Pruitt, Jr., MD, Colonel, MC

Advancements in medical and surgical management of burn injury have resulted in decreased mortality. Hypertrophic scarring is a major long-term complication for burn injury survivors. Research into methods for preventing or managing burn wound scarring may enhance the quality of life of burn injury survivors. The objective of this study is to evaluate the efficacy of silicone gel (Silastic™ gel sheeting) in the prevention of hypertrophic burn scar formation.

A CLINICAL STUDY OF THE EFFICACY OF TOPICAL SILICONE GEL (Silastic™ GEL SHEETING) IN THE PREVENTION OF HYPERTROPHIC BURN SCAR FORMATION

There has been interest in the use of silicone materials for the treatment of burns for many years. There have been reports of silicone oil and silicone dressings in the treatment of hand burns (1-3). In 1988, Dr. Ohmori of Tokyo reported successful treatment of keloid scars with Silastic™ (silicone) sheeting. Dow-Corning-Wright has developed and is now marketing a Silastic™ gel sheeting for clinical use with hypertrophic scars. The product, Silastic™ gel sheeting, is a 3.5-mm thick polyethyleneterephthalate-mesh, reinforced silicone gel sheet. Silicone gel sheets have been found to be bacteriologically inert and to have mechanical extensibility similar to skin (4). The mechanism of action of silicone gel on hypertrophic scar is unknown. After studying silicone gel sheets, Quinn et al (5) concluded that its mechanism of action in altering scar tissue is not secondary to pressure, temperature, oxygen tension, skin hydration, or occlusion and may therefore involve a chemical factor. Previous studies (5,6) have demonstrated that applications of silicone gel sheets soften, flatten, and increase the extensibility of existing hypertrophic burn scars. With the exception of a five-patient clinical trial of silicone gel as a partial-thickness burn injury dressing (7), there have been no studies to assess its effect as treatment for the prevention of burn scar formation.

The objective of this study is to evaluate the efficacy of silicone gel (Silastic™ gel sheeting) in the prevention of hypertrophic burn scar formation. A within subjects, repeated measures and between groups research design involving recently skin-grafted patients will be used. The results of this preliminary study will determine if subsequent investigations into the mechanism of action of silicone gel are feasible within an acute care setting.

MATERIALS AND METHODS

Study Design. This study utilizes patients who have undergone meshed, split-thickness skin grafting of at least one upper extremity. When the graft heals so that the interstices are closed, a 4-cm^2 patch of Silastic™ gel sheeting is applied to a grafted area and secured with surgical netting (Surginet^R). The silicone gel sheeting remains in contact with the graft for a total of 23 h a day and is removed for 30 min twice a day for cleaning, skin inspection, and hygiene. Treatment continues for 3 weeks. Treatment will be discontinued in the event of pruritus, pain, maceration, ulceration, or other tissue degradation.

Patient Criteria. Forty-two patients will be enrolled in this study. Properly signed and witnessed DA Forms 5303-R, Volunteer Agreement Affidavit, are obtained from each patient prior to initiation of the study.

Patient Inclusion. Patients meeting the following criteria are eligible for enrollment in this study:

1. Male or female patients ≥ 18 yr. Female patients must have been surgically sterilized, be postmenopausal (> 45 yr and lack of menstrual periods for > 1 yr), or have a negative pregnancy test before initiation into the study.

2. Patients with burns > 10% of the total body surface area (the presence of an inhalation injury not being exclusionary).

Patient Exclusion. Patients meeting the following criteria are excluded from participation in this study:

1. Patients < 18 yr.

2. Patients with burns < 10% of the total body surface area.

3. Patients who are pregnant or nursing.

4. Patients with toxic epidermal necrolysis.

Description of Procedures. A test site is selected from a skin-grafted area. The procedure begins when the attending physician determines that the skin graft is sufficiently healed to allow application of the Silastic™ gel sheeting. Prior to application of the sheeting, the test site is photographed, the softness or pliability of the graft is measured with a tonometer, and the color and texture of the graft is rated by a panel of five burn center personnel. Next, a 4-cm^2 piece of Silastic™ gel sheeting is placed on one-half of the test site and secured in place with surgical netting. The sheeting is removed twice a day for cleaning but otherwise remains in place for 3 weeks. The pliability of the test site is measured once a week. At the end of the 3-week period, the test site is again photographed and rated for color and texture by the same panel of five burn center personnel. The pliability of the test site continues to be measured once a week until the patient is discharged from the Institute.

Determination of Number of Subjects Required. A total number of 42 patients will be required based on an expected treatment effect difference of 25%, a 5% type I error, and a 10% type II error.

Data Collection. Initial data collected on each patient include the patient's admission number, age, sex, burn size, date of burn, location of target burn/graft test area, depth of burn at target area, type of excision, type of skin graft, and date of skin grafting. The effect of treatment is be assessed by pre- and posttreatment color (see Table 1) and texture ratings (see Table 2). These ratings are conducted by a panel of five disinterested observers. In addition, weekly measurements of scar pliability are taken using a modified tonometer (see Table 3) as described by Esposito et al (8). The test sites and adjacent areas are photographed before and after treatment for later comparison. Weekly measurements to assess the effect of treatment discontinuation continue until the patient's discharge from the Institute.

Data Analysis Plan. Data from treated and untreated sites of each patient will be subjected to a one-way, within subjects ANOVA. Data groups will be formed for treated and untreated sites and will undergo a one-way, between patients ANOVA.

RESULTS

Twenty-nine patients have been enrolled in this study to date, 18 during this reporting period. Immediate clinical and tactile improvement of the treatment sites has been noted. Risks have been minimal with no resultant problems.

DISCUSSION

When the projected total of 42 patients have completed the study, the data will be analyzed as to the efficacy of Silastic™ gel sheeting in the prevention of hypertrophic burn scar formation.

PRESENTATIONS/PUBLICATIONS

None.

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TABLE 1. Graft Color Difference Between Treated and Control Sites

Subject	Pretreatment	Posttreatment*
1		
.		
.		
42		

Rating Scale:	Less Color (Paler)		Same		More Color (Darker)
	1	2	3	4	5

*Week 4

TABLE 2. Graft Texture Difference Between Treated and Control Sites

Subject	Pretreatment	Posttreatment*
1		
.		
.		
42		

Rating Scale:	Less Texture (Smoother)		Same		More Texture (Raised/Rough)
	1	2	3	4	5

*Week 4

TABLE 3. Weekly Tonometric Measurements (Graft Pliability)

Subject	Pre Tx	1	2	3	Post Tx	4	5	6
1	X C	X C	X C		X C	X C	X C	
.								
.								
42								

X indicates treated site; C, control site.

5. Quinn KJ, Evans JM, Courtney JM, et al: Non-pressure treatment of hypertrophic scars. *Burns* 12:102-8, 1985.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

ACCESSION: DA335466

SUMMARY DATE: 921001 **SUMMARY KIND:** D **PREV DATE:** 911001 **DISTRIBUTION:** CX

PROGRAM #: 62787A **PROJ #:** 30162787A874 **TASK AREA:** BJ **WORK UNIT:** 177

TITLE: A Clinical Study of the Efficacy of Low-Dose Dopamine Therapy in Hospitalized Burn Patients

SUBJ1: 060500 - Medicine and Medical Research

START DATE: 9007 **END DATE:** 9309 **PERFORMANCE METHOD:** C

CONTRACT/GRANT NO:

CONT TOTAL:	$
CUM TOTAL:	$
TOTAL LAB FUNDS:	$

RESOURCES ESTIMATE

FY	WORK YRS	$(Thousands)
91	0.5	$39
92	0.5	$43
93	0.5	$55

RESPONSIBLE DOD ORGANIZATION: 404885
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PERFORMING ORGANIZATION: 404885
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210-221-8440

ASSOC1: HEIRONIMUS, J D

ASSOC2: VAUGHAN, G M

FOREIGN INTELLIGENCE CONSIDERED: Y **CIVILIAN APPLICABILITY:** M

KEYWORDS: RA II; Volunteers; Adults; Burns (Injuries); Dopamine; Therapy

OBJECTIVE: A DTIC literature search was conducted under DTIC request numbers W6K26L/W6K33L dated 9 February 1990. The objective of this work is to evaluate the efficacy of low-dose (2-5 μg/kg/min) dopamine therapy in burn patients. Better understanding of the effect of such compounds in patients after thermal injury will allow refinement of resuscitation techniques.

APPROACH: Effective renal plasma flow, glomerular filtration rate, sodium and potassium excretion, and free water clearance will be measured using radiolabelled tracers before and during a continuous intravenous dopamine infusion in 20 consecutive burn patients with burns greater than 30% of the total body surface area and 10 normal volunteers. Results will be evaluated using data tables prepared to compare both burn and control populations in terms of effective renal plasma flow, glomerular filtration rate, sodium and potassium osmolar excretion, serum and plasma variables, free water clearance, and cardiac output. ANOVA will include hour of infusion, burn size, age, and postburn day as main sources of variation for measured variables.

PROGRESS: 9110-9209. Eleven burn patients and 6 control subjects have been enrolled in this study to date, 4 burn patients during this reporting period. Patients exhibited a significant chronotropic effect of low-dose dopamine which increased cardiac output and renal plasma flow. A natriuretic effect was inconsistently seen, thus limiting the usefulness of this therapy. For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report* for fiscal years 1990 through 1992.

ABSTRACT

PROJECT NUMBER: 3M162787A874-177, Applied Research and Exploratory Development

PROJECT TITLE: A Clinical Study of the Efficacy of Low-Dose Dopamine Therapy in Hospitalized Burn Patients

INSTITUTION: US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas 78234-5012,[1] Nuclear Medicine Department, Brooke Army Medical Center, Fort Sam Houston, San Antonio, Texas 78234-6200,[2] and US Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland 21701-5011[3]

PERIOD COVERED IN THIS REPORT: 1 October 1991 - 30 September 1992

INVESTIGATORS: William G. Cioffi, Jr., MD, Major, MC[1]
James D. Heironimus, MD, Lieutenant Colonel, MC[2]
George M. Vaughan, MD, Colonel, MC[1]
Laura W. Pratt, MD, Captain, MC[3]
William F. McManus, MD, Colonel, MC[1]
Basil A. Pruitt, Jr., MD, Colonel, MC[1]

The renal effects of low-dose dopamine therapy in hyperdynamic thermally injured patients are unknown. We investigated the renal effects of low-dose dopamine therapy in 10 patients with thermal injury and 6 control subjects. Low-dose dopamine therapy significantly increased GFR, ERPF, sodium excretion, and urine flow in controls, and ERPF, urine flow, heart rate, and cardiac index in patients.

The chronotropic effect of dopamine appears to be a principal contributor in the patients' increased effective renal plasma flow. Sodium excretion was increased by low-dose dopamine therapy only in those patients in whom the predopamine sodium excretion exceeded 5 mEq/h. Lack of a consistent natriuretic effect and the consistent chronotropic effect suggest that the routine use of low-dose dopamine in burn patients is unwarranted. The side effects that attend the desired response determine clinical use, i.e., the potential for blood flow redistribution and increased cardiac work demands must be balanced against an increased renal plasma flow and natriuresis.

A CLINICAL STUDY OF THE EFFICACY OF LOW-DOSE DOPAMINE THERAPY IN HOSPITALIZED BURN PATIENTS

Dopamine (3,4-dihydroxyphenylethylamine) is a precursor of norepinephrine and epinephrine. It is found in the sympathetic nerves and ganglia, most prominently in the brain, heart, kidney, vasculature, and intestines. Depending upon the dosage used and thus the type of receptor stimulated, it has a wide variety of pharmacological actions. At low doses, 0.5-1 μg/kg/min, dopamine-1 (DA1) receptors are primarily activated. The DA1 receptors are located in the renal, mesenteric, cerebral, and coronary vasculature (1). Their stimulation leads primarily to vasodilation. Additionally, stimulation of the DA1 receptors located in the renal juxtaglomerular apparatus and zona glomerulosa leads to inhibition of sodium transport in the kidney. At slightly higher doses, 2-5 μg/kg/min, the beta-1 receptors are also activated (1). This exerts a positive inotropic effect on the myocardium, with a subsequent increase in cardiac output. At doses of approximately 10 μg/kg/min, alpha-1 and alpha-2 receptors in the peripheral vasculature are activated, leading to vasoconstriction and increased systemic vascular resistance (1). The combined renal and cardiovascular effects of "low-dose" dopamine therapy (2-5 μg/kg/min) result in increased renal blood flow, with an associated increased glomerular filtration rate (GFR), increased sodium excretion, and increased urine output (1-4).

Excretion rates determine the dopamine half-life of 1-2 min when it is administered parenterally. Monoamine oxidase rapidly metabolizes dopamine to sulfates and glucuronides by conjugation. This rapid elimination necessitates that dopamine be administered via a continuous infusion, using a metered pump for strict control of rate of flow. In addition, the dose-related pharmacological effects of dopamine occur gradually and the clinical response is variable from patient to patient. Therefore, careful monitoring of blood pressure, cardiac output, and urine flow is mandatory (5).

Side effects are rarely seen when low-dose dopamine infusion is administered. The renal clearance of other drugs which are eliminated primarily by glomerular filtration is increased and dosage adjustment may be required. The clearance of those drugs eliminated by hepatic degradation is likewise augmented secondary to mesenteric vasodilation and increased hepatic flow. Therefore, drugs eliminated by this route must also be closely monitored. Extravasation of dopamine into the subcutaneous tissue can lead to an intense local vasoconstriction and subsequent necrosis. Therefore, dopamine should be administered via a large central vein. Phosphate levels must also be monitored due to increased urinary excretion. Hypoxemia must be avoided, since dopamine suppresses the ventilatory response to low oxygen tension at the carotid body. At higher doses, dopamine can cause tachycardia,

arrhythmias, and ischemia. As a result, continuous cardiac monitoring is mandatory (1,5).

Dopamine's effect on renal perfusion and urine flow has been documented in a wide variety of clinical situations (3,6-9). Using radiolabeled tracers, it has been shown to increase both ERPF and GFR with a resultant increase in urine output (1,2). These effects have been attributed to both the renal vasodilation and increased cardiac output observed with "low-dose" dopamine therapy.

Low-dose dopamine therapy is also occasionally utilized in burn patients when the fluid resuscitation requirements exceed the predicted rates; however, the efficacy of this therapy in burn patients has never been documented. It is uncertain whether the effects of low-dose dopamine are the same in burn patients with associated hypermetabolic response and elevated levels of aldosterone and antidiuretic hormone as in nonburned patients. The purpose of this study is to document the effect of low-dose dopamine therapy on the ERPF, GFR, solute excretion, and free-water clearance in burn patients and to compare these with previously documented effects seen in various other clinical situations (2,3,6-9).

MATERIALS AND METHODS

Study Design. This protocol will study 20 consecutive burn patients with burns > 30% of the total body surface area and 10 normal volunteers. ERPF and GFR of each patient is measured utilizing radiolabeled tracers, both before and during a continuous intravenous dopamine infusion between 1 and 30 days after injury.

The radiopharmaceuticals administered in this study include ^{99m}Tc-diethylenetriamine penta-acetic acid (Tc-DTPA) and ^{131}I-hippuran (I-HIP), used to measure GFR and ERPF, respectively. These radiopharmaceuticals are administered in a loading dose and a continuous infusion, delivering minimal radiation exposure (less than a standard chest roentgenogram) and allowing for precise quantitation by gamma counting. The doses of Tc-DTPA and I-HIP do not exceed 4 mCi and 0.35 mCi, respectively. Clearance is calculated by measurement of both plasma and urine levels of each radiopharmaceutical (10,11).

The patients receive a priming bolus injection and sustaining infusion of each radiopharmaceutical which is estimated based upon body size and renal function. The loading dose is estimated to yield (after distribution) plasma levels of < 40,000 cpm/min/ml for the Tc-DTPA and < 1,000 cpm/min/ml for the I-HIP. The patient is then begun on a continuous infusion of the two compounds to sustain these serum levels. After a 1-h equilibration period (during which distribution and adjustment in serum tracer levels are expected to occur), the infusion is then continued for an 8-h study period (T=0-8 h). Blood is collected from another site every 15 min for

the first hour (T=0–1 h), then every 30 min during the remaining 7 h. The plasma is separated and a measured volume is counted in a well-type gamma counter for Tc-DTPA and I-HIP simultaneously within a few hours of collection. Timed urine samples, 1 h each, are collected for 8 h. Aliquots of the urine samples are counted for radioactivity in volumes and tubes equivalent to those used for plasma. Diluted proportions of the injectate are counted to determine the dose actually given and to permit correction of spillover of counts for the ^{131}I channel into the ^{99m}Tc channel of the detector. A special computer program was written to correct for physical decay. From blood and urine samples, GFR, ERPF, clearances of Na+, total osmolytes, H_2O, and creatinine are determined, and changes in serum thyrotropin and plasma dopamine.

Description of Procedures. Treatment does not exceed 11 days for burn patients and 3 days for control subjects for the purpose of this study, including clinical observations after the infusion of renal function tracers and dopamine.

Burn Patient Inclusion. Individuals meeting the following criteria are eligible for enrollment in the study. Properly witnessed DA Forms 5303-R, Volunteer Agreement Affidavits, are obtained from each patient, or their legal guardian, before beginning the study.

1. Male or female patients ≥ 18 yr. Female patients must have been surgically sterilized, be postmenopausal (> 45 yr and lack of menstrual periods for at least 1 yr), or have a negative pregnancy test before initiation into the study.

2. Patients admitted to the US Army Institute of Surgical Research within 72 h postburn.

3. Patients with burns > 30% of the total body surface area.

Burn Patient Exclusion. Patients meeting any of the following criteria are excluded from participation in this study:

1. Patients < 18 yr.

2. Patients not admitted to the US Army Institute of Surgical Research within 72 h postburn.

3. Patients with burns < 30% of the total body surface area.

4. Patients who are pregnant or nursing.

5. Patients with evidence of tachyarrhythmias, ventricular fibrillation, or evidence of cardiac ischemia on admission EKG.

6. Patients with uncorrected hypoxemia.

7. Patients with uncorrected hypovolemia as assessed by clinical and Swan-Ganz indices.

8. Patients with preexisting renal disease or a creatinine level > 2.

9. Patients being treated with a monoamine oxidase inhibitor before injury.

10. Patients with known dopamine or sulfite allergy or sensitivity.

11. Patients with known pheochromocytomas.

12. Patients with occlusive vascular disease, i.e., Raynaud's disease, diabetic endarteritis, and Buerger's disease.

Control Subject Inclusion. Control subjects meeting all of the following criteria are eligible for enrollment in this study. Properly signed and witnessed DA Forms 5303-R, Volunteer Agreement Affidavit, are obtained from each subject before beginning the study.

1. Male or female volunteers ≥ 18 yr old. Female patients must have been surgically sterilized, be postmenopausal (> 45 yr and lack of menstrual periods for at least 1 yr), or have a negative pregnancy test before initiation into the study.

2. Volunteers with no chronic medical problems and not currently on any medications.

3. Volunteers obtained from the US Army Medical Research Institute of Infectious Diseases (Fort Detrick, Frederick, MD).

Control Subject Exclusion. Control subjects meeting any of the following criteria are excluded from participation in this study:

1. Subjects < 18 yr old.

2. Subjects who are pregnant or nursing.

3. Subjects with evidence of tachyarrhythmias, ventricular fibrillation, or evidence of cardiac ischemia on EKG.

4. Subjects with preexisting renal disease or a creatinine level > 2.

5. Subjects being treated with a monoamine oxidase inhibitor.

6. Subjects with known dopamine or sulfite allergy or sensitivity.

7. Subjects with known pheochromocytomas.

8. Subjects with occlusive vascular disease, i.e., Raynaud's disease, diabetic endarteritis, and Buerger's disease.

Procedures Before Study

1. Medical history.

2. Physical examination.

3. ECG.

4. Arterial blood gases (as part of routine care, only in burn patients).

5. Laboratory profiles to include standard chemistries (phosphate included), hematology, and urine analysis.

6. A central venous cordis catheter is placed in burn patients only, with pulmonary artery catheter advanced to measure cardiac output, central venous pressure, and pulmonary capillary wedge pressure. Control subjects have a large bore peripheral intravenous catheter placed.

7. A Foley catheter is placed in each burn patient and hourly urine output before treatment is recorded. Control subjects stand to void and no catheter is placed.

8. The burn patient's and control subject's weight in kilograms is obtained upon admission.

9. A second intravenous catheter is placed, at a site distant from the central venous cordis catheter, with a 3-way stopcock in place for blood withdrawal.

10. The patient is given an infusion (2 ml/kg/h D5W plus part of the previous ongoing fluid therapy) in order to produce a urine flow of 2 ml/min. The added infusion volume includes the amount of fluid (as D5W) that later (beginning at T=4 h) contains the dopamine.

11. A 2 ml/min urine flow is established prior to time 0, at which time the bladder is flushed with 100 ml of air. This air flush technique is used to end all urine collections. Control subjects stand to void and do not have a urinary catheter nor undergo bladder air flush.

12. The urine produced during each hour of the 8-h study is collected for electrolyte and osmolality determinations and a plasma sample, collected from the midpoint, is likewise analyzed.

13. One hour before time 0, the priming bolus of the Tc-DTPA and I-HIP is injected into the infusion port of the central venous cordis catheter in burn patients and the catheter flushed with 5 ml of saline (T=0). Control subjects have the same dosages injected into the large peripheral intravenous catheter. The constant infusion of the Tc-DTPA and I-HIP is then begin.

14. Beginning at time 0, 2 ml of blood is collected in heparinized tubes at 15-min intervals for 1 h, then every 30 min for 7 h and placed on wet ice for gamma counting of plasma later in a well-type gamma counter. At the midpoint of each hour, a 3-ml blood sample is taken for determination of serum electrolytes, creatinine, and osmolality. At time 0 and at each hour up to 8 h, a 6-ml blood sample is taken for determination of serum thyrotropin, a marker of dopamine effect, and plasma dopamine.

Dosage and Administration. At T=240 min, dopamine is administered at a rate of 3 µg/kg/min by continuous intravenous infusion. It is infused through a central venous catheter, placed in one of the patient's central veins (femoral, subclavian, or internal jugular). The rate of infusion is controlled by the use of a metered infusion pump. The weight used to calculate the dosage is the patient's preburn weight. Control subjects are weighed immediately prior to participating in the study. The infusion volume of dopamine (in D5W) replaces a portion of the infusion rate of D5W that has been ongoing.

Procedures During the Study.

1. Continuous blood pressure monitoring is performed.

2. Continuous cardiac monitoring is performed using telemetry.

3. Urine is collected and the volume recorded each hour, using the bladder air flush technique previously described. Control subjects stand to void.

4. Cardiac output, central venous pressure, and pulmonary capillary wedge pressure are measured and recorded each hour in the burn patients.

5. Continuous pulse oximetry is performed.

6. Dopamine infusion is continued for 4 h (time 4-8 h), so that GFR, ERPF, all clearances, serum thyrotropin, plasma dopamine, and hemodynamic parameters over the 4-h interval of dopamine infusion can be compared with those taken in the preceding 2 h

without dopamine infusion. A preinfusion period of 4 h is necessary because of hourly variation in urine residual volume (even with a urinary catheter).

7. The dopamine infusion is discontinued at T=480 min.

Procedures After the Study.

1. Continuous blood pressure monitoring is continued for 24 h poststudy.

2. Continuous cardiac telemetry is continued for 24 h poststudy.

3. Hourly urine outputs are recorded for 24 h poststudy.

4. Cardiac output, central venous pressure, and pulmonary capillary wedge pressure are measured and recorded each hour for 4 h poststudy.

5. A laboratory profile, including standard chemistry and phosphate level, are obtained immediately poststudy and the following morning.

6. The clinical effectiveness of dopamine infusion is evaluated using a standard statistical comparison of ERPF and GFR and other measurements pretreatment and during treatment in both burn patients and normal control groups.

Determination of Number of Subjects Required. This study was designed as a pilot study and there were no previous studies of its kind for reference. It was felt that 20 consecutive burn patients and 10 normal volunteers would provide an adequate number of subjects to assess the clinical effects of low-dose dopamine therapy in burn patients and provide some estimate of variation in measured variables with burn size and time postburn.

Data Analysis Plan. Predopamine infusion data were compared between the control and patient populations utilizing a two-tailed t test and nonparametric analysis (Wilcoxon Rank Sum test) when variances differed. The effect of dopamine therapy within each population was analyzed using a paired t test. Comparison of variables was by correlation analysis. All analyses were performed using BMDP software (Los Angeles CA).

RESULTS

Ten patients with thermal injury and 6 control subjects have been enrolled in this study to date. Table 1 contains demographic data for both groups:

TABLE 1. Demographics (Mean ± SEM)

Group	n	Age (Yr)	Sex (M/F)	Postburn Day of Study	Burn Size (% TBSA)
Burn patients	10	30.2 ± 3.3 (19–53)	8/2	19.5 ± 2.5 (9–32)	53.4 ± 7.0 (30–91)
Control subjects	6	20.2 ± 0.5 (19–22)	6/0	–	–

( ) indicates range; TBSA, total body surface area.

Table 2 contains predopamine hemodynamic and fluid administration data for both groups. Patients had significantly higher heart rates and mean arterial pressures, a reflection of the anticipated hemodynamic response to injury typical of thermally injured patients. Urine flow was significantly less in the patients, despite a fluid administration rate which was significantly greater. This is considered to reflect both the increased circulating levels of antidiuretic hormone we have previously reported in thermally injured patients (12) and an elevated evaporative water loss. Table 3 contains further hemodynamic data confirming the presence of a hyperdynamic response in the patients.

Table 4 contains preinfusion renal function data for both groups. As expected, patients had significantly higher GFR and ERPF and lower free water clearance and fractional excretion of sodium than controls.

Renal dose dopamine significantly increased GFR, ERPF, urine flow, osmolar clearance, sodium excretion, and fractional excretion of sodium in the controls, while mean arterial pressure, heart rate, and free water clearance were unchanged (figs 1–4, Table 5). In the patients, renal dose dopamine significantly increased ERPF, urine flow, free water clearance, fractional excretion of sodium, cardiac index, and heart rate while decreasing mean arterial pressure and systemic vascular resistance (figs 1–5, Tables 3 and 6). In the patients, there was no univariate correlation between burn size and the change in ERPF, GFR, urine flow, sodium excretion, or cardiac function. Changes in urine output did not reflect changes in GFR in either population. However, changes in urine output did accurately predict changes in sodium excretion in the patients ($R = 0.811$, $P = 0.004$). Finally, natriuresis did not correlate with changes in renal blood flow.

TABLE 2. Comparison of Predopamine Hemodynamics and Fluid Status (Mean ± SEM)

Group	Mean Arterial Pressure (mmHg)	Heart Rate (beats/min)	Urine Flow (ml/h/1.73 m^2)	Fluid Infusion Rate (ml/h)
Burn patients	82.5 ± 2.6*	125 ± 2.7*	111 ± 22*	388 ± 26*
Control subjects	74 ± 2.8	61.4 ± 1.8	204 ± 30	239 ± 5

*P < 0.05 vs control subjects.

TABLE 3. Hemodynamic Effects of Dopamine in Patients with Thermal Injury (Mean ± SEM)

	Heart Rate (beats/min)	Mean Arterial Pressure (mmHg)	Stroke Volume (ml)	Systemic Vascular Resistance (dyn/sec/cm^2)	Cardiac Index (l/min/m^2)	Pulmonary Artery Occlusion Pressure (mmHg)
Predopamine	125 ± 2.7	82.5 ± 2.6	119 ± 4	205 ± 11.0	7.6 ± 0.3	9.4 ± 1.2
Dopamine	131 ± 2.9*	77.4 ± 2.6*	122 ± 4	183 ± 10.3*	8.03 ± 0.34*	9.0 ± 0.8

*P < 0.05 vs predopamine.

TABLE 4. Predopamine Renal Function (Mean ± SEM)

Group	Glomerular Filtration Rate (ml/min/1.73 m^2)	Effective Renal Plasma Flow (ml/min/1.73 m^2)	Osmolar Clearance (osmol/min/1.73 m^2)	Free Water Clearance (ml/min/1.73 m^2)	Fractional Excretion of Sodium (%)
Burn patients	151 ± 7*	678 ± 54*	3.23 ± 0.4	−1.38 ± 0.34*	0.7 ± 0.3*
Control subjects	118 ± 4.5*	511 ± 25	2.91 ± 0.4	0.48 ± 0.21	1.2 ± 0.2

*$P < 0.05$ vs control subjects.

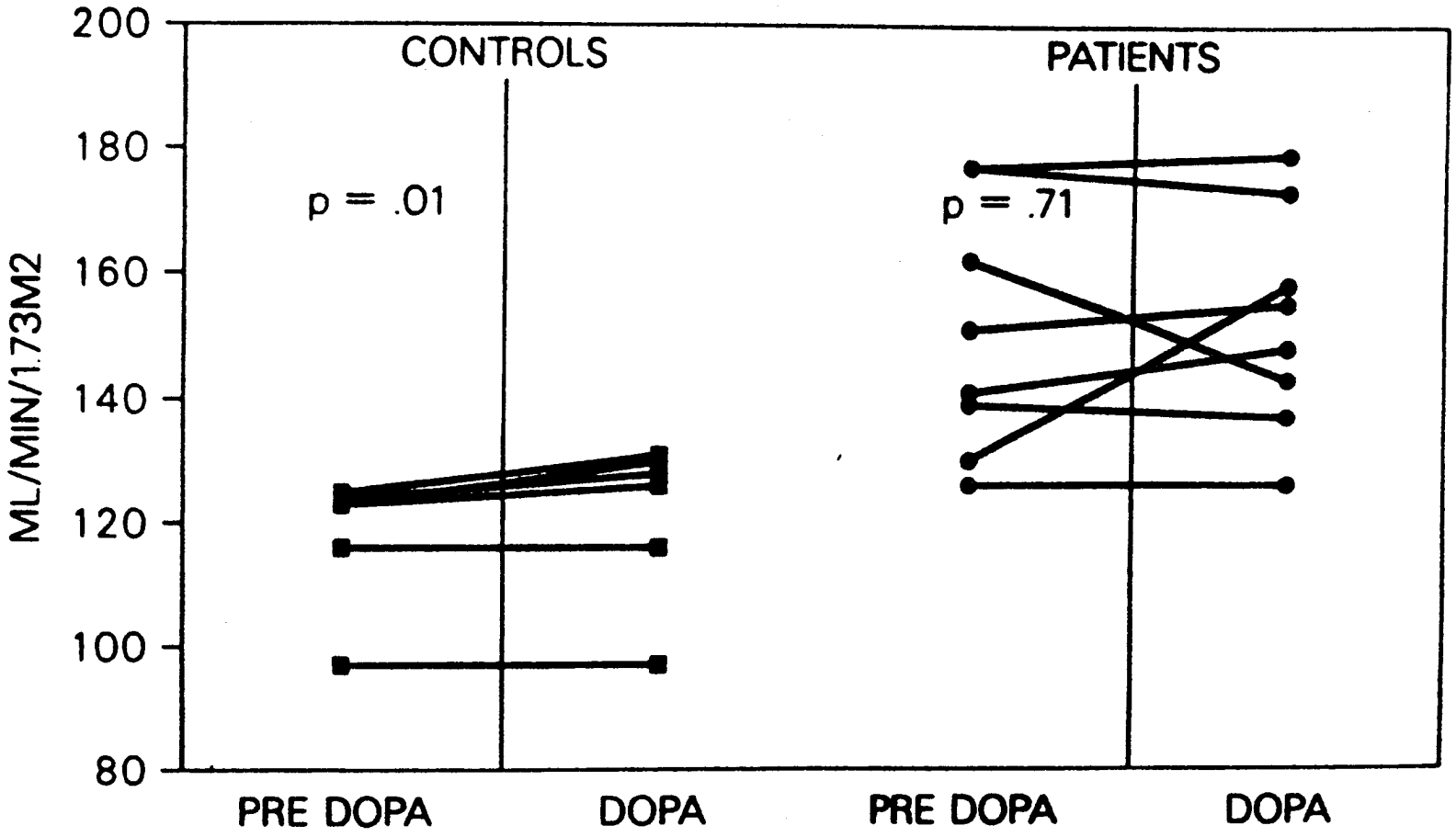


FIGURE 1. Glomerular filtration rate (ml/h/1.73 m^2) for each control subject (n=6) and burn patient (n=8) is depicted before and during dopamine therapy. The GFR was consistently increased by dopamine in the control subjects, but not in the burn patients.

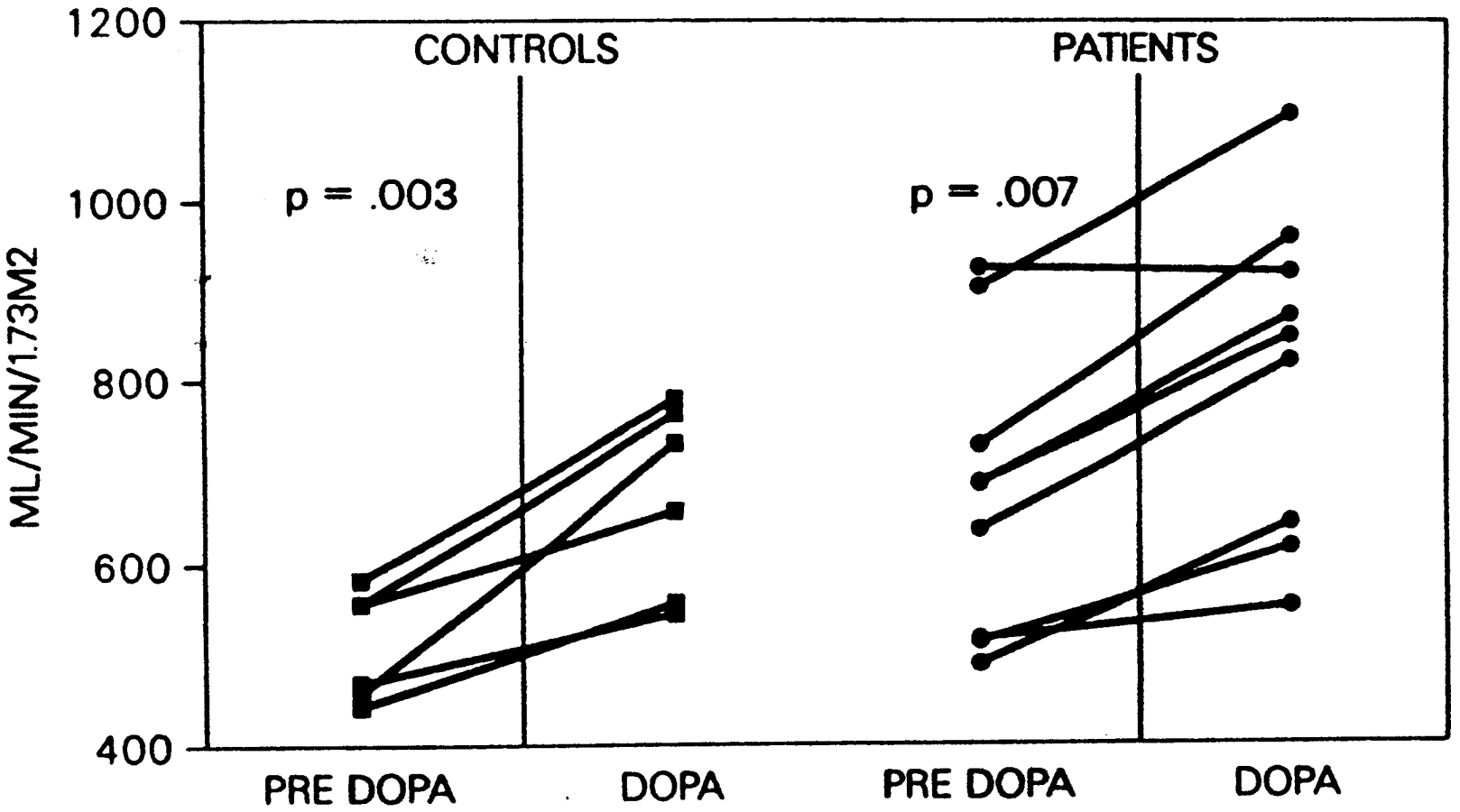


FIGURE 2. Renal plasma flow (ml/min/1.73 m^2) for each control subject (n=6) and burn patient (n=9) is depicted before and during dopamine therapy. Low-dose dopamine significantly increased renal plasma flow in both populations.

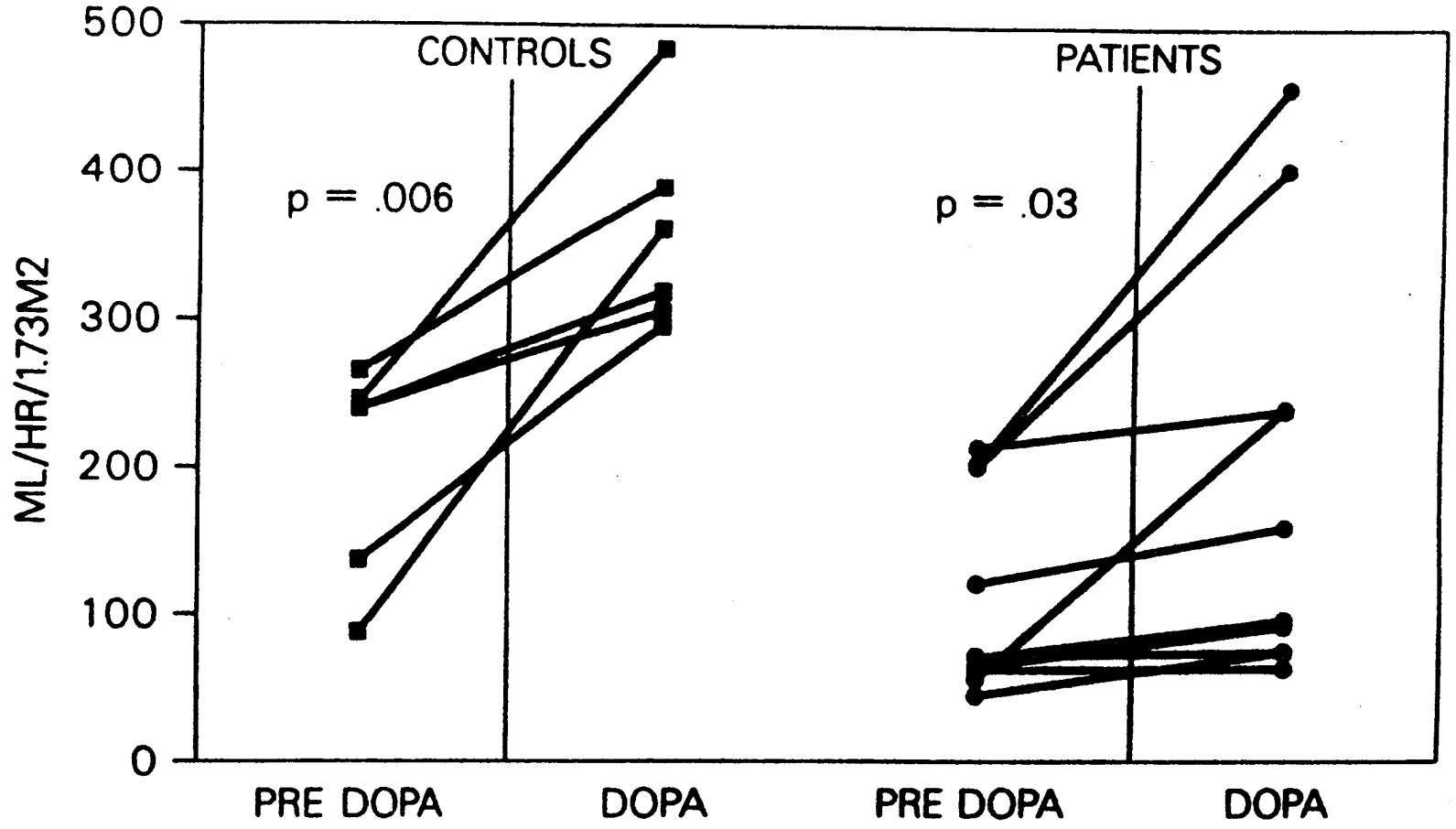


FIGURE 3. Urine output (ml/h/1.73 m^2) for each control subject (n=6) and burn patient (n=10) is depicted before and during dopamine therapy. Low-dose dopamine significantly increased urine flow in both populations, although the effect was more pronounced in the control subjects.

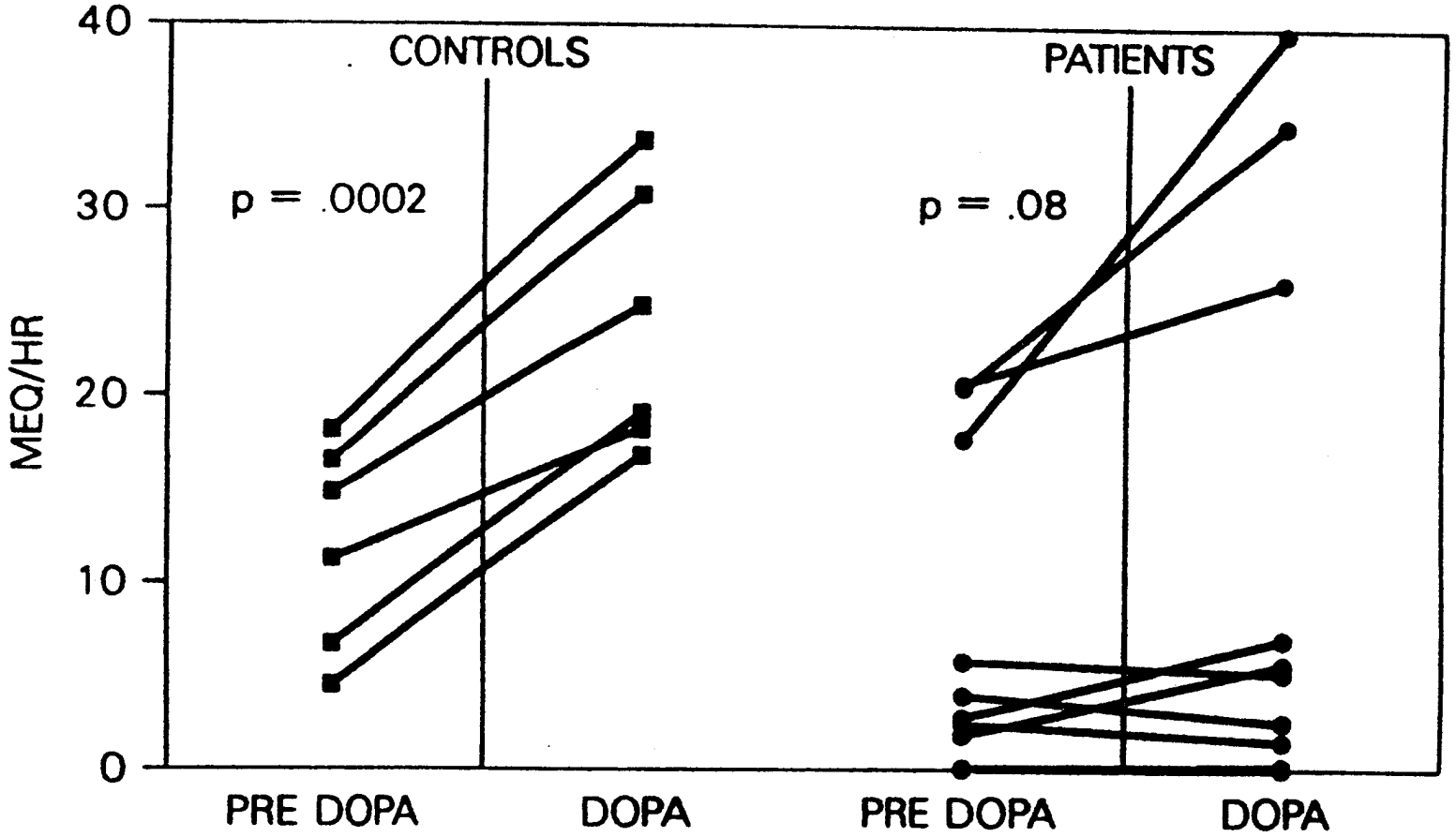


FIGURE 4. Sodium excretion (mEq/h) for each control subject (n=6) and burn patient (n=10) is depicted before and during dopamine therapy. All control subjects demonstrated a marked increase in sodium excretion with dopamine. The effect was inconsistent in the burn patients, with majority showing none or a relatively small increase in sodium excretion with dopamine.

TABLE 5. Effect of Dopamine in Control Subjects (Mean ± SEM)

	Glomerular Filtration Rate (ml/min/1.73 m^2)	Effective Renal Plasma Flow (ml/min/1.73 m^2)	Urine Flow (ml/h/1.73 m^2)	Osmolar Clearance (ml/min/1.73 m^2)	Fractional Excretion of Sodium (%)	Sodium Excretion (mEq/h)	Mean Arterial Pressure (mmHg)	Heart Rate (beats/min)
Predopamine	118 ± 4.5	511 ± 25	204 ± 30	2.9 ± 0.4	1.25 ± 0.2	12.0 ± 2.3	74 ± 2.4	61.5 ± 1.8
Dopamine	122 ± 5.3*	673 ± 42*	360 ± 29*	5.2 ± 0.6*	2.40 ± 0.2*	23.9 ± 2.9*	71 ± 2.9	65.0 ± 2.9

*$P < 0.05$ vs predopamine.

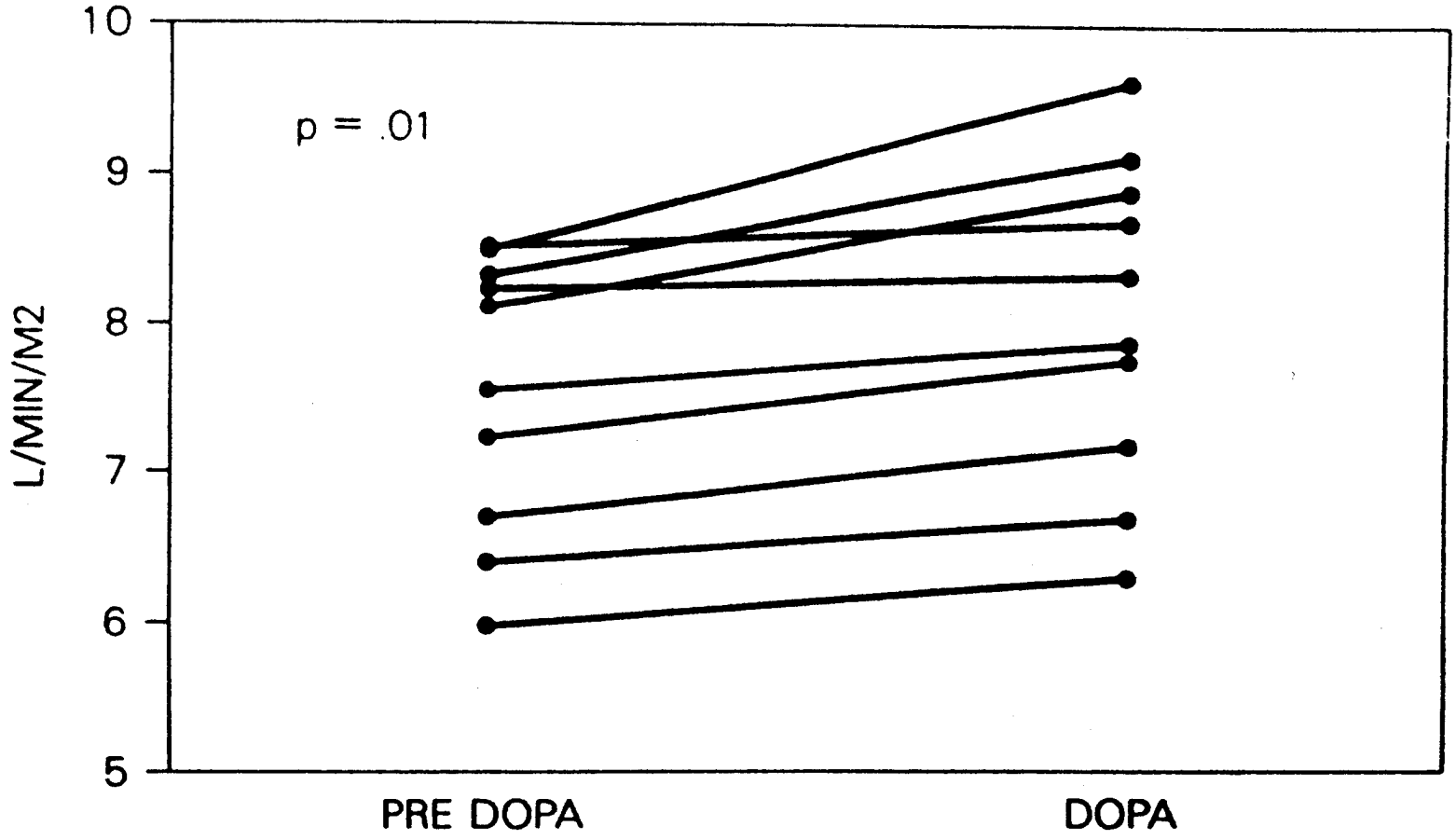


FIGURE 5. Cardiac index (l/min/m^2) for each burn patient (n=10) is depicted before and during dopamine therapy. Cardiac output was not measured in the control population. All patients demonstrated a significant increase in cardiac output with the infusion of low-dose dopamine at 3 μg/kg/min.

TABLE 6. Effect of Dopamine on Renal Function in Patients with Thermal Injury (Mean ± SEM)

	Glomerular Filtration Rate (ml/min/1.73 m^2)	Effective Renal Plasma Flow (ml/min/1.73 m^2)	Urine Flow (ml/h/1.73 m^2)	Osmolar Clearance (ml/min/1.73 m^2)	Sodium Excretion (mEq/h)	Fractional Excretion of Sodium (%)	Free Water Clearance (ml/min/1.73 m^2)
Predopamine	151 ± 7	678 ± 54	111 ± 22	3.2 ± 0.4	7.6 ± 2.7	0.7 ± 0.2	-1.38 ± 0.33
Dopamine	152 ± 5.3	816 ± 59*	190 ± 45*	3.96 ± 0.6	12.2 ± 4.8	1.1 ± 0.4*	-0.786 ± 0.35*

*$P < 0.05$ vs predopamine.

DISCUSSION

The use of low doses of dopamine to maintain renal perfusion in critically ill patients is common, despite the relative lack of data to support its use. We have demonstrated that dopamine infused at a rate of 3 μg/kg/min increases renal blood flow in postresuscitative thermally injured patients, although the mechanism does not appear to be solely dependent on dopamine receptor stimulation at the level of the kidney.

Several hemodynamic variables differed significantly between the two groups in a manner consistent with the hyperdynamic response to injury. The patients as a group had significantly greater GFR, ERPF, and cardiac index than controls, which is in agreement with previous studies (12,13). Despite the presence of a hyperdynamic circulation, patients also had a negative free water clearance and significantly lower fractional excretion of sodium than controls, values consistent with the paradoxical blood volume deficit we have previously reported to be present in burn patients during the postresuscitative phase (12). This difference was further characterized by a lack of significant correlation between ERPF and urine flow in the patients which was present in the controls ($R = 0.788$, $P < 0.05$). Controls reacted to low-dose dopamine in a manner consistent with published reports (2-5). Effective renal plasma flow, urine flow, and sodium excretion were significantly increased, findings previously reported to be secondary to DA1 receptor stimulation. Renal plasma flow increased in both groups, and remained elevated for the entire 3-h dopamine infusion. The mechanism responsible for this increase appears to involve alterations in cardiac function and the renal vascular bed. Cardiac output was significantly increased in all patients during dopamine infusion. Dopamine at 3 μg/kg/min had a significant chronotropic effect, contributing to the increase in cardiac output. Stroke volume was not increased, suggesting a negligible effect of the decrease in afterload and the lack of a pure inotropic effect of the dopamine.

The significant chronotropic response at this low infusion rate of dopamine suggests an increased sensitivity of cardiac beta-adrenergic receptors in spite of the elevated beta-adrenergic activity previously documented in thermally injured patients. Other studies have failed to document a chronotropic effect of this dose of dopamine; rather, they have demonstrated an increase in cardiac output primarily mediated by left ventricular after load reduction (14,15). Despite the decrease in systemic vascular resistance, the chronotropic effect may impose increased work on the already hyperdynamic myocardium. A similar hemodynamic profile with a chronotropic effect has been documented during infusion of higher doses of dopamine, which have resulted in an increased systemic oxygen consumption measured by computerized indirect calorimetry (15).

To determine if the increase in effective renal plasma flow of the patients was mediated by changes in cardiac output, we compared the percent of cardiac output represented by renal plasma flow before and during dopamine therapy. While on dopamine, this mean value increased from 5.24% to 5.98% ($P = 0.01$). However, as depicted in Figure 6, this ratio did not change in 5 of the 10 patients, indicating that the augmented cardiac output contributed to the increase in effective renal plasma flow in the majority of these patients.

It was not possible to predict the change in ERPF or GFR from the urine flow or natriuretic response to dopamine in either the patients or controls; a similar finding was reported by Smit et al (16) in normal man. Thus, the absence of a diuresis does not preclude an increase in GFR or ERPF in this sample of thermally injured patients. Baseline ERPF did not correlate with ERPF response to dopamine in our controls or patients. This is in contrast to previously published data from Schwartz et al (17) and Beukhof et al (8), who studied vascular surgery and glomerulopathy patients, respectively. Both of these patient groups consisted of a large number of individuals with low baseline ERPF and significant renal dysfunction, making them distinctly different from our patients and possibly explaining this discrepancy.

The natriuretic effect of low-dose dopamine in the patients was quite variable. There was a strong positive correlation between the predopamine sodium excretion and the increase in sodium excretion while receiving dopamine ($R = 0.758$, $P = 0.011$). Those patients with predopamine sodium excretions < 5 mEq/h/1.73 m^2 did not appreciably increase sodium excretion with dopamine therapy. Those patients may have had a relative intravascular volume deficit, whereby other control mechanisms may obscure a direct inhibitory effect of dopamine on proximal tubule sodium transport (12,18).

We did not document a significant effect of low-dose dopamine on osmolar clearance in this patient population. Parker and associates (19) documented a significant increase in osmolar clearance in conjunction with increased GFR in a group of critically ill oliguric patients. Presumably, the lack of a significant increase in the already elevated GFR prevented this desirable effect in our group of hypermetabolic burn patients.

Low-dose dopamine has been reported to effect deleteriously the distribution of microcirculatory blood flow in the liver and skeletal muscle of normal rats despite a reduction in systemic vascular resistance (20). Lundberg et al (21) have reported a failure of low-dose dopamine to increase mesenteric blood flow despite a drop in systemic vascular resistance in the setting of elevated sympathetic nervous system activity, a milieu known to exist in burn patients (13). The redistribution of flow suggests the possibility of either a steal phenomenon in which DA-1 receptor

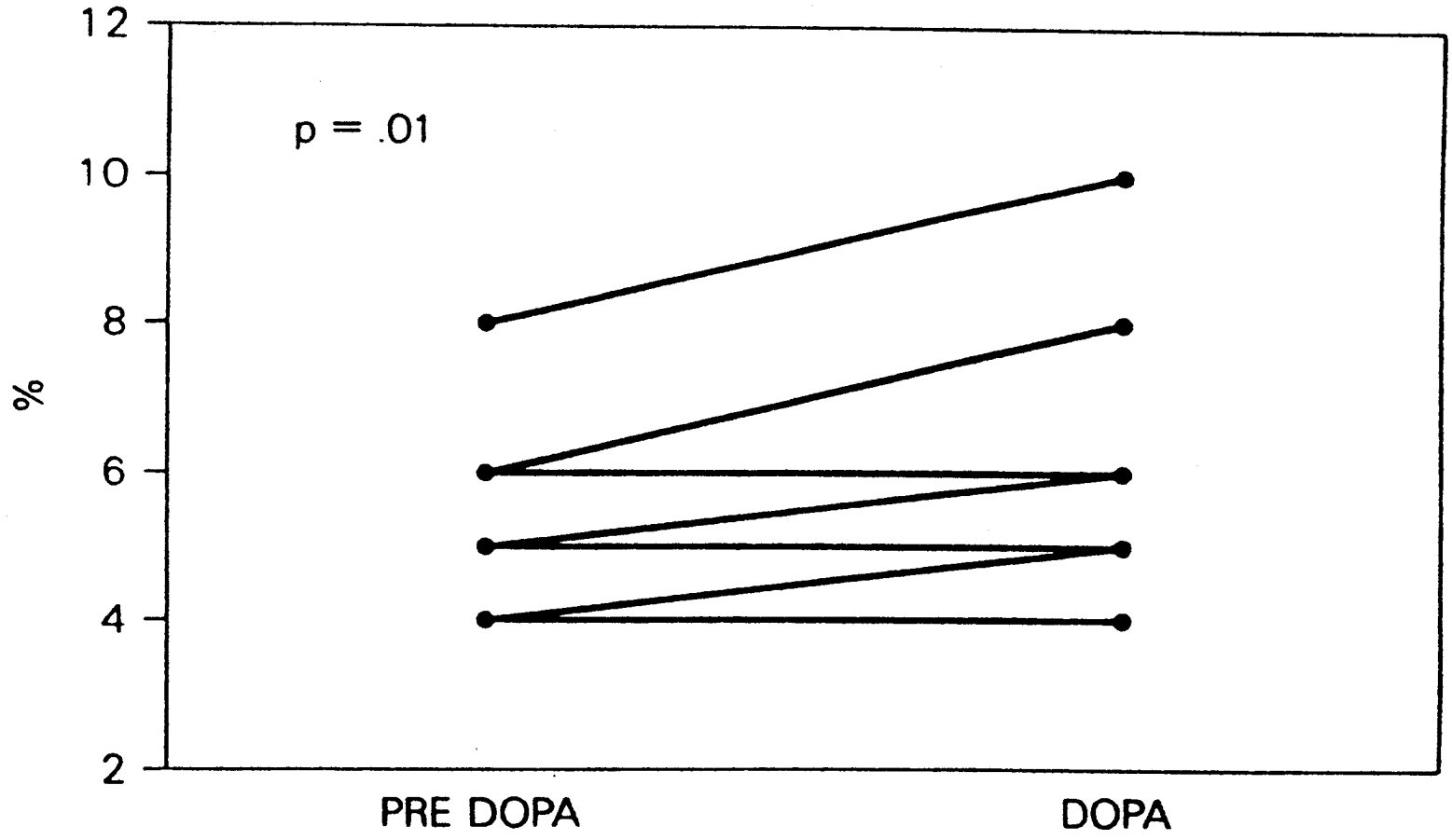


FIGURE 6. Renal plasma flow/cardiac index ratio (%) for each burn patient (n=9) is depicted before and during dopamine therapy. Although the difference was significantly (P = 0.01), 5 patients demonstrated no increase in this ratio, suggesting that the increase in renal plasma flow contributed to the increase in cardiac output. The two lowest horizontal lines each represent data from 2 burn patients.

mediated vasodilatation in some beds results in decreased flow to those vascular beds without these receptors or altered alpha receptor sensitivity to low-dose dopamine. Although altered alpha receptor sensitivity has not been documented in normal man while receiving low-dose dopamine, it has been documented to occur in preterm neonates (22). Whether such alpha receptor changes were present in our patients is unknown. Our finding of altered beta-receptor sensitivity with the associated potential for maldistribution of blood flow and increased oxygen consumption suggests that low-dose dopamine therapy may not be innocuous in thermally injured patients. Further studies delineating the effect of this therapy on distribution of cardiac output and alterations in the oxygen availability ratio are necessary to document its safety. Finally, the benefit of maintaining an effective renal plasma flow at levels higher than those normally present in the thermally injured patient remains to be proven.

PRESENTATIONS/PUBLICATIONS

Graves TA, Cioffi WG, Vaughan GM, Pratt L, Heironimus JD, McManus WF, and Pruitt BA Jr: The renal effects of low dose dopamine in thermally injured patients. Presented as part of the Resident's Program of the American Association for the Surgery of Trauma, Cincinnati, Ohio, 15 February 1992.

Graves TA, Cioffi WG, Vaughan GM, Pratt L, Heironimus JD, McManus WF, and Pruitt BA Jr: The renal effects of low dose dopamine in thermally injured patients. Presented at the 53rd Annual Meeting of the American Association for the Surgery of Trauma, Louisville, Kentucky, 17 September 1992.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

ACCESSION: DA335883

SUMMARY DATE: 921001 **SUMMARY KIND:** D **PREV DATE:** 911001 **DISTRIBUTION:** CX

PROGRAM #: 62787A **PROJ #:** 30162787A874 **TASK AREA:** ED **WORK UNIT:** 178

TITLE: A Study to Evaluate the Effects of Heparinized Flush Solutions on the Patency of Arterial Pressure Monitoring Lines

SUBJ1: 060500 - Medicine and Medical Research
SUBJ2: 061500 - Pharmacology

START DATE: 9105 **END DATE:** 9309 **PERFORMANCE METHOD:** C

CONTRACT/GRANT NO:

CONT TOTAL: $
CUM TOTAL: $
TOTAL LAB FUNDS: $

RESOURCES ESTIMATE

FY	WORK YRS	$(Thousands)
91	0.2	$10
92	0.3	$5
93	0.3	$5

RESPONSIBLE DOD ORGANIZATION: 404885
MRDC INSTITUTE OF SURGICAL RESEARCH USAISR
FT SAM HOUSTON, TX 78234
PRUITT, B A
210-221-2720

PERFORMING ORGANIZATION: 404885
MRDC INSTITUTE OF SURGICAL RESEARCH USAISR
FT SAM HOUSTON, TX 78234
DRISCOLL, D M
210-221-8482

ASSOC1: MCMANUS, W F

ASSOC2:

FOREIGN INTELLIGENCE CONSIDERED: Y **CIVILIAN APPLICABILITY:** M

KEYWORDS: RA II; Volunteers; Adults; Burns (Injuries); Heparin; Sodium Chloride; Catheters

OBJECTIVE: A DTIC literature search was conducted under DTIC request numbers W6L54M/W6L56K dated 13 March 1991. The objectives of this work are to determine if there is a significant difference in survival curves for patency of arterial pressure monitoring lines maintained with heparinized or nonheparinized flush solutions as measured by acceptable square waveform test and free backflow of blood every 4 h after insertion of the line until the line is removed and to determine the relationship of potentially confounding variables such as site of insertion, length of catheter, and gauge of catheter to survival curves of arterial pressure monitoring lines maintained with heparinized and nonheparinized flush solutions. Currently, the arterial line protocol at this Institute dictates the use of 1:1 solution of heparin and 0.9% sodium chloride as a means of maintaining arterial line patency. If a nonheparinized solution of 0.9% sodium chloride is proven equally effective in maintaining arterial line patency in the burn population, the protocol may be changed, thus eliminating the risk of heparin administration and reducing costs.

APPROACH: The length of time the arterial line remains patent after insertion will be compared using survival analysis techniques. The time of line patency for the heparinized flush solution group will be compared with the time of line patency for the nonheparinized flush solution group using appropriate log rank tests on product limit survival estimates. If groups differ significantly, hazard regression analysis using proportional

hazard models will be used to determine significance. Stratified analyses will be used to control for the effects of covariates.

PROGRESS: 9110-9209. Thirteen patients have been enrolled in this study to date, 8 during this reporting period. Upon completion of enrollment, data will be analyzed as indicated. For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report* for fiscal years 1991 through 1992.

ABSTRACT

PROJECT NUMBER: 3M162787A874-178, Applied Research and Exploratory Development

PROJECT TITLE: A Study to Evaluate the Effects of Heparinized Versus Nonheparinized Flush Solutions on the Patency of Arterial Pressure Monitoring Lines

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

PERIOD COVERED IN THIS REPORT: 1 October 1991 - 30 September 1992

INVESTIGATORS: Dennis M. Driscoll, RN, Captain, AN
Norman D. Warren, LPN, Staff Sergeant
William F. McManus, MD, Colonel, MC
Basil A. Pruitt, Jr., MD, Colonel, MC

Arterial cannulation presents risk of thrombus formation which practitioners have attempted to reduce with the addition of heparin to the arterial flush solutions. However, addition of heparin may contribute to the development of thrombi in some individuals. Research suggests that nonheparinized solutions are as effective as heparinized solutions in maintaining patency in peripheral access devices while eliminating the risks of heparin administration. Preliminary studies in other areas, including arterial pressure lines, suggest that a randomized trial of heparinized and nonheparinized flush solutions in a large sample is warranted.

Currently, the arterial line protocol at this Institute dictates the use of 1:1 solution of heparin and 0.9% sodium chloride as a means of maintaining arterial line patency. If a nonheparinized solution of 0.9% sodium chloride is proven equally effective in maintaining arterial line patency in the burn population, the protocol may be changed, thus eliminating the risks of heparin administration.

Sixteen patients have been enrolled in this study to date, 10 during this reporting period. There have been no complications associated with the patency of arterial lines in these patients. The small number of patients limits the validity of statistical manipulation of the data at this point.

A STUDY TO EVALUATE THE EFFECTS OF HEPARINIZED VERSUS NONHEPARINIZED FLUSH SOLUTIONS ON THE PATENCY OF ARTERIAL PRESSURE MONITORING LINES

Recommendations to nurses regarding the maintenance of arterial lines from 1971 to the present have included use of heparinized flush solutions (1-5). Institutional standards of practice reflect the recommendations. In a recent survey conducted by the American Association of Critical Care Nurses (AACN), 96% of the 1,072 randomly selected critical care nurses responding indicated flush solutions used for arterial lines in their institution were routinely heparinized (6).

Heparin use, however, is not without risk. In those individuals who are immunologically sensitive, heparin-induced thrombocytopenia (HITP) can lead to life-threatening thromboembolic events (7-12). In reviewing the literature on 600 reported cases of HITP, Becker and Miller (13) found that 50% of the cases involved thromboembolic events. Prospectively, they estimate that 10% of individuals receiving heparin will develop HITP and 10% of those will have thromboembolic events, or 1-2% of those receiving heparin. Warkentin and Kelton (10) estimate the incidence of HITP to be 5% in their review; Scott (14) states the incidence of HITP ranges between 3% and 7%.

Mortality rates associated with thromboembolic events have been reported in several retrospective reviews of patients with HITP. In many instances, low-dose heparin in amounts used to flush solutions or heparin-bonded catheters is implicated (15-19). Chang (20) reviewed 23 cases with a diagnosis of white clot syndrome with HITP and found a mortality rate of 40%. Of the 23 patients, 8 received low doses of heparin of < 15,000 U total. A review of patients who developed HITP after receiving heparin in support of cardiac surgery (12 patients), in support of arterial reconstructive surgery (3 patients), or for prevention of embolism (6 patients) showed mortality rates from HITP ranging from 25% to 40% depending on the reason for heparin administration (21). In a study of 16 patients with thrombocytopenia or new thrombotic complications during heparin administration, the mortality rate was reported to be 18.8% (17). Two patients in the group received heparin from heparin flushes only. Another review of 169 patients with HITP completed over a 3-yr period showed that 91 of the patients (54%) had received low-dose heparin; the mortality rate was 12.4% (18).

Most of the studies comparing the effects of heparinized and nonheparinized solutions on maintaining patency of catheters have been completed in populations requiring intravenous peripheral access devices. The following double-blind studies all evaluated the effects of heparinized and nonheparinizing 0.9% sodium chloride solutions on patency and other selected variables. A study of 412

patients over 1,448 patient days of "heparin-lock" therapy evaluated site loss due to loss of patency, phlebitis, or infiltration (22). No significant differences occurred among the groups on any of the outcome variables. Hamilton et al (23) studied 160 patients over 307 observations. They found no differences in catheter patency or phlebitis incidence. In a third study of 147 patients, the authors concluded that nonheparinized saline was as effective as heparinized saline in terms of phlebitis incidence and patency loss; they do report an absolute loss of site at 61% in nonheparinized solution group and 53% in the heparinized solution group (24). A recent report of a study of 32 patients also indicates that sodium chloride alone is as effective as heparinized sodium chloride in maintaining patency of peripheral access devices and preventing phlebitis (25).

Results from other reports comparing or discussing heparinized and nonheparinized flush solutions for peripheral access devices vary. Most authors report no difference in outcome variables of patency, phlebitis, and/or infiltrations in heparinized and nonheparinized lines (26-29). One study reports better results with heparinized solutions (30). Recently, a meta-analysis of 20 published and unpublished studies identified no significant different between heparin flushes and saline flushes in peripheral access devices based on low average effect size (0.076 ± 0.14) and the qualitative data from the studies (unpublished data).

Only one study comparing the effects of heparinized and nonheparinized flush solutions on the patency of arterial lines has been reported. In the study, heparinized and nonheparinized lactated Ringer's solutions were compared in a group of 50 cardiovascular surgery patients (31). No significant differences were found between the groups in patency variables, although the authors reported anecdotally more frequent dampening of waveforms and three incidences of clot formation in the nonheparin group.

As reported in the AACN *Survey of Current Practice* concerning flush solutions for maintenance of arterial pressure monitoring lines, 96% of the respondents indicated that heparinized flush solutions were used at their institutions. However, 4% of the respondents indicated that use of nonheparinized flush solutions was standard protocol at their institutions (6). Normal saline without heparin was used as the continuous flush solution in an early single-site evaluation of clinical issues arising from intra-arterial monitoring in 2,500 intensive care unit patients (32).

Problems of maintenance of patency have been linked to catheter size, length, and gauge with an inverse relationship between the size of the catheter and likelihood of loss of patency (33). Site of artery placement is also of concern. Kaye (34) reports that 50% of radial artery cannulations experience thrombosis.

The necessity to monitor and maintain the pressure within the solution bag has been demonstrated in two reports. Pressure within the continuous arterial flush solution systems has been shown to vary with the quantity of solution in the bag (35). Decreasing volume decreases pressure, promoting inconsistency in flow rate. Further, in studies of the Pharmaseal™ continuous flushing device, driving pressure made a significant difference in flow while arterial and venous pressure did not make a significant difference (36).

The objectives of this study are to determine if there is a significant difference in survival curves for patency of arterial pressure monitoring lines maintained with heparinized or nonheparinized flush solutions as measured by acceptable square waveform test and free backflow of blood every 4 h for 72 h after insertion of the line or until the line is removed, whichever comes first, and to determine the relationship of potentially confounding variables such as site of insertion, length of catheter, and gauge of catheter to survival curves of arterial pressure monitoring lines maintained with heparinized and nonheparinized flush solutions.

MATERIALS AND METHODS

Study Design. The effects of heparinized and nonheparinized flush solutions on the patency of arterial pressure lines will be evaluated using a two-group, randomized clinical trial design. Study design and protocols were developed by the AACN Thunder Project™ Task Force and pretested in a three-part pretesting scheme to assure safety of the protocol and efficiency in data collection.

Patient Criteria. Thirty patients will be enrolled in this study. Properly signed and witnessed DA Forms 5303-R, Volunteer Agreement Affidavit, is obtained from each patient prior to beginning the study.

Patient Inclusion. Patients meeting all of the following criteria are eligible for enrollment in this study:

1. Male or female patients ≥ 18 yr. Female patients must have been surgically sterilized, be postmenopausal (> 45 yr and lack of menstrual periods for > 1 yr), or have a negative pregnancy test prior to initiation into the study.

2. Patients who require insertion of an arterial line for purpose of monitoring arterial pressure and/or drawing blood.

Patient Exclusion. Patients meeting any of the following criteria are excluded from participation in this study:

1. Patients < 18 yr.

2. Patients who are pregnant or nursing.

3. Patients with known sensitivity to heparin or whose physician has excluded heparin from the treatment plan.

4. Patients with a platelet count < 100,000.

5. Patients enrolled in this study at a previous time.

Determination of Number of Subjects Required. This protocol will involve a study of 30 patients with indwelling arterial catheters as part of a multicenter study.

Data Collection. The Institute continues data collection on each subject until the arterial line is discontinued or until the subject's attending physician chooses to withdraw the subject from the study. The following information is recorded:

1. Site, length, and gauge of the catheter.

2. Nearest hour to the time of catheter insertion.

3. Patency check data.

4. Indication as to whether or not the patient receives heparin or thrombolytics from sources other than the arterial line.

5. If the line is removed prior to 72 h, the reason for removal.

Data Analysis Plan. The null hypothesis states that there will be no significant difference in survival curves for patency of arterial pressure monitoring lines maintained with heparinized or nonheparinized flush solutions. The length of time the arterial line remains patent after insertion as measured by acceptable square waveform test and free backflow of blood will be compared using survival analysis techniques. Time of line patency for the heparinized flush solution group will be compared with time of line patency for the nonheparinized flush solution group using appropriate log rank tests on product limit survival estimates (37).

If groups differ significantly, hazard regression analysis using proportional hazard models will be used to determine significance. Stratified analyses will be used to control for the effects of covariates such as length of catheter, size of catheter, or site of insertion if there are significant differences in survival rates based on these covariates (37).

Data will be analyzed at 3 months and again at 6 months to determine the incidence rate of problems associated with heparinized versus nonheparinized arterial line flush solutions.

RESULTS

Thirteen patients have been enrolled in this study to date, 8 during this reporting period. There have been no complications associated with the patency of arterial lines in these patients.

DISCUSSION

The small number of patients precludes statistical manipulation of the data at this point.

PRESENTATIONS/PUBLICATIONS

Driscoll DM: Evaluation of the effect of heparinized and nonheparinized flush solutions on the patency of arterial pressure monitoring lines: The AACN Thunder Project. *Am J Crit Care* 2(1):3-15, 1992.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

ACCESSION: DA335924

SUMMARY DATE: 921001 **SUMMARY KIND:** H **PREV DATE:** 911001 **DISTRIBUTION:** CX

PROGRAM #: 62787A **PROJ #:** 30162787A874 **TASK AREA:** DA **WORK UNIT:** 179

TITLE: A Clinical Study of the Safety and Efficacy of DermaGraft™ Dermal Replacement

SUBJ1: 060500 - Medicine and Medical Research

START DATE: 9106 **END DATE:** 9208 **PERFORMANCE METHOD:** C

CONTRACT/GRANT NO:

CONT TOTAL: $
CUM TOTAL: $
TOTAL LAB FUNDS: $

RESOURCES ESTIMATE

FY	WORK YRS	$(Thousands)
91	0.2	$27
92	0.2	$1
93	0.0	$0

RESPONSIBLE DOD ORGANIZATION: 404885
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ASSOC1: CIOFFI, W G

ASSOC2: DRISCOLL, D M

FOREIGN INTELLIGENCE CONSIDERED: Y **CIVILIAN APPLICABILITY:** M

KEYWORDS: RA II; Volunteers; Adults; Burns (Injuries); Skin Grafts; Fibroblasts; Healing; Morbidity

OBJECTIVE: A DTIC literature search was conducted under DTIC request numbers W6031J/W6032J dated 15 April 1991. The objective of this work is to assess the short- and long-term usefulness of DermaGraft™ dermal replacement on burn patients.

APPROACH: Thirty consecutive patients were authorized for enrollment in this study as part of a multicenter study. Each patient's wounds were excised in the operating room and one treatment wound site and one control site anatomically comparable as to location and of approximate equal dimensions were identified. A biopsy for quantitative microbial culture and histology was taken from each excised wound bed before application of grafts. DermaGraft™ was then placed onto the chosen site. Split-thickness autograft was placed over the treatment and control sites and secured and dressed using standard surgical techniques. Treatment and control wounds were examined and photographed on postoperative days 5, 7, and 14 and at the time of discharge. Four-millimeter full-thickness punch biopsies were taken from the center of the treatment and control sites on postoperative day 14. The wounds were assessed for percent take, pigmentation, vascularity, and pliability and patients were queried about pain and itching.

PROGRESS: 9110-9209. This study was approved by the USAISR Research Council, the US Army Institute of Surgical Research Human Use Committee, and The Surgeon General's Human Subjects Research Review Board during the third quarter of fiscal year 1991. Four patients were enrolled in this study. There were no adverse reactions or side effects noted. The manufacturer of DermaGraft™

is currently modifying the produce to produce a thicker mesh. A new manufacturer IDE application will be required. Therefore, this study was terminated. For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report* for fiscal years 1991 through 1992.

ABSTRACT

PROJECT NUMBER: 3M162787A874-179, Applied Research and Exploratory Development

PROJECT TITLE: A Clinical Study of the Safety and Efficacy of DermaGraft™ Dermal Replacement

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

PERIOD COVERED IN THIS REPORT: 1 October 1991 - 24 August 1992

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Autograft skin is the standard for permanent closure of full-thickness wounds. Intact areas of the patient's skin, or donor sites, are harvested tangentially to yield split-thickness autografts, comprised of the whole epidermis and part of the dermis. The lower portion of the dermis is left on the donor site. However, severely burned patients frequently do not have enough intact donor sites to allow complete coverage of their wounds with split-thickness autografts. DermaGraft™ was developed to provide a dermal replacement. The purpose of this study is to assess the short- and long-term usefulness of the DermaGraft™ dermal replacement on burn patients.

This project was approved by the USAISR Research Council, the US Army Institute of Surgical Research Human Use Committee, and The Surgeon General's Research Review Board during the third quarter of fiscal year 1991. Four patients were enrolled in this study. Preliminary results demonstrated slightly less graft take on DermaGraft™ wounds than control wounds.

The manufacturer of DeramGraft™ is currently modifying the product to produce a thicker mesh. A new manufacturer IDE application will be required. Therefore, this study was terminated.

A CLINICAL STUDY OF THE SAFETY AND EFFICACY OF DERMAGRAFT™ DERMAL REPLACEMENT

Autograft skin is the standard for permanent closure of full-thickness wounds. Intact areas of the patient's skin, or donor sites, are harvested tangentially to yield split-thickness autografts, comprised of the whole epidermis and part of the dermis. The lower portion of the dermis is left on the donor site. It provides a base for rapid reepithelization and healing of the donor site. Autografts are sutured onto excised wound beds where they generally "take" rapidly, vascularizing and becoming incorporated into the wound.

Severely burned patients frequently do not have enough intact donor sites to allow complete coverage of their wounds with split-thickness autografts. In such cases, some of the wound surface is covered with available autograft and the rest with a temporary biologic dressing, usually cadaver allograft skin. Allograft skin takes well, but is rejected within a few weeks. Donor sites may be reharvested after they heal to provide additional split-thickness autograft. Reharvesting and grafting may be repeated over weeks to months, until all of the wounds have been permanently closed with split-thickness autografts. This process is lifesaving, but entails multiple surgical procedures and prolonged hospitalization for the patient.

In order to decrease the number of donor site harvests and grafting procedures, available split-thickness sheets can be incised and expanded to produce a lattice, or "meshed" pattern. Meshed split-thickness grafts allow wider wound coverage from each donor site; they have played a key role in allowing surgeons to cover large full-thickness burn areas more rapidly, but only a small portion of the wound is covered by dermis from the meshed graft. Dermis, unlike epidermis, does not regenerate spontaneously; the majority of the wound surface heals by migration of epidermal cells from the meshwork across the interstices of the graft.

Cosmetic and functional results of meshed grafts are relatively poor, particularly when the ratio of interstices to tissue is 3:1 or greater. The skin is thin and prone to breakdown in the early stages. After healing, the skin has decreased compliance and an unpleasant "cobblestone" appearance. Often, there are severe hypertrophic scars and wound contraction. Patients with these outcomes may require repeated surgical revisions and extended physical therapy.

The poor quality of skin resulting from epithelization over meshed grafts is due, at least in part, to the lack of a dermal layer. Skin grafts heal best when a thick dermal layer is present, as in a thick split-thickness, or a full-thickness sheet graft.

But, if thick grafts are taken from the donor sites, the donor sites themselves lack sufficient dermis to heal well. The patient may therefore have additional scars. Reharvesting of the donor sites is also delayed, which can prolong hospitalization until sufficient autograft skin can be obtained. In summary, meshing can extend the immediate supply of split-thickness autograft and reduce reharvesting of donor sites and hospitalization time, but at a significant sacrifice in the quality of healing.

Several groups have developed manufactured skin substitutes to try to address the problems of inadequate supplies of autograft skin. Cultured autologous keratinocyte sheets have been used to close full-thickness burn wounds. Such sheets lack a dermis and have been observed to lead to extensive scar formation and skin that is thin and shears easily.

Various biosynthetic, full-thickness skin substitutes have been studied in animal and clinical trials. All of these, to date, appear to have both possible advantages and limitations. None is yet commercially available. A few groups have employed cadaver dermis with overlying autologous keratinocytes, either cultured into sheets or seeded from suction blisters of intact areas of the patient's skin. Clinical outcomes have been reported to be equivalent to those obtained with split-thickness sheet grafts. Allogeneic dermis has been observed to be less immunogenic than other tissues.

DermaGraft™ (Marrow-Tech Incorporated, 10933 North Torrey Pines Road, La Jolla, CA 92037) was developed to provide a dermal replacement. This device consists of neonatal dermal fibroblasts which have been cultured in vitro onto a biodegradable mesh of polyglactin (Vicryl™). The mesh is hydrolyzed after implantation. As the fibroblasts proliferate on the mesh, they secrete collagens and create a more natural dermal matrix. Preclinical trials in rats, pigs, and nude mice indicate that DermaGraft™ takes rapidly onto full-thickness wounds and vascularizes well. DermaGraft™ will support vascularization of overlying mesh split-thickness skin grafts in these animal models.

The ability to replace thermally injured dermis could result in a better functional result as compared to standard mesh skin grafting techniques. The purpose of this study was to assess the short- and long-term usefulness of the DermaGraft™ dermal replacement on burn patients.

MATERIALS AND METHODS

Study Design. Thirty consecutive patients meeting admission criteria were authorized for enrollment in this study as part of a multicenter study. Four patients were enrolled in this study. After informed consent was obtained, each patient's wounds were

excised in the operating room and one treatment wound site and one control site were identified.

Criteria for Admission to the Study. Patients admitted to the US Army Institute of Surgical Research were offered the opportunity to participate in this study. Properly signed and witnessed DA Forms 5303-R, Volunteer Agreement Affidavit, were obtained prior to initation of the study.

Patient Inclusion. Patients meeting the following criteria were enrolled in the study upon giving written informed consent:

1. Male or female patients ≥ 18 yr. Female patients must have been surgically sterilized, postmenopausal (> 45 yr and lack of menstrual periods for > 1 yr), or have had a negative pregnancy test before initiation into the study.

2. Patients with donor sites that had not been previously harvested and were sufficiently large for coverage of treatment and control areas.

3. Patients with full-thickness wounds after excision which were each sufficiently large to allow two grafts of at least 4" X 4" on comparable body sites (wounds may be adjacent to one another).

4. Patients where meshed autograft was medically indicated for treatment of wound sites.

5. Patients whose wounds could be excised within 2 weeks of burn injury.

6. Patients with burn sites to be treated with meshed split-thickness grafts located on the anterior or lateral trunk or extremities or on any aspect of the calves or forearms. Head and neck were excluded. The back was included.

7. Patients who were available for the 1-yr follow-up period.

Patient Exclusion: Patients meeting any of the following criteria were excluded from participation in the study:

1. Patients < 18 yr.

2. Patients who were pregnant or nursing.

3. Patients with any clinically significant medical condition, including endocrine, renal, pulmonary, hematologic, neurologic, immune, or infectious disease, as defined by the primary investigator.

4. Patients with a psychiatric condition which compromises the patient's ability to complete this study.

5. Patients who had previous excisional therapy of the wounds considered for evaluation.

6. Patients with an electrical burn or toxic epidermal necrolysis.

7. Patients using immunotherapy, cytotoxic chemotherapy, or investigational drugs within 1 month preceding study entry or anticipated use of any of these therapies during the course of the study.

8. Patients using immunosuppressants within 1 month preceding study entry or anticipated requirement for it during the course of this study.

Description of Procedures. Patients underwent a baseline evaluation prior to beginning the study. The following information was documented: history and physical examination, estimate of burn size, blood and urine cultures, standard hematologic studies, electrolytes, liver function studies, and urinalysis. Thirty cubic centimeters of blood were archived for future serum or WBC studies. A pregnancy test was performed on all child-bearing age women.

After full written informed consent was obtained, the patient was scheduled for excisional treatment within 14 days postburn. After the excision was complete, treatment and control sites were chosen using a randomization scheme provided by Marrow-Tech Incorporated (Table 1). Treatment and control sites were of approximately equal dimensions and anatomically comparable as to their location. They could be adjacent. A biopsy for quantitative microbial culture and histology was taken from each excised wound bed prior to application of the graft. Also, wound bed photographs were taken prior to application of grafts.

DermaGraft™ was then prepared. It was supplied frozen in 4" X 6" sheets enclosed in a Teflon bag for transport. Prior to grafting, the sheets were thawed (within 2 h of application), rinsed, and removed from the Teflon™ bags. The DermaGraft™ was then placed onto the chosen site. Split-thickness autograft measuring between 3 and 12/1000s of an inch meshed 1.5:1 or greater were then prepared using standard surgical techniques. Graft was placed over the treatment and control areas and secured and dressed using standard surgical techniques.

Treatment and control wounds were then examined on postoperative days 5, 7, and 14 and at the time of discharge. They were assessed for percent take, pigmentation, vascularity, and pliability. The patients were queried about pain and itching. Photographs were obtained on postoperative days 5, 7, and 14 and at

TABLE 1. Randomization Scheme

Patient	Treatment Assignment		Order Assignment		Choice of Placement, If Available								
	Site A	Site B	Site A	Site B									
1	Control	NeoDerm	First	Second	Ca	AFA	AL	T	UA	PFA	F	Ch	Ab
2	Control	NeoDerm	First	Second	Ca	Ab	AL	Ch	F	AFA	UA	T	PFA
3	Control	NeoDerm	First	Second	F	Ca	AFA	T	UA	AL	Ab	PFA	Ch
4	NeoDerm	Control	Second	First	UA	T	AFA	Ch	PFA	Ca	Ab	F	AL
5	NeoDerm	Control	First	Second	PFA	Ca	AL	UA	AFA	Ab	Ch	T	F
6	Control	NeoDerm	First	Second	Ch	Ca	F	AFA	T	UA	Ab	AL	PFA
7	Control	NeoDerm	Second	First	T	Ca	PFA	UA	AFA	F	AL	Ch	Ab
8	NeoDerm	Control	First	Second	Ca	Ab	UA	Ch	F	T	AFA	PFA	AL
9	Control	NeoDerm	Second	First	Ch	PFA	T	UA	AFA	F	AL	Ca	Ab
10	Control	NeoDerm	First	Second	PFA	AL	Ab	UA	Ca	T	AFA	Ch	F
11	Control	NeoDerm	Second	First	PFA	Ch	T	AL	Ab	F	UA	AFA	Ca
12	Control	NeoDerm	First	Second	Ch	F	Ab	AL	UA	Ca	T	PFA	AFA
13	Control	NeoDerm	First	Second	Ch	AL	PFA	Ca	T	AFA	Ab	UA	F
14	Control	NeoDerm	Second	First	AL	T	UA	Ab	PFA	F	Ca	Ch	AFA
15	Control	NeoDerm	First	Second	AL	T	Ca	Ab	UA	PFA	AFA	F	Ch
16	NeoDerm	Control	Second	First	Ca	Ch	UA	PFA	F	T	Ab	AL	AFA
17	NeoDerm	Control	First	Second	PFA	Ca	AL	Ab	Ch	T	F	UA	AFA
18	NeoDerm	Control	Second	First	F	T	Ca	Ab	PFA	UA	AL	Ch	AFA
19	NeoDerm	Control	First	Second	PFA	AL	Ab	Ca	T	F	UA	Ch	AFA
20	NeoDerm	Control	Second	First	Ab	AFA	UA	T	AL	Ch	F	Ca	PFA
21	Control	NeoDerm	Second	First	Ch	T	Ca	F	AL	Ab	UA	PFA	AFA
22	NeoDerm	Control	Second	First	F	T	UA	AFA	AL	PFA	Ca	Ch	Ab
23	NeoDerm	Control	Second	First	F	Ab	PFA	Ch	T	UA	AFA	Ca	AL
24	Control	NeoDerm	First	Second	AL	Ab	Ch	PFA	Ca	F	AFA	UA	T
25	Control	NeoDerm	Second	First	Ca	T	Ch	F	AL	Ab	AFA	PFA	UA
26	NeoDerm	Control	First	Second	PFA	Ab	T	UA	Ca	Ch	AFA	AL	F
27	NeoDerm	Control	Second	First	F	PFA	UA	T	AL	Ab	AFA	Ch	Ca
28	Control	NeoDerm	First	Second	Ab	UA	AFA	AL	Ca	Ch	PFA	F	T
29	NeoDerm	Control	Second	First	Ca	Ab	AL	F	PFA	UA	AFA	T	Ch
30	Control	NeoDerm	Second	First	Ca	F	AL	Ch	AFA	UA	Ab	PFA	T

Ab indicates abdomen; AFA, anterior forearm; AL, anterior leg; Ca, calf; Ch, chest; F, foot; PFA, posterior forearm; T, thigh; and UA, upper arm.

the time of discharge. Four-millimeter full-thickness punch biopsies were taken from the center of the treatment and control sites on postoperative day 14.

Determination of Number of Subjects Required. Thirty patients were authorized for enrollment in this study as part of a multicenter effort. Four patients were enrolled at this Institute.

Data Collection. Safety and efficacy data collected during this study included percent of graft take, percent of epithelization, pigmentation, vascularity, pliability, pain, itching, and overall outcome. All these parameters were measured at multiple time periods.

Data Analysis Plan. Comparisons of wound healing rates between treatment and control sites were made using Wilcoxon's rank sum test on both the percent of graft take and the percent of epithelialization. Comparisons of pigmentation between treatment and control sites were made using categorical log-linear models. Pliability, vascularity, pain, itching, and overall outcome were measured on scales with a natural ordering. Comparisons between treatment and control sites for these parameters were made using categorical log-linear models with ordinal (ordered) responses (1).

Time comparisons for pigmentation, vascularity, pliability, scar height, pain, itching, and overall outcome were also made. If there were responses for all these parameters at all time periods, then the repeated measures analysis was to be incorporated into the categorical log-linear analyses (2). If there are missing data, then separate analyses were to be made for each time period and a Bonferroni correction applied to correct for multiple testing.

RESULTS

This study was approved by the USAISR Research Council and US Army Institute of Surgical Research Human Use Committee during the third quarter of fiscal year 1991. Four patients were enrolled in this study. No adverse reactions or side effects were noted. Preliminary results demonstrated slightly less graft take on wounds covered with DermaGraft™ than on control wounds.

DISCUSSION

The manufacturer of DeramGraft™ is currently modifying the product to produce a thicker mesh. A new manufacturer IDE application will be required. Therefore, this study was terminated.

PRESENTATIONS/PUBLICATIONS

None.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

ACCESSION: DA335923

SUMMARY DATE: 921001 **SUMMARY KIND:** D **PREV DATE:** 911001 **DISTRIBUTION:** CX

PROGRAM #: 62787A **PROJ #:** 30162787A874 **TASK AREA:** ED **WORK UNIT:** 180

TITLE: Study of the Effects of Weak Direct Current (DC) on Donor Site Healing in the Thermally Injured Patient

SUBJ1: 060500 - Medicine and Medical Research
SUBJ2: 061200 - Medical Facilities, Equipment, and Supplies

START DATE: 9106 **END DATE:** 9309 **PERFORMANCE METHOD:** C

CONTRACT/GRANT NO:

CONT TOTAL: $
CUM TOTAL: $
TOTAL LAB FUNDS: $

RESOURCES ESTIMATE

FY	WORK YRS	$(Thousands)
91	0.1	$53
92	0.1	$24
93	0.1	$50

RESPONSIBLE DOD ORGANIZATION: 404885
MRDC INSTITUTE OF SURGICAL RESEARCH USAISR
FT SAM HOUSTON, TX 78234
PRUITT, B A
210-221-2720

PERFORMING ORGANIZATION: 404885
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ASSOC1: CHU, C S

ASSOC2: MCMANUS, A T

FOREIGN INTELLIGENCE CONSIDERED: Y **CIVILIAN APPLICABILITY:** M

KEYWORDS: RA II; Volunteers; Adults; Burns (Injuries); Direct Current; Low Voltage; Healing; Skin Grafts

OBJECTIVE: A DTIC literature search was conducted under DTIC request numbers W6P35E/W6P37F dated 4 March 1991. The objective of this work is to evaluate the effects of weak DC on the healing of partial-thickness skin graft donor sites in patients with thermal injury. Since burns comprise about 10% to 15% of combat casualties, efforts leading to an improved outcome would be of military benefit.

APPROACH: This study will be conducted in two phases. Phase I will directly compare the time of reepithelization between a donor site treated with DC applied through silver-nylon dressings and a donor site located in a comparable anatomic area treated with fine-mesh gauze. A group of 20 patients requiring partial-thickness grafts and with sufficient unburned skin to allow initial harvesting of two donor sites will be utilized. If a patient requires reharvesting of the same donor sites, the patient will undergo a second comparison of treatments. If positive results are observed during Phase I, a second phase will be conducted in another group of 20 patients to determine if DC is the active principle. The same comparisons will be made as during Phase I except that the silver-nylon dressing without applied DC will serve as the parallel control.

PROGRESS: 9110-9209. Phase I of this study has been completed. The use of low-intensity DC appears to be safe and well tolerated. Upon completion of Phase II, the data will be analyzed as indicated. For technical reports, refer to the *US Army Institute of Surgical*

Research Annual Research Progress Report for fiscal years 1991 through 1992.

ABSTRACT

PROJECT NUMBER: 3M162787A874-180, Applied Research and Exploratory Development

PROJECT TITLE: Study of the Beneficial Effects of Weak Direct Current (DC) on Donor Site Healing in the Thermally Injured Patient

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

PERIOD COVERED IN THIS REPORT: 1 October 1991 - 30 September 1992

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Previous animal work at this Institute indicated that partial-thickness donor sites treated with low-intensity direct current applied through silver-nylon dressings heal faster. The present study was designed to apply those encouraging results in the clinical arena. To this end, 21 patients requiring burn wound excision and grafting were prospectively enrolled in Phase I of this study. Paired donor sites were harvested in each patient for comparison. One donor site, serving as the control, was treated conventionally with fine-mesh gauze and the other, the test site, with silver-nylon.

The use of low-intensity DC appeared to be safe and well tolerated. SN-treated donor sites remained painless throughout the study period while MG-treated sites were painful for the first postoperative week. No clinical signs of infection were observed at either site.

Upon completion of Phase II, the data will be analyzed as indicated.

STUDY OF THE BENEFICIAL EFFECTS OF WEAK DIRECT CURRENT (DC) ON DONOR SITE HEALING IN THE THERMALLY INJURED PATIENT

Major burns are uncompromising and the burned host, having lost the protective skin barrier, is defenseless in dealing with potential sources of wound infection. From the very outset of illness, the burned victim is at a disadvantage. With an impaired immune system and a resultant proclivity for systemic and local wound infection, this is further compounded when a dearth of unburned skin prevents wound closure in the extensively burned individual. The devitalized thermally injured skin often is the source, as well as the point of ingress, for bacterial assault that leads to infectious complications, prolonged hospital stay, and ultimately, accentuated mortality.

The belief that prompt excision and burn wound closure shorten hospital stay seems justified, at least in patients with smaller burns, and the notion that early excision and grafting in major burns may improve patient survival seems plausible. Regardless of the rationale for and timing of wound closure, one must reckon with the verity that a full-thickness skin injury, almost always, and deep partial-thickness burns, most of the time, require skin replacement.

At present, excision and autografting constitute standard surgical burn wound therapy. However desirable it may seem, autografting is difficult to accomplish effortlessly, particularly when demand outstrips the supply of unburned skin. Over the years, several commendable techniques have evolved that permit an expeditious wound closure. Definitive methods of closure of the excised burn wound rely exclusively on the availability of autogenous skin and fall under three broad categories, i.e., autografting with split-thickness skin grafts, autografting combined with dermal augmentation, and wound coverage using cultured epidermal keratinocytes.

In autografting major burns, one heavily relies on harvesting the finite unburned donor areas multiple times. This entails about a 2-week delay for a previously used donor site to heal sufficiently and yield, once again, to recropping. Should healing be impaired as a result of, for example, complicated postoperative donor site care or a local wound infection, donor site readiness for reharvesting is delayed even further.

In using artificial skin, the same donor site limitations apply to the acquisition of autologous skin needed to replace the outer Silastic™ membrane covering of the dermal analogue. Although it is true that the ultra-thin skin grafts employed with the dermal analogue shorten donor site healing time by a 3- to 4-day margin (1) compared to conventional split-thickness skin grafts, this gain

is lost to the minimum 2-week delay imposed by the requirement of vascularization before the dermal analogue can accept a skin graft.

The prospect of wound coverage with contemporary techniques employing epidermal keratinocytes alone (2,3) or in conjunction with human allogeneic dermis (4) is promising, since epidermal cells can be cultured with relative ease and in a quantity sufficient to cover the entire body surface of a patient (5,6). Once again, there is a 3- to 4-week delay between the initial skin biopsy and the availability of the cultured cells for clinical use. In patients with large burns, however, it appears that autografting with either split-thickness skin grafts or with cultured epidermal keratinocytes lags behind the resurfacing needs of a patient by several weeks, chiefly due to relative autologous skin shortage.

The use of allogeneic material as a substitute for burned skin is another area that has been explored, but with controversial results. Skin allografts, under an umbrella of immunosuppressive therapy which subdues allograft rejection, have been used for long-term wound coverage in children (7). However, the results of that study have defied verification by other investigators. In a similar vein, another group has used cultured allogeneic epidermal keratinocytes in humans and reported success (8,9). Factually, however, all the evidence to date suggests that the cultured allogeneic epidermal keratinocytes serve more as a biologic dressing than true skin substitute and are eventually replaced by the recipient's epidermis (10-12). Allogeneic epidermis, whether cultured or not, seems unsuitable at present for permanent wound closure.

With burn injury, it is sad and ironic that regardless of the technique employed, it is the patient who ultimately must provide skin to cover his wounds and save his life. The present methods of burn wound closure are restricted for want of autologous skin suitable for grafting. Measures that will stretch the available skin to meet the individual patient's need are, therefore, welcome. Aside from the prevention of burns that need no cure, a suitable response to the persistent dilemma of skin shortage in patients with major burns would be to devise a means to accelerate healing of the burn wound itself so that a lesser burned area needs grafting. A panacea to that effect is not at hand. However, a methodology that expedites reepithelialization, makes donor sites available for multiple skin harvesting, and shortens the inter-harvest period is seemingly within our grasp. The technique offers promise of making skin available in relative abundance. Research in laboratory animals at this Institute indicates that the phenomenon of wound healing can be harnessed and the reparative processes modified. The work (13,14) leading to these conclusions is summarized below.

In a group of Hartley guinea pigs, a partial-thickness scald injury over 18% of the dorsal trunk of animals was created by the

Walker-Mason technique (15). The burn wound was dressed with a silver-nylon (SN) meshed fabric that received an anodal current. A SN contact point over the animal's abdomen received the cathodal current. Animals in the treatment group received a constant DC, 1 $\mu A/cm^2$ for 2 days and 0.5 $\mu A/cm^2$ for 3 days. Animals in the control group received no current, though their wounds were dressed with SN fabric. Another group of 140 animals received scald burns. After complete healing in these animals, a continuous strip of split-thickness skin graft was harvested from the previously scalded area, located cephalad, and from the contiguous unburned skin of similar dimensions over the caudal aspect of animals's back. The harvest from the scalded area was discarded and that from the caudal unburned skin was utilized to graft the raw donor surface of the scalded area. In this manner, the scalded area donor site served as the recipient site for the unburned skin. One hundred and twenty animals received DC and 20 received no DC. After complete healing, 60 of the 120 animals underwent a second harvest and grafting procedure and received DC treatment. DC was studied for its effects on three discrete endpoints of healing, i.e., wounds, donor sites, and grafts. To assess the depth of microcirculation, the animals were injected with india ink through the superior mesenteric artery and, upon sacrifice of the animals, biopsies were taken from central portions of the grafts and examined microscopically for deposition of carbon particles as a measure of the extent of microcirculation.

In the first study, the time to complete healing of the burn was 12 days for 10 animals in the treatment group and 16 days for animals in the control group. Skin biopsies revealed that the dermis and its appendages were largely preserved in the treated animals, while in control animals, approximately one-third of the dermis was replaced with granulation tissue and the surviving hair follicles were sparse. In the second study, the autografts in the treated animals were firmly adherent to the wound bed by the 4th postoperative day and were fully vascularized as evidenced by the deposition of carbon particles. An exuberant outgrowth of epithelium spawned from the remnants of wound bed hair follicles and appeared to link the partial-thickness skin graft above to the wound bed below. Stratum corneum appeared by day 12 in the graft, giving it the appearance and texture of normal skin. Even after the second harvest, the normal histologic appearance and skin texture was well preserved in treated animals. In control animals, in contrast, the skin graft adherence was minimal and graft vascularization was delayed for 7 days, by which time most of the hair follicles had already degenerated. A marked graft contraction and hair loss were obvious at 3 months in the control animals.

The striking finding in the treated group was the remarkable attenuation of inflammatory response at the nonburned donor sites and minimal subepidermal fibrosis. With treatment, surface migration of the epithelial cells of hair follicle origin was demonstrable by 48 h after harvesting, the neodermis attained 3- to

8-cell thickness by 72 h, and complete healing occurred by 14 days. Wound contraction was undetectable during the 3-month observation period, since both the donor sites and the grafted areas in the treated animals increased in size parallel with the growth of the whole animal. The hair density in the treated animals was reduced, but only minimally. The donor sites of the control animals, on the other hand, developed an intense inflammatory response by 48 h after harvesting, the epithelial growth from the hair follicles proceeded with great difficulty under the inflammatory exudate, reepithelialization took over 3 weeks to complete, and donor site wounds showed marked fibrosis, contracture, and hair loss. More importantly, the donor sites were rendered unusable after the very first harvest in untreated animals.

In a subsequent study in the guinea pig, the advantageous effects of DC on the maturation of split-thickness skin grafts placed on a tangentially excised deep partial-thickness burn were confirmed. The grafts in the treated animals revascularized in 2 days and adhered firmly to the underlying wound bed in 4 days; in comparison, it took 7 days for the wounds of control animals to reach that stage of graft maturity. The epithelial proliferation was much more pronounced in treated animals compared to control animals. In control animals, the skin grafts showed mild contraction, moderate hair loss, and extensive subepidermal fibrosis at 3 months. In treated animals, the graft sites' enlargement was proportionate to the entire animal growth and the animals exhibited a lavish fur coat and minimal subepidermal fibrosis.

These data indicate that DC offers a means of accelerating wound healing. The extremely low voltage used in these studies seems safe for human use and has had no discernable adverse effects on the animals studied. The output from many FDA-approved devices, such as nerve stimulators and electric cautery, used in clinical practice is several thousand-fold greater compared to the device proposed for this clinical protocol. In humans, low-intensity DC has been used with reported success to promote healing of indolent ischemic skin ulcers (16-21), chronic granulating burns, and chronic osteomyelitis and nonhealing fractures (22).

Based on our animal data, we postulate that the application of weak DC will accelerate wound healing, improve donor site quality, and allow repetitive use of limited donor sites in patients with extensive full-thickness burns. Therefore, the objective of this study is to evaluate the effects of weak DC on the healing of partial-thickness skin graft donor sites in patients with thermal injury.

MATERIALS AND METHODS

Study Design. This prospective study seeks human confirmation of the utility of DC for improving the time of reepithelization and

the quality of wound healing of partial-thickness donor sites. The study will be conducted in two phases. The first phase will directly compare the time of reepithelization between a donor site treated with DC applied through SN dressings and a donor site located at a parallel body location, e.g., opposite upper extremity, treated with fine-mesh gauze dressings (the standard method of care at this Institute). A group of 20 patients requiring partial-thickness grafts and with available unburned skin to allow initial harvesting of two donor sites will be utilized. If a patient requires reharvesting of the same donor sites, the patient will undergo a second comparison of treatments. If positive results are observed during the first phase, a second phase will be conducted in another group of 20 patients to determine if DC is the active principle. The same comparisons will be made as during the first phase except that the SN dressing without applied DC will serve as the parallel control.

Selection of Patients. Forty patients will be enrolled in this study. Properly signed and witnessed DA Forms 5303-R, Volunteer Agreement Affidavit, will be obtained from each patient before beginning the study.

Patient Inclusion. Male or female patients ≥ 18 yr with burns (the presence of inhalation injury not being exclusionary) requiring excision and grafting.

Patient Exclusion. Patients meeting any of the following criteria will be excluded from participation in this study:

1. Patients < 18 yr.
2. Patients who are pregnant or nursing.
3. Patients with toxic epidermal necrolysis syndrome.

Study Groups. Two modalities of donor site treatment, i.e., fine-mesh gauze and SN dressing, will be compared to the application of low-intensity DC to assess wound healing. Initial studies in 20 subjects (Group I) requiring excision and grafting will attempt to verify donor site healing times with fine-mesh gauze on one site and with electric current therapy on another. Each patient will serve as his own control. To differentiate between the local and remote effects of DC, the donor sites in 20 patients (Group II) will receive SN dressing with and without DC and each patient will serve as his own control. Further, in patients with large burns requiring multiple harvestings, the potential of the DC-treated and untreated donor sites for secondary harvest will be determined clinically prior to a reharvest procedure.

Description of Procedures. Forty thermally injured patients requiring excision and grafting of their burn wounds will be

enrolled in the study after giving informed consent. Donor site selection will be based on the clinical needs of the patient as modified by the individual surgeon's choice. Presterilized SN fabric (Style A-2589-5, Swift Textile Metalizing Corporation, Hartford, CT) will be cut to the size of the wound and placed directly over the raw donor site surface. The SN fabric is a heavy ripstop, knit nylon fabric weighing 84.8 gm/m^2 and containing 22.6 gm/m^2 of silver. The dressing will be secured with several layers of fine-mesh gauze moistened with normal saline, laparotomy pads, and a tubular Kerlix™ bandage. The dressings will be kept moist with normal saline instillation on a regular basis. A constant voltage, constant current power supply (Electrowave Systems, Inc., San Antonio, TX) will be used to deliver 1 $\mu A/cm^2$ DC for 5 days to selected donor sites through the SN fabric. The SN donor site will be connected to the anode. The opposite electrode will be established by placing a SN dressing over the opposite extremity of the patient or on the same extremity on a surface directly opposite from the donor site or on the same extremity as the donor site but at a remote location. Control donor sites will be dressed with fine-mesh gauze. During the second phase of the investigation, SN fabric will be placed directly on the control wound bed and secured with fine-mesh gauze moistened with normal saline, laparotomy pads, and a tubular Kerlix™ bandage. These dressings will also be kept moist on a regular basis with normal saline instillation. The first dressing change will occur on the 3rd to 5th postoperative day and daily thereafter until healing. At the time of each dressing change, control and treatment donor sites will be evaluated for wound healing and maturation of wounds.

Beginning at 24-48 h postoperatively and at 1-2 day intervals, healing will be assessed clinically by visual inspection of DC- and SN-treated donor sites and documented by photography. Fine-mesh gauze-treated donor sites will be considered healed upon complete separation of the gauze. The characteristics of the healed donor site will be verified on physical examination by appraisal of the wound texture, wound surface regularity and smoothness, and wound bed pliability. On the 7th postoperative day, punch biopsies from the margins and central portions of selected donor and graft sites will be obtained under local anesthesia and sent to the pathology laboratory for histopathologic examination.

Determination of Number of Subjects Required. This protocol involves a study of 40 thermally injured patients, all receiving direct anodal current to one donor site and either fine-mesh gauze or SN dressing on the other donor site. Since each patient may receive multiple dressings at one sitting and the participating patients are expected to be studied more than once, it is anticipated that the number of observations made in 40 patients will be sufficient to permit meaningful evaluation of the efficacy of DC in wound healing.

Data Collection. Initial data collected on each patient will include the patient's admission number, age, burn size, date of burn, location of target donor site test area. All excision and grafting dates will be recorded as they occur. The effect of treatment will be assessed clinically. Photographs will be taken immediately after harvest, at the time of the first dressing change, and at the time of complete separation of the gauze. Histologic data from the punch biopsies will also be recorded. End points will be developed and will include time to healing, visual (color) aspects of the graft surface, and extensiveness of skin appendage regeneration.

Data Analysis. Using paired tests, comparisons will be made for the time to healing of the gauze and SN-treated donor sites and also for complications between SN and control sites.

RESULTS

Phase I of the study was completed during this reporting period. The use of low-intensity DC appears to be safe and well tolerated. SN-treated donor sites remained painless throughout the study period while MG-treated sites were painful for the first postoperative week. No clinical signs of infection were observed with either site.

DISCUSSION

Upon completion of Phase II, the data will be analyzed as indicated.

PRESENTATIONS/PUBLICATIONS

None.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

ACCESSION: DA0G6971

SUMMARY DATE: 921001 **SUMMARY KIND:** D **PREV DATE:** 911001 **DISTRIBUTION:** CX

PROGRAM #: 61102A **PROJ #:** 30161102BS14 **TASK AREA:** EA **WORK UNIT:** 301

TITLE: Studies of Infection and Microbiologic Surveillance of Patients with Thermal Injury

SUBJ1: 060500 - Medicine and Medical Research
SUBJ2: 061300 - Microbiology
SUBJ3: 061500 - Pharmacology

START DATE: 7610 **END DATE:** 9909 **PERFORMANCE METHOD:** C

CONTRACT/GRANT NO:

CONT TOTAL: $
CUM TOTAL: $
TOTAL LAB FUNDS: $

RESOURCES ESTIMATE

FY	WORK YRS	$(Thousands)
91	2.8	$105
92	2.8	$111
93	2.8	$143

RESPONSIBLE DOD ORGANIZATION: 404885
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ASSOC1:

ASSOC2:

FOREIGN INTELLIGENCE CONSIDERED: Y **CIVILIAN APPLICABILITY:** M

KEYWORDS: RA II; Volunteers; Adults; Lab Animals; Rats; Mice; Burns (Injuries); Virulence; Septicemia; Pseudomonas

OBJECTIVE: A DTIC literature search was conducted under DTIC request numbers W6L50C/W6L09M dated 19 October 1989. The objectives of this work are to perform epidemiologic studies, study the response of significant species to topical chemotherapy modalities, and determine the relationship of antibiotic usage to sepsis control.

APPROACH: 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.

PROGRESS: 9110-9209. During calendar year 1991, microbiologic surveillance was carried out on 212 of the 218 admitted and discharged burn patients. More than 7888 isolates were identified from 14457 specimens. Gram-negative organisms represented less than 44% of ioslates. *Neisseria sicca* was the most common gram-negative isolate. The most common blood isolate was *Staphylococcus aureus*. *Pseudomonas aeruginosa* was isolated from the blood cultures of four patients. No *Pseudomonas aeruginosa* wound infections were identified. For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report* for fiscal years 1977 through 1992.

ABSTRACT

PROJECT NUMBER: 3M161102BS14-301, Research

PROJECT TITLE: Studies of Infection and Microbiologic Surveillance of Troops with Thermal Injury

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

PERIOD COVERED IN THIS REPORT: 1 January 1991 - 31 December 1991

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During calendar year 1991, 212 burned patients were cultured and 7888 isolates were identified. Gram-negative organisms accounted for less than 44% of the bacterial isolates, which is a continuation of a 10-yr trend. This was also reflected in an increase in Gram-positive organisms in blood cultures. *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Staphylococcus saprophyticus* represented 43.2% of the bacteremia cases. The computerized microbial culture surveillance system now contains infection control and antibiotic usage data bases. This system is being evaluated for its use in predicting infecting organisms on the basis of sites of colonization and antibiotic usage.

STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE OF TROOPS WITH THERMAL INJURY

This report is produced from microbiology data collected for patients admitted during calendar year 1991. Data were collected from admission through disposition. These culture results are presented in concert with the annual research progress report produced by the Clinical Division for the same patient population.

AUTOMATED MICROBIOLOGY DATA BASE

The microbiology data base now contains complete surveillance data for over 2,000 burn patient admissions. Epidemiologic use of these data has resulted in several publications. The microbiology data base has been aligned with antibiotic use and infection control data bases. This has improved the utility of the system for prospective use in identifying outbreaks and aiding empiric therapy by predicting on a statistical basis the probable antibiotic sensitivity patterns of infecting organisms.

ANTIBIOTIC SENSITIVITY DETERMINATION

The 1991 antibiotic testing panels are presented in Table 1. Bacterial organisms were tested by agar overlay disc diffusion. Broth dilution minimal inhibitory concentrations and minimal bactericidal concentrations were available upon specific request. The protocol for selecting organisms for in vitro sensitivities was isolation from blood cultures, predominant organisms in biopsy cultures, predominant Gram-negative organisms in sputum and urine cultures with $> 10^5$ cfu/ml, all *Staphylococcus aureus* isolates, all *Pseudomonas aeruginosa* isolates, and any other organisms as requested.

MICROBIAL SURVEILLANCE

The microbial surveillance protocol established during fiscal year 1983 was continued during calendar year 1991. Cultures were obtained from the wound, sputum, urine, and rectum of each patient upon admission. Thereafter, sputum and urine were cultured three times weekly and stools and wound surfaces twice weekly. Patients transferred to the convalescent ward and hospitalized more than 30 days were cultured weekly. Gentamicin-resistant Gram-negative organisms from sputum or stool specimens were screened by plating on MacConkey agar containing gentamicin sulfate (25 µg/ml).

MICROBIOLOGIC FINDINGS IN BURN PATIENTS

A total of 212 patients admitted during 1991 were cultured. Species isolated and number of patients yielding each species are

TABLE 1. In Vitro Sensitivity Panels (1991)

Enteric Organisms	Nonenteric Gram-Negative Organisms	Gram-Positive Organisms
Amikacin sulfate[a,b]	Amikacin sulfate[a,b]	Amikacin sulfate[a,b]
Ampicillin sodium[a]	Azlocillin sodium[a]	Ampicillin sodium
Aztreonam	Aztreonam	Cefoperazone sodium
Cefamandole nafate[a]	Cefoperazone sodium	Cefotaxime sodium
Cefoperazone sodium	Cefotaxime sodium[a]	Ceftazidime[a,b]
Cefotaxime sodium[a]	Ceftazidime[a,b]	Ceftriaxone sodium
Cefoxitin sodium[a]	Ceftriaxone sodium	Cephalothin[a]
Ceftazidime[a,b]	Cefsulodin[a]	Chloramphenicol palmitate[a]
Ceftriaxone sodium[a]	Chloranphenicol palmitate	Clindamycin hydrochloride[a]
Chloramphenicol palmitate	Colistin sulfate	Erythromycin[a]
Gentamicin sulfate[a,b]	Gentamicin sulfate[a,b]	Gentamicin sulfate[a,b]
Imipenem-cilastatin sodium[b]	Imipenem-cilastatin sodium[b]	Imipenem-cilastatin sodium[b]
Kanamycin sulfate	Kanamycin sulfate	Mezlocillin sodium[b]
Mezlocillin sodium[a,b]	Mezlocillin sodium[a,b]	Moxalactam[b]
Nalidixic acid	Moxalactam[a,b]	Oxacillin sodium[a]
Netilmicin sulfate[a]	Netilmicin sulfate[a]	Penicillin[a]
Norfloxacin	Norfloxacin	Piperacillin sodium[a,b]
Piperacillin sodium[a,b]	Piperacillin sodium[a,b]	Sulfadiazine
Streptomycin sulfate	Sulfadiazine[a]	Streptomycin sulfate
Sulfadiazine	Tetracycline hydrochloride	Tetracycline hydrochloride
Tetracylcine hydrochloride	Ticarcillin disodium[a]	Tobramycin sulfate
Ticarcillin disodium[a]	Ticarcillin disodium-clavulanate potassium	Vancomycin hydrochloride[a,b]
Trimethoprim	Tobramycin sulfate	
Trimethoprim-sulfadiazine		

[a]Reported on daily clinical microbiology report (hard copy).
[b]Reported on computer screen from patient data base.

presented in Table 2. Because of the decreased host resistance of the patient population, no organism is considered "normal" flora and all isolated organisms are reported to the physician. A summary of the 10 most common isolates is presented in Table 3. The table contains 78.3% of the species identified. The relative frequencies of sites of isolation are presented in Figure 1. The relative frequencies of sites of isolation of Gram-negative organisms, Gram-positive organisms, and yeast are shown in Figure 2.

FLORA RECOVERED FROM RESPIRATORY SYSTEM SPECIMENS

A total of 4211 organisms were recovered from respiratory system specimens. The majority of these were sputum cultures

TABLE 2. Distribution by Organism (1991)

Organism	Number of Isolates	Number of Patients Colonized	Organism	Number of Isolates	Number of Patients Colonized
Acinetobacter anitratus	113	16	*Propionibacterium acnes*	1	1
Acinetobacter lwoffii	3	3	*Proteus mirabilis*	342	27
Acinetobacter species	3	3	*Proteus* species	6	6
Aeromonas hydrophila	18	5	*Proteus vulgaris*	2	1
Alcaligenes faecalis	2	2	*Providencia rettgeri*	4	2
Allescheria species	2	2	*Providencia stuartii*	4	2
Aspergillus flavus	3	3	*Pseudomonas aeruginosa*	869	42
Aspergillus fumigatus	5	4	*Pseudomonas fluorescens*	18	5
Aspergillus terreus	5	4	*Pseudomonas maltophila*	5	2
Bacillus species	65	40	*Pseudomonas putida*	1	1
Branhamella catarrhalis	4	2	*Pseudomonas* species	7	6
Candida albicans	69	22	*Pseudomonas stutzeri*	1	1
Candida tropicalis	14	2	*Salmonella* species	1	1
Citrobacter diversus	23	11	*Serratia liquefaciens*	7	5
Citrobacter freundii	21	8	*Serratia marcescens*	200	13
Cladosporium species	4	4	*Serratia odorifera*	4	1
Corynebacterium species	1	1	*Staphylococcus aureus*	1196	101
Enterobacter aerogenes	165	39	*Staphylococcus epidermidis*	569	158
Enterobacter agglomerans	23	17	*Staphylococcus saccharolyticus*	2	2
Enterobacter cloacae	217	33	*Staphylococcus saprophyticus*	165	66
Enterobacter species	8	7	*Staphylococcus* species	16	14
Escherichia coli	444	67	Alpha *Streptococcus*	18	14
Fusarium species	7	4	Beta hemolytic *Streptococcus*	1	1
Hafnia alvei	16	2	Beta hemolytic *Streptococcus*, not Group A, B, or D	83	35
Haemophilus influenzae	9	6			
Klebsiella oxytoca	14	7	Group A nonhemolytic beta *Streptococcus*	7	6
Klebsiella planticola	1	1			
Klebsiella pneumoniae	530	67	Group B *Streptococcus*	2	2
Klebsiella species	8	7	Group C *Streptococcus*	1	1
Kingella dentrificans	2	1	Group D *Streptococcus*, not *Enterococcus*	59	39
Lactobacillus species	1	1			
Micrococcus species	4	4	Group D *Enterococcus*	322	52
Morganella morganii	24	13	Group F *Streptococcus*	1	1
Mucor species	1	1	Nonhemolytic *Streptococcus*	8	7
Neisseria lactamica	1	1	Nonhemolytic *Streptococcus*, not Group D	580	157
Neisseria sicca	272	95			
Neisseria species	1	1	*Streptococcus pneumoniae*	10	7
Neisseria subflava	1	1	*Streptococcus viridans*	1229	176
Pencillin species	2	2	True fungi species (other)	38	21
Peptostreptococcus species	1	1	Yeast species (other)	2	2

Total Number of Isolates = 7888

Total Number of Cultured Patients = 212

TABLE 3. Ten Most Frequent Isolates (1991)

Organism	Number of Patients Colonized	% Patients	Number of Isolates	% Total Isolates
Streptococcus viridans	176	83.0	1229	15.6
Staphylococcus epidermidis	158	74.5	569	7.2
Nonhemolytic *Streptococcus*, not Group D	157	74.1	580	7.3
Staphylococcus aureus	101	47.6	1196	15.2
Neisseria sicca	95	44.8	272	3.4
Klebsiella pneumoniae	67	31.6	530	6.7
Escherichia coli	67	31.6	444	5.6
Staphylococcus saprophyticus	66	31.1	165	2.1
Group D *Enterococcus*	52	24.5	322	4.1
Pseudomonas aeruginosa	42	19.8	869	11.0

Total Number of Patients Cultured = 212
Total Number of Isolates = 7888

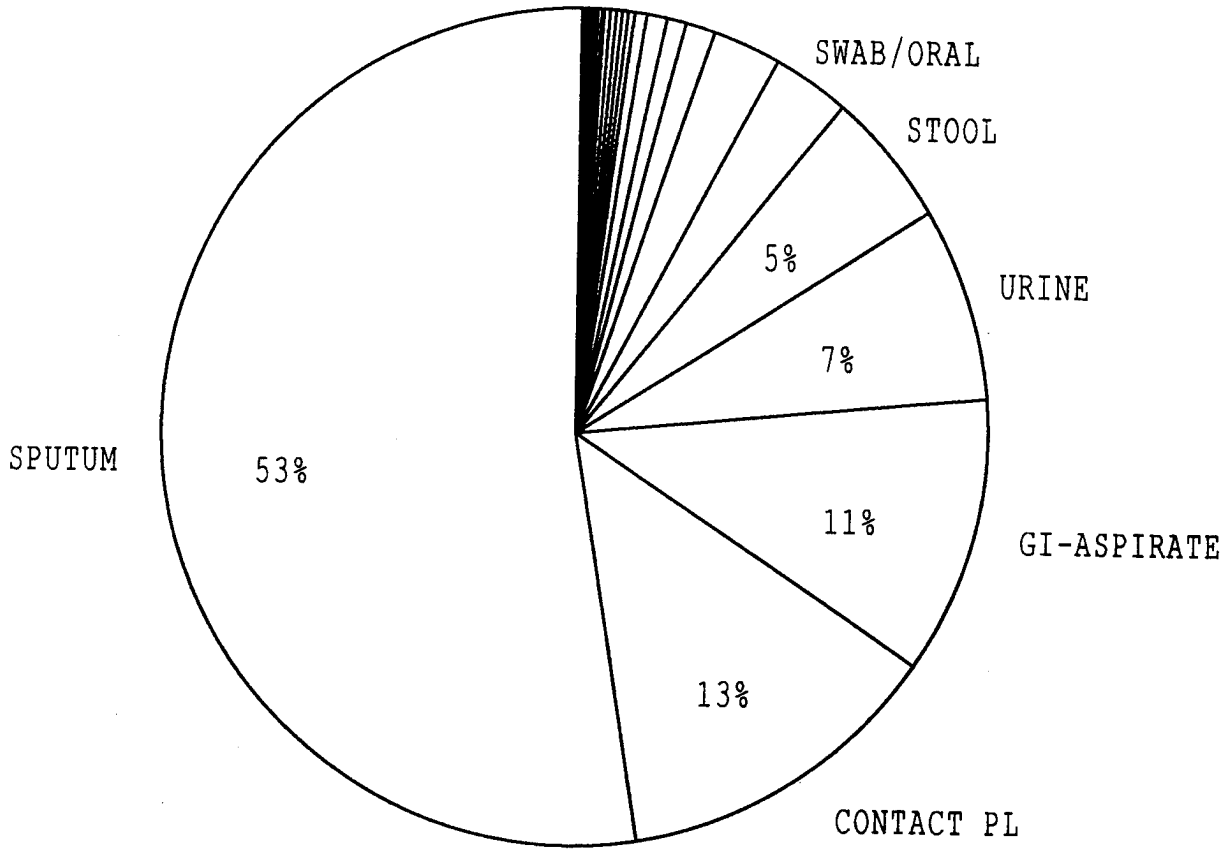


FIGURE 1. Display of the relative frequency of specimen sources yielding isolates in 1991.

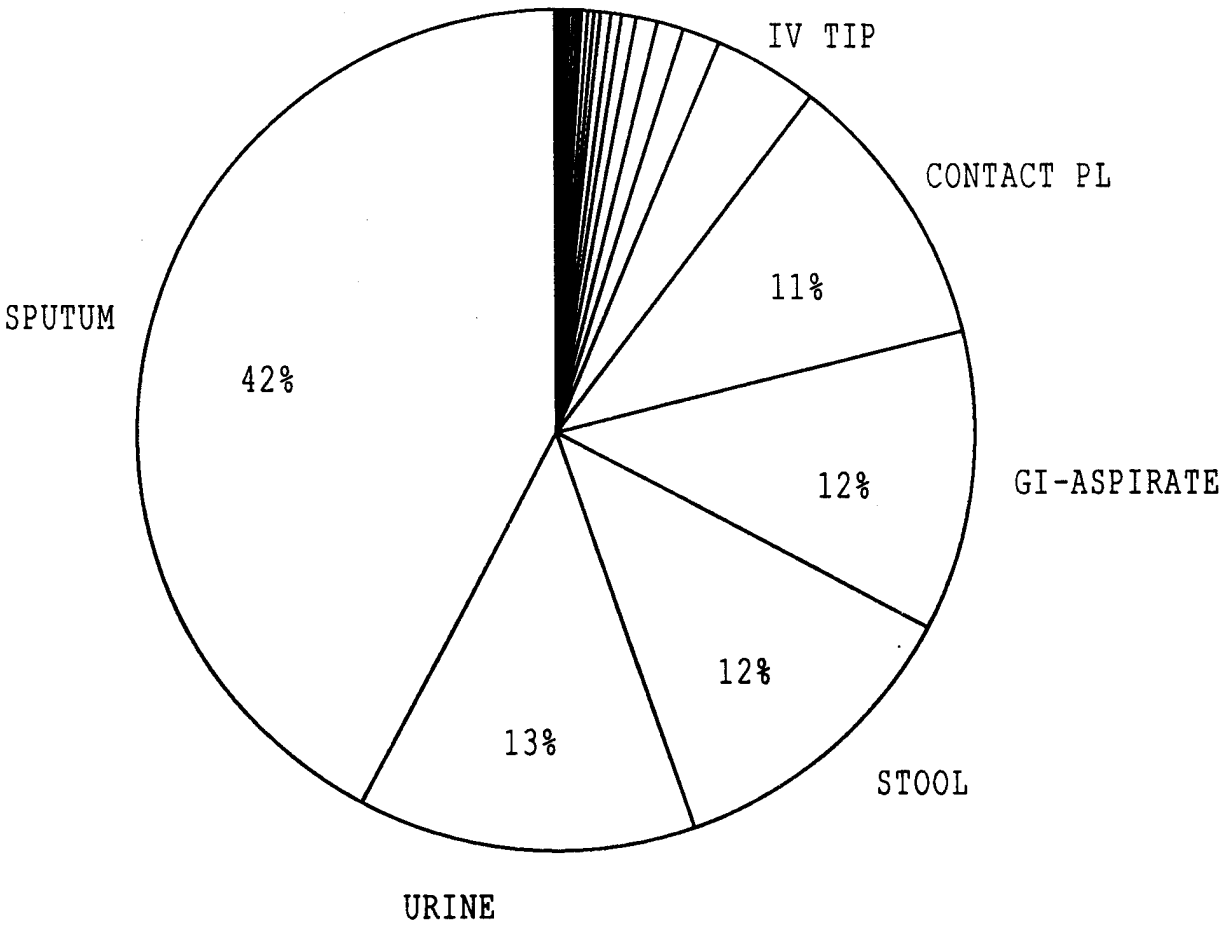


FIGURE 2A. Display of the relative frequency of specimen sources yielding Gram-negative organisms.

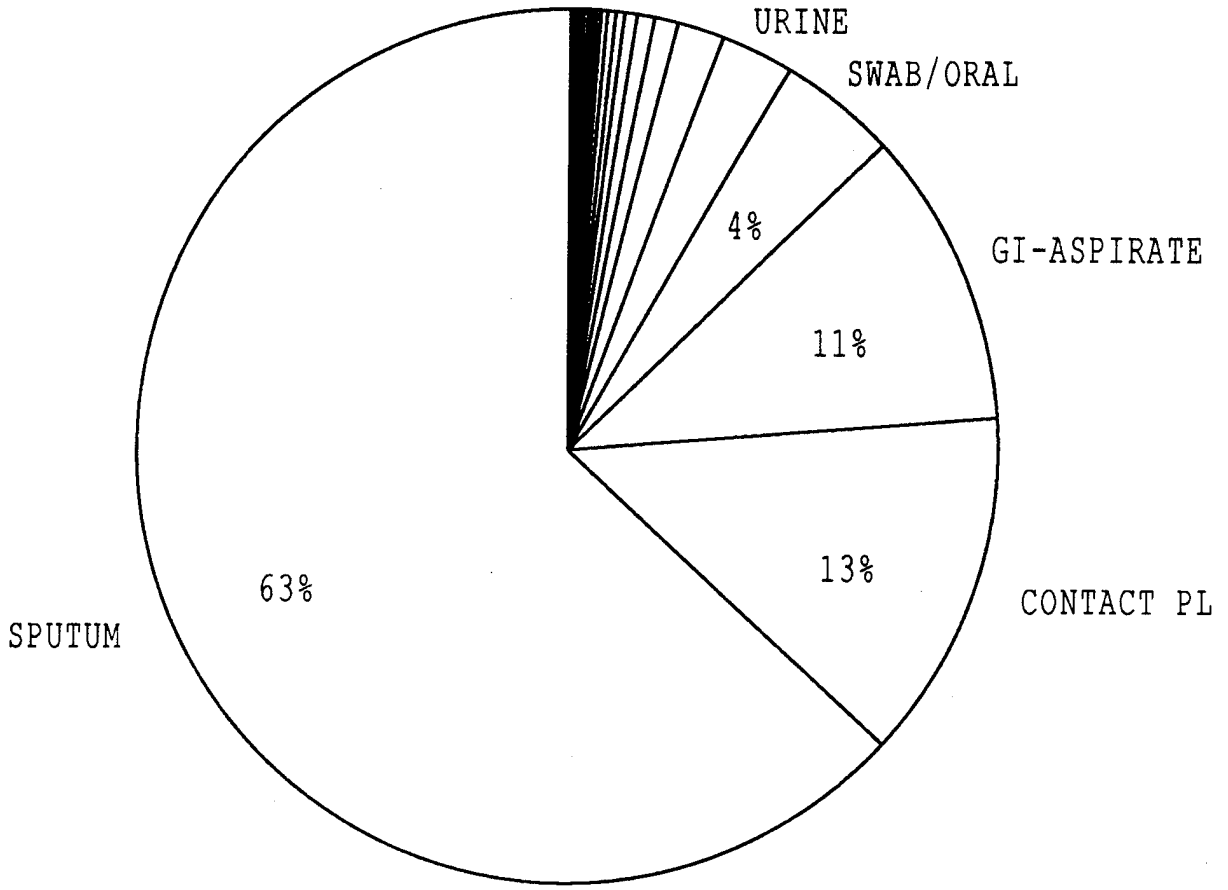


FIGURE 2B. Display of the relative frequency of specimen sources yielding Gram-positive organisms.

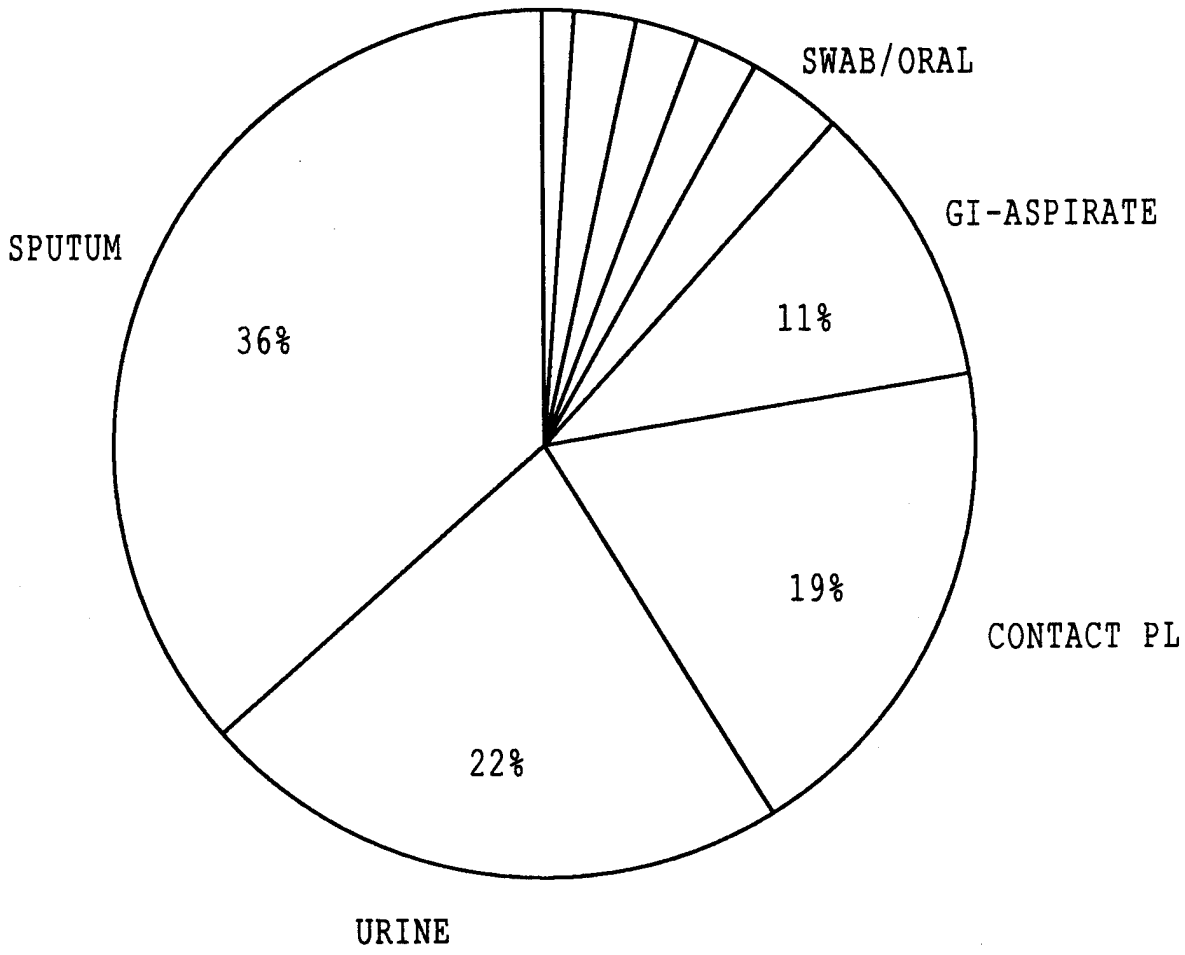


FIGURE 2C. Display of the relative frequency of specimen sources yielding yeast-like organisms.

TABLE 4. Ten Most Frequent Isolates from Respiratory Sources (1991)

Organism	Number of Patients Colonized	% Patients	Number of Isolates	% Total Isolates
Streptococcus viridans	158	96.3	998	23.7
Nonhemolytic *Streptococcus*, not Group D	133	81.1	441	10.5
Neisseria sicca	83	50.6	230	5.5
Staphylococcus aureus	78	47.6	776	18.4
Staphylococcus epidermidis	73	44.5	221	5.2
Klebsiella pneumoniae	32	19.5	157	3.7
Beta hemolytic *Streptococcus*, not Group A, B, or D	32	19.5	63	1.5
Group D *Streptococcus*, not *Enterococcus*	31	18.9	47	1.1
Staphylococcus saprophyticus	27	16.5	62	1.5
Pseudomonas aeruginosa	19	11.6	372	8.8

Total Number of Patients Cultured = 164
Total Number of Isolates = 4211

collected in the surveillance program. The 10 most frequent species are presented in Table 4, which represents 80% of the respiratory isolates. Gram-negative organisms continued to account for more than 20% of the respiratory isolates. *Pseudomonas aeruginosa* colonized 19 of the 164 patients with an isolate. This was just under an 8% increase from previous years.

FLORA RECOVERED FROM WOUND SURFACE SPECIMENS

A total of 1918 contact plate surface cultures were taken and 1033 isolates were made. Relative frequencies of isolated species are presented in Figure 3. Subsurface flora, as measured by biopsy specimens, was measured in 634 biopsies taken from 122 patients. Organisms were recovered from 38 of the biopsied patients. The 10 most common organisms are presented in Table 5. Filamentous fungi remained the principal isolate, with Fusarium being the most common fungal genus. *Pseudomonas aeruginosa* was recovered from biopsies taken from 8 patients. The continued decrease in recovery of wound bacteria is best correlated with the decrease in resistance to topical and parenteral antimicrobial agents. The loss of competitive bacterial flora is a reasonable basis for the increased frequency of fungal isolates.

FLORA RECOVERED FROM URINARY TRACT SPECIMENS

Urine specimens from 202 patients yielded 586 isolates. The 10 most common species are presented in Table 6. The top 10 organisms isolated from urine specimens with $> 10^5$ cfu/ml are presented in Table 7.

FLORA RECOVERED FROM BLOOD CULTURES

Blood cultures were obtained from 84 patients for a total of 700 cultures. All organisms recovered are listed in Table 8. Positive cultures were obtained from 23 patients and 68 isolates were made from 65 positive cultures. Thirty-six cases of bacteremia were noted. A case of bacteremia was defined as isolation of an organism once or more than once within a 30-day period.

Intravenous catheter tips were cultured from 65 patients. Two hundred and sixteen organisms were isolated from 48 patients. Data are presented in Table 9. These data show a continued high incidence of contamination.

SUMMARY OF ANTIBIOTIC TESTING

A total of 3794 bacterial isolates were tested for in vitro sensitivity to antibiotics. A comparison of sources of tested strains is presented in Figure 4. The relative frequency of tested organisms is presented in Figure 5.

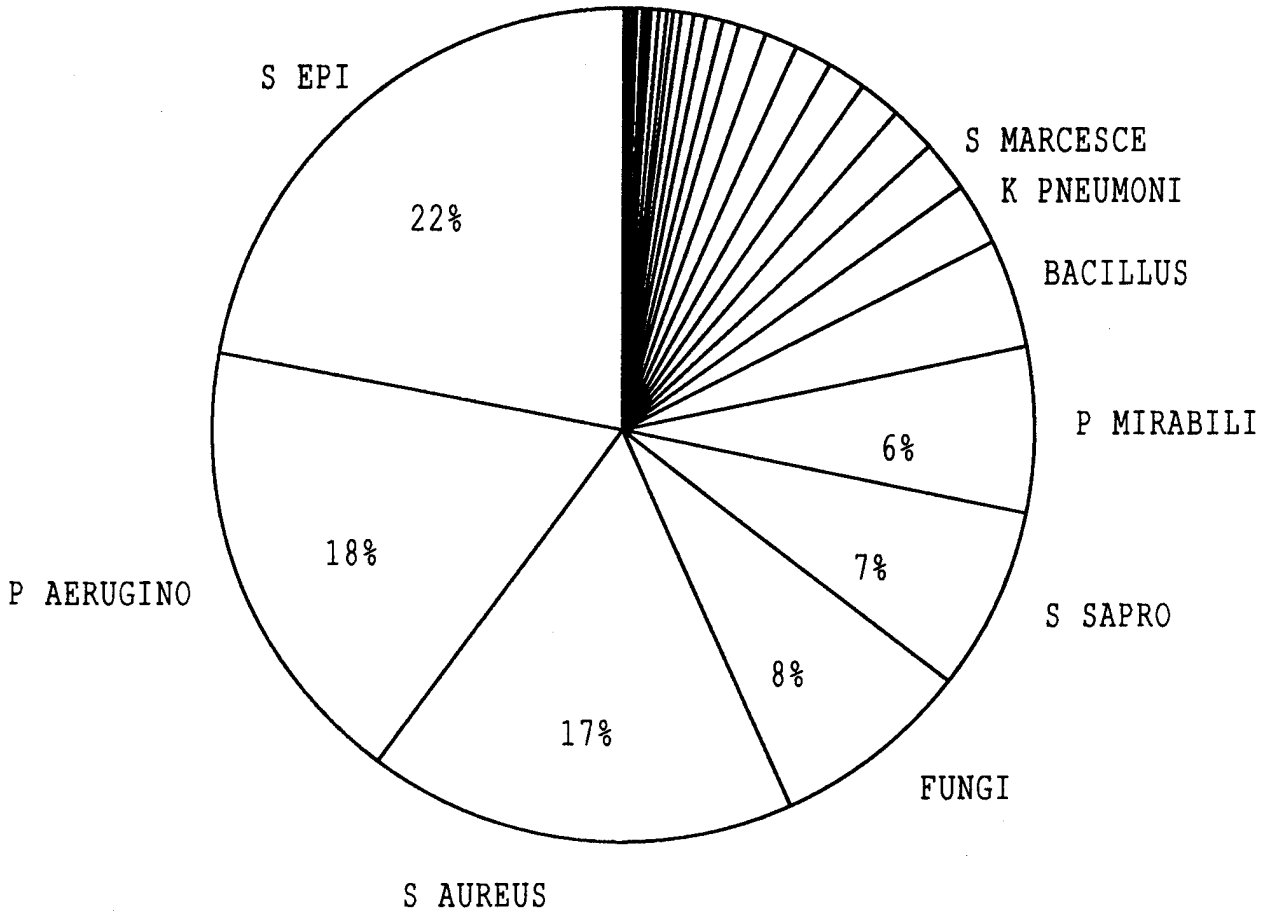


FIGURE 3. Display of the relative frequency of organism types isolated from surface wound cultures.

TABLE 5. Principal Organisms Recovered in Biopsy Specimens (1991)

Organism	Number of Patients Colonized	% Patients	Number of Isolates	% Total Isolates
Filamentous fungi	24	19.7	66	41.0
Pseudomonas aeruginosa	8	6.6	21	13.0
Bacillus species	8	6.6	8	5.0
Escherichia coli	7	5.7	12	7.5
Staphylococcus epidermidis	7	5.7	8	5.0
Staphylococcus aureus	6	4.9	9	5.6
Nonhemolytic Streptococcus, not Group D	6	4.9	6	3.7
Group D *Enterococcus*	3	2.5	10	6.2
Streptococcus viridans	3	2.0	3	1.8
Proteus mirabilis	2	1.6	3	1.8

Total Number of Patients Biopsied = 122
Total Number of Isolates = 161
Biopsies Taken = 634

TABLE 6. Ten Most Frequent Organisms from Urinary Specimens (1991)

Organism	Number of Patients Colonized	% Patients	Number of Isolates	% Total Isolates
Klebsiella pneumoniae	32	15.8	85	14.5
Escherichia coli	30	14.9	70	11.9
Group D *Enterococcus*	30	14.9	60	10.2
Staphylococcus epidermidis	19	9.4	26	4.4
Proteus mirabilis	17	8.4	66	11.3
Enterobacter aerogenes	15	7.4	33	5.6
Pseudomonas aeruginosa	14	6.9	83	14.2
Enterobacter cloacae	10	5.0	17	2.9
Nonhemolytic *Streptococcus*, not Group D	10	5.0	11	1.9
Morganella morganii	8	4.0	12	2.0

Total Number of Patients Cultured = 202
Total Number of Isolates = 586

TABLE 7. Ten Most Frequent Organisms from Urinary Specimens with ≥ 10^5 cfu (1991)

Organism	Number of Patients Colonized	% Patients	Number of Isolates	% Total Isolates
Group D *Enterococcus*	18	33.3	28	10.4
Escherichia coli	17	31.5	34	12.6
Klebsiella pneumoniae	15	27.8	36	13.3
Pseudomonas aeruginosa	11	20.4	49	18.1
Proteus mirabilis	9	16.7	30	11.1
Enterobacter aerogenes	8	14.8	19	7.0
Staphylococcus epidermidis	6	11.1	10	3.7
Enterobacter cloacae	4	7.4	4	1.5
Serratia marcescens	3	5.5	6	2.2
Citrobacter diversus	3	5.5	5	1.9
Morganella morganii	3	5.5	5	1.9

Total Number of Patients with ≥ 10^5 cfu/ml = 54
Total Number of Isolates = 270

TABLE 8. Organisms Found in Blood Cultures (1991)

Organism	Number of Patients	% Patients Cultured	% Cases	Number of Isolates	% Total Isolates
Staphylococcus aureus	11	13.1	29.7	30	45.5
Pseudomonas aeruginosa	4	4.8	10.8	12	18.2
Staphylococcus epidermidis	4	4.8	10.8	4	6.1
Bacillus species	3	3.6	8.1	3	4.5
Acinetobacter anitratus	2	2.4	5.4	3	4.5
Staphylococcus saccharoyticus	2	2.4	5.4	2	3.0
Candida albicans	1	1.2	2.7	2	3.0
Enterobacter aerogenes	1	1.2	2.7	1	1.5
Escherichia coli	1	1.2	2.7	1	1.5
Klebsiella pneumoniae	1	1.2	2.7	1	1.5
Peptostreptococcus species	1	1.2	2.7	1	1.5
Propionibacterium acnes	1	1.2	2.7	1	1.5
Proteus mirabilis	1	1.2	2.7	1	1.5
Serratia marcescens	1	1.2	2.7	1	1.5
Staphylococcus saprophyticus	1	1.2	2.7	1	1.5
Streptococcus viridans	1	1.2	2.7	1	1.5

Total Number of Patients Cultured = 84
Total Number of Isolates = 65
Total Number of Cases* = 36

Total Number of Cultures = 700
Total Number of Patient Positives = 23

*Indicates number of different organisms isolated per patient, regardless of the number of times any one specific organism is isolated.

TABLE 9. Ten Most Frequent Organisms from Intravenous Catheters (1991)

Organism	Number of Patients Colonized	% Patients	Number of Isolates	% Total Isolates
Staphylococcus epidermidis	20	30.8	28	13.0
Group D *Enterococcus*	15	23.1	18	8.3
Staphylococcus aureus	11	16.9	14	6.5
Klebsiella pneumoniae	10	15.4	16	7.4
Pseudomonas aeruginosa	7	10.8	54	25.0
Enterobacter aerogenes	7	10.8	8	3.7
Proteus mirabilis	6	9.2	12	5.6
Serratia marcescens	5	7.7	17	7.9
Acinetobacter anitratus	5	7.7	8	3.7
Escherichia coli	5	7.7	7	3.2

Total Number of Patients Cultured = 65
Total Number of Isolates = 216

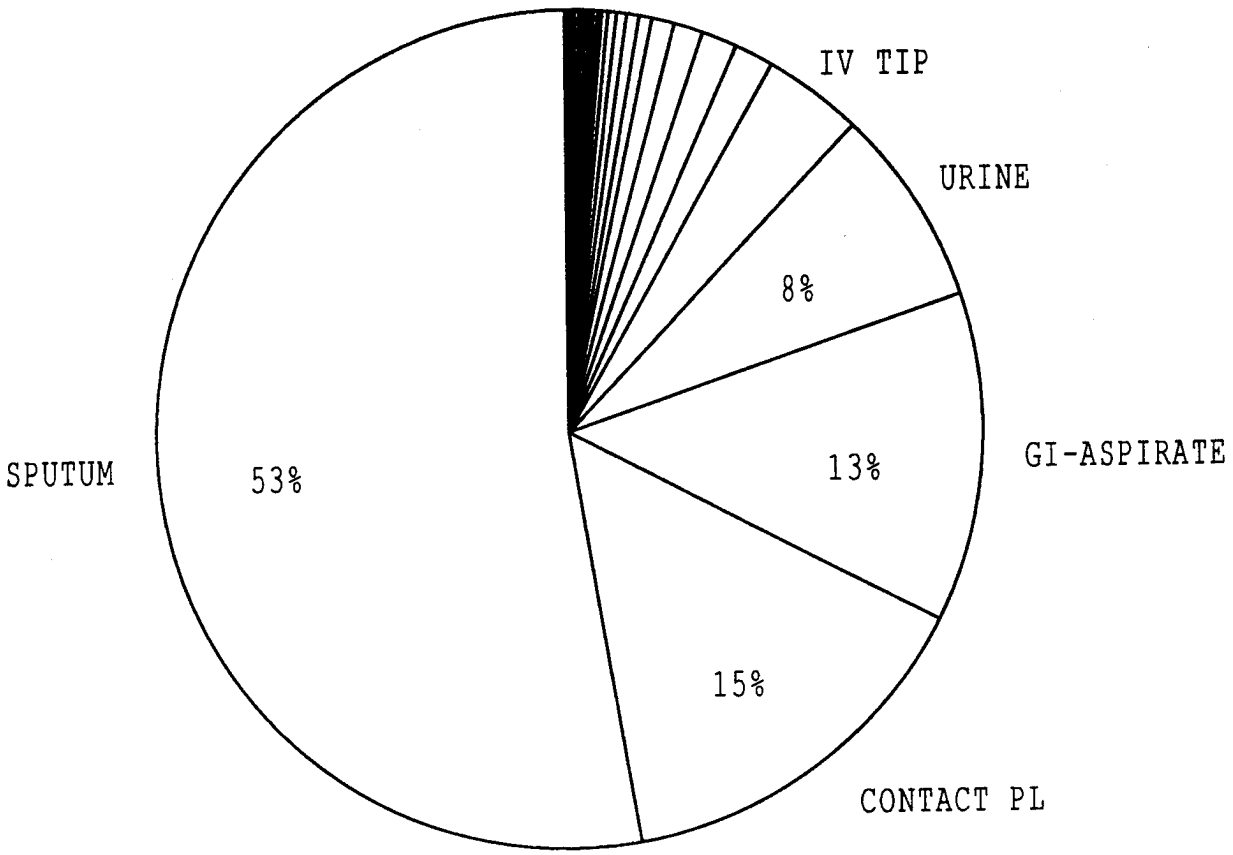


FIGURE 4. Display of the relative frequency of sources yielding organisms tested for in vitro sensitivity to antibiotics in 1991.

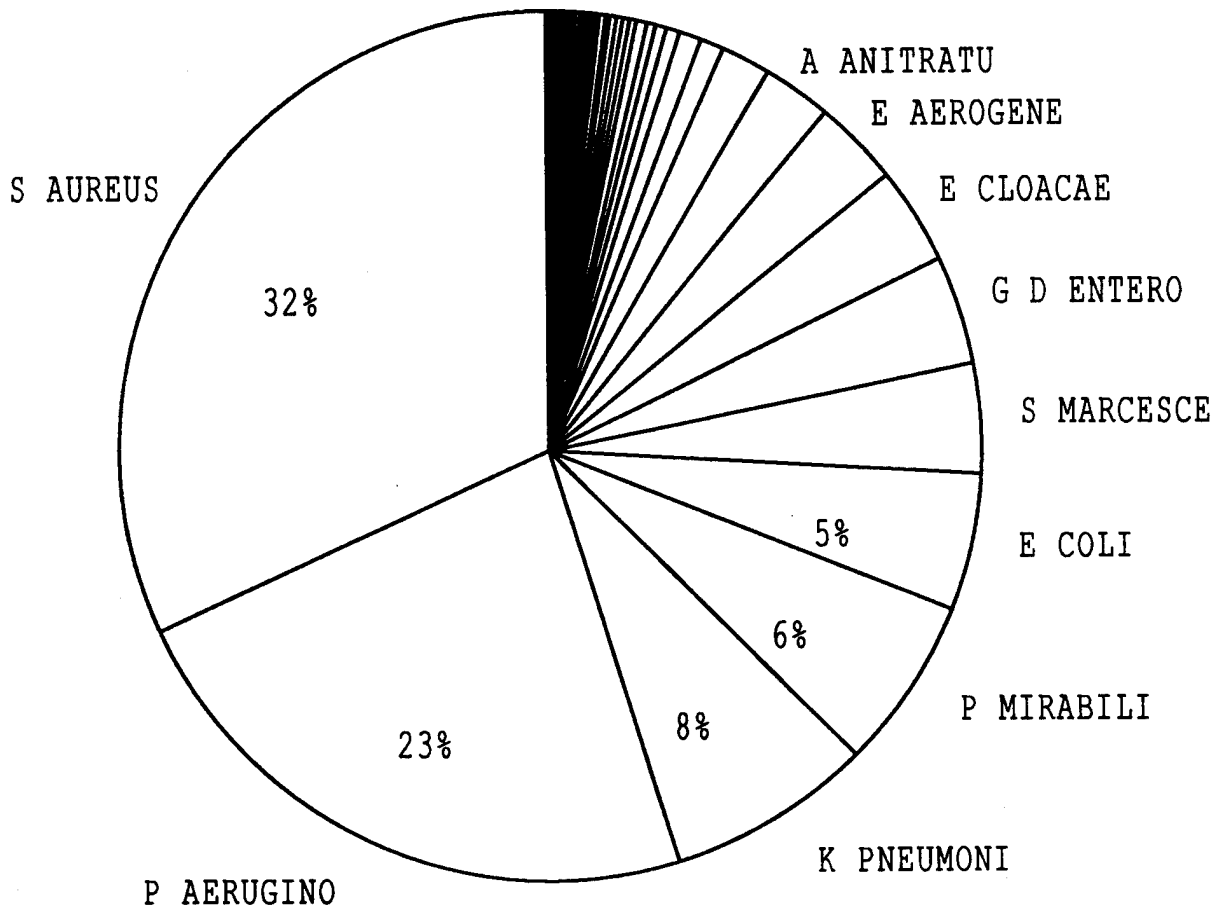


FIGURE 5. Display of the relative frequency of organisms tested for in vitro sensitivity to antibiotics in 1991.

Gentamicin resistance was again used as a plasmid surveillance marker. Testing was done on 3416 isolates. Figure 6 displays the relative frequency of tested organisms. Figure 7 displays the frequency of resistant species. *Staphylococcus aureus* represented 18.3% of the gentamicin-resistant isolates. Only 457 Gram-negative isolates of 2213 strains tested were resistant to gentamicin (20.7%). This continues to be a low percentage and is a direct marker of the success of infection control isolation techniques in preventing the accumulation of a resistant Gram-negative flora.

Staphylococcus aureus. The sources of *Staphylococcus aureus* strains tested for in vitro activity are presented in Figure 8. The incidence of multiply resistant *Staphylococcus aureus* decreased from 34% in 1990 to only 1.7% of isolates in 1991 while the incidence of oxicillin resistance remained high. The resistant strains are multiply resistant, with expression of gentamicin, erythromycin, oxacillin, and streptomycin resistance. Multiply resistant *Staphylococcus aureus* and gentamicin-sensitive strains are displayed separately in Table 10 and histograms are shown in Figure 9.

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

Klebsiella pneumoniae. A total of 240 isolates were tested for in vitro sensitivities to antibiotics. The sources of isolation for tested strains are presented in Figure 14. The results of in vitro antibiotic testing are presented in Table 12. Histogram displays of the distributions of zone sizes for selected antibiotics are presented in Figure 15.

Proteus mirabilis. A total of 205 isolates were tested for in vitro sensitivities to antibiotics. The sources of isolation for tested strains are presented in Figure 16. The results of in vitro antibiotic testing are presented in Table 13. Histogram displays of the distributions of zone sizes for selected antibiotics are presented in Figure 17.

Escherichia coli. A total of 177 isolates were tested for in vitro sensitivity to antibiotics. The sources of isolation for tested strains are presented in Figure 18. The results of in vitro antibiotic testing are presented in Table 14. Histogram displays of the distributions of zone sizes for selected antibiotics are presented in Figure 19.

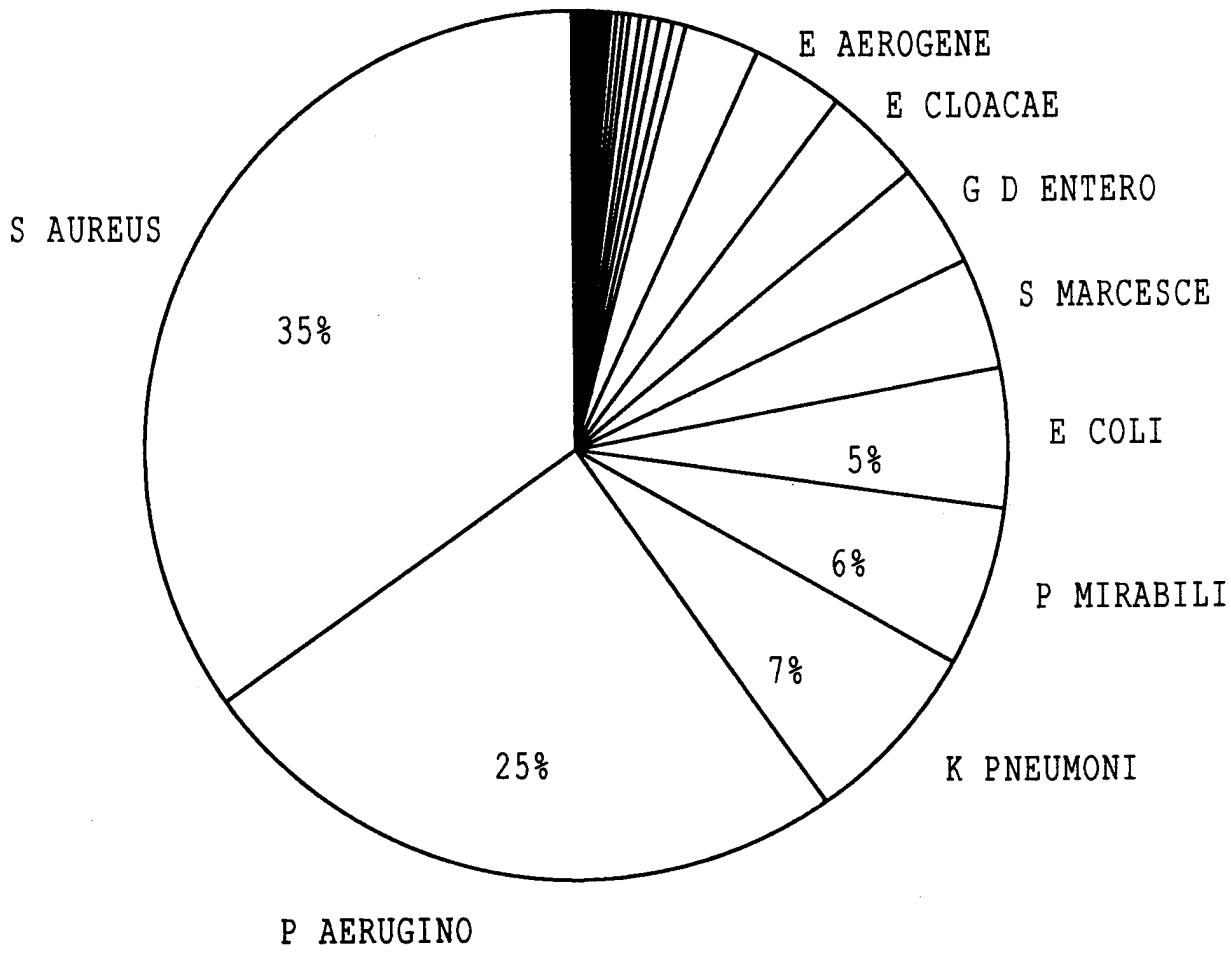


FIGURE 6. Display of the relative frequency of organisms tested for in vitro sensitivity to gentamicin in 1991.

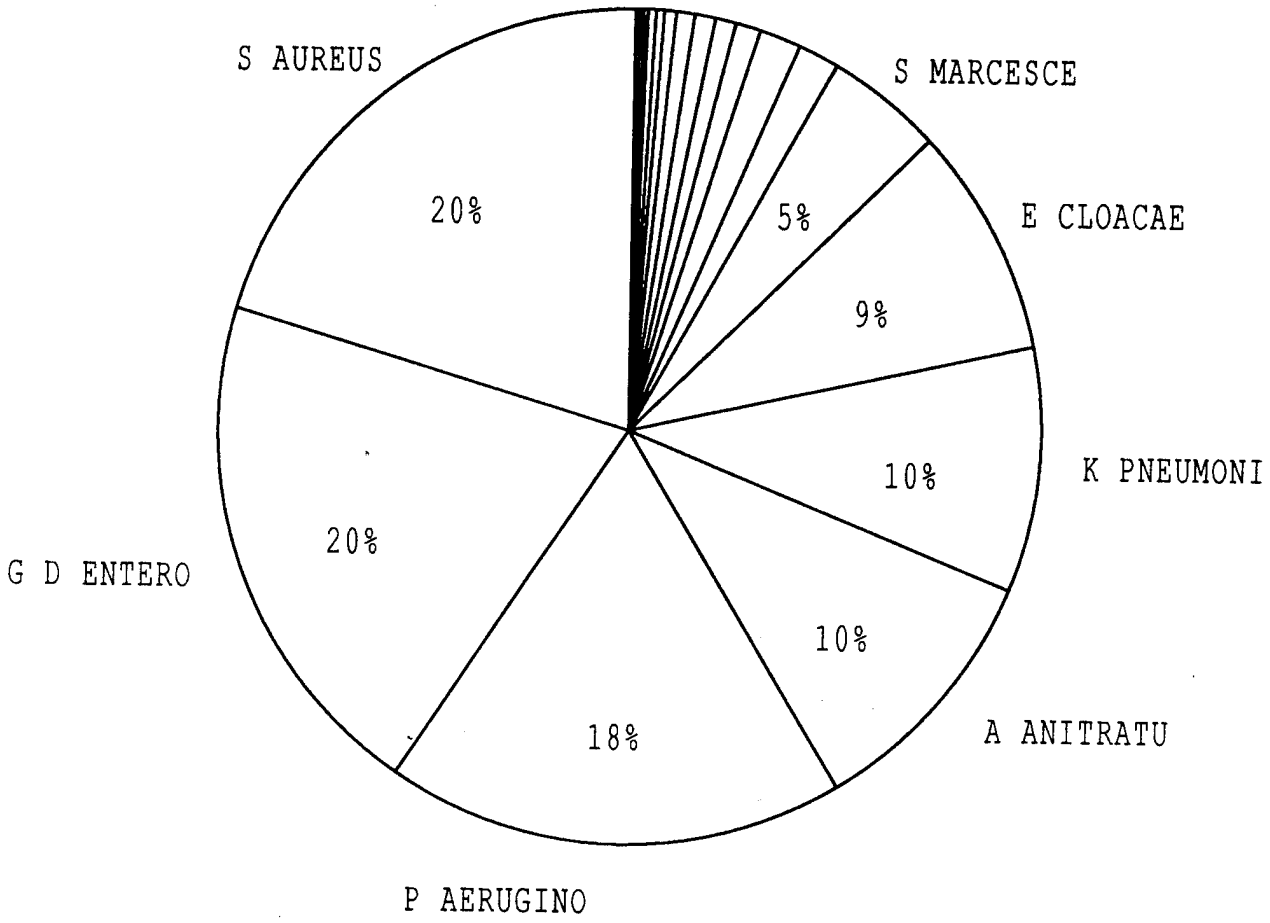


FIGURE 7. Display of the relative frequency of gentamicin-resistant organisms isolated in 1991.

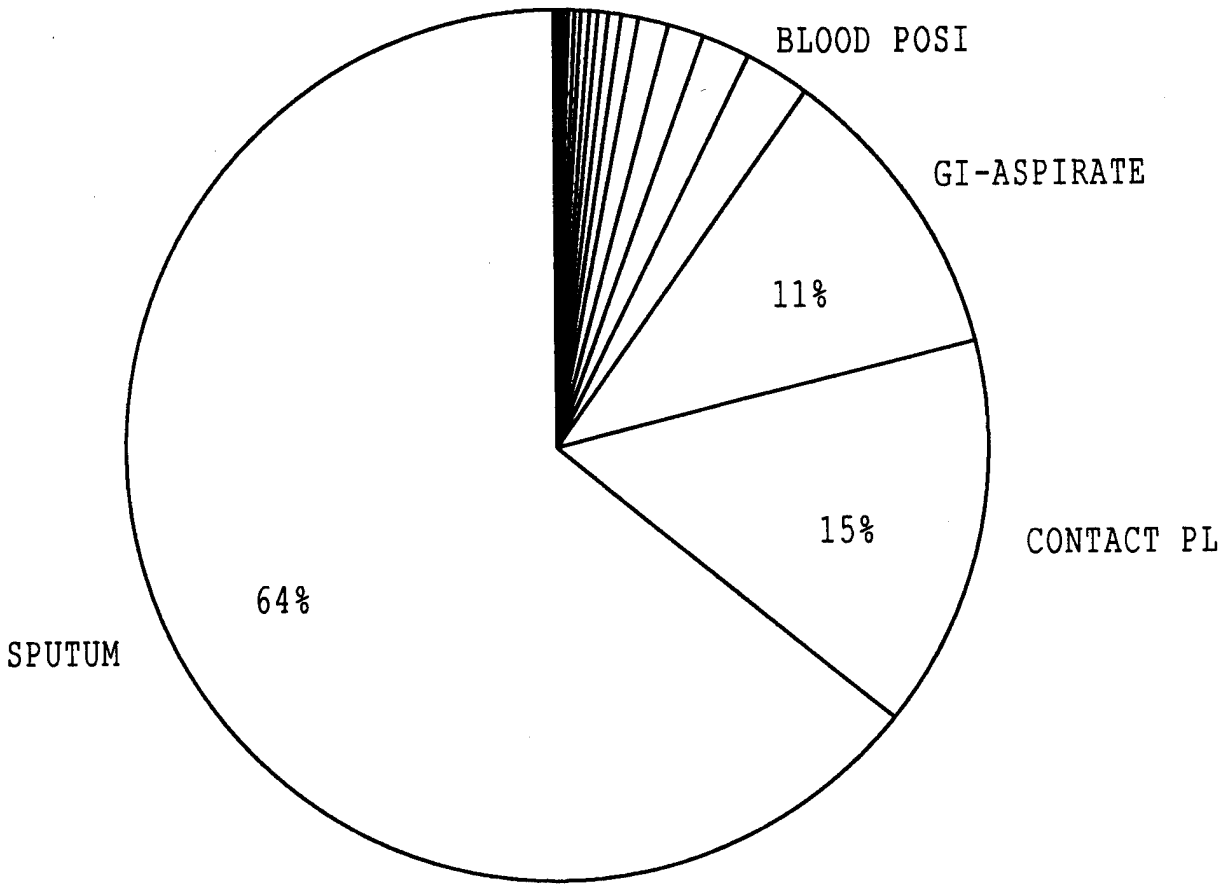


FIGURE 8. Display of the relative frequency of sources yielding *Staphylococcus aureus* tested for in vitro sensitivity to antibiotics in 1991.

TABLE 10A. Antibiotic Sensitivity Data for *Staphylococcus aureus* Sensitive to Gentamicin (1991)

Antibiotic	RESISTANT		INTERMEDIATE		SENSITIVE		Total
	%	Number	%	Number	%	Number	Number
Amikacin sulfate	3.98	42	25.14	265	70.87	747	1054
Ampicillin sodium	30.32	319	4.47	47	65.21	686	1052
Cefoperazone sodium	31.65	307	1.55	15	66.80	648	970
Cefotaxime sodium	29.94	315	0.76	8	69.30	729	1052
Cefsulodin	–	–	–	–	100.00	4	4
Ceftazidime	30.46	321	5.12	54	64.42	679	1054
Ceftriaxone sodium	–	–	–	–	100.00	1052	1052
Cephalothin	30.07	316	0.38	4	69.55	731	1051
Chloramphenicol palmitate	0.38	4	0.19	2	99.43	1048	1054
Ciprofloxacin	–	–	–	–	100.00	91	91
Clindamycin hydrochloride	28.83	292	0.69	7	70.48	714	1013
Erythromycin	36.91	389	0.47	5	62.62	660	1054
Gentamicin sulfate	–	–	–	–	100.00	1054	1054
Imipenem-cilastatin sodium	20.23	213	1.33	14	78.44	826	1053
Methicillin sodium	40.00	2	–	–	60.00	3	5
Mezlocillin sodium	30.70	323	20.44	215	48.86	514	1052
Moxalactam	31.72	334	14.06	148	54.23	571	1053
Mupirocin	–	–	–	–	100.00	83	83
Oxacillin sodium	31.62	332	0.86	9	67.52	709	1050
Penicillin	90.70	956	5.88	62	3.42	36	1054
Piperacillin sodium	30.45	320	21.03	221	48.53	510	1051
Streptomycin sulfate	3.61	38	43.36	457	53.04	559	1054
Sulfadiazine	7.68	80	4.99	52	87.33	910	1042
Tetracycline hydrochloride	3.23	34	1.52	16	95.26	1004	1054
Tobramycin sulfate	29.93	305	1.08	11	68.99	703	1019
Vancomycin hydrochloride	0.47	5	–	–	99.53	1048	1053

TABLE 10B. Antibiotic Sensitivity Data for *Staphylococcus aureus* Resistant to Gentamicin (1991)

Antibiotic	RESISTANT		INTERMEDIATE		SENSITIVE		Total
	%	Number	%	Number	%	Number	Number
Amikacin sulfate	60.74	82	33.33	45	5.93	8	135
Ampicillin sodium	70.37	95	5.19	7	24.44	33	135
Cefoperazone sodium	13.18	17	49.61	64	37.21	48	129
Cefotaxime sodium	31.11	42	42.96	58	25.93	35	135
Ceftazidime	54.81	74	28.89	39	16.30	22	135
Ceftriaxone sodium	–	–	–	–	100.00	135	135
Cephalothin	9.63	13	2.96	4	87.41	118	135
Chloramphenicol palmitate	3.70	5	0.74	1	95.56	129	135
Ciprofloxacin	–	–	–	–	100.00	9	9
Clindamycin hydrochloride	14.07	19	–	–	85.93	116	135
Erythromycin	18.52	25	1.48	2	80.00	108	135
Gentamicin sulfate	93.28	125	6.72	9	–	–	134
Imipenem-cilastatin sodium	3.70	5	0.74	1	95.56	129	135
Mezlocillin sodium	73.33	99	8.15	11	18.52	25	135
Moxalactam	80.74	109	8.15	11	11.11	15	135
Mupirocin	–	–	–	–	100.00	9	9
Oxacillin sodium	74.81	101	3.70	5	21.48	29	135
Penicillin	94.07	127	2.96	4	2.96	4	135
Piperacillin sodium	72.59	98	6.67	9	20.74	28	135
Streptomycin sulfate	87.41	118	7.41	10	5.19	7	135
Sulfadiazine	78.79	104	4.55	6	16.67	22	132
Tetracycline hydrochloride	11.11	15	2.22	3	86.67	117	135
Tobramycin sulfate	96.99	129	1.50	2	1.50	2	133
Vancomycin hydrochloride	–	–	–	–	100.00	134	134

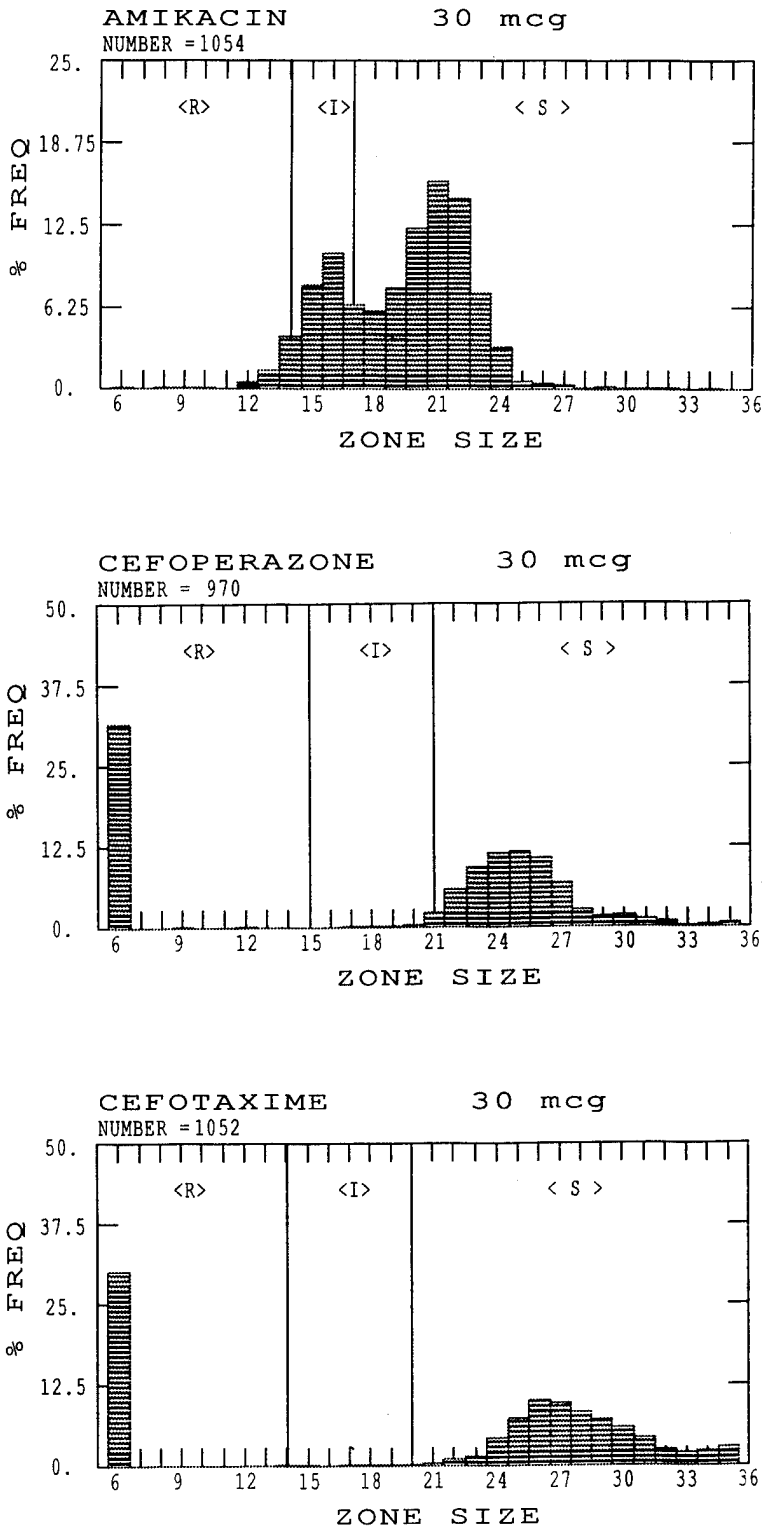
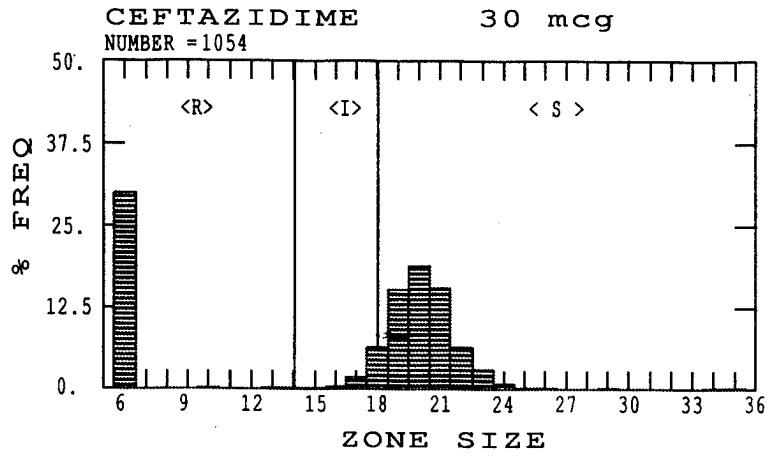
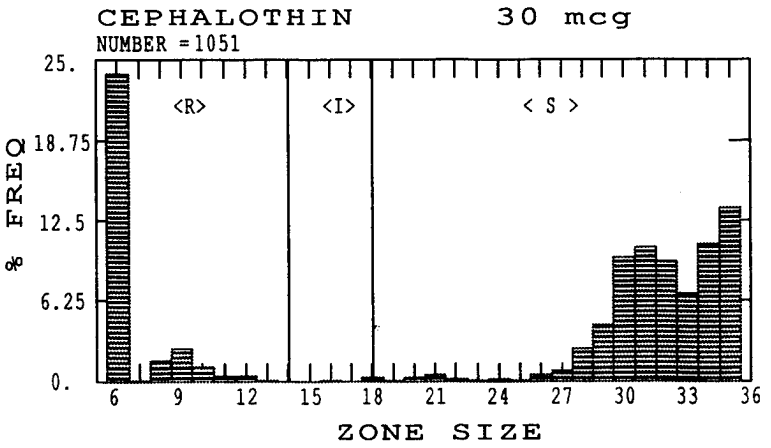


FIGURE 9A. Histogram display of the distribution of zones of inhibition of growth of multiply-resistant *Staphylococcus aureus.*





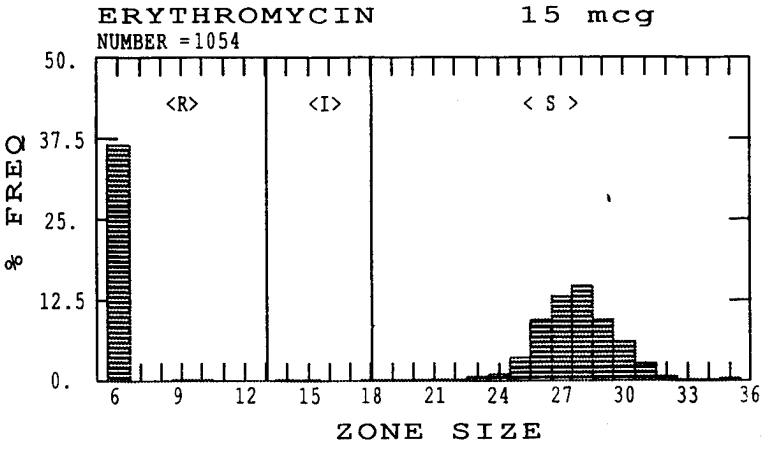
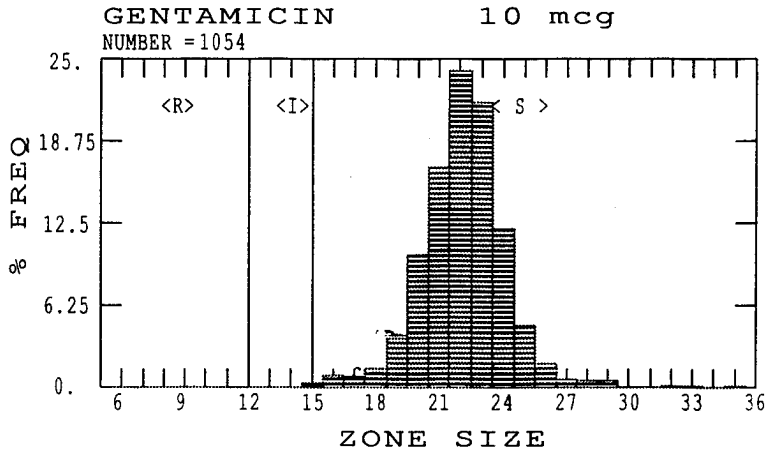
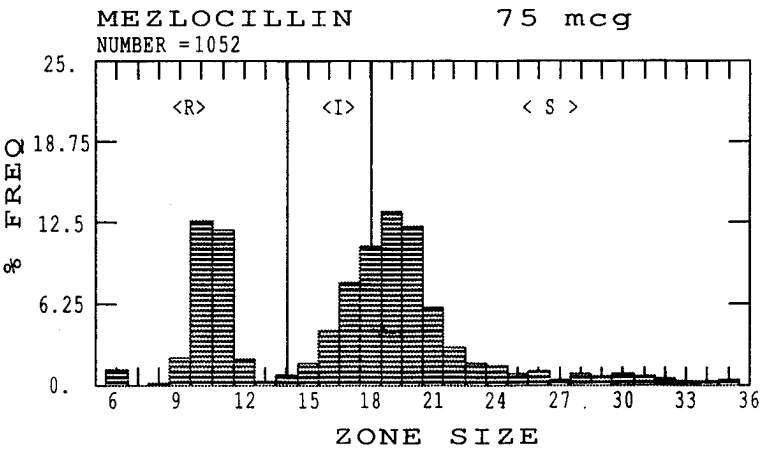


FIGURE 9A. Histogram display of the distribution of zones of inhibition of growth of multiply-resistant *Staphylococcus aureus* (continued).





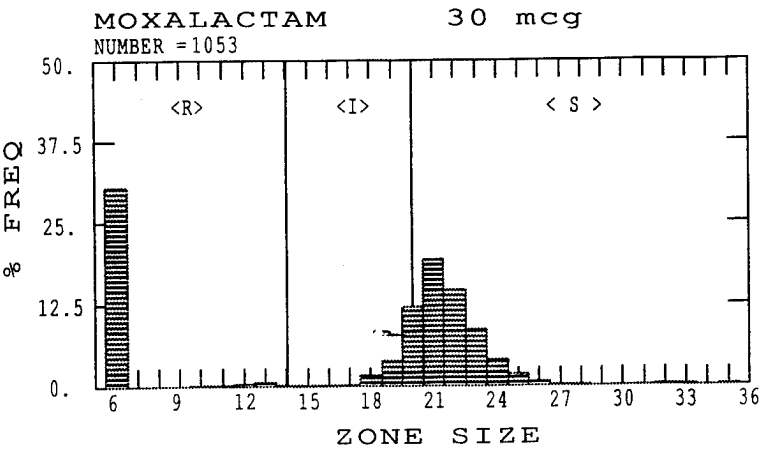


FIGURE 9A. Histogram display of the distribution of zones of inhibition of growth of multiply-resistant *Staphylococcus aureus* (continued).

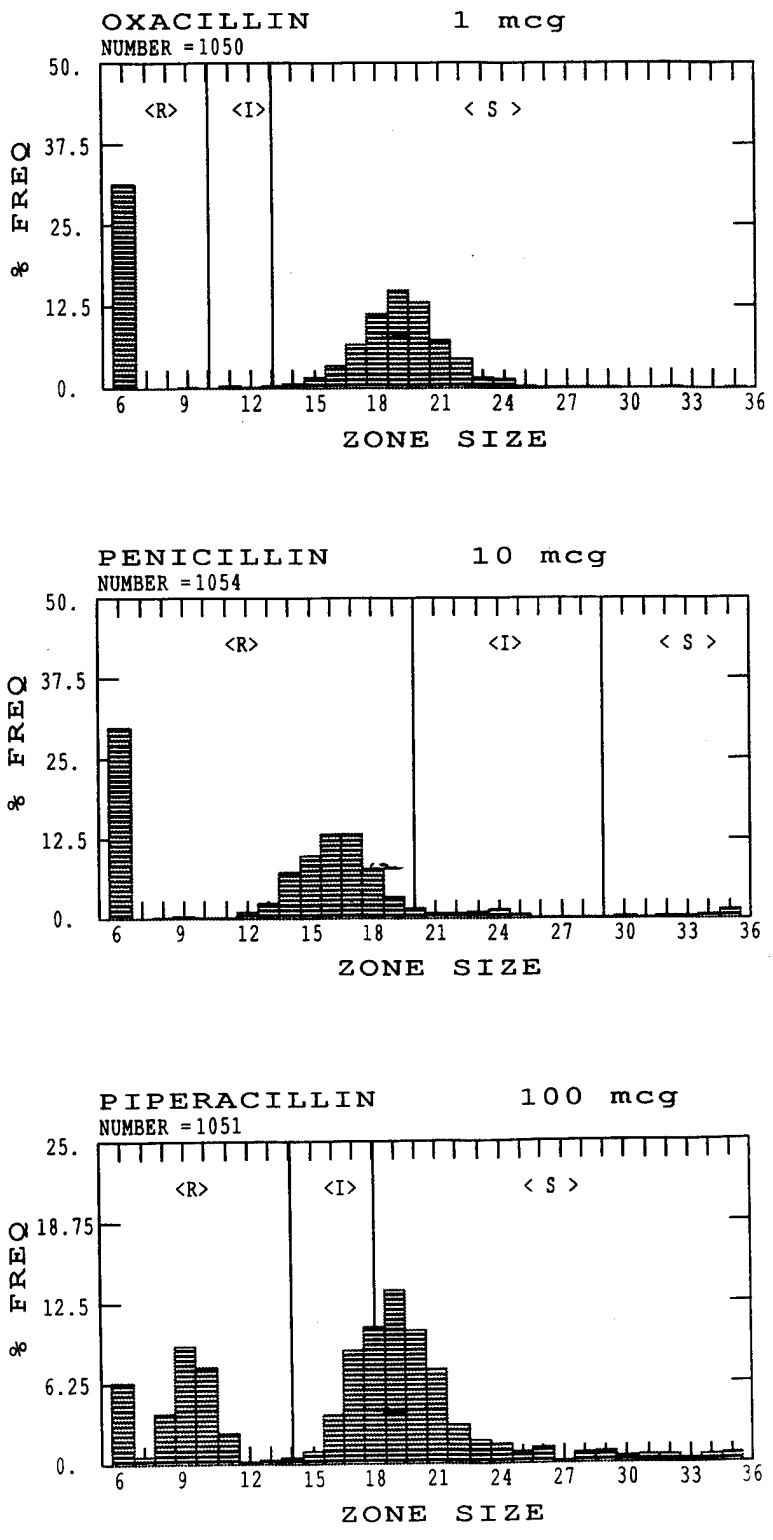
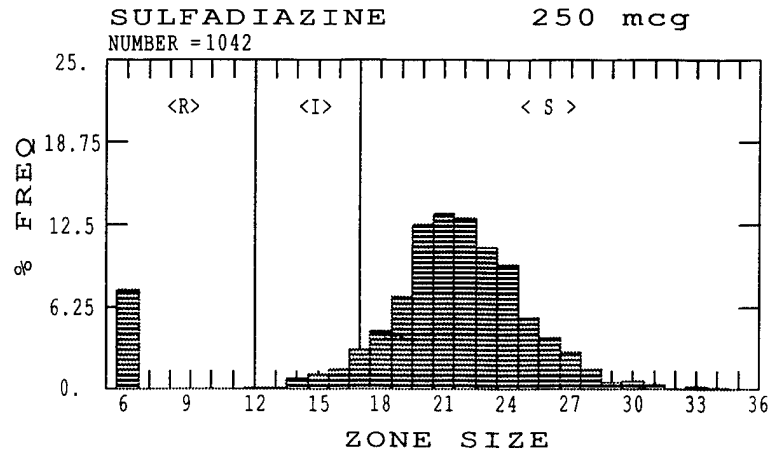
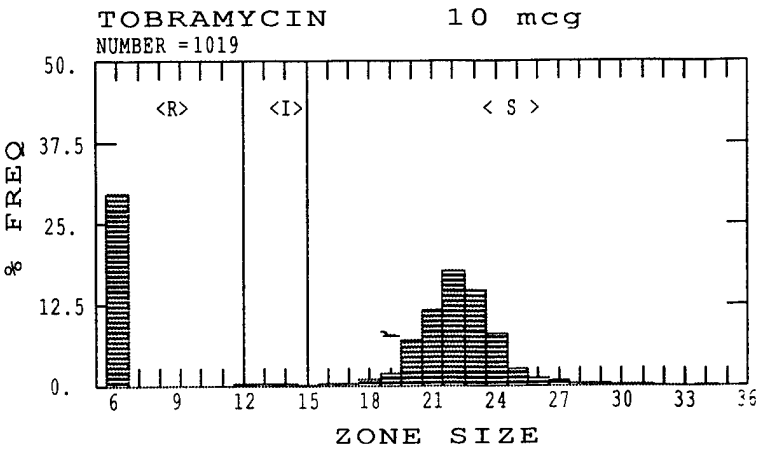


FIGURE 9A. Histogram display of the distribution of zones of inhibition of growth of multiply-resistant *Staphylococcus aureus* (continued).





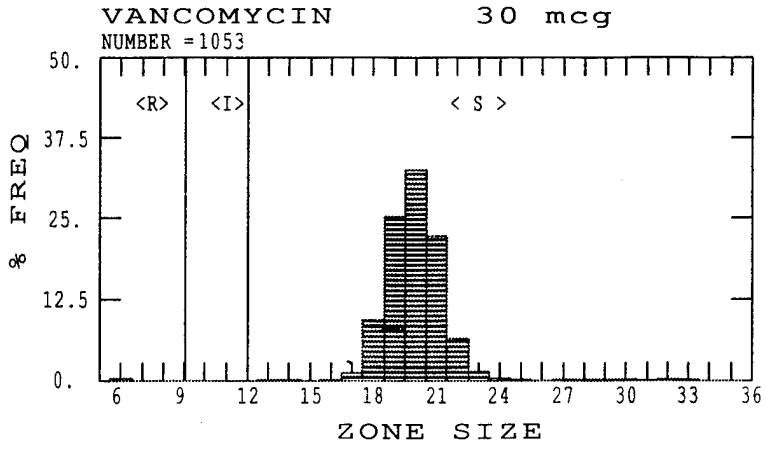


FIGURE 9A. Histogram display of the distribution of zones of inhibition of growth of multiply-resistant *Staphylococcus aureus* (continued).

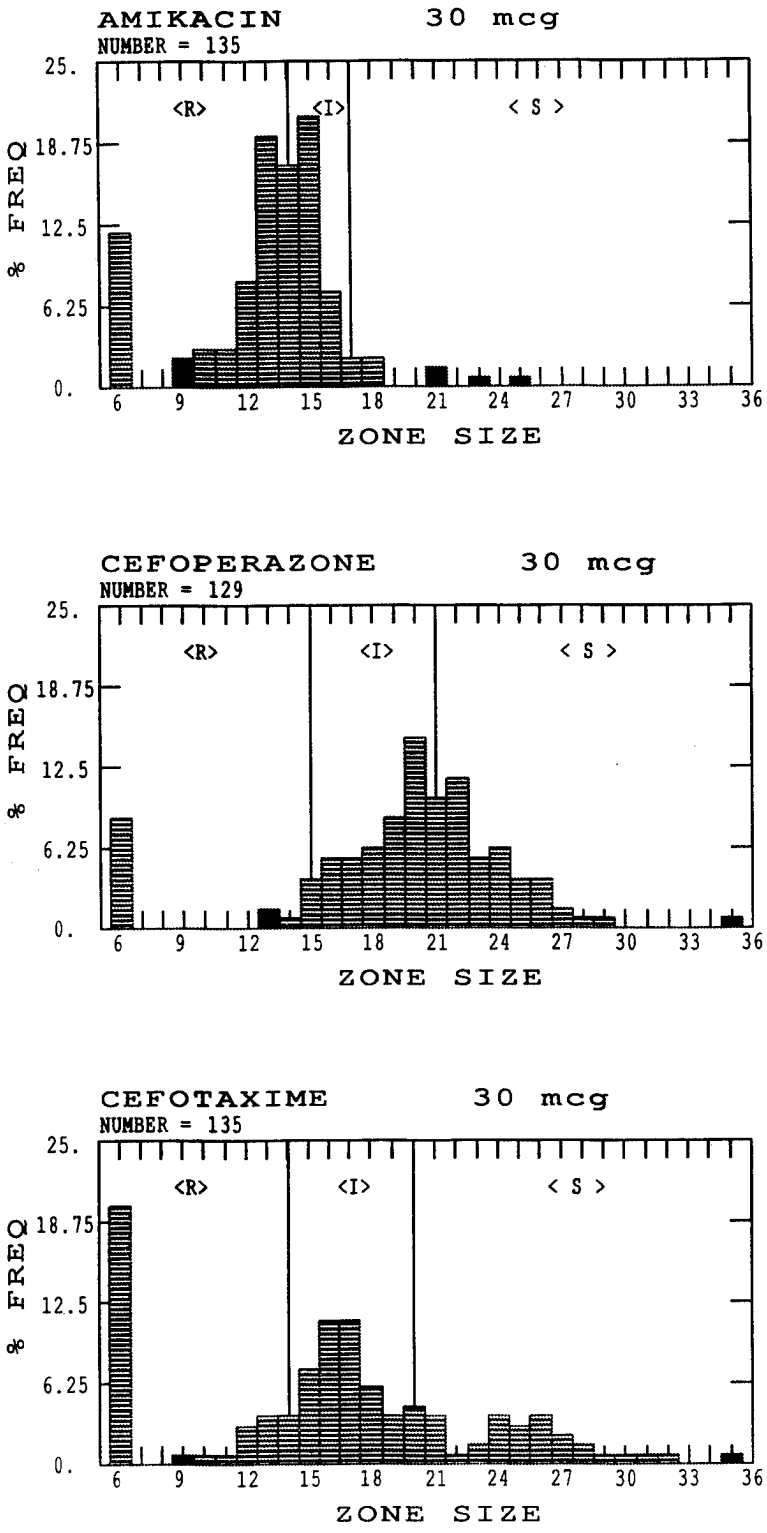
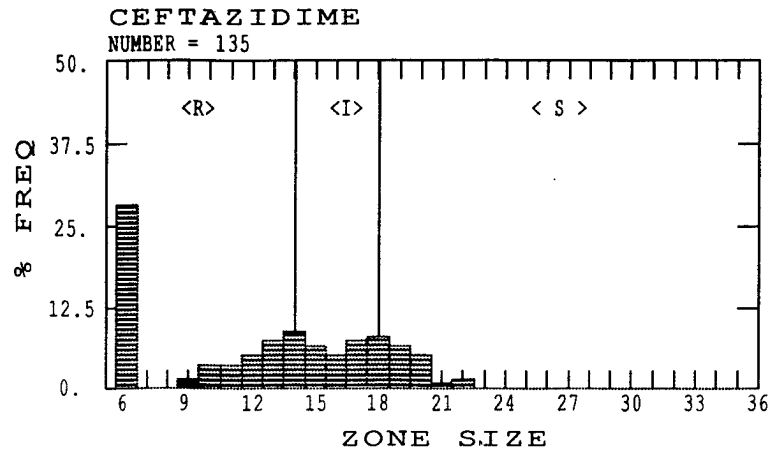
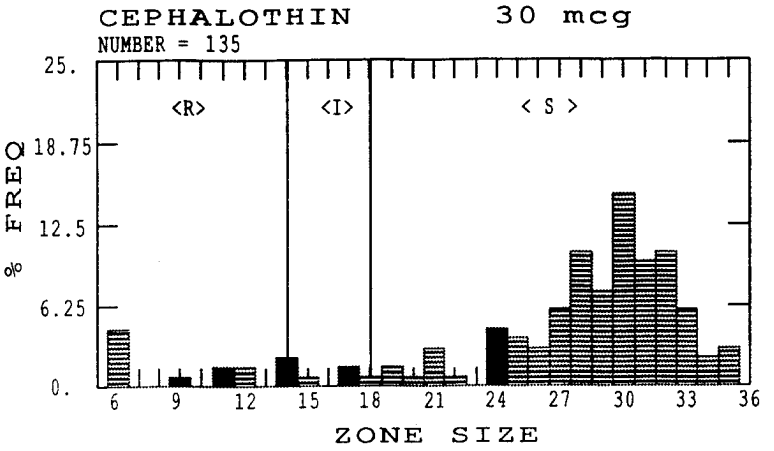


FIGURE 9B. Histogram display of the distribution of zones of inhibition of growth of multiply-sensitive *Staphylococcus aureus*.





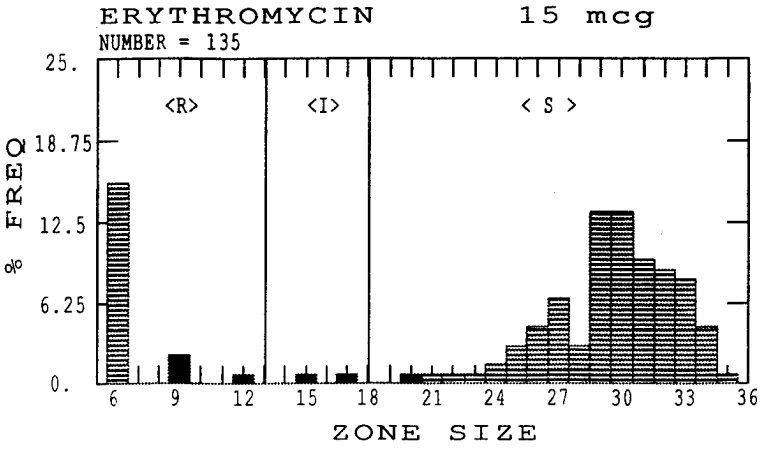
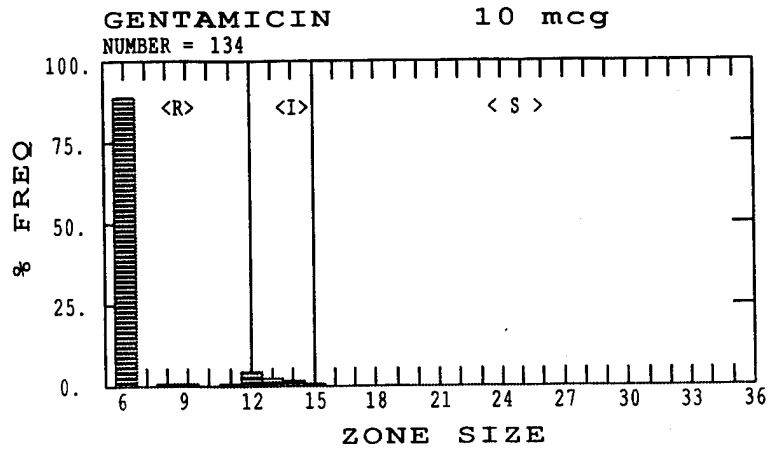
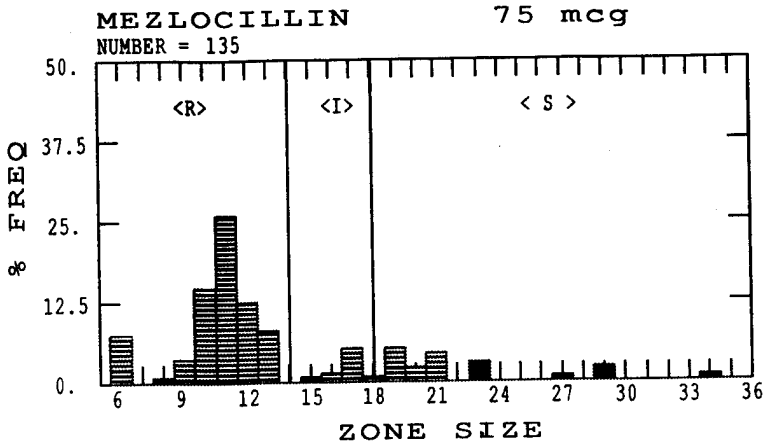


FIGURE 9B. Histogram display of the distribution of zones of inhibition of growth of multiply-sensitive *Staphylococcus aureus* (continued).





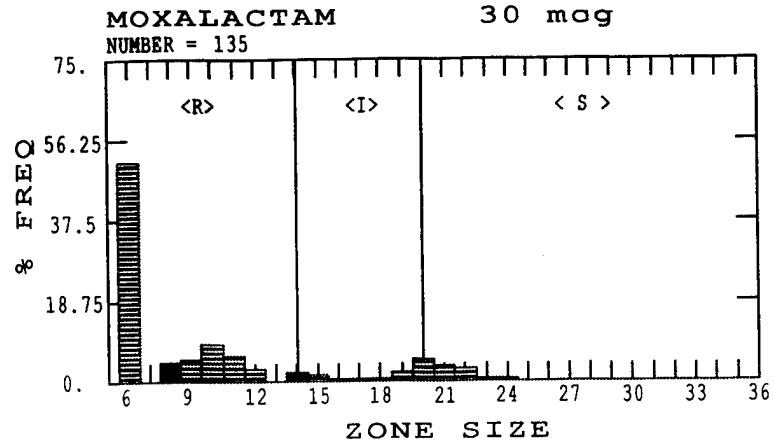
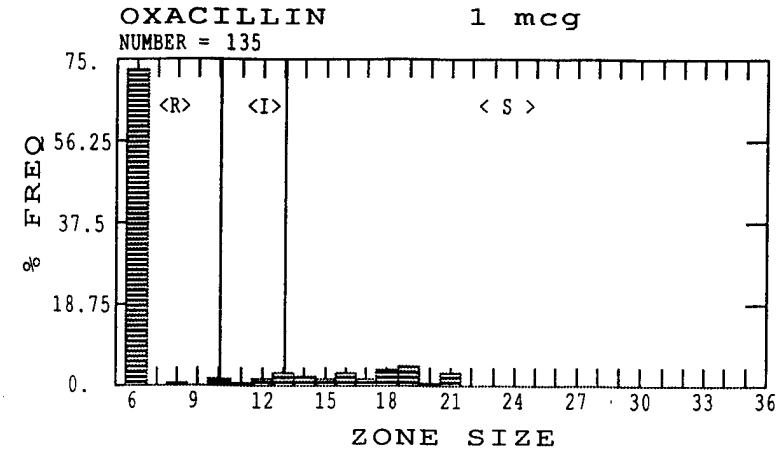
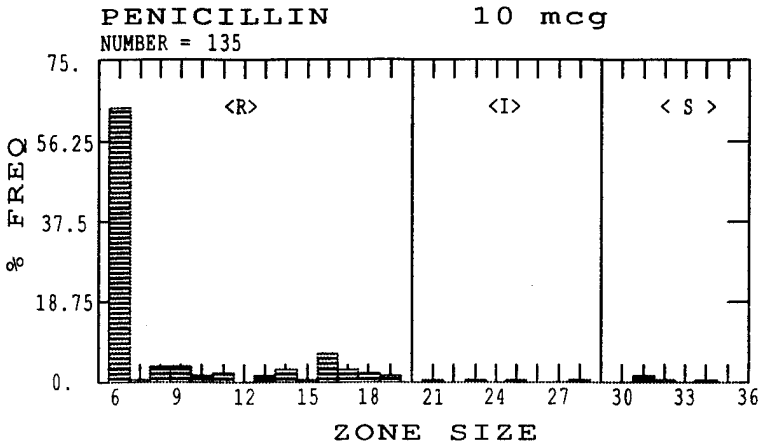


FIGURE 9B. Histogram display of the distribution of zones of inhibition of growth of multiply-sensitive *Staphylococcus aureus* (continued).





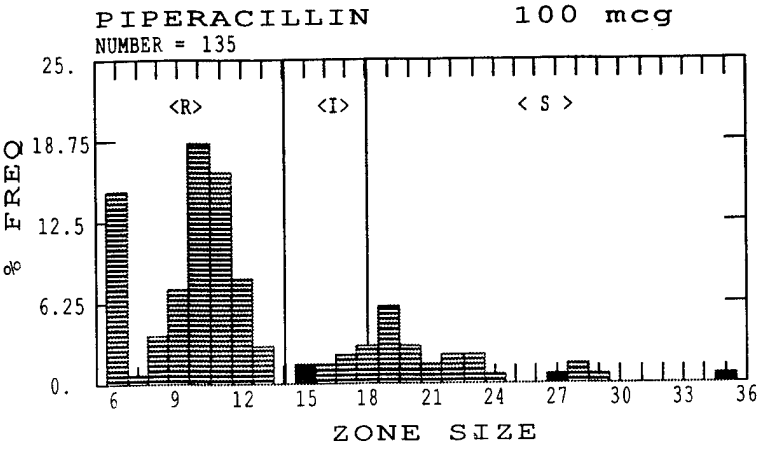


FIGURE 9B. Histogram display of the distribution of zones of inhibition of growth of multiply-sensitive *Staphylococcus aureus* (continued).

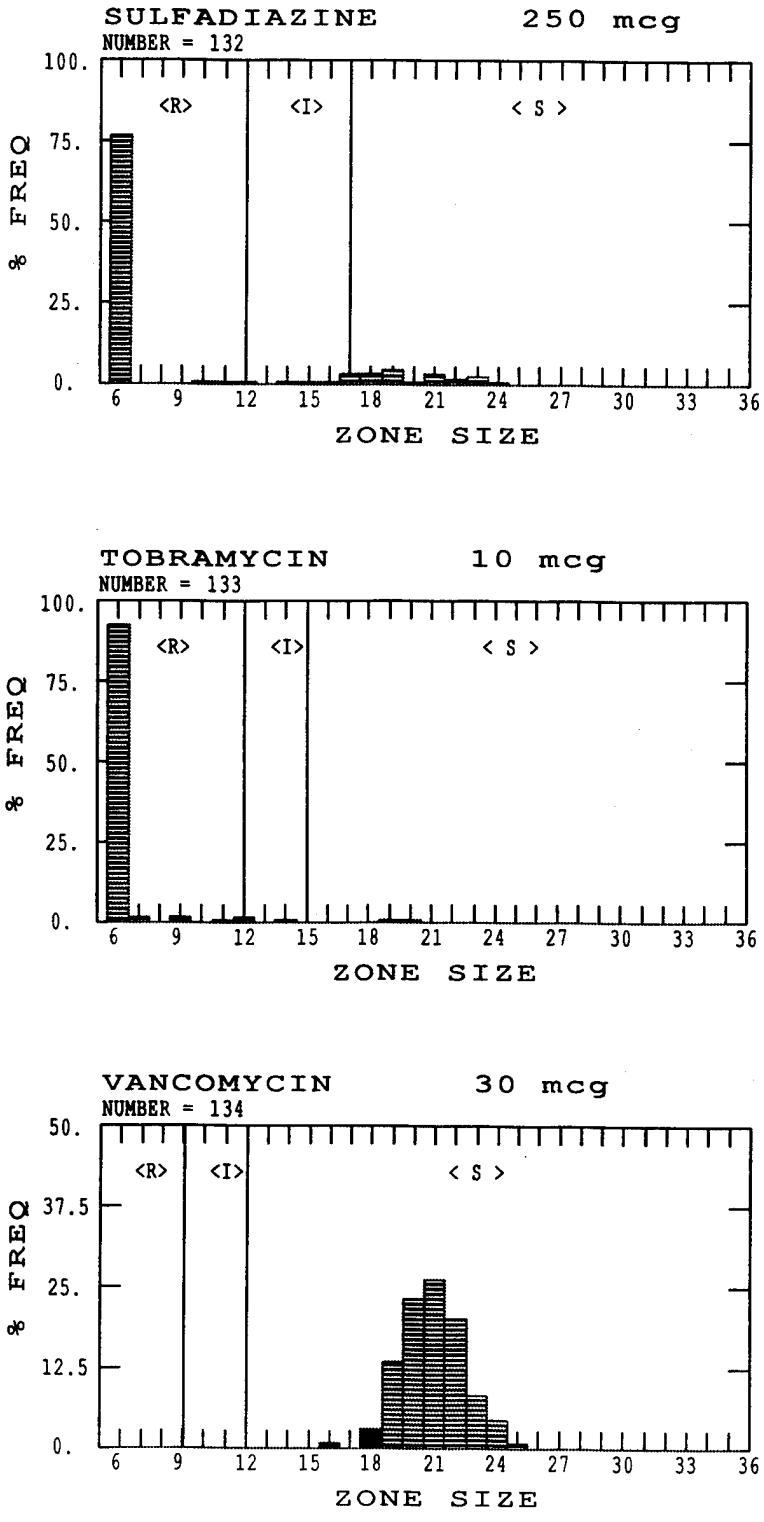


FIGURE 9B. Histogram display of the distribution of zones of inhibition of growth of multiply-sensitive *Staphylococcus aureus* (continued).

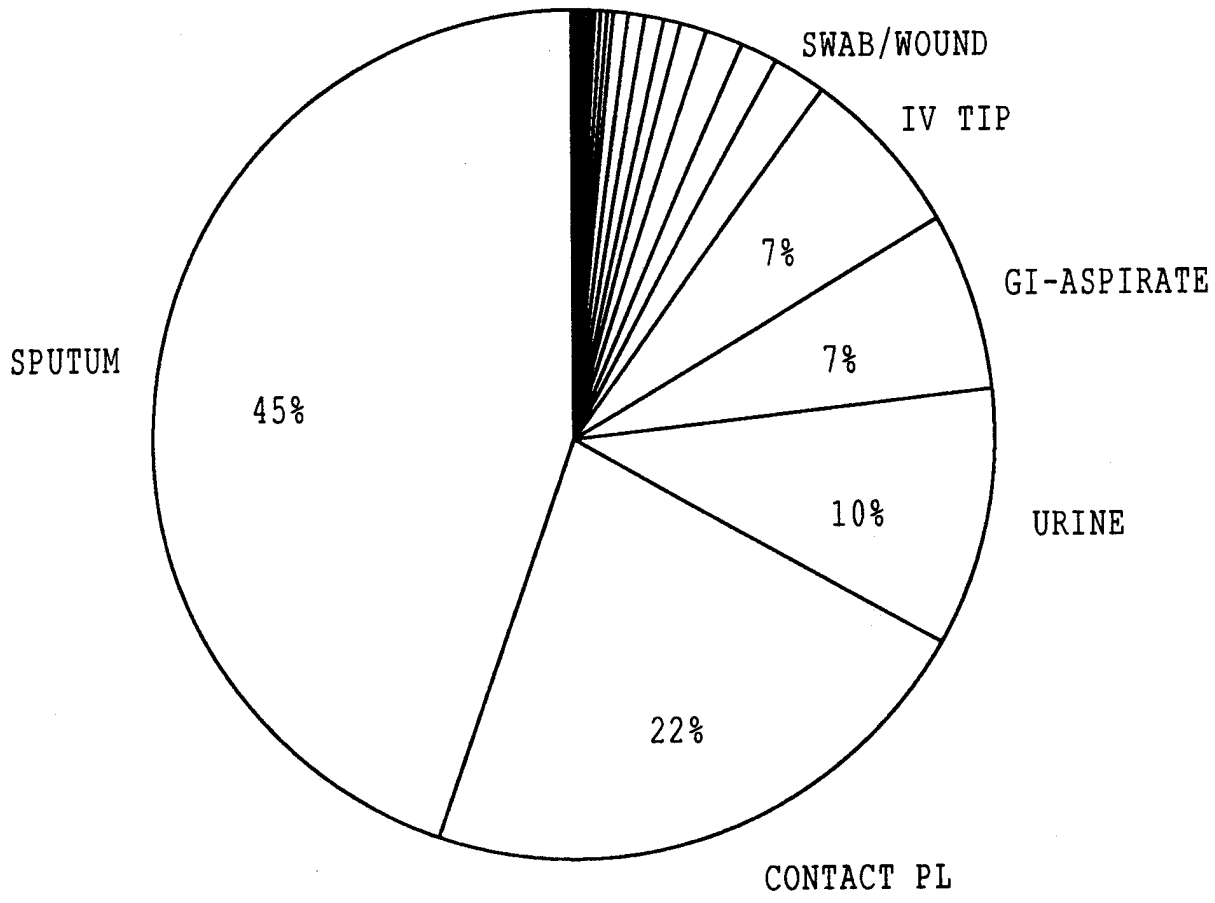


FIGURE 10. Display of the relative frequency of sources yielding *Pseudomonas aeruginosa* tested for in vitro sensitivity to antibiotics in 1991.

TABLE 11. Antibiotic Sensitivity Data for *Pseudomonas aeruginosa* (1991)

Antibiotic	RESISTANT		INTERMEDIATE		SENSITIVE		Total
	%	Number	%	Number	%	Number	Number
Amikacin sulfate	10.25	85	8.93	74	80.82	670	829
Azlocillin sodium	48.37	401	8.44	70	43.18	358	829
Aztreonam	45.14	367	25.95	211	28.91	235	813
Cefoperazone sodium	45.29	317	13.86	97	40.86	286	700
Cefotaxime sodium	71.62	593	26.45	219	1.93	16	828
Cefsulodin	50.00	1	-	-	50.00	1	2
Ceftazidime	44.31	366	4.84	40	50.85	420	826
Ceftriaxone sodium	-	-	-	-	100.00	827	827
Chloramphenicol palmitate	87.82	728	11.94	99	0.24	2	829
Ciprofloxacin	-	-	-	-	100.00	115	115
Colistin sulfate	0.70	5	0.14	1	99.16	707	713
Gentamicin sulfate	12.70	105	10.40	86	76.90	636	827
Imipenem-cilastatin sodium	4.23	35	3.26	27	92.51	766	828
Kanamycin sulfate	98.55	817	1.09	9	0.36	3	829
Mezlocillin sodium	56.60	465	16.28	134	27.22	224	823
Moxlactam	55.97	464	34.02	282	10.01	83	829
Mupirocin	-	-	-	-	100.00	115	115
Netilmicin sulfate	10.01	83	4.70	39	85.28	707	829
Norfloxacin	1.11	9	2.96	24	95.93	778	811
Piperacillin sodium	45.83	379	2.42	20	51.75	428	827
Sulfadiazine	44.90	361	21.02	169	34.08	274	804
Tetracycline hydrochloride	50.85	421	44.57	369	4.59	38	828
Ticarcillin disodium	46.14	382	6.16	51	47.71	395	828
TIM-85	43.00	356	8.82	73	48.19	399	828
Tobramycin sulfate	8.33	69	2.78	23	88.89	736	828

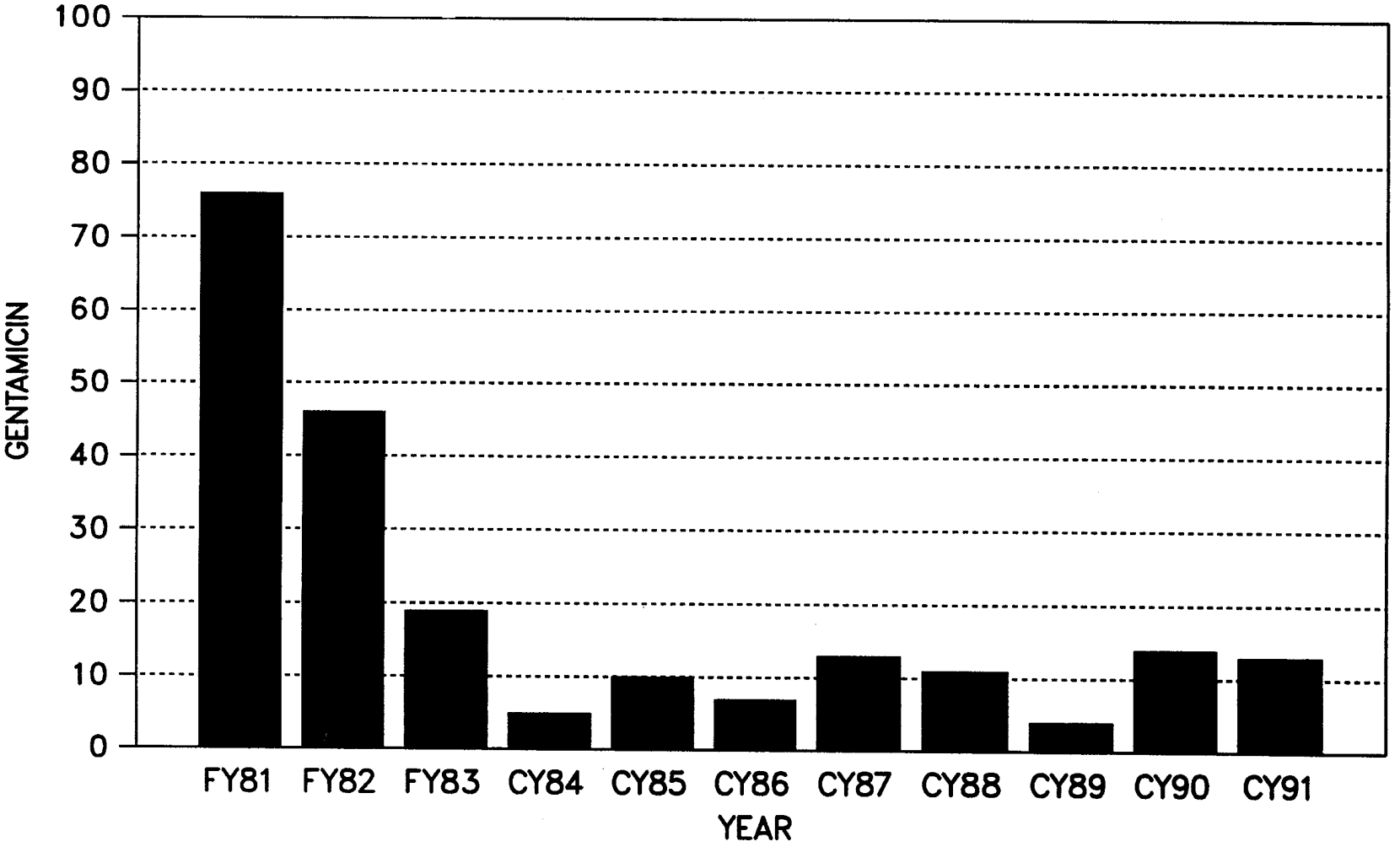


FIGURE 11. Relative frequency (%) of *Pseudomonas aeruginosa* resistant to gentamicin for fiscal years 1981-3 and calendar years 1984-91.

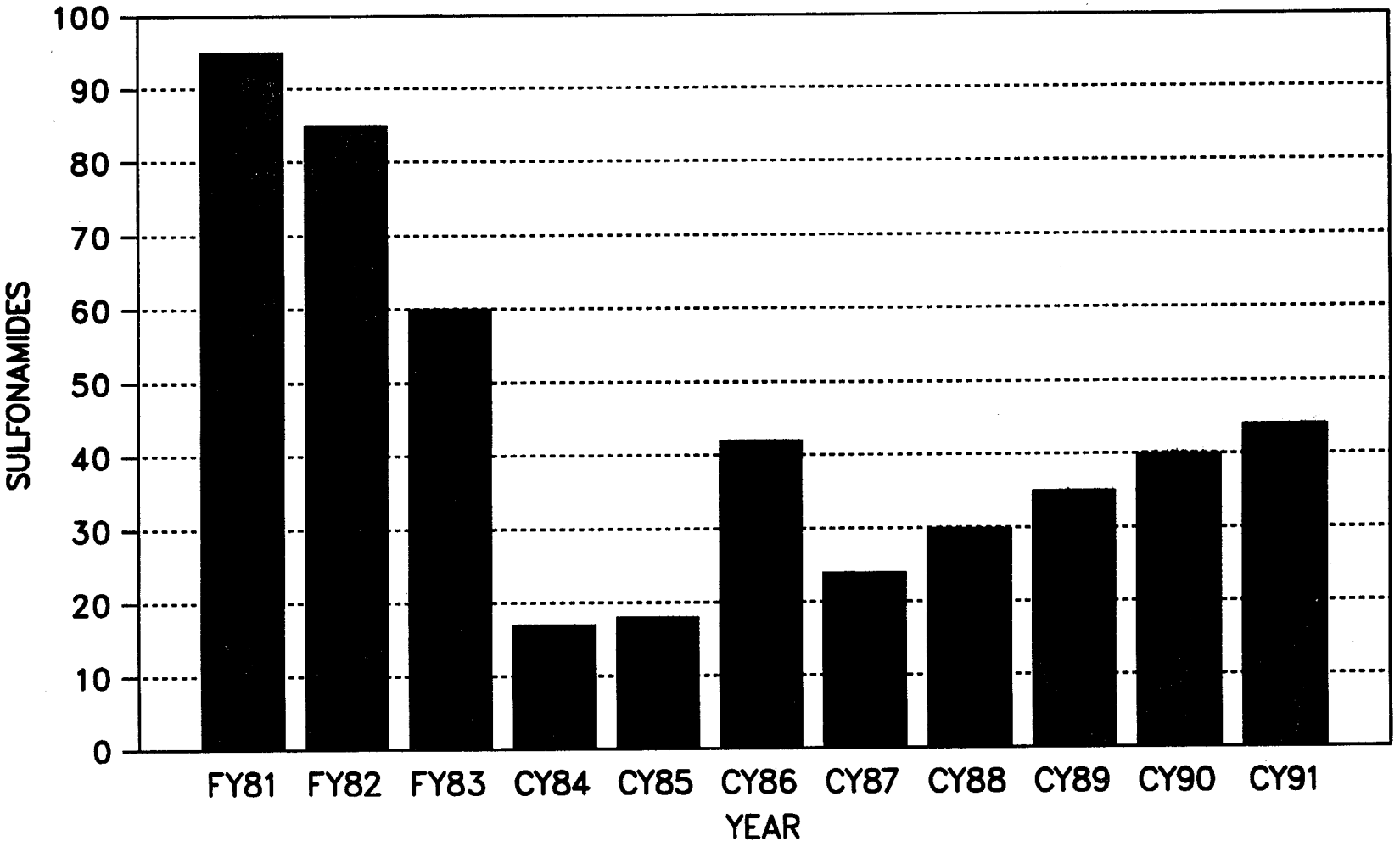


FIGURE 12. Relative frequency (%) of *Pseudomonas aeruginosa* resistance to sulfonamides for fiscal years 1981-3 and calendar years 1984-91.

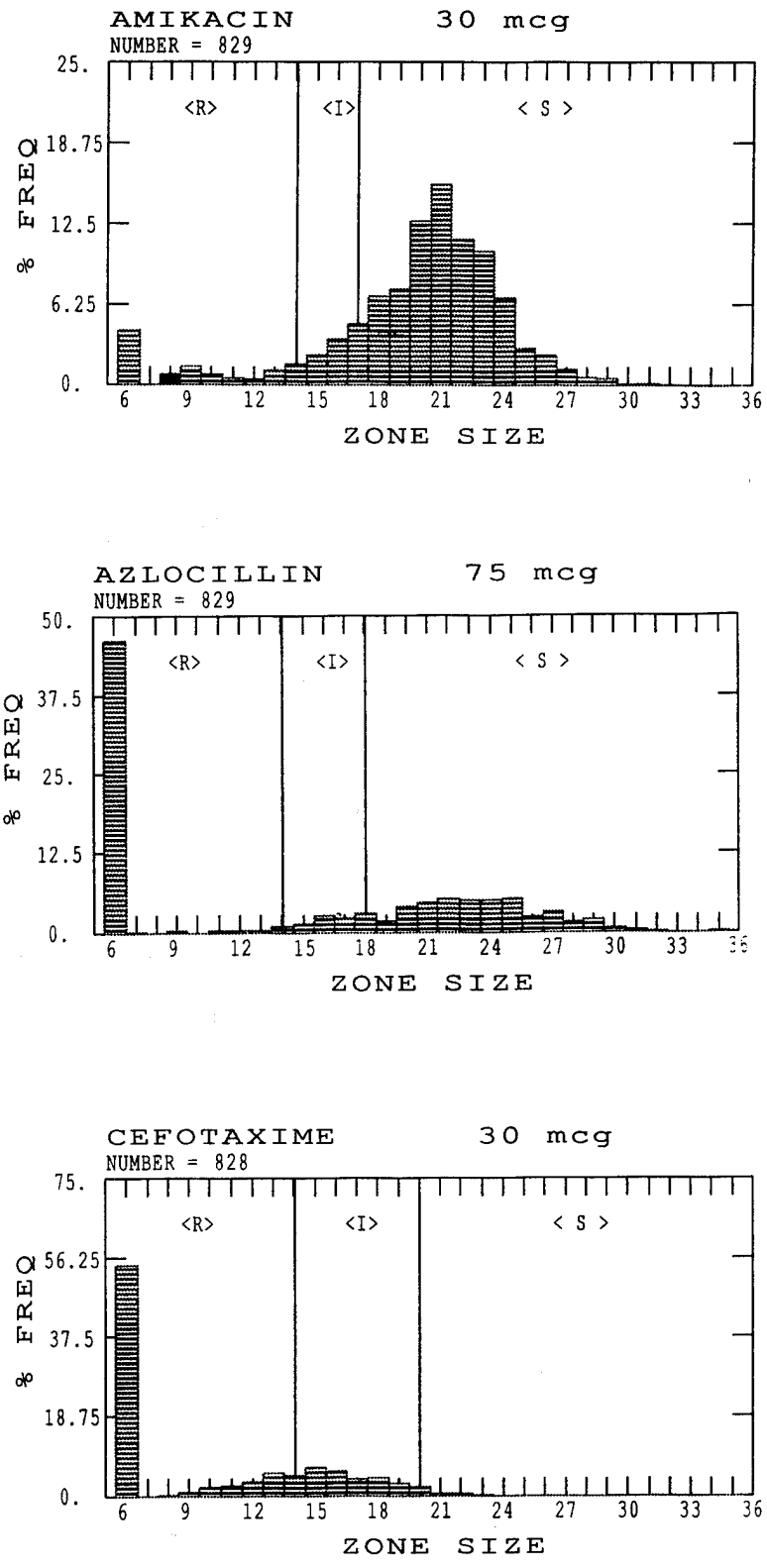


FIGURE 13. Histogram display of the distribution of zones of inhibition of growth of *Pseudomonas aeruginosa*.

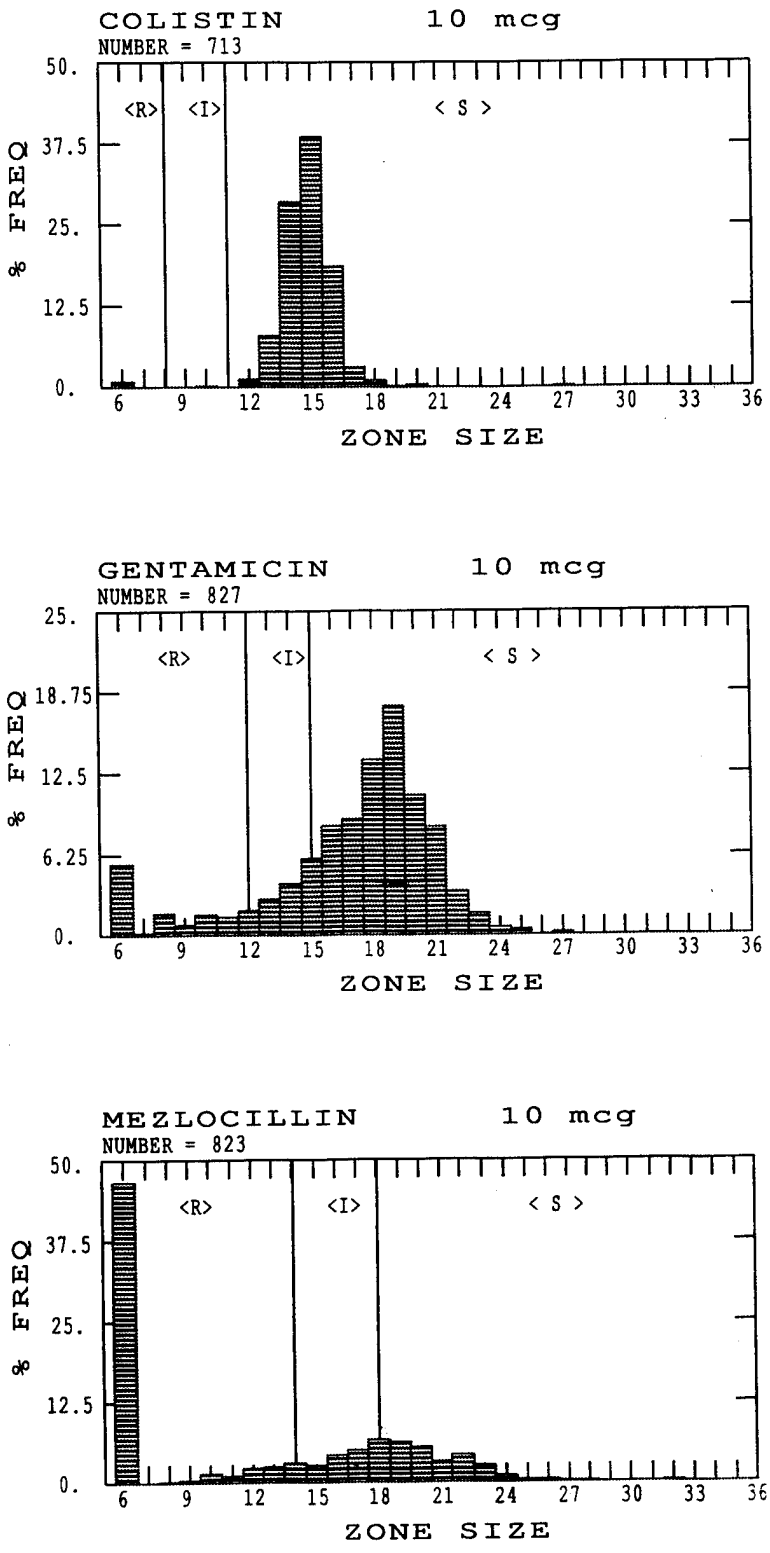


FIGURE 13. Histogram display of the distribution of zones of inhibition of growth of *Pseudomonas aeruginosa* (continued).

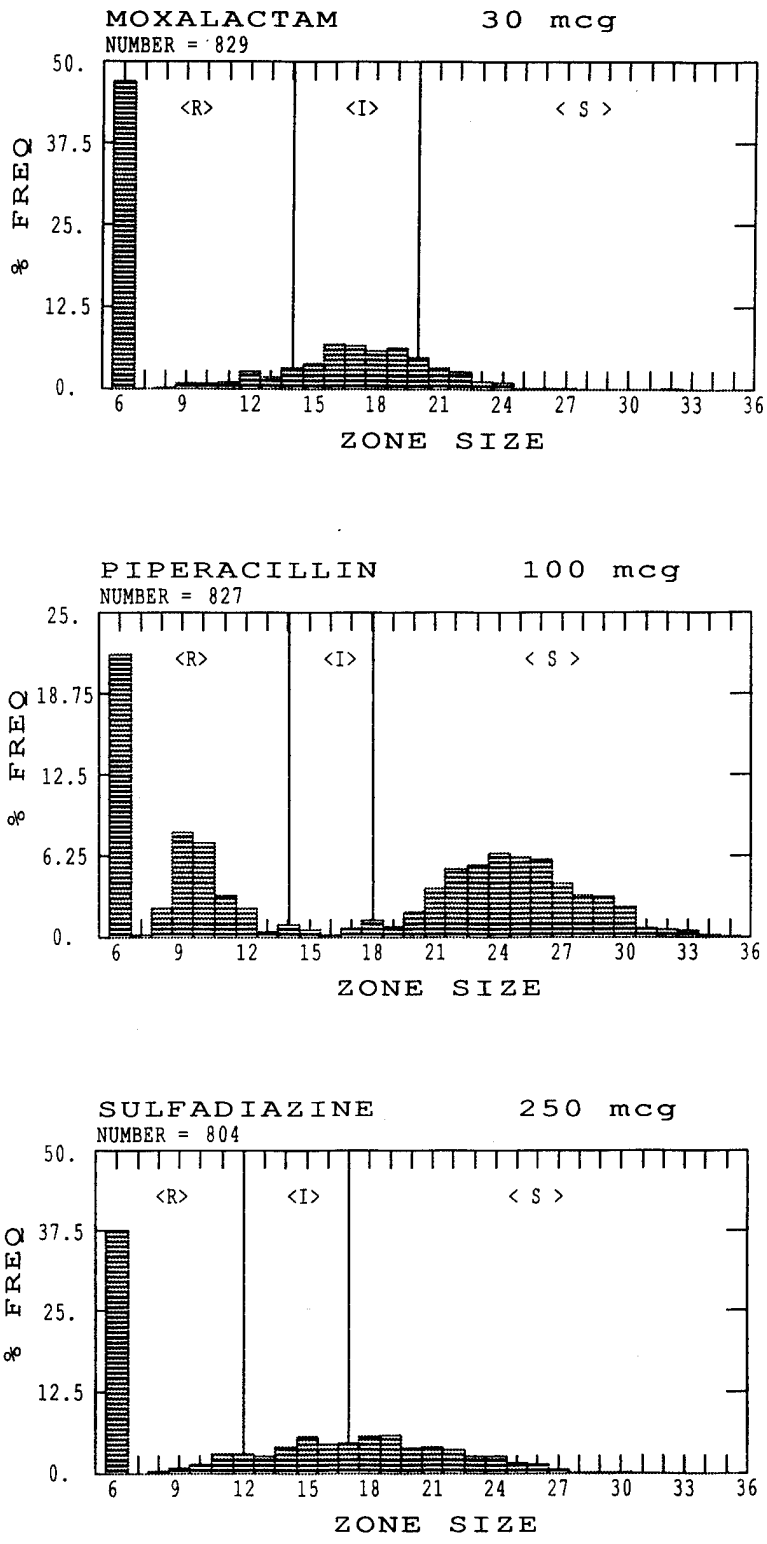
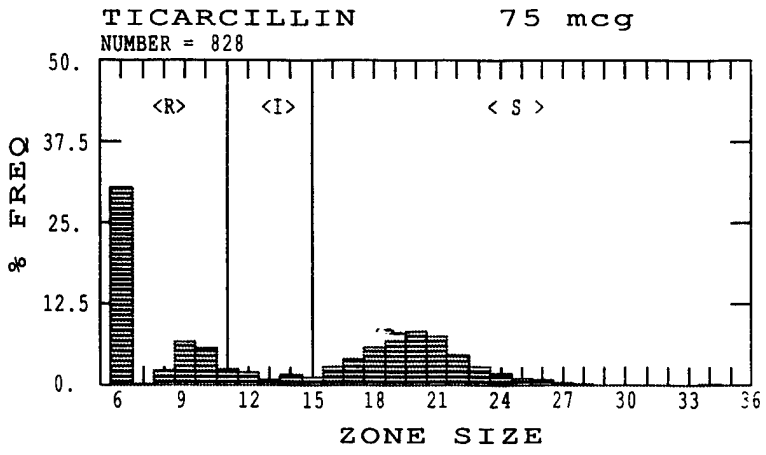


FIGURE 13. Histogram display of the distribution of zones of inhibition of growth of *Pseudomonas aeruginosa* (continued)



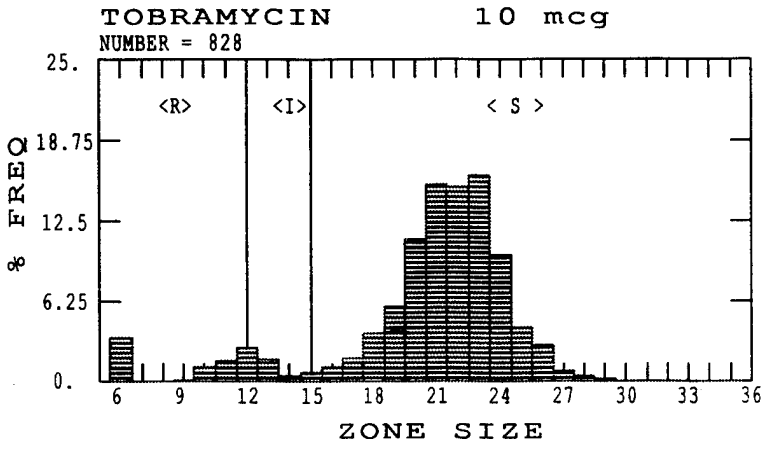


FIGURE 13. Histogram display of the distribution of zones of inhibition of growth of *Pseudomonas aeruginosa* (continued)

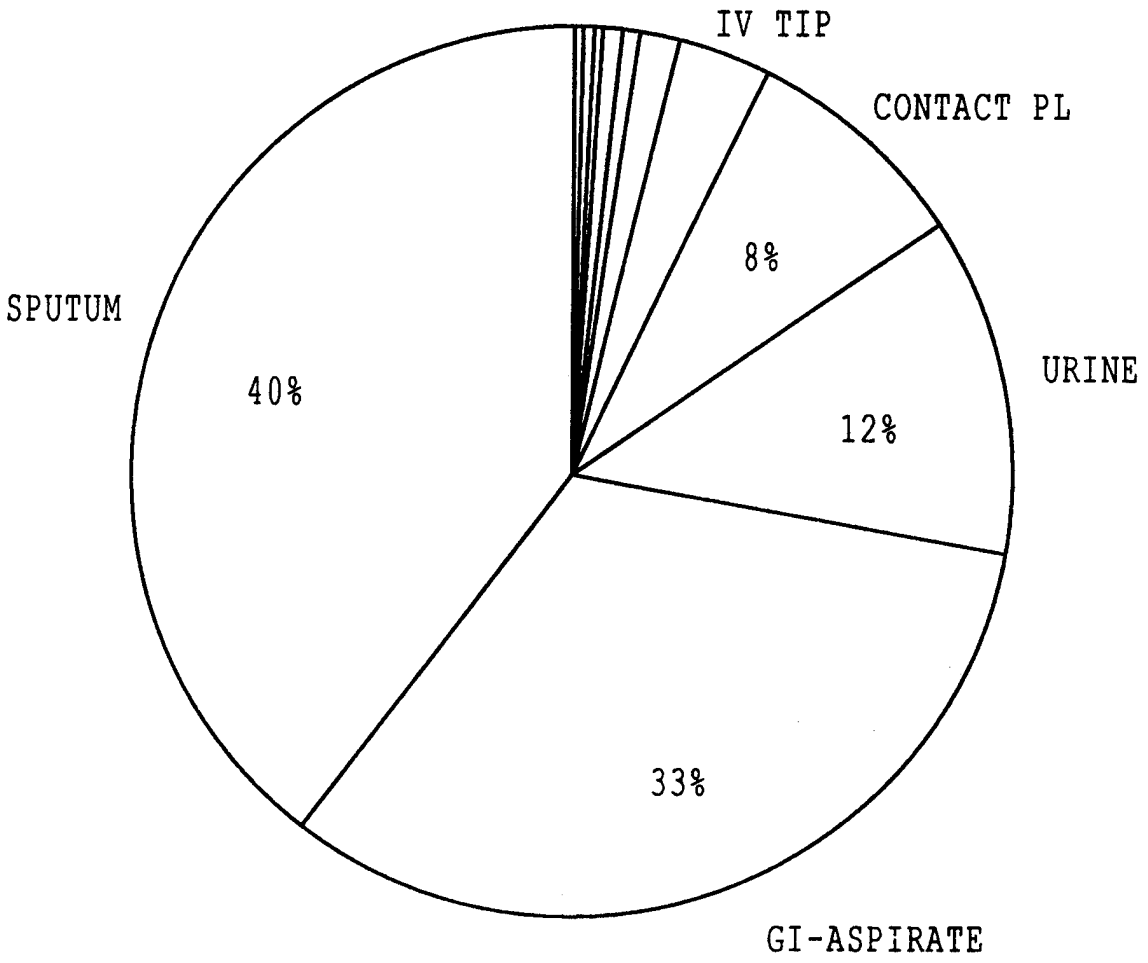


FIGURE 14. Display of the relative frequency of sources yielding *Klebsiella pneumoniae* tested for in vitro sensitivity to antibiotics in 1991.

TABLE 12. Antibiotic Sensitivity Data for *Klebsiella pneumoniae* (1991)

Antibiotic	RESISTANT		INTERMEDIATE		SENSITIVE		Total
	%	Number	%	Number	%	Number	Number
Amikacin sulfate	0.42	1	9.58	23	90.00	216	240
Ampicillin sodium	93.75	225	4.17	10	2.08	5	240
Aztreonam	2.93	7	2.09	5	94.98	227	239
Cefamandole nafate	7.50	18	9.58	23	82.92	199	240
Cefoperazone sodium	2.04	4	18.37	36	79.59	156	196
Cefotaxime sodium	2.50	6	2.08	5	95.42	229	240
Cefoxitin sodium	10.00	24	2.92	7	87.08	209	240
Ceftazidime	2.92	7	2.08	5	95.00	228	240
Ceftriaxone sodium	–	–	–	–	100.00	240	240
Chloramphenicol palmitate	8.75	21	2.08	5	89.17	214	240
Ciprofloxacin	–	–	–	–	100.00	11	11
Gentamicin sulfate	24.58	59	0.42	1	75.00	180	240
Imipenem-cilastatin sodium	0.42	1	–	–	99.58	239	240
Kanamycin sulfate	25.00	60	14.58	35	60.42	145	240
Mezlocillin sodium	33.64	72	19.16	41	47.20	101	214
Nalidixic acid	7.08	17	0.83	2	92.08	221	240
Netilimicin sulfate	0.83	2	15.00	36	84.17	202	240
Norfloxacin	1.25	3	1.67	4	97.08	233	240
Piperacillin sodium	29.58	71	12.50	30	57.92	139	240
Streptomycin sulfate	9.58	23	43.75	105	46.67	112	240
Sulfadiazine	58.33	140	15.42	37	26.25	63	240
Tetracycline hydrochloride	12.50	30	2.92	7	84.58	203	240
Ticarcillin disodium	83.68	200	8.37	20	7.95	19	239
Trimethoprim	9.09	18	1.52	3	89.39	177	198
Trimethoprim-sulfadiazine	7.98	19	4.62	11	87.39	208	238
Tobramycin sulfate	63.64	7	–	–	36.36	4	11

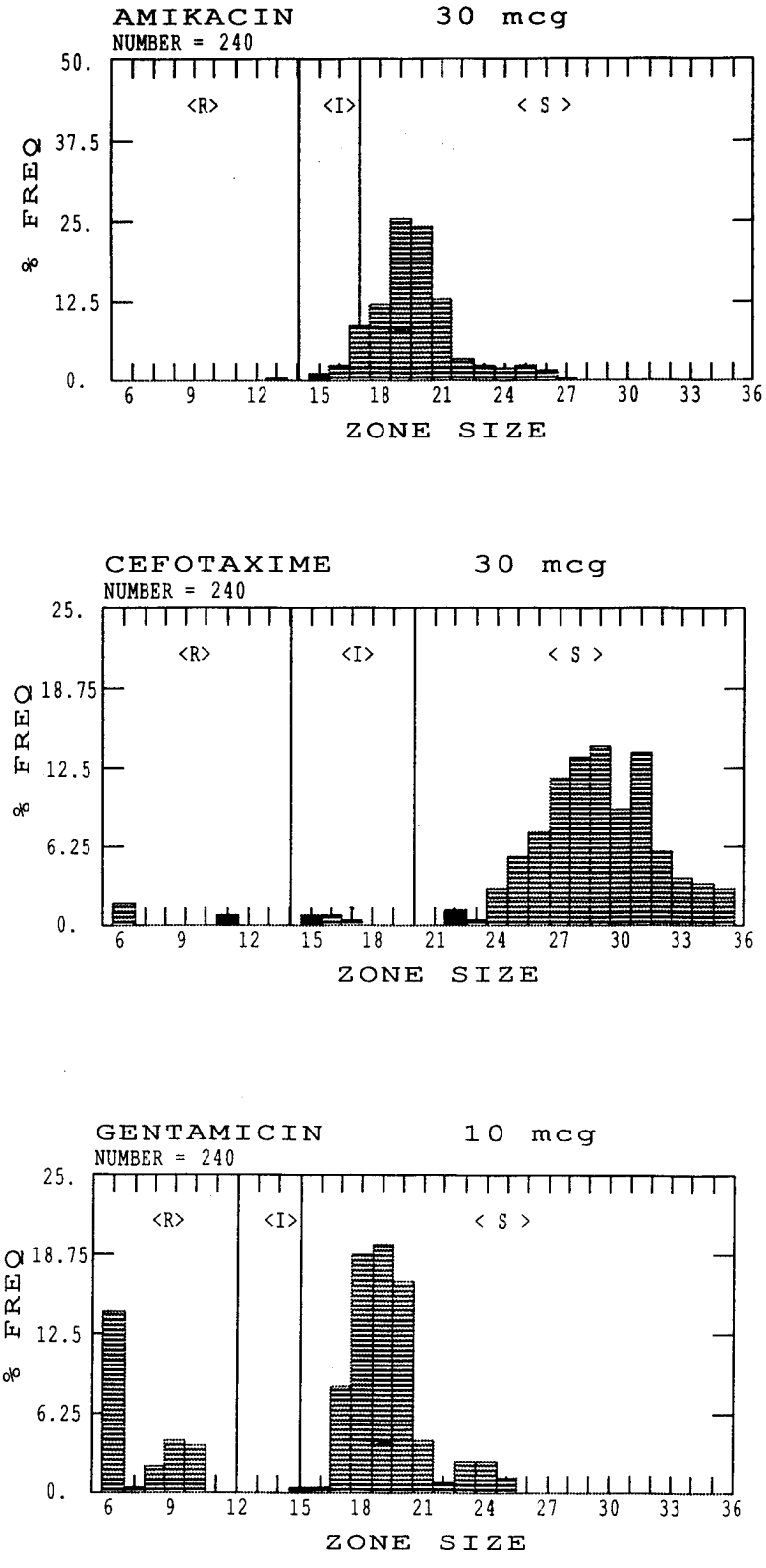


FIGURE 15. Histogram display of the distribution of zones of inhibition of growth of *Klebsiella pneumoniae*.

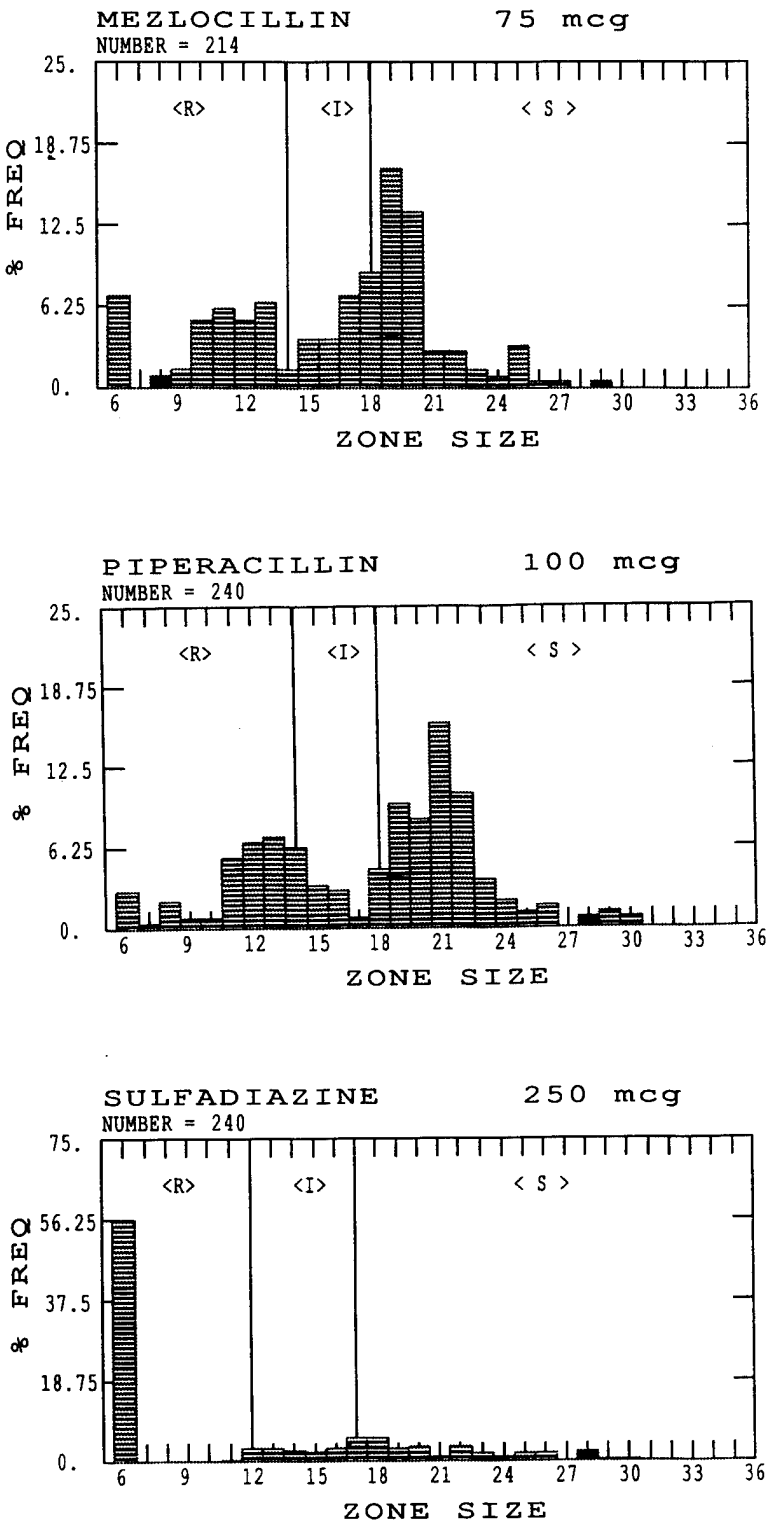


FIGURE 15. Histogram display of the distribution of zones of inhibition of growth of *Klebsiella pneumoniae* (continued).

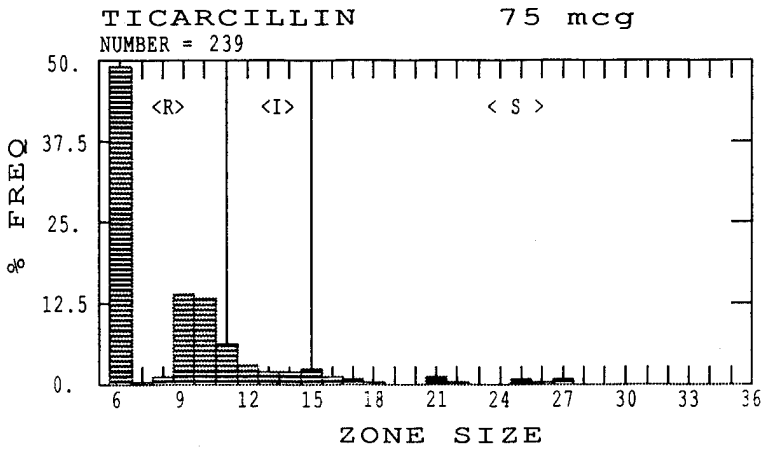


FIGURE 15. Histogram display of the distribution of zones of inhibition of growth of *Klebsiella pneumoniae* (continued).

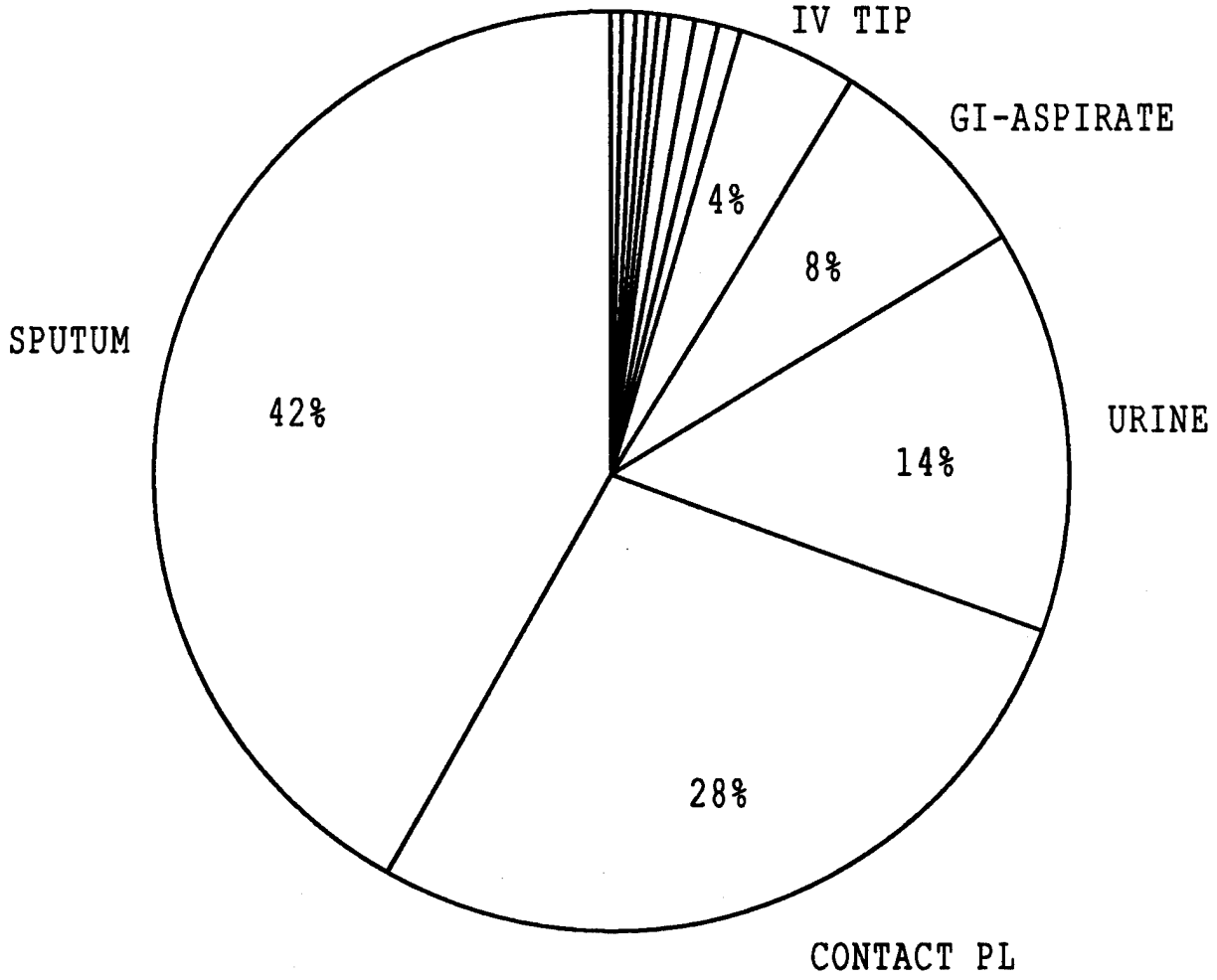


FIGURE 16. Display of the relative frequency of sources yielding *Proteus mirabilis* tested for in vitro sensitivity to antibiotics in 1991.

TABLE 13. Antibiotic Sensitivity Data for *Proteus mirabilis* (1991)

Antibiotic	RESISTANT		INTERMEDIATE		SENSITIVE		Total
	%	Number	%	Number	%	Number	Number
Amikacin sulfate	0.98	2	1.95	4	97.07	199	205
Ampicillin sodium	26.83	55	1.46	3	71.71	147	205
Aztreonam	1.00	2	5.47	11	93.53	188	201
Cefamandole nafate	9.27	19	6.34	13	84.39	173	205
Cefoperazone sodium	1.30	2	7.79	12	90.91	140	154
Cefotaxime sodium	0.49	1	0.98	2	98.54	202	205
Cefoxitin sodium	12.20	25	7.32	15	80.49	165	205
Ceftazidime	2.93	6	0.49	1	96.59	198	205
Ceftriaxone sodium	–	–	–	–	100.00	205	205
Chloramphenicol palmitate	8.33	17	19.12	39	72.55	148	204
Ciprofloxacin	–	–	–	–	100.00	28	28
Gentamicin sulfate	1.95	4	0.98	2	97.07	199	199
Imipenem-cilastatin sodium	1.48	3	–	–	98.52	200	203
Kanamycin sulfate	11.27	23	12.75	26	75.98	155	204
Mezlocillin sodium	3.36	5	3.36	5	93.29	139	149
Nalidixic acid	8.33	17	4.41	9	87.25	178	204
Netilimicin sodium	0.49	1	0.49	1	99.02	203	205
Norfloxacin	1.98	4	0.50	1	97.52	197	202
Piperacillin sodium	4.39	9	2.44	5	93.17	191	205
Streptomycin sulfate	7.92	16	61.39	124	30.69	62	202
Sulfadiazine	33.00	67	13.30	27	53.69	109	203
Tetracycline hydrochloride	95.59	195	0.98	2	3.43	7	204
Ticarcillin disodium	4.88	10	1.46	3	93.66	192	205
Trimethoprim	16.67	19	19.30	22	64.04	73	114
Trimethoprim-sulfadiazine	10.34	21	2.96	6	86.70	176	203
Tobramycin sulfate	7.69	2	3.85	1	88.46	23	26

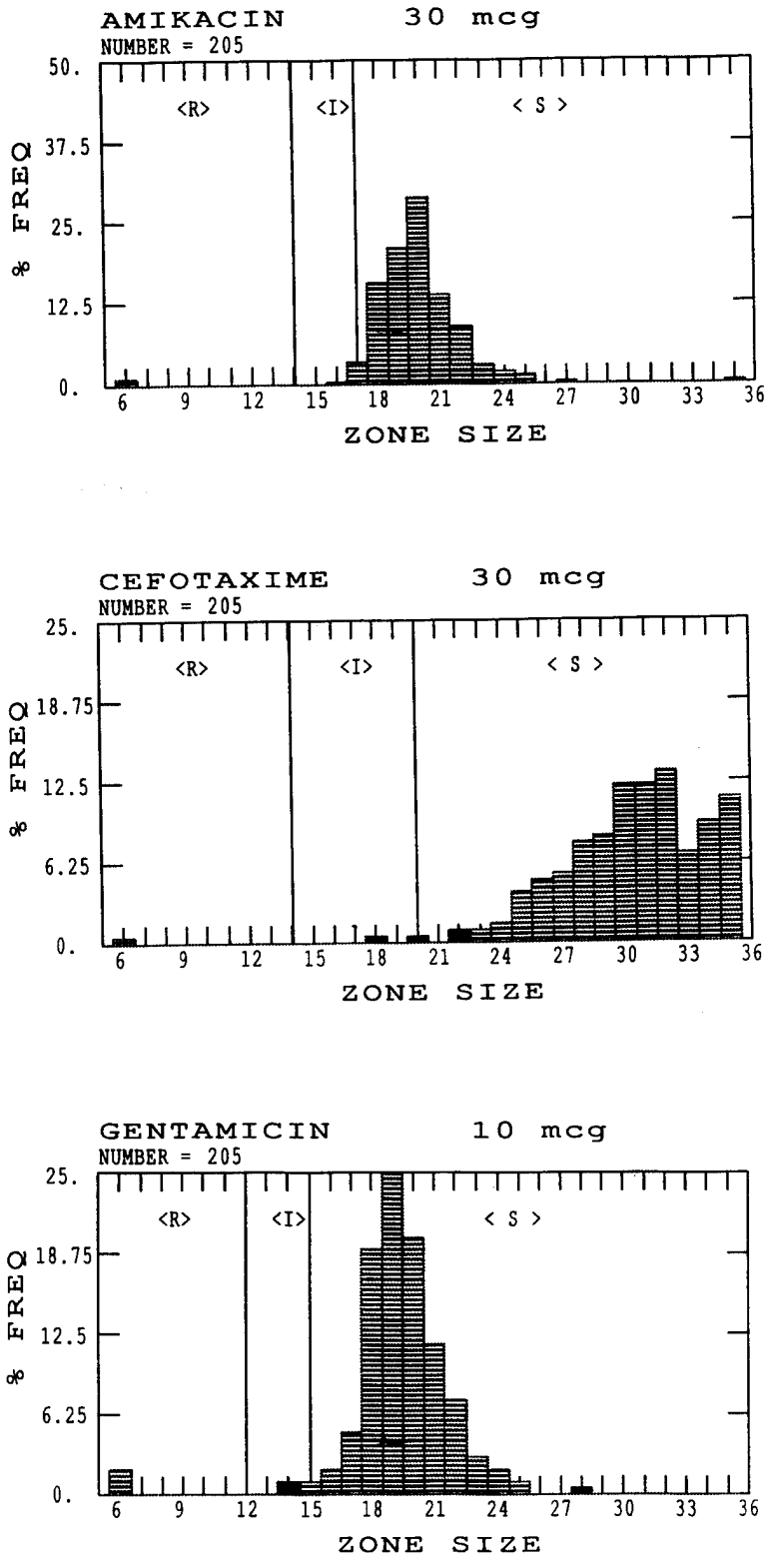
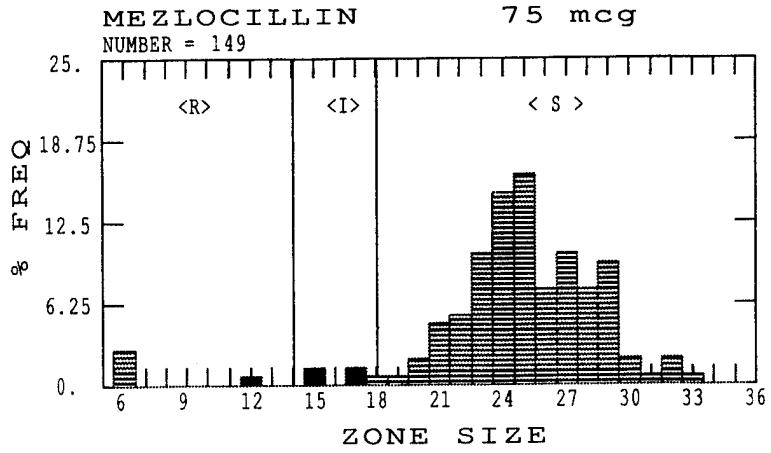
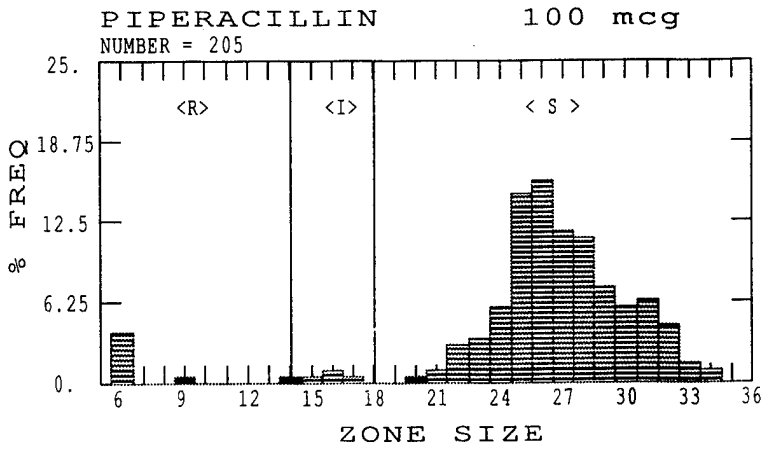


FIGURE 17. Histogram display of the distribution of zones of inhibition of growth of *Proteus mirabilis*.





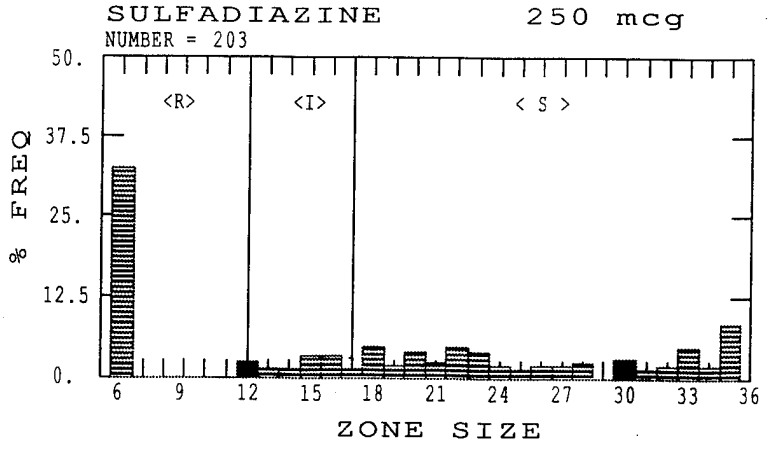


FIGURE 17. Histogram display of the distribution of zones of inhibition of growth of *Proteus mirabilis* (continued).

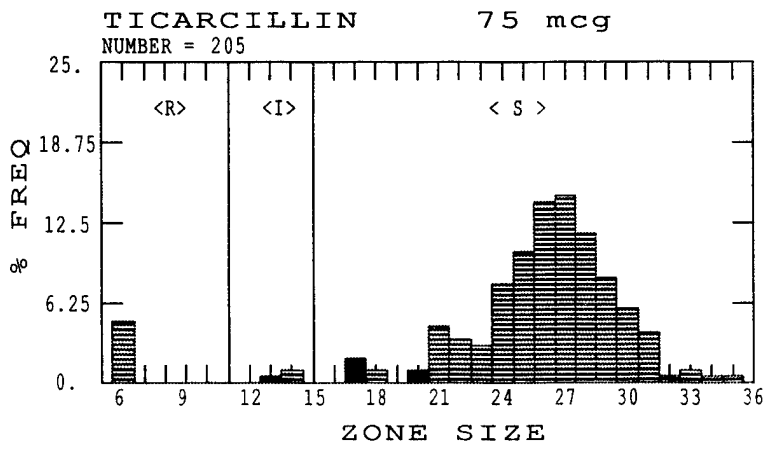


FIGURE 17. Histogram display of the distribution of zones of inhibition of growth of *Proteus mirabilis* (continued).

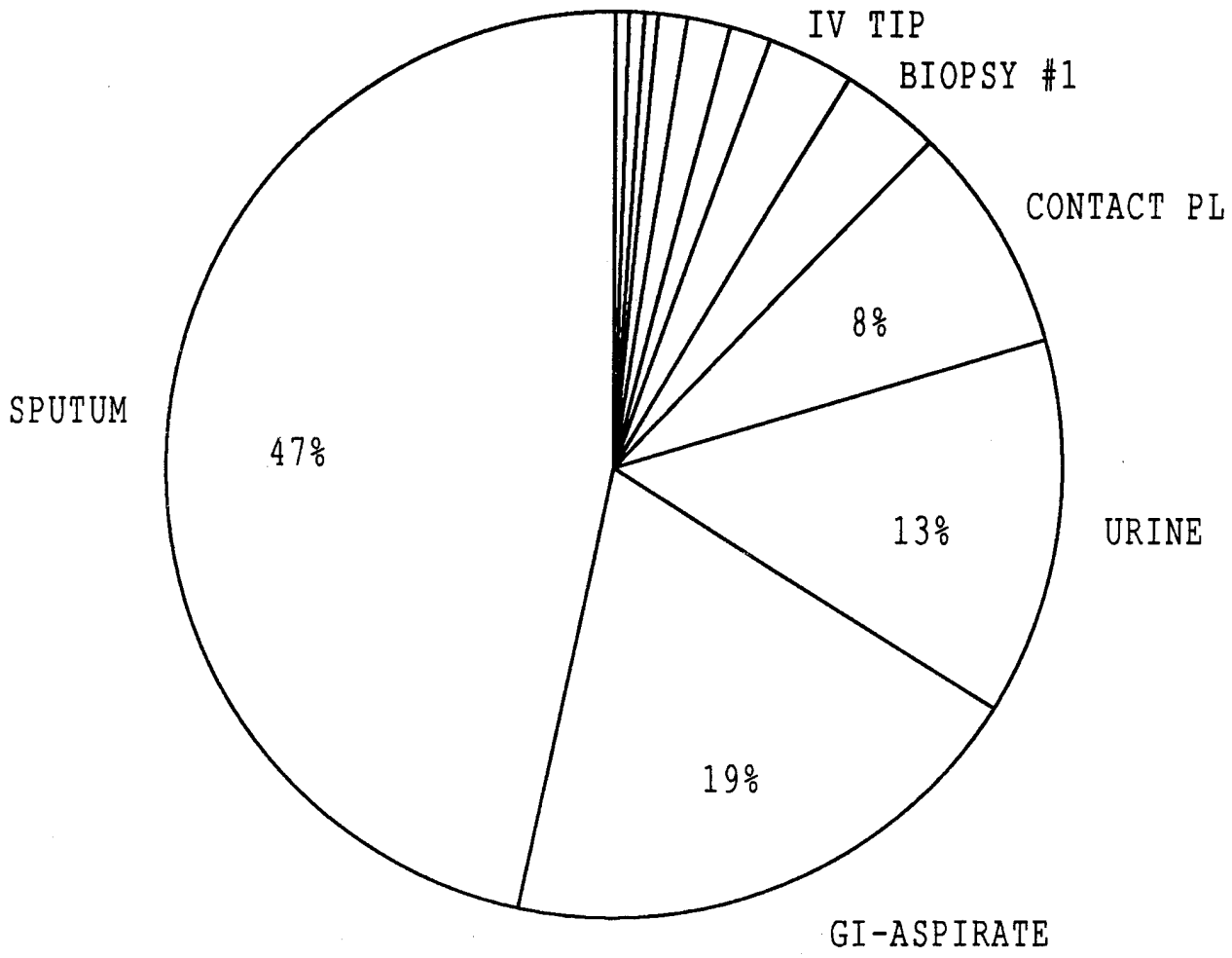


FIGURE 18. Display of the relative frequency of sources yielding *Escherichia coli* tested for in vitro sensitivity to antibiotics in 1991.

TABLE 14. Antibiotic Sensitivity Data for *Escherichia coli* (1991)

Antibiotic	RESISTANT %	RESISTANT Number	INTERMEDIATE %	INTERMEDIATE Number	SENSITIVE %	SENSITIVE Number	Total Number
Amikacin sulfate	1.13	2	6.21	11	92.66	164	177
Ampicillin sodium	44.32	78	0.57	1	55.11	97	176
Aztreonam	0.57	1	1.70	3	97.73	172	176
Cefamandole nafate	4.55	8	14.20	25	81.25	143	176
Cefoperazone sodium	2.10	3	6.99	10	90.91	130	143
Cefotaxime sodium	1.13	2	–	–	98.87	175	177
Cefoxitin sodium	2.82	5	5.65	10	91.53	162	177
Ceftazidime	0.56	1	–	–	99.44	176	177
Ceftriaxone sodium	–	–	–	–	100.00	175	175
Chloramphenicol palmitate	7.91	14	12.43	22	79.66	141	177
Ciprofloxacin	–	–	–	–	100.00	3	3
Gentamicin sulfate	5.65	10	1.69	3	92.66	164	177
Imipenem-cilastatin sodium	–	–	1.14	2	98.86	174	176
Kanamycin sulfate	20.34	36	27.12	48	52.54	93	177
Mezlocillin sodium	27.61	45	13.50	22	58.90	96	163
Nalidixic acid	–	–	2.82	5	97.18	172	177
Netilmicin sulfate	1.13	2	0.56	1	98.31	174	177
Norfloxacin	0.56	1	–	–	99.44	176	177
Piperacillin sodium	25.99	46	13.56	24	60.45	107	177
Streptomycin sulfate	45.20	80	36.16	64	18.64	33	177
Sulfadiazine	68.36	121	6.21	11	25.42	45	177
Tetracycline hydrochloride	55.68	98	0.57	1	43.75	77	176
Ticarcillin disodium	40.91	72	1.70	3	57.39	101	176
Tobramycin sulfate	–	–	–	–	100.00	3	3
Trimethoprim	32.43	48	0.68	1	66.89	99	148
Trimethoprim-sulfadiazine	27.43	48	0.57	1	72.00	126	175

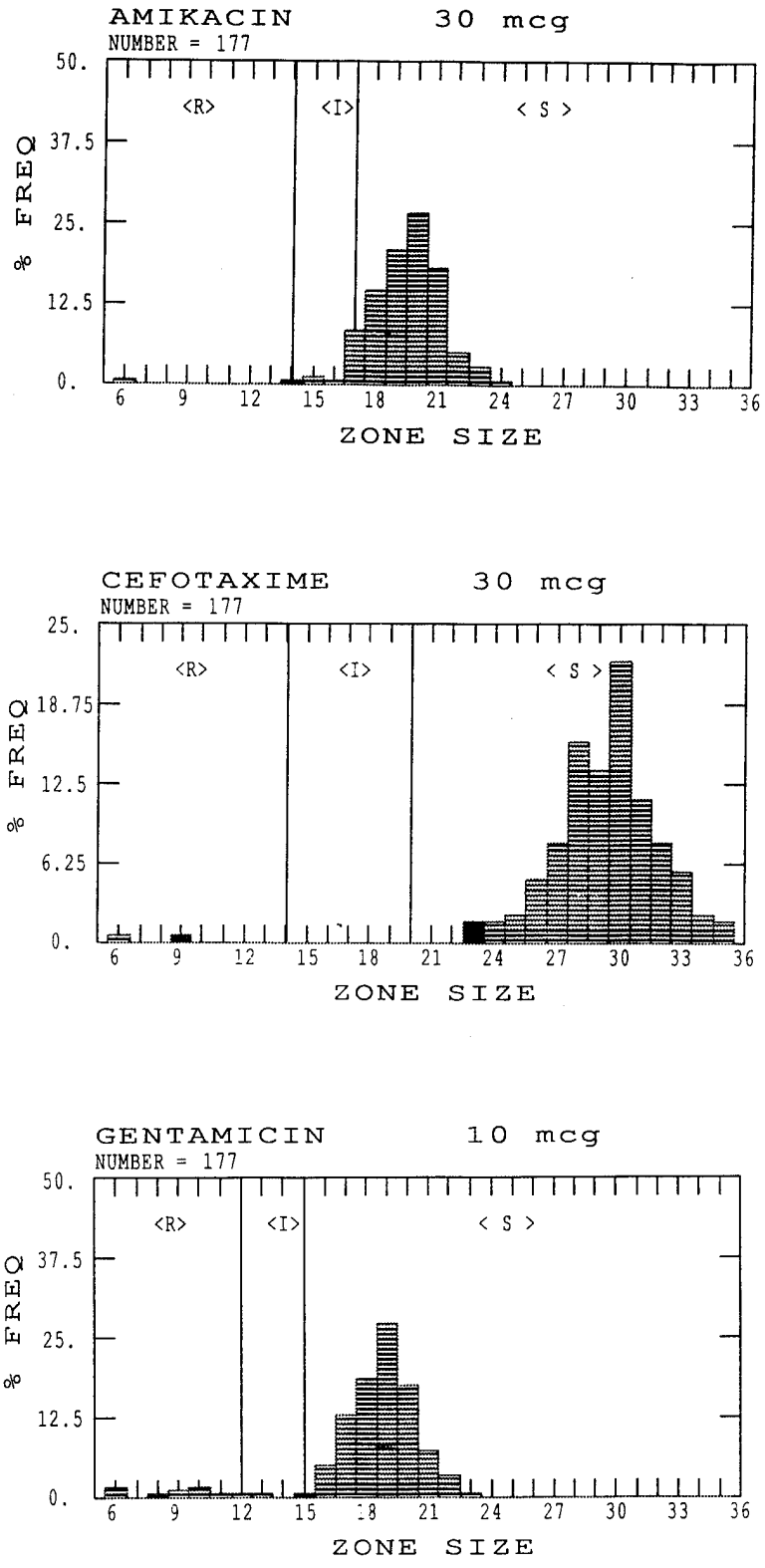


FIGURE 19. Histogram display of the distribution of zones of inhibition of growth of *Escherichia coli*.

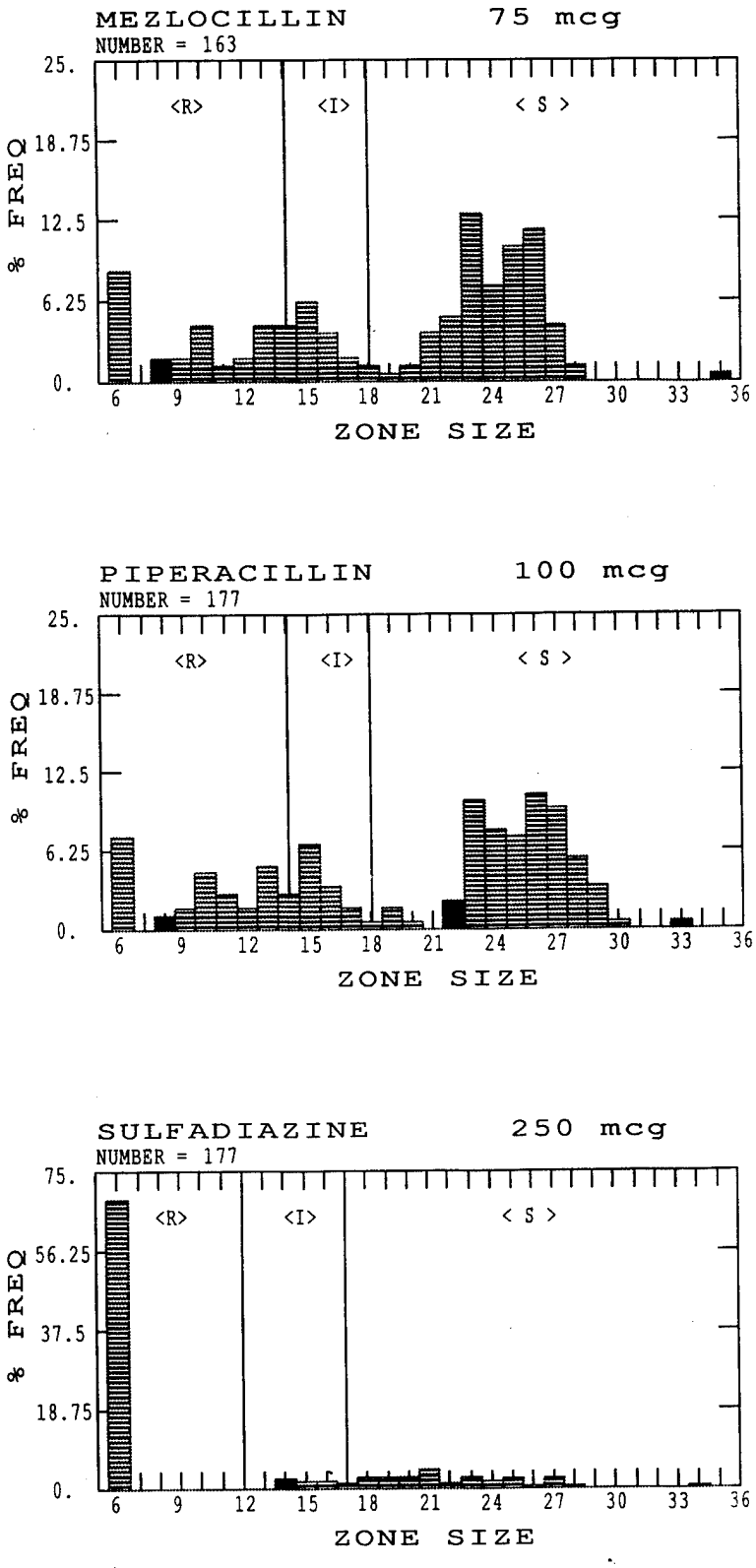


FIGURE 19. Histogram display of the distribution of zones of inhibition of growth of *Escherichia coli* (continued).

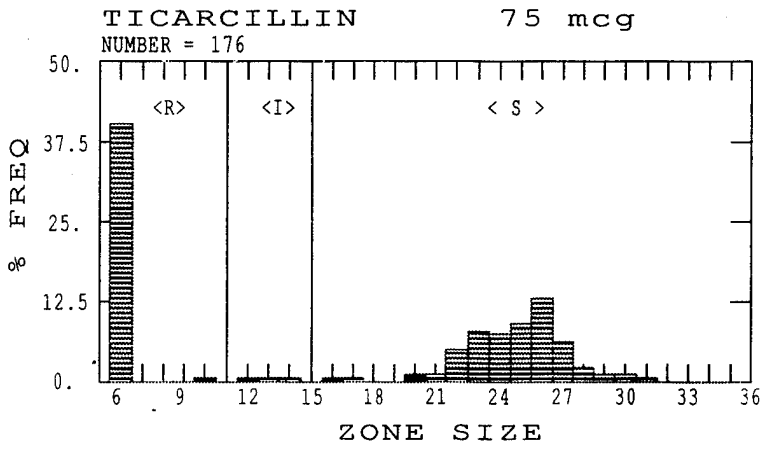


FIGURE 19. Histogram display of the distribution of zones of inhibition of growth of *Escherichia coli* (continued).

PRESENTATIONS

McManus AT: Improved survival in infection burn patients: association with patient isolation. Presented at the 23rd Annual Meeting of the American Burn Association, Baltimore, Maryland, 5 April 1991.

Denton CL: Identification of endemic *Serratia-marcescens* in a burn ICU by pulsed field electrophoresis. Presented at the 91st General Meeting of the American Society for Microbiology, Dallas, Texas, 6 May 1991.

Guymon CL: Yeast colonization and infection in seriously burned patients. Presented at the 91st General Meeting of the American Society for Microbiology, Dallas, Texas, 8 May 1991.

McManus AT: Control of *Pseudomonas aeruginosa* infection in burned patients. Presented at the 5th Annual Meeting of the Surgical Infection Society - Europe, Athens, Greece, 25 May 1991.

McManus AT: The frequency of *Pseudomonas aeruginosa* infections in burned patients may indicate more about hospital conditions than about immunosuppression. Presented at the International Symposium on Pseudomonads: Biology and Biotechnology, Trieste, Italy, 18 June 1991.

PUBLICATIONS

Becker WK, Cioffi WG Jr, McManus AT, Kim SH, McManus WF, Mason AD, and Pruitt BA Jr: Fungal burn wound infection. A 10-year experience. *Arch Surg* 126(1):44-8, January 1991.

McManus AT, Mason AD Jr, McManus WF, and Pruitt BA Jr: Control of *Pseudomonas aeruginosa* infection in burn patients (abstr). *Surg Res Commun* 10(Suppl 1):27, September 1991.

McManus AT: Causes and risks of wound infection. In Davis JM, Shires GT (eds): Principles and Management of Surgical Infections. Philadelphia: JB Lippincott, 1991, Chap 13, pp 313-21.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

ACCESSION: DA335673

SUMMARY DATE: 921001 **SUMMARY KIND:** D **PREV DATE:** 911001 **DISTRIBUTION:** CX

PROGRAM #: 61102A **PROJ #:** 30161102BS14 **TASK AREA:** BA **WORK UNIT:** 302

TITLE: In Vitro Antigen-Presenting Capacity of Macrophages from Burned Rats and In Vivo Assessment of Immunological Consequences of Defective Antigen Presentation

SUBJ1: 060100 - Biochemistry
SUBJ2: 060500 - Medicine and Medical Research

START DATE: 9010 **END DATE:** 9309 **PERFORMANCE METHOD:** C

CONTRACT/GRANT NO:

CONT TOTAL: $
CUM TOTAL: $
TOTAL LAB FUNDS: $

RESOURCES ESTIMATE

FY	WORK YRS	$(Thousands)
91	1.9	$109
92	1.9	$86
93	1.9	$90

RESPONSIBLE DOD ORGANIZATION: 404885
MRDC INSTITUTE OF SURGICAL RESEARCH USAISR
FT SAM HOUSTON, TX 78234
PRUITT, B A
210-221-2720

PERFORMING ORGANIZATION: 404885
MRDC INSTITUTE OF SURGICAL RESEARCH USAISR
FT SAM HOUSTON, TX 78234
BURLESON, D G
210-221-4858

ASSOC1: KOPPENHEFFER, T L

ASSOC2: MC MANUS, A T

FOREIGN INTELLIGENCE CONSIDERED: Y **CIVILIAN APPLICABILITY:** M

KEYWORDS: RA II; Lab Animals; Rats; Burns (Injuries); Lymphocytes; Antigens; Immunosuppression

OBJECTIVE: A DTIC literature search was conducted under DTIC request numbers W6R38K/W6R39J dated 9 January 1990. The objectives of this work are to compare macrophage antigen presentation capacity in the presence and absence of burn injury and to determine whether defective antigen presentation in vivo is merely a passive event or whether it leads to specific anergy. Improvement of treatment regimens will reduce morbidity and mortality of patients with thermal injury.

APPROACH: At selected times postburn, spleens will be removed from burned and sham-burned Lewis rats and a group of nonburned Brown Norway (BN) rats. MLR cultures will be set for individual Lewis antigen-presenting cell (APC) preparations (stimulator cells) and pooled BN splenocytes (responder cells). Cultures will be maintained for 3 days, after which time ^{3}H-thymidine will be added to the cultlres for measuring the proliferative response. Cells will be harvested and counted. Supernatants and cells will be harvested from additional cultures for measurement of IL2 production by stimuatlion of a murine CTL line and IL2β expression by flow cytometry. Splenic macrophages will also be isolated from burned and sham-burned Lewis rats and individual nonburned BN rats will be given intravenous inoculation of one of the splenic macrophage preparations from the Lewis rats while another group of BN rats will receive pooled APCs from sham-burned Lewis rats. One week later, spleen cells will be isolated from each BN rat

recipient and tested for the ability to respond in the MLR against normal Lewis APCs.

PROGRESS: 9110-9209. Purification of splenic macrophages by gradient techniques led to inconsistent assay results. Splenic adherent cells had the ability to inhibit the uptake of tritiated thymidine. Since this inhibition could be reversed by the readdition of rat RBCs or hemoglobin, it was postulated that the inhibition occurred due to release of nitric oxide. Hemoglobin has the ability to absorb excess nitric oxide. This was independently confirmed by the partial reversal of the inhibition by addition of n-methyl-arginine, a competitive inhibitor of nitric oxide synthesis. Future assays will employe the use of autologous red cells to absorb excess nitric oxide. For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report* for fiscal years 1991 through 1992.

ABSTRACT

PROJECT NUMBER: 3M161102BS14-302, Research

PROJECT TITLE: In vitro Antigen-Presenting Capacity of Macrophages from Burned Rats and in vivo Assessment of Immunological Consequences of Defective Antigen Presentation

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

PERIOD COVERED IN THIS REPORT: 1 October 1991 - 30 September 1992

INVESTIGATORS: David G. Burleson, PhD, Colonel, MS
Thomas L. Koppenheffer, PhD
Adriana C. Drost, MS
Albert T. McManus, PhD
William G. Cioffi, Jr., MD, Major, MC

The goal of this study is to evaluate the role of macrophages in the immune response of burned rats. Initial studies have involved developing macrophage purification procedures and the rat MLR as an assay of antigen-presenting capacity. In order to definitively evaluate macrophage function, the isolation of macrophages in pure form was required. To accomplish this, an isolation technique was needed that would specifically isolate macrophages from other WBCs in high yield. Specificity was to be provided by one of a number of partially characterized monoclonal antibodies to rat macrophages. These antibodies were evaluated by flow cytometry morphological staining techniques. High-yield purification was obtained using magnetic microbeads, which can be attached to the monoclonal antibodies and used to bind nonmacrophage cells for indirect isolation. Preliminary evaluation of this technique shows it is feasible if an appropriate macrophage specific monoclonal antibody can be determined.

IN VITRO ANTIGEN-PRESENTING CAPACITY OF MACROPHAGES FROM BURNED RATS AND IN VIVO ASSESSMENT OF IMMUNOLOGICAL CONSEQUENCES OF DEFECTIVE ANTIGEN PRESENTATION

Numerous immunological changes have been reported in burn victims and none have been accurately linked to the cause of the infection susceptibility seen in burned patients. One such change is found in the function of antigen-presenting cells (APCs) such as macrophages. Since the macrophage plays a central role in the immune response to infection, it is important to more fully characterize the function of macrophages after burn injury and determine the relationship of changes in function to infection susceptibility.

The delivery of appropriate costimulatory signals by APCs is critical to the function of T cells and other lymphocytes (1). Jenkins and Schwartz (2) demonstrated with mouse T-cell clones that a state of proliferative unresponsiveness was induced when antigen is presented by chemically fixed macrophages in vitro. The absence of costimulatory signals which are normally delivered by accessory cells at the time of antigen presentation led to intracellular changes that induced an anergic state in T cells (3). A similar phenomenon was observed in vivo (4,5) where intravenous injection of allogeneic, nonantigen-presenting, Ia-bearing cells in mice induced a state of unresponsiveness to the "immunizing" antigens, whereas spleen cells from untreated mice produced a vigorous proliferative response in the MLR.

Defects have also been observed in antigen presentation by macrophages/monocytes from humans and animals with burn injuries. These findings include reduced monocyte activity in generating a cellular response (6), defective antigen-presenting capacity of macrophages that can be partly restored by exogenous IL1 in mice (7) and reduced MLR stimulatory capacity of monocytes from human burn victims (8). In the latter studies, both humans (10) and animals (9) had variably reduced MLR responses after burn injury, whereas the ability of mononuclear cells from humans to provide stimulation was completely lost during the first nine days postburn and partially restored thereafter.

Altered macrophage function after burn injury may contribute to infection susceptibility. This report describes our current attempts to develop a macrophage purification procedure that will facilitate the characterization of macrophage function after thermal injury.

MATERIALS AND METHODS

Cell Preparations. Lewis and Brown Norway (BN) rats were anesthetized with sodium pentobarbital (35 mg/kg IP) and exsanguinated by bleeding from the portal vein. The spleens and

lymph nodes removed aseptically. The spleens were minced with sterile forceps and passed through 60-mesh wire screens. Lymph nodes were minced with surgical scissors, disrupted with forceps, and pressed through 40-mesh stainless steel wire screens. After the cells were collected, they were washed three times in HBSS to remove debris. The cells were separated by specific binding to monoclonal antibodies as described below. The stimulator cells were treated with mitomycin C (50 μg/ml final concentration incubated for 45 min at 37°C) or irradiation to prevent them from incorporating the ^{3}H-thymidine.

MLR Procedure. Mixed-lymphocyte cultures were generated in 96-well tissue culture dishes by mixing stimulator spleen cells with preparations of lymph node cells from a histoincompatible rat. Cultures were maintained for 3 days at 37°C in a humidified 5% CO_2 environment, after which time ^{3}H-thymidine was added to cultures for measuring the proliferative response. Cells were harvested and the amount of thymidine incorporation determined 12 h later as a measure of DNA synthesis occurring in the activated responder cells.

To absorb excreted nitric oxide, which inhibits the proliferation of lymphocytes in this system, RBCs or rat hemoglobin was added to the MLR culture. Heparinized blood drawn during exsanguination was diluted 1:2 in isotonic saline, spun at 3000 rpm for 10 min at 4°C and the dilute serum and buffy coat removed with a pasteur pipette. The RBCs were resuspended at the previous concentration and washed 3 times in cold saline. Hemoglobin was prepared by freezing packed RBCs in a acetone-dry ice bath. The cell preparation was frozen and thawed 4 times (0.5 min freeze, 1.5 min thaw). The ghosts were removed by centrifugation and the hemoglobin concentration in the supernatant was determined by reading the absorbance at 540 nm in a spectrophotometer.

Analysis of Monoclonal Antibodies. Several macrophage binding antibodies are available commercially. The antibodies were bound to red cell free spleen cell preparations and analyzed by flow cytometry to determine the percentage of positive cells and by wright stain for a determination of the proportion of macrophages by morphology. The monoclonal antibodies used with specificity to nonmacrophage cells were OX19, a T-cell marker binding to CD5, MARL4 and MARK4 antibodies against B-cell antibody kappa and lambda light chains, respectively, and W3/13 which reacts CD43 present on T cells and PMNs. Macrophage markers included ED2, ED3, OX41 and OX42.

Purification of Macrophages by Magnetic Microbeads. Macrophages were purified indirectly by adding nonmacrophage (lymphocytic and PMN specific) biotinylated antibodies. Washed cells from disrupted rat spleens were labeled with the biotinylated monoclonal antibody for 15 min on ice. Excess antibody was removed by a wash in isolation medium (phosphate-buffered saline (PBS)

0.288 ml of PBS containing magnetic microbeads (Becton Dickinson) labeled with streptavidin. The cell preparation was incubated for another 15 min, washed once with isolation medium and resuspended in 0.5 ml isolation medium. The suspension was underlayed with 0.5 ml fetal calf serum, centrifuged, the supernatant discarded and the cell pellet resuspended in 0.5 ml PBS containing 1% bovine serum albumin (PBS-BSA). The cell-microbead mixture was then added to a special eluting column with the magnet turned on. The PBS-BSA was passed through the column to elute cells not bound to magnetic microbeads. Subsequently, the magnet was turned off and the cells bound to the microbeads were eluted with additional PBS-BSA. The cells eluting from the column were evaluated by further binding to monoclonal antibodies and analysis on the flow cytometer.

RESULTS

Evaluation of Magnetic Microbead Columns for Purification of Macrophages. MACS™ columns (Becton Dickinson) were first evaluated for the appropriate size and grid matrix for our cell system. The percentage of cells recovered on different MACS™ columns are shown in Table 1. Column B2 is was best for large scale separations and Column A1 for small scale separations in this type of cells and culture system.

TABLE 1. Recovery of Rat Splenocytes Applied to MACS™ Columns

Experiment Number	Column Type	Cells Applied	Cells Recovered	Percent Recovery
1	B2	3 X 108	2.95 X 108	98
2	A1	1 X 107	8.70 X 106	87
3	B1	4 X 107	1.36 X 106	35
4	A2	1.63 X 107	1.18 X 107	72

Lewis rat spleen cells were teased from the spleen, counted, stained with nonmacrophage antibodies and the number of cells appropriate for the size of column were applied. Cells were eluted first with the magnet turned on and then with the magnet turned off. The recovered cell fractions were counted and the percent recovery was calculated from the total cells eluted under both conditions.

Evaluation of Rat Monoclonal Antibodies to Macrophages. In order for accurate judgements to be made as to the effectiveness of the purification techniques, monoclonal antibodies against

the purification techniques, monoclonal antibodies against different cell types were compared between the two strains of rats being used for this study. Cells were analyzed by flow cytometry. The comparative results are shown in Table 2. Strain-specific differences in the cell subpopulations are evident. Cell purification can only be compared within strains and not between strains.

The percentage of macrophages found in the preparation varied according to the antibody used to define the macrophage population. The mean value for our experiments ranged from 1.36% for ED2 to 6.74% for OX41 in Lewis rats and 1.11% for ED2 to 7.81% for OX41 in BN rats.

Spleen cells were isolated from Lewis or BN rats by teasing the spleen tissue. Cells were washed, stained, and analyzed by flow cytometry as indicated in the previous section.

Evaluation of MACS™ Indirect Purification of Macrophages. Cell preparations were simultaneously stained for T cells, B cells, NK cells, and PMNs by adding the appropriate biotinylated monoclonal antibodies. Magnetic microbeads were added and the cell preparation placed on the column with the magnet turned on. Cells were eluted with cell medium, the magnet was turned off, and the cells binding to magnetic beads were eluted. Both aliquots of eluted cells were stained with fluoroscein isothiocyanate-labeled OX42 as a macrophage marker and analyzed by flow cytometry. Table 3 shows the results of that experiment. Based on OX42 as a marker of macrophages, the MACS™ procedure enriched the cells from the Lewis rats by fourfold and the BN rats by sixfold.

Spleen cells were isolated from Lewis or BN rats. Cells were purified by negative selection. Cells not labeled with monoclonal antibody (macrophages or null cells) would not be retained by the column when the magnet was turned on.

Evaluation of Purified Macrophage Function in MLR. Purified macrophages were used as stimulator cells in MLR assays. Starting at 4×10^5 cells per well, progressively fewer cells were added to subsequent wells to determine the optimum number of cells needed for a strong proliferative response. As can be seen in Figure 1, the optimum number of cells per well was 2500. This is 16-fold less cells than needed for optimum stimulation with unpurified spleen cell preparations.

DISCUSSION

Although present as a relatively low percentage of leukocytes in lymphoid organs, macrophages play a central role in regulating the immune response. Understanding the exact nature of this role in the immune response will require that pure cell populations be

TABLE 2. Comparison of Splenocytes from Lewis and Brown-Norway Rats (%)

Antibody	Lewis			Brown-Norway		
	Experiment 1	Experiment 2	Experiment 3	Experiment 1	Experiment 2	Experiment 3
Isotype control	-	-	0.31	-	-	0.28
CD5 (OX19 T cell)	57.18	52.01	56.88	39.10	34.73	23.52
CD43 (T cell + PMN)	67.45	57.79	63.88	44.33	44.10	40.20
B cells (ML4/MK4)	9.95	10.90	8.86	23.59	9.96	10.53
Macrophages						
ED2	2.22	0.66	1.19	0.77	0.77	1.78
OX41	Not done	3.94	0.54	Not done	6.13	9.48
ED3	Not done	0.75	2.82	Not done	0.36	2.65
OX42	2.60	5.02	0.95	2.40	6.83	1.35

TABLE 3. Purification of Rat Macrophages by MACS™ (%)

Antibody	Lewis			Brown-Norway		
	Unpurified Cells	Unretained Cells	Retained Cells	Unpurified Cells	Unretained Cells	Retained Cells
Isotype control	-	0.36	0.87	-	0.30	3.50
OX 42	5.02	19.76	7.47	6.83	47.32	5.66

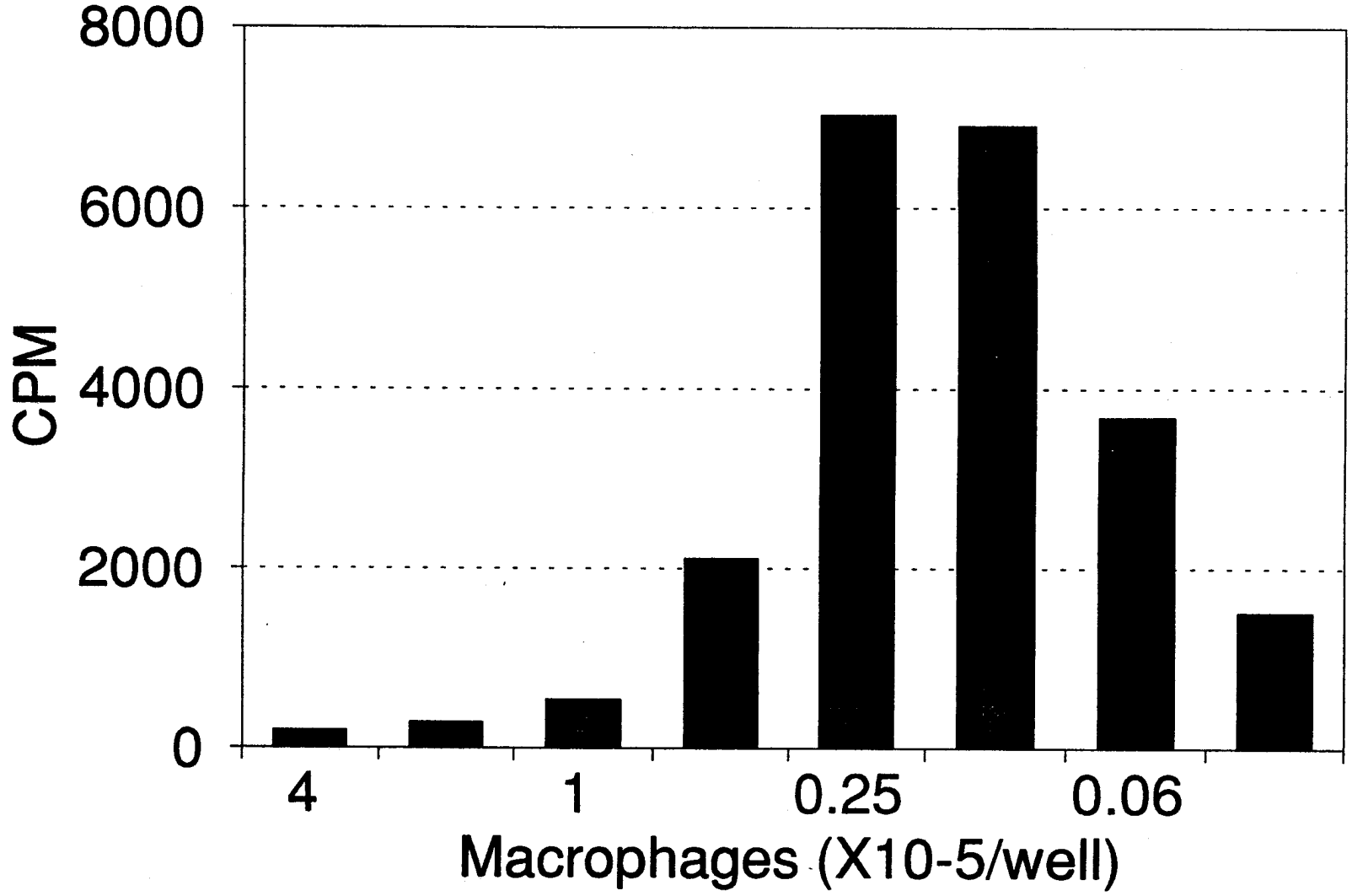


FIGURE 1. Determination of optimum number of spleen cells in rat MLR using purified macrophages. The MLR procedure was performed using varying numbers of MACS™-purified Lewis rat macrophages as stimulator cells.

obtained for manipulation so that effects of interactions with other cells can be observed. The best large scale purification technique for the number of cells required in immune function studies is a technique using the specificity of monoclonal antibodies and the efficiency of a passive restraint system that can be easily manipulated. Such a system is provided by the magnetic bead separation technique. Many variables affect the purity and recovery of the cells purified with this technique, so a considerable amount of development is required for each cell system for which it is used.

The biggest obstacle to obtaining macrophages of high purity using this system is the lack of well defined monoclonal antibodies for the surface antigens of rat macrophages. The commercially available monoclonal antibodies for rat macrophages (ED2, ED3, OX41, OX42) are very poorly defined compared to those available for human or mouse macrophages. The ED antibodies have specificity for tissue macrophage antigens. OX41 has some cross-reactivity for PMNs. OX42 appears to bind a rat analog of the human iC3b receptor. An antibody to CD14, the principal marker for macrophages/monocytes in the human/mouse systems have not been found in the rat. The results of our purification efforts using these antibodies have been highly variable. It may be necessary to find a suitable reference macrophage marker such as morphological staining in combination with specific enzyme markers or electron micrograph morphology to verify the specificity and accuracy of these commercially available monoclonal antibodies. At this point, the enzyme marker (nonspecific esterase) most useful in the human and mouse system have proved to be unsuitable for the rat (data not shown). Electron micrograph morphology studies are the next logical step in our attempts to verify the macrophage antibodies.

PRESENTATIONS/PUBLICATIONS

None.

REFERENCES

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2. Jenkins MK, Schwartz RH: Antigen presentation by chemically modified splenocytes induces antigen-specific T cell unresponsiveness in vitro and in vivo. *J Exp Med* 165:302-19, 1987.

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4. Mueller DL: Do tolerant T cells exist? *Nature* 339:513–4, 1989.

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9. Kupper TS, Green DR: Immunoregulation after thermal injury: sequential appearance of $I\text{–}J^+$, Ly–1 T suppressor inducer cells and Ly–2 suppressor effector cells following thermal trauma in mice. *J Immunol* 133:3047–53, 1984.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

ACCESSION: DA0G6968

SUMMARY DATE: 921001 **SUMMARY KIND:** D **PREV DATE:** 911001 **DISTRIBUTION:** CX

PROGRAM #: 61102A **PROJ #:** 30161102BS14 **TASK AREA:** BA **WORK UNIT:** 303

TITLE: Alteration of Host Resistance in Burned Patients

SUBJ1: 060500 - Medicine and Medical Research
SUBJ2: 061300 - Microbiology
SUBJ3: 061500 - Pharmacology

START DATE: 7610 **END DATE:** 9909 **PERFORMANCE METHOD:** C

CONTRACT/GRANT NO:

CONT TOTAL: $
CUM TOTAL: $
TOTAL LAB FUNDS: $

RESOURCES ESTIMATE

FY	WORK YRS	$(Thousands)
91	3.0	$200
92	3.0	$84
93	3.0	$220

RESPONSIBLE DOD ORGANIZATION: 404885
MRDC INSTITUTE OF SURGICAL RESEARCH USAISR
FT SAM HOUSTON, TX 78234
PRUITT, B A
210-221-2720

PERFORMING ORGANIZATION: 404885
MRDC INSTITUTE OF SURGICAL RESEARCH USAISR
FT SAM HOUSTON, TX 78234
MC MANUS, A T
210-221-3411

ASSOC1:

ASSOC2:

FOREIGN INTELLIGENCE CONSIDERED: Y **CIVILIAN APPLICABILITY:** M

KEYWORDS: RA II; Volunteers; Adults; Lab Animals; Rats; Burns (Injuries); Infectious Disease Transmission; Immunity; Virulence; Antibiotics

OBJECTIVE: A DTIC literature search was conducted under DTIC request numbers W6K56A/W6K56F dated 20 October 1989. The objectives of this work are to define the microbial basis of opportunistic infection in susceptible burn patients, identify specific mechanisms of decreased host resistance that are targeted by opportunistic pathogens, and develop and evaluate countermeasures.

APPROACH: The effect of in vitro alterations of specific microbial characteristics on infection will be investigated. Specific antimicrobial and immunostimulatory therapies will be examined.

PROGRESS: 9110-9209. The novel compound, mafenide phosphanilate (ISR-55), has been submitted for United States and international patents. This compound has been found to have significantly improved colligative properties that allow high formulation concentrations without the osmotic difficulties associated with previous mafenide salts. For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report* for fiscal years 1976 through 1992.

ABSTRACT

PROJECT NUMBER: 3M161102BS14-303, Research

PROJECT TITLE: Alteration of Host Resistance in Burned Soldiers

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

PERIOD COVERED IN THIS REPORT: 1 October 1991 - 30 September 1992

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Specific strain identification is the cardinal requirement for microbial epidemiology. We are evaluating a single technical approach, pulsed-field gel electrophoresis of endonuclease digested whole-cell DNA, for this purpose. We have examined 82 consecutive *Staphylococcus aureus* blood isolates from 29 of 667 burn patients admitted to a single burn center during a 3-yr period. The bacteremic group included 27 burn patients and two with toxic epidermal necrolysis syndrome. Using Sma I digest of whole-cell DNA, 26 distinct gel patterns (strains) were identified. Patient-unique strains were identified in 24 patients. Multiple bacteremic episodes occurred in 5 patients. Evidence for cross-infection was observed with 45 strains, among which single patient-to-patient cross-contamination was observed with two, single-patient transfer to two other patients with one, and transfer to more than two other patients with one. The effectiveness of the pulsed-field gel electrophoresis technique was validated by identifying the latter strain in 7 patients in which it caused five single-strain bacteremias.

New Antibiotics in Clinical Use. The parenteral antibiotic agents, ceftazidime, ceftriaxone sodium, and aztreonam, were used clinically for the third year. The fluoroquinolone agents, ciprofloxacin and norfloxacin, were used for the second year. Results of in vitro testing are presented in Table 1.

TABLE 1. Activity of Newly Released Antibiotics for Fiscal Years 1987–92

Fiscal Year	1987	1988	1989	1990	1991	1992
			Aztreonam[a]			
Resistant	119 (10.5)	540 (25.3)	602 (36.8)	669 (30.4)	966 (27.7)	691 (29.1)
Sensitive	1009	1593	1035	1529	2518	1682
			Ceftazidime[b]			
Resistant	248 (12.5)	480 (15.4)	683 (22.3)	531 (15.9)	764 (22.6)	872 (25.6)
Sensitive	1731	2633	2374	2805	2610	2536
			Ceftriaxone Sodium[b]			
Resistant	267 (13.5)	843 (27.0)	786 (25.8)	885 (28.2)	1,497 (44.2)	1139 (33.4)
Sensitive	1708	2279	2265	2449	1892	2271
			Norfloxacin[b]			
Resistant	24 (1.6)	41 (1.9)	198 (12.1)	129 (5.9)	416 (17.2)	98 (4.2)
Sensitive	1472	2158	1439	2070	2000	2217

( ) = Percent resistant. [a]Against Gram-negative aerobic flora. [b]Against all flora except oxacillin-resistant *Staphylococcus aureus*.

Experimental Topical Agents. Mafenide acetate was examined for in vitro activity against *Pseudomonas aeruginosa* isolated from 48 burn patients. Agar dilution minimal inhibitory concentration

(MIC) assays were completed on 306 strains. The median MIC was 0.078 g/100 ml, a decrease from the Fiscal Year 1991 value of 0.156 g/100 ml. Data comparing the past five reporting periods are presented in Table 2 and the cumulative display of MIC for Fiscal Year 1992 is presented at Figure 1.

TABLE 2. Minimal Inhibitory Concentration for *Pseudomonas aeruginosa* Strains to Mafenide Acetate for Fiscal Years 88-92

Mafenide Acetate Concentration (g/100 ml)	Number of Strains				
	1988	1989	1990	1991	1992
0.019	10	24	2	13	22
0.039	16	11	18	24	54
0.078	36	26	15	31	98
0.156	39	50	38	51	106
0.312	42	59	61	32	25
0.625	13	23	36	7	1
1.250	2	–	6	–	–
Total Number of Strains	**158**	**193**	**176**	**158**	**306**

Delayed Infection Studies Using Piperacillin Sodium. Investigation of the possible therapeutic effects of the antipseudomonal penicillin, piperacillin sodium, in delayed treatment of *Pseudomonas aeruginosa* (Strain 59-1244) was continued. Animals were infected within 1 h postburn and twice daily antibiotic treatment (50 mg/kg SC) was initiated at 72, 96, 120, and 144 h postinoculation and continued for 15 days (instead of 7 days as previously tested). It appears that increasing the duration of parenteral piperacillin sodium therapy is ineffective in increasing the time delay for commencement of therapy for effective treatment of *Pseudomonas* burn wound infection (see Table 3). It was previously shown that wound excision with parenteral piperacillin sodium at 72 h postinoculation did not extend the therapeutic effect of piperacillin sodium (1). Table 4 shows that wound excision with piperacillin sodium therapy at 48, 72, 96, 120, and 144 h postinoculation also did not improve the therapeutic effectiveness of piperacillin sodium alone.

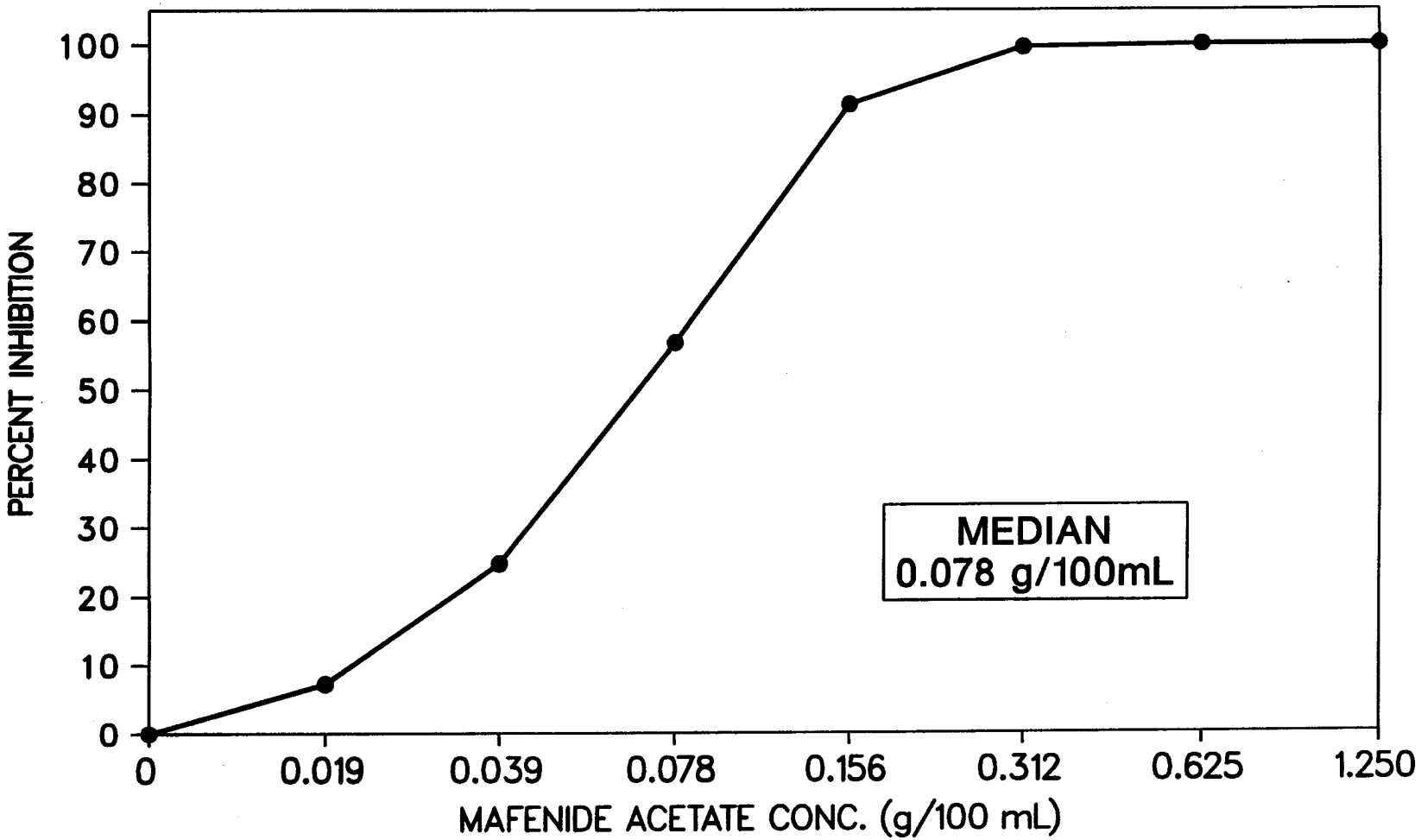


FIGURE 1. Cumulative distribution of minimal inhibitory concentration for mafenide acetate against *Pseudomonas aeruginosa*.

TABLE 3. Examination of Piperacillin Sodium in *Pseudomonas aeruginosa*-Infected Rats

Group	Number of Animals Total	Number of Animals Dead	Mortality (%)
Burn control	10	–	–
Infection control	10	10	100
72 h postinoculation	10	7	70
96 h postinoculation	10	10	100
120 h postinoculation	10	9	90
144 h postinoculation	10	10	100

TABLE 4. Examination of Piperacillin Sodium After Wound Excision in *Pseudomonas aeruginosa*-Infected Rats

Group	Number of Animals Total	Number of Animals Dead	Mortality (%)
Burn control	6	–	–
Infection control	9	9	100.0
48 h postinoculation	9	2	22.2
72 h postinoculation	9	5	55.6
96 h postinoculation	9	9	100.0
120 h postinoculation	9	9	100.0
144 h postinoculation	8	8	100.0

Serologic Types of *Pseudomonas aeruginosa* Isolated from Burn Patients. *Pseudomonas aeruginosa* isolates from burned patients were serotyped using the Difco International Typing Sera™ set and autoclaved bacterial suspensions. Strains were selected on the basis of having a distinct antibiotic sensitivity pattern for each patient. Data are presented at Figure 2 as total number of

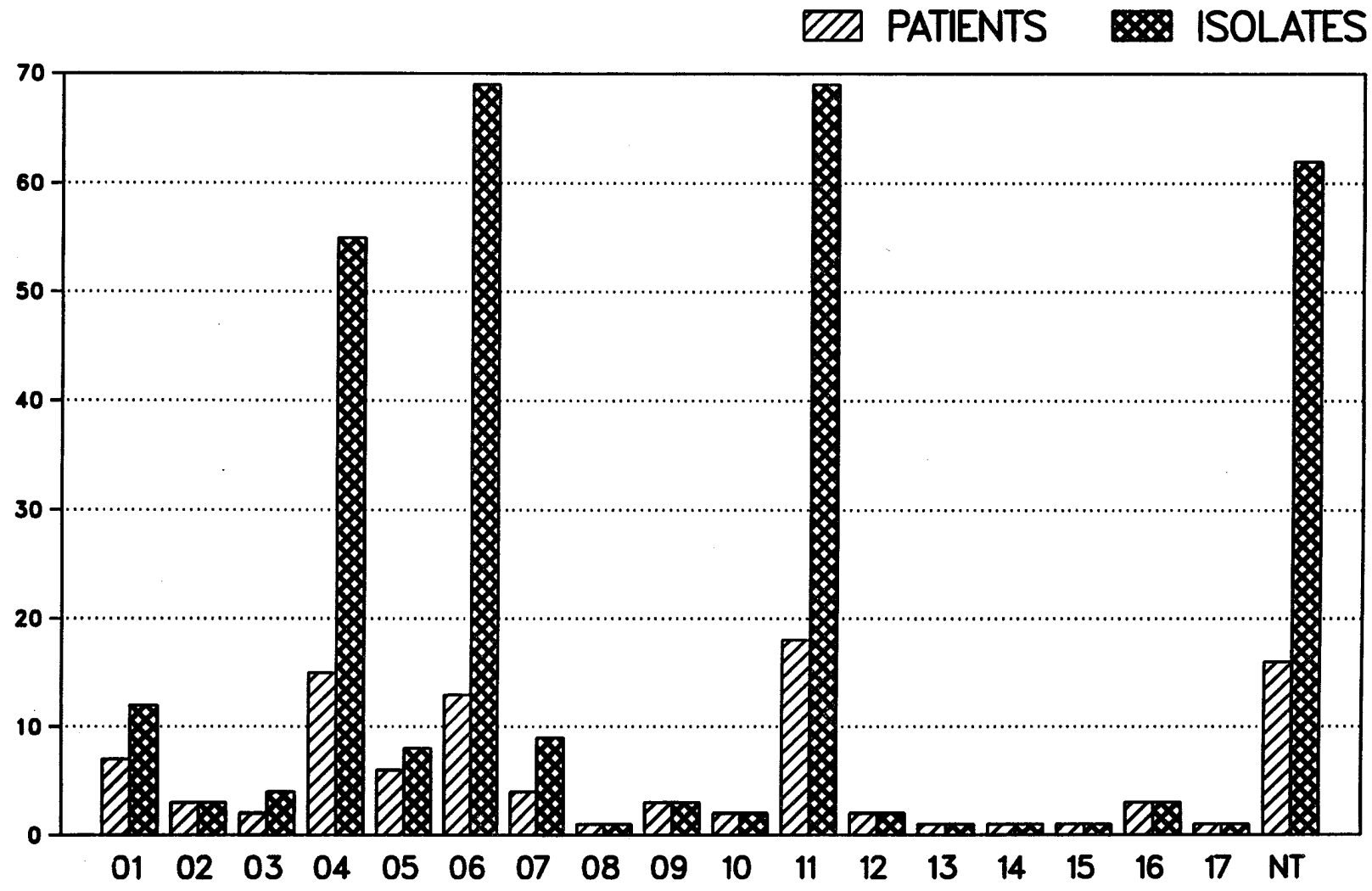


FIGURE 2. Histogram display of the frequency of *Pseudomonas aeruginosa* O-serotypes (Difco International Typing Sera™) by number of patients and isolates. NT indicates all strains not typeable by the standard 17 typing sera.

patients with each serotype and the total number of isolates per serotype. Figure 3 summarizes the number of patient isolates per serotype by year from 1 January 1984 through 30 September 1991.

***Pseudomonas aeruginosa* Bronchopneumonia Model.** A bronchopneumonia model was developed using the In-Tox™ nose-only aerosol exposure system. On the day of the study, Harlan Sprague-Dawley rats were administered 20% total body surface area full-thickness scald burns and then exposed to an aerosol of *Pseudomonas aeruginosa* (Strain VA-134) for 30, 60, or 90 min. The 90-min exposure time produced the best results, with over a 78% mortality rate (see Table 5). To determine if the animals were being inoculated via the respiratory tract or the burn wound, animals were sacrificed at 1, 2, 3, 4, 5, and 6 days after aerosol exposure. Quantitative bacterial counts were performed on the blood, left lung, and a 25-mm^2 section of the burn wound or dorsal skin. Quantitative bacteriology found no organisms present in the blood of either the burn or nonburn group. Organisms were present in the lungs and wound of the burn group on all six days of the study, while positive cultures were detected in the nonburned group on only one of the six days (see Table 6). At the same time the quantitative bacteriology study was conducted, the animal's heart, right lung, liver, spleen, gastrointestinal tract, kidneys, and a section of the burn wound or dorsal skin were taken for histological examination. Histological examination found lung inflammation on days 1 through 5 after aerosol exposure. Bacteria were not detected on the burn wound until day 4 after aerosol exposure, with bacteria being found in the deep muscle of the wound at day 3 after aerosol exposure.

Aerosolized Mafenide Acetate Treatment of *Pseudomonas aeruginosa* Bronchopneumonia. Harlan Sprague-Dawley rats were administered a 20% total body surface area full-thickness scald wound. The next day, the animals were exposed to an aerosol of *Pseudomonas aeruginosa* (Strain VA-134). Aerosolized mafenide acetate treatment was initiated at 2, 12, or 24 h postexposure and repeated again after 24 and 48 h. Each animal received three treatments of 15 min each. At each time tested, aerosolized mafenide acetate produced results less than 50% of the time (see Table 7).

PRESENTATIONS/PUBLICATIONS

None.

REFERENCES

1. McManus AT, English VC, Denton CL, et al: Alteration of host resistance. In *US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1992*. San Antonio: US Government Printing Office, 1992, pp 330-7.

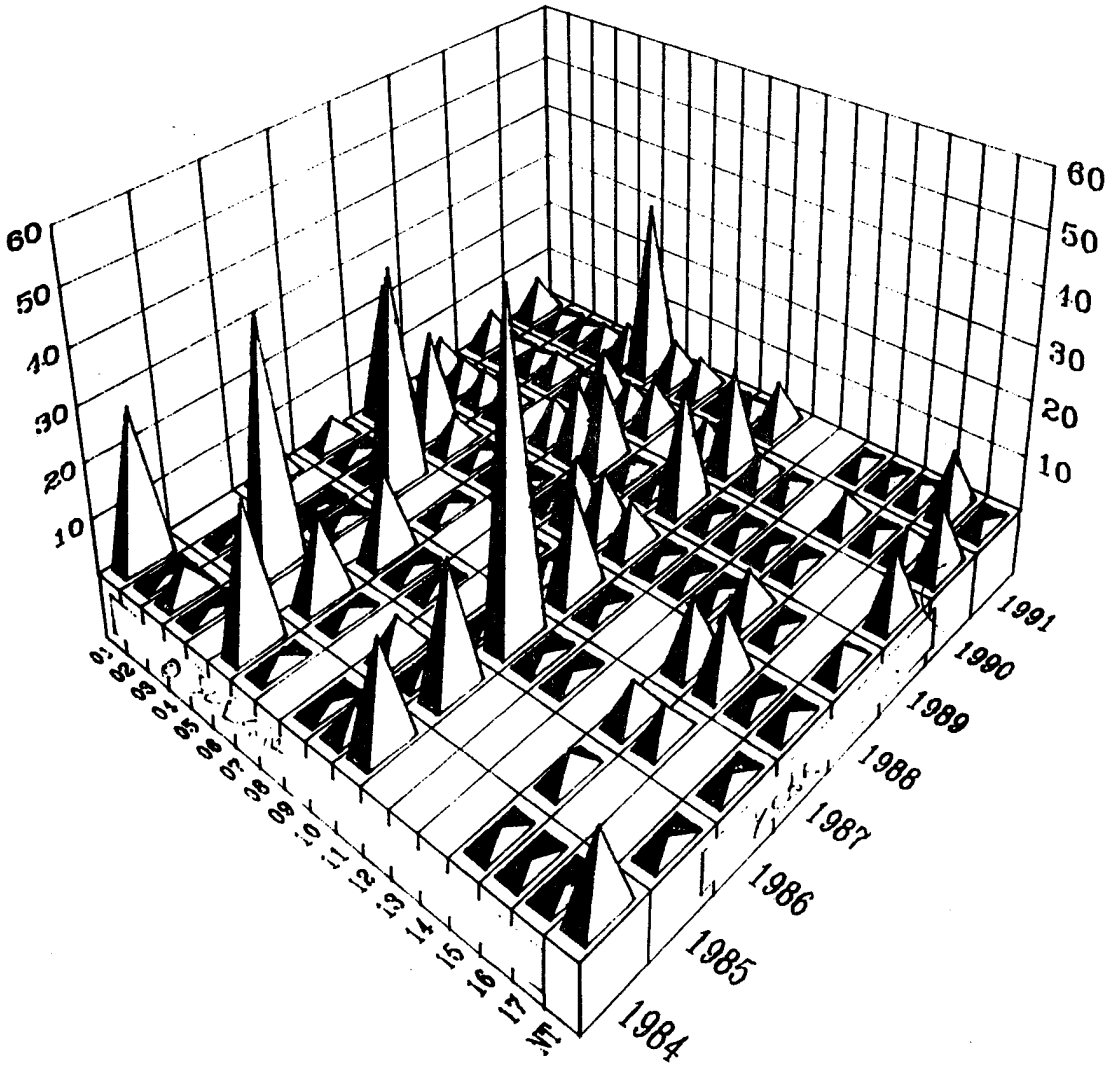


FIGURE 3. Frequency of number of patients per *Pseudomonas aeruginosa* O-serotypes (Difco International Typing Sera™) by year from 1 January 1984 through 30 September 1992. NT indicates all strains not typeable by the standard 17 typing sera.

TABLE 5. Exposure Time Versus Mortality in a Rat Model of Bronchopneumonia

Group	Exposure Time (min)	Number of Animals		Mortality (%)
		Total	Dead	
Sham burn	30	10	–	–
	60	20	–	–
	90	18	–	–
Burn	30	20	12	60.0
	60	29	18	62.1
	90	28	22	78.6

TABLE 6. Detection of Bacteria by Histological Examination in a Rat Model of Bronchopneumonia (Infected/Total)

Group	Day of Sacrifice*	Specimen		
		Blood	Lung	Skin
Burn	1	0/10	10/10	1/10
	2	0/10	7/10	9/10
	3	0/10	9/10	9/10
	4	0/10	8/10	10/10
	5	0/10	3/10	3/10
	6	0/10	4/8	7/8
Sham burn	1	0/10	7/10	4/10
	2	0/10	4/10	2/10
	3	0/10	0/10	3/10
	4	0/10	1/10	0/10
	5	0/10	2/10	2/10
	6	0/10	0/8	0/8

*Indicates day after exposure.

TABLE 7. Examination of Aerosolized Mafenide Acetate in a Rat Model of Bronchopneumonia (Dead/Total)

Group	Time of Treatment (h)	Number of Animals Total	Dead	Mortality (%)
Sham burn	2	29	–	–
	12	20	1	5.0
	24	30	3	6.7
Infection control	2	28	23	82.1
	12	20	17	85.0
	24	30	26	86.7
Mafenide acetate	2	29	17	58.6
	12	30	16	53.3
	24	30	18	60.0
Sterile water	2	10	9	90.0
	12	9	8	88.9
	24	20	14	70.0

ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3M161102BS14-303, Research

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

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

PERIOD COVERED IN THIS REPORT: 1 October 1991 - 30 September 1992

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Previous studies had indicated that the presence of neopterin in serum might be useful as an early indicator of infection in patients with thermal injury. We have measured serum neopterin and urinary neopterin excretion in more than 250 burn patients. Neopterin was measured in 24-h urine samples and corresponding serum samples by HPLC once weekly during the postburn recovery period. Since renal impairment influences both serum and urine neopterin by altering excretion, samples were screened to exclude specimens from patients with creatinine clearances of < 50 ml/min. Mean serum neopterin and 24-h neopterin excretion were similar for infected and noninfected patients with similar burn sizes. The pattern of mean serum neopterin concentration and 24-h neopterin excretion were also similar during the postrecovery period for both infected and noninfected burn patients. Mean serum concentration did not change significantly with respect to time of infection diagnosis in infected patients whereas urine neopterin decreased just before and shortly after the diagnosis of infection. In contrast to serum neopterin levels and 24-h excretion, the pattern of neopterin clearance during the postburn recovery period was different for infected and noninfected patients. When plotted versus time of infection diagnosis, neopterin clearance in infected patients was decreased before the diagnosis of infection, increased to the normal range during the time of infection diagnosis, and decreased again after the diagnosis and treatment in comparison to neopterin clearance in noninfected patients . Rather than use neopterin clearance which requires a 24-h urine collection, a similar pattern with respect to the time of infection diagnosis could be obtained from a ratio of serum neopterin/serum creatinine and urine neopterin/urine creatinine. This ratio should be useful for monitoring neopterin clearance from a "spot" urine sample.

CHARACTERIZATION OF BIOCHEMICAL INDICATORS OF INFECTION IN THE THERMALLY INJURED

The massive response to severe thermal injury is frequently accompanied by fever and high leukocyte counts in burn patients. The distortion in these two classical indicators of infection can make systemic infection difficult to diagnose. An easily measured biochemical metabolite of immunoreactive cells might give a more objective measure of the presence of systemic infection and have diagnostic utility in these patients. Such a metabolite would have to differentiate the host response to infection from the host response to trauma. Neopterin is a pteridine derived from GTP as a byproduct of the synthesis of tetrahydroneopterin. Its precursor, dihydroneopterin triphosphate, is a precursor of tetrahydrobiopterin. Tetrahydrobiopterin has been shown to be a cofactor in the hydroxylation of phenylalanine to form tyrosine, the precursor of serotonin and the catecholamines. In addition, tetrahydrobiopterin appears to be a cofactor for nitric oxide synthetase (1–3) in the production of nitric oxide from arginine. Nitric oxide has been to shown to have many physiological effects, including modulation of the immune response. There is no known biological function for neopterin, but it is secreted in large amounts by macrophages (4) and B cells (5) stimulated with cytokines, especially γ–interferon. These leukocytes appear to have a block in the biosynthetic pathway of tetrahydrobiopterin, leading them to accumulate neopterin when the activity of the cyclohydrolase which forms neopterin from GTP is increased.

Neopterin is secreted in increased amounts by patients with viral infection, including HIV (6–9), tuberculosis (10), and sepsis (11–14). Neopterin has been used as a prognostic indicator for certain kinds of cancer (15,16) and as a measure of allograft rejection (17).

The previous report for this project noted a general increase in the excretion of neopterin in burn patients over nonburned controls. Yet in the group of patients experiencing systemic infection, there was a decreased level of urinary neopterin compared to patients who did not experience infection (18). This report discloses the results of further investigation of the potential usefulness of neopterin measurement in serum and urine in the diagnosis of infection in burn patients.

MATERIALS AND METHODS

Study Design. A total of 803 serum and urine samples drawn for clinical purposes were used for this study. The size of burn in this group of patients ranged from 5.5% to 89.5% of the total body surface area, with an average of 49.4%. Samples were obtained from 250 different patients on approximately a weekly basis. Blood samples were drawn at 0600 at the end of a 24–h urine collection

period. Neopterin and creatinine were determined in each sample. Neopterin values were reported as neopterin per milligram creatinine to compensate for small variations in the urine flow. Since serum and urine neopterin concentrations could be affected by renal function, creatinine clearance was calculated for each patient to permit screening for renal sufficiency. Infections were diagnosed according to the criteria reported previously (19). Systemic infections were considered to be pneumonia, bacteremia, and wound infection. All other infections (urinary tract, bronchitis, cellulitis) were not considered to be systemic and were considered to be samples from noninfected patients.

Neopterin levels were determined in serum (300 µl) that was deproteinized by adding 200 µl of 0.12N potassium phosphate buffer (final pH 4.5) and incubating at 100°C in an oil bath for 20 min. The mixture was centrifuged at 20000 g for 20 min and filtered through a 0.45-µ filter (Rainin, Woburn, MA). A 200-µl aliquot of the supernatant was injected directly on the HPLC. Urine samples were prepared by a dilution of 1:10 with water. Cloudy samples were filtered with a 0.45-µ filter. A 10-µl sample was injected directly onto the HPLC.

HPLC Analysis of Neopterin. A Model 1090 (Hewlett-Packard, 3200 Hillview Avenue, Palo Alto, CA 94304) liquid chromatograph with a Biophase™ ODS reverse phase 4.6 X 250 mm column (Bioanalytical Systems, West Lafayette, IN) was used. The mobile phase consisted of 0.05M ammonium acetate (Ph 7.0). Column temperature was maintained at 45°C and the flow rate was 1.0 ml/min. The HPLC was equipped with a Kratos™ fluorescence detector (Model 980, Kratos Analytical, Ramsey, NJ) with a 25-µl flow cell. The excitation monochronometer was set at 350 nm and the emission cutoff filter was at 389 nm. The retention times for standard neopterin (Sigma Chemical Company, St. Louis, MO) were determined using 10 µl of a standard solution of pterins (10 ng/ml). The amount of neopterin was determined using a Model 3392A integrator (Hewlett-Packard).

The potential problems in data interpretation caused by accumulation of serum neopterin due to insufficient renal excretion were addressed by eliminating from consideration all samples with a creatinine clearance rate of < 50 ml/min. Creatinine clearance is a more direct measure of renal function than serum creatinine and thus a better indication of sample integrity.

RESULTS

Our previous studies had shown that serum neopterin and urine neopterin values were increased after thermal injury reaching a maximum at 5 to 6 weeks postburn. There was no correlation between neopterin urine or serum concentrations and burn size. There was also no correlation between negative nitrogen balance (which is

associated with the hypermetabolic response) and serum or urine neopterin concentration (unpublished data).

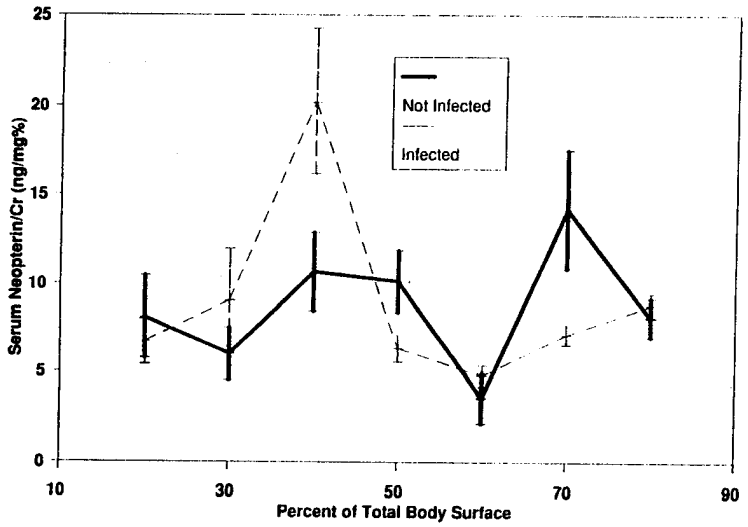
The effect of infection on serum and urine concentration was analyzed as follows. Neopterin levels in samples from patients with a systemic infection were compared to neopterin values of samples taken from patients who experienced no systemic infection during their hospital stay. Figures 1 and 2 show that systemic infection did not systematically change the pattern of serum or urine neopterin levels in relation to burn size. Figure 3 depicts serum neopterin levels as a function of time postburn. With increasing time postburn, urine neopterin levels from infected patients were consistently lower than in noninfected patients while serum levels were not significantly different.

Samples from infected patients were grouped according to the time of infection diagnosis. Figure 4 depicts the comparison of these grouped samples to the range of neopterin levels in noninfected patients. There is no change in serum neopterin levels before or after infection diagnosis compared to the samples from noninfected patients. Urinary levels are similar to noninfected levels more than a week before infection diagnosis. Levels begin to decrease below those of noninfected patients during the week before infection diagnosis and reach a low point approximately 1 week after diagnosis.

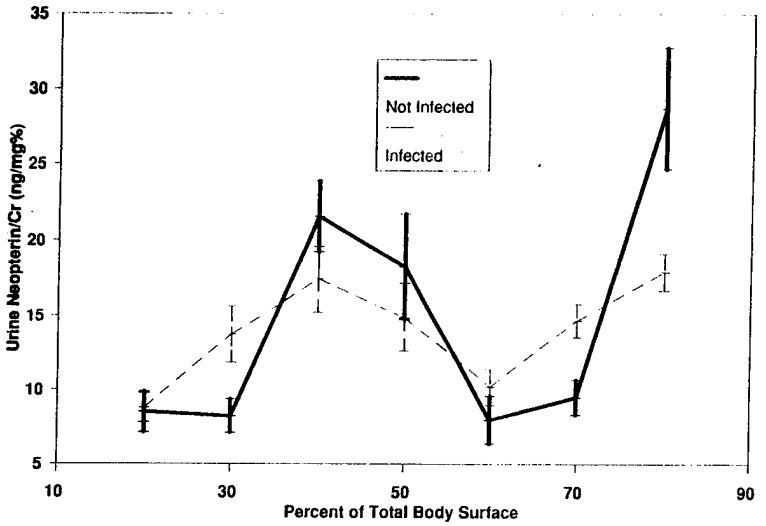
Neopterin clearance from the circulation can be calculated in the same manner as creatinine clearance. Creatinine is principally excreted by glomerular filtration and creatinine clearance gives a reasonable estimate of the GFR, which averages about 125 ml/min for normal individuals. Neopterin clearance is approximately three to four times that of creatinine clearance in burn patients. This is evidence that the excess neopterin production in burn patients is excreted in an active process via the renal tubules rather than by glomerular filtration. The temporal relationship of neopterin clearance in burn patients is shown in Figure 5. Neopterin clearance in infected patients is frequently below and never significantly above that of noninfected patients during the postburn recovery period.

Neopterin clearance from infected patients was compared to that of noninfected patients in relation to the time of diagnosis of infection. Figure 6 illustrates that even several weeks before infection diagnosis, neopterin clearance is decreased in patients that will become infected. Neopterin clearance rises abruptly during the week previous to the diagnosis of infection and enters the normal range for approximately the week before and after infection diagnosis. Neopterin clearance then decreases again in the postinfection period.

Determination of neopterin clearance is labor-intensive because a 24-h urine collection is required. A similar result was obtained

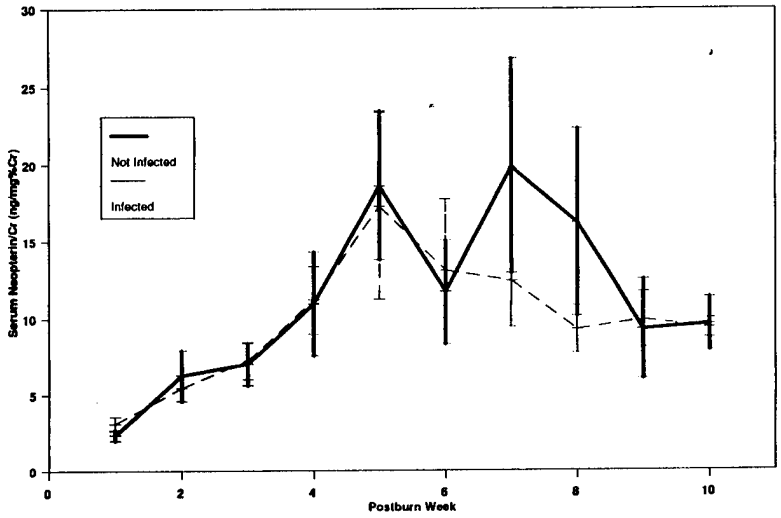


A

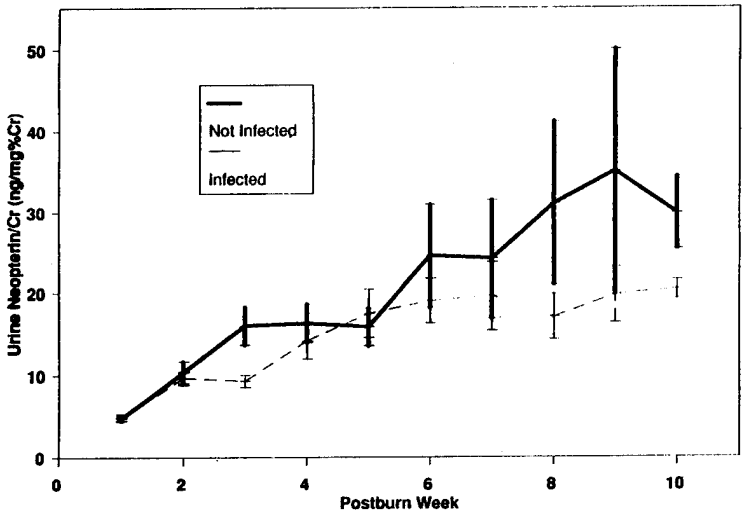


B

FIGURE 1. Mean serum (A) and urine (B) neopterin values in infected and noninfected sufficient burn patients within a particular range of total body surface area burn sizes are plotted against the range of burn sizes, e.g., 10 = burn size range from 10% to 19%. Error bars represent mean ± SEM.

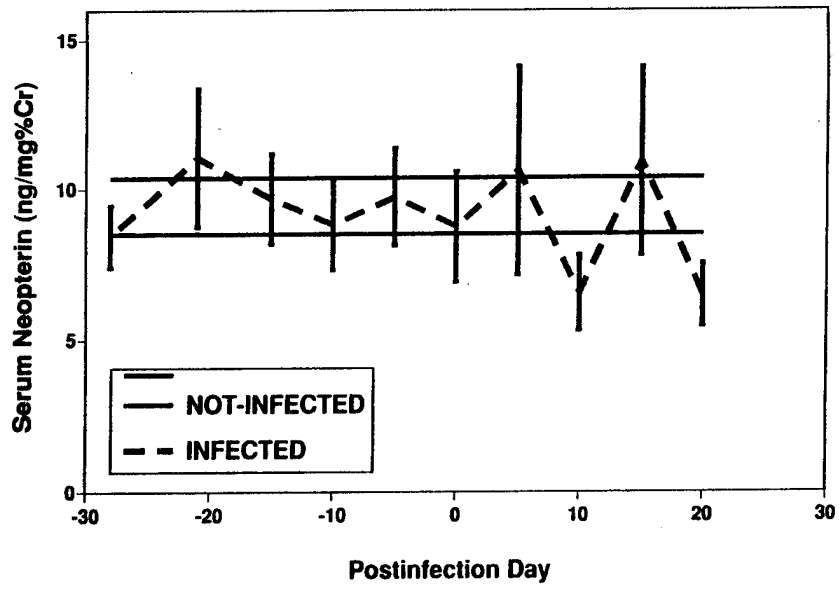


A

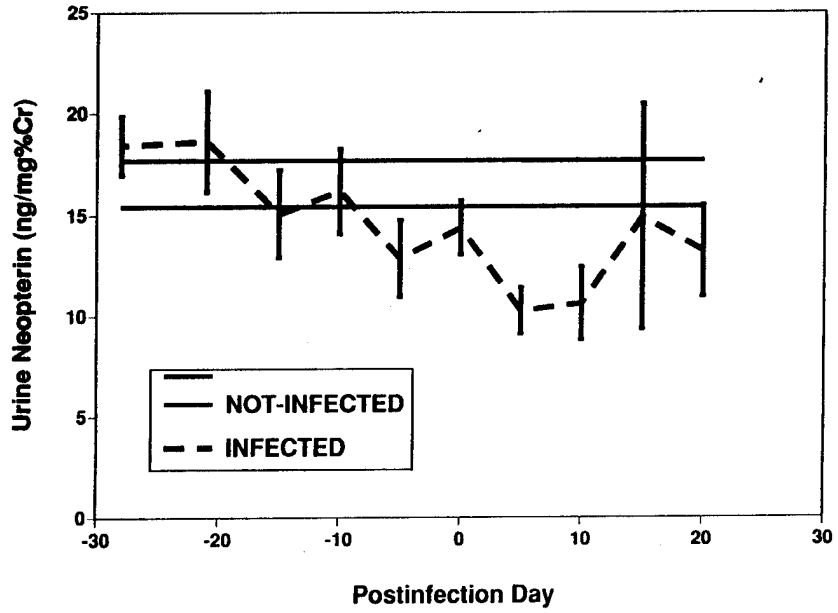


B

FIGURE 2. Mean serum (A) and urine (B) neopterin values in infected and noninfected sufficient burn patients for all serum and urine samples taken during each week postinjury are plotted against the postburn week. Error bars represent mean ± SEM.



A



B

FIGURE 3. Mean serum (A) and urine (B) neopterin values in sufficient infected burn patients for all serum and urine samples taken from infected patients before or after infection diagnosis are plotted against the time postinfection and compared to noninfected patients. Error bars and the range for noninfected patients represent mean ± SEM.

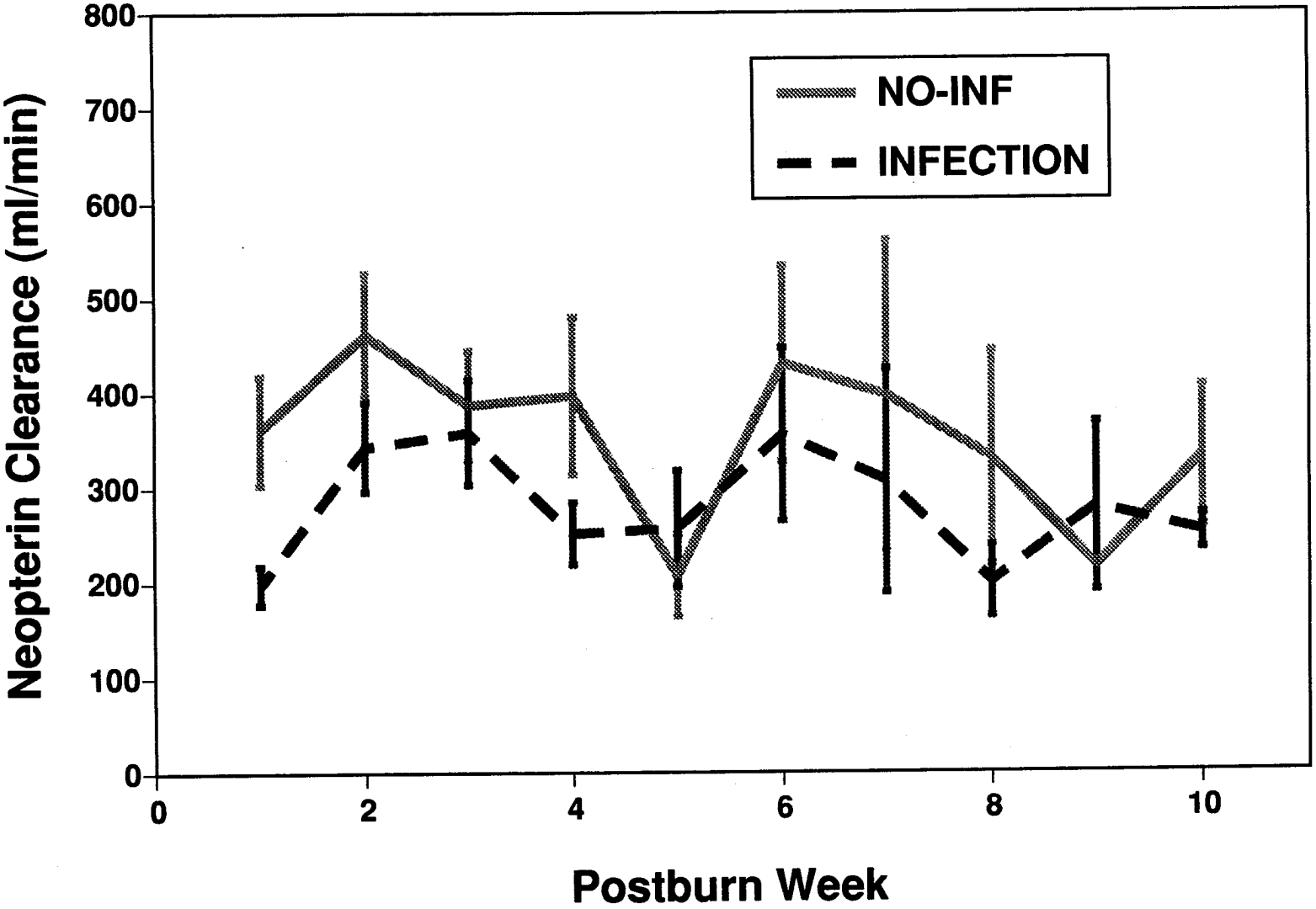


FIGURE 4. Mean neopterin clearance in infected and noninfected sufficient burn patients versus postburn week. Neopterin clearance was calculated in the same manner as creatinine clearance for all patients. Error bars represent mean ± SEM.

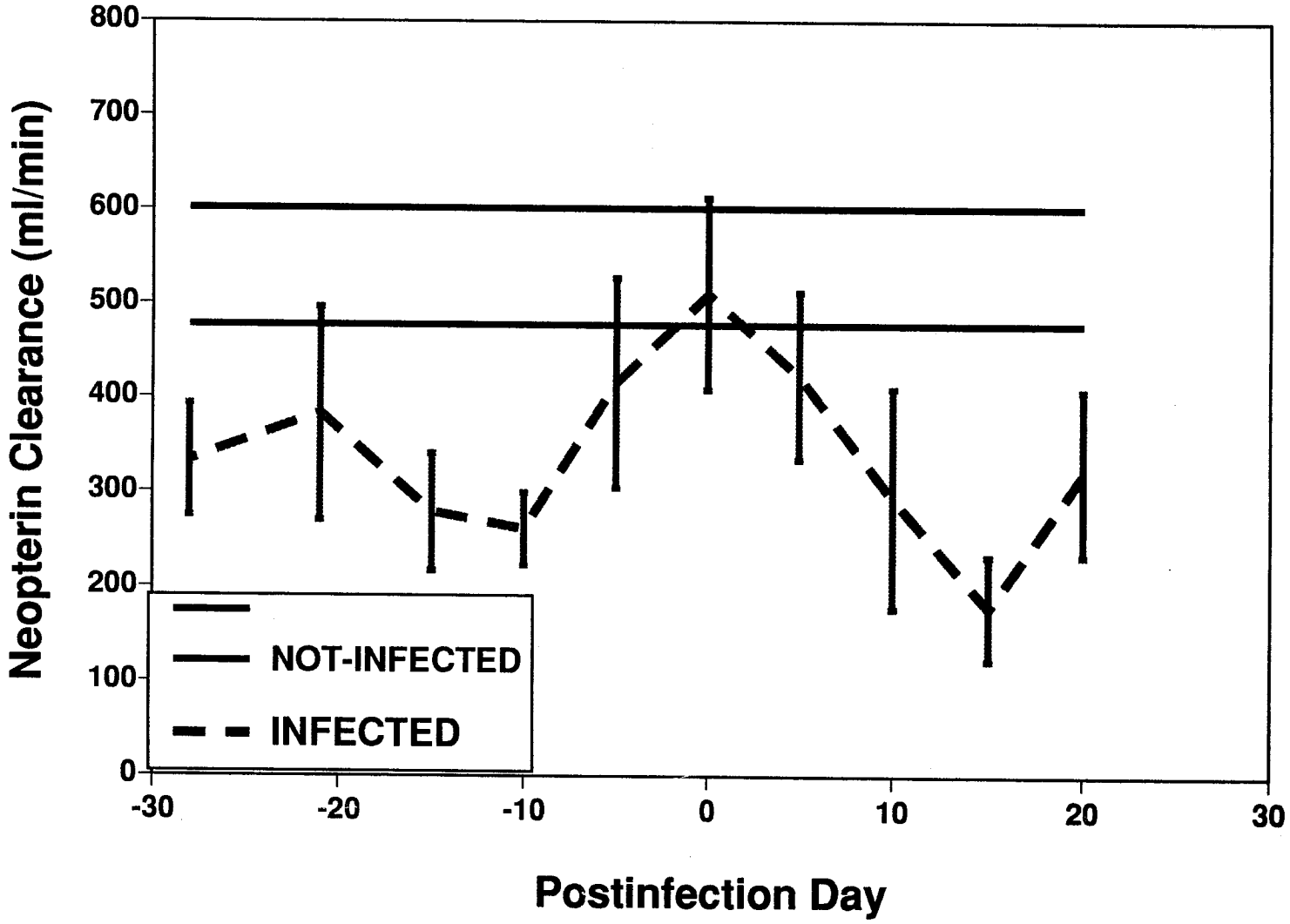


FIGURE 5. Mean neopterin clearance in infected sufficient burn patients versus time of infection diagnosis. Mean neopterin clearance was calculated for all infected patients in the study and grouped by the times indicated and plotted versus time with respect to the day of diagnosis. Error bars and the range for noninfected patients represent mean ± SEM.

using a more easily determined ratio between neopterin and creatinine in serum and urine. This value can be obtained by dividing the ratio of serum neopterin/serum creatinine by that of urine neopterin/urine creatinine obtained using a "spot" urine. A 24-hr urine collection "averages" the variation of glomerular filtration that occurs over shorter periods of time. In a "spot" urine collection, the creatinine concentration in serum and urine helps to compensate for the variation in excretion rate that may occur at a point in time. Figure 6 depicts the temporal relationship between this ratio and the time of diagnosis of infection compared to the range of ratios from noninfected patients. The curve of the ratio is very similar to that obtained using neopterin clearance. During the two weeks before the diagnosis of infection, the clearance ratio is below the range of the noninfected patients. The ratio slips within the normal range by the day of infection diagnosis and drops below the range again after the next week. Since the ratio is easier to obtain than neopterin clearance and gives a similar result, it may be more practical to use to predict those patients who are susceptible to infection.

DISCUSSION

Two other laboratories have now reported that serum neopterin levels are increased in burn patients (20,21). They surmise that the increased levels are due to the general stimulation of immunological activity resulting from the release of cytokines after thermal injury. The increase in serum neopterin they report agrees with that reported here, reaching a maximum at 3 to 5 weeks postburn and roughly corresponding to the increased metabolic rate seen in burn patients during that time.

Our results indicate that patients who are subsequently diagnosed with infection have decreased production (urine excretion) a week before infection diagnosis compared to those patients who remain free of systemic infection. Of the parameters measured, neopterin clearance seems to be the potentially valuable predictor for infection. Since the sample necessary to determine neopterin clearance is difficult to obtain, another predictor is proposed. The ratio of serum neopterin concentration to serum creatinine concentration divided by the ratio of urine neopterin concentration to urine creatinine concentration is easily obtainable and has a predictive profile virtually identical to that of neopterin clearance. The equation may be rearranged as follows:

$$\frac{\frac{S_{Neop}}{S_{Cr}}}{\frac{U_{Neop}}{U_{Cr}}} = \frac{S_{Neop} * U_{Cr}}{U_{Neop} * S_{Cr}}$$

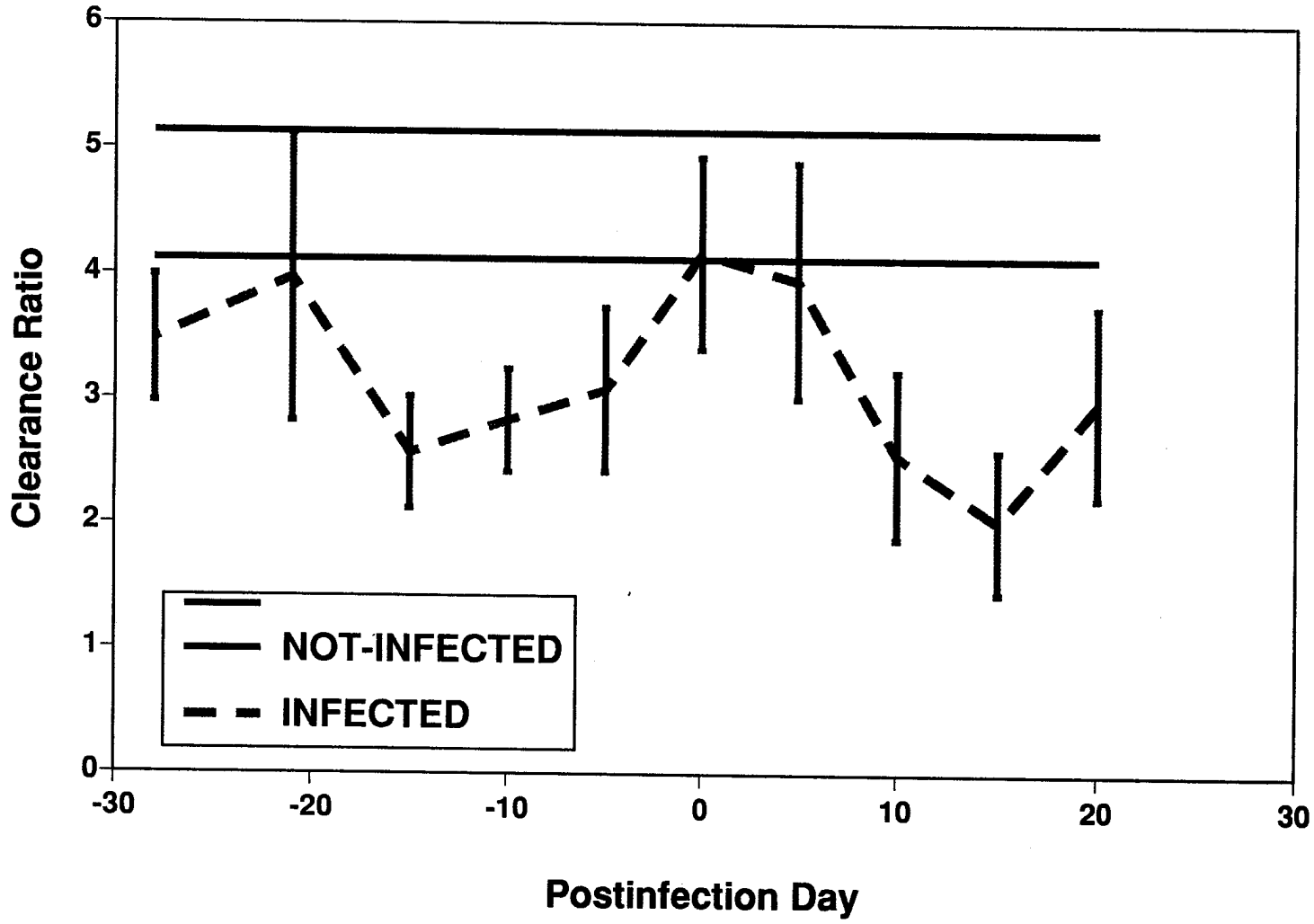


FIGURE 6. Mean neopterin clearance/creatinine clearance ratio versus time of infection diagnosis. This figure depicts the temporal relationship between the ratio and the time of diagnosis of infection and is compared to the mean ratios ± SEM from noninfected patients. During the two weeks before the diagnosis of infection, the clearance ratio is below the range of the noninfected patients.

The studies to this point have been performed on excess clinical specimens (24-h urine) that are collected only once per week. A new phase of the study is now required that will measure sequential daily or triweekly serum and urine samples in order to more closely correlate neopterin excretion to the clinical status of the patient.

PRESENTATIONS

Burleson DG: Neopterin in burned patients. Presented at Henning Berlin Laboratories and the Urban Hospital, Berlin Germany, 12 September 1992.

PUBLICATIONS

Burleson DG, Johnson A, Salin M, Mason AD Jr, Pruitt BA Jr: Identification of neopterin as a potential indicator of infection in burned patients. *Proc Soc Exp Biol Med* 199:305-10, March 1992.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

ACCESSION: DA0G1842

SUMMARY DATE: 921001 **SUMMARY KIND:** D **PREV DATE:** 911001 **DISTRIBUTION:** CX

PROGRAM #: 61102A **PROJ #:** 30161102BS14 **TASK AREA:** BG **WORK UNIT:** 304

TITLE: Thyroid Hormones as Neurotransmitters or Neuromodulators

SUBJ1: 060100 - Biochemistry
SUBJ2: 060500 - Medicine and Medical Research

START DATE: 7908 **END DATE:** 9909 **PERFORMANCE METHOD:** C

CONTRACT/GRANT NO:

CONT TOTAL:	$
CUM TOTAL:	$
TOTAL LAB FUNDS:	$

RESOURCES ESTIMATE

FY	WORK YRS	$(Thousands)
91	1.5	$99
92	1.5	$55
93	1.5	$106

RESPONSIBLE DOD ORGANIZATION: 404885
MRDC INSTITUTE OF SURGICAL RESEARCH USAISR
FT SAM HOUSTON, TX 78234
PRUITT, B A
210-221-2720

PERFORMING ORGANIZATION: 404885
MRDC INSTITUTE OF SURGICAL RESEARCH USAISR
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ASSOC1:

ASSOC2:

FOREIGN INTELLIGENCE CONSIDERED: Y **CIVILIAN APPLICABILITY:** M

KEYWORDS: RA II; Volunteers; Adults; Lab Animals; Rats; Hamsters; Burns (Injuries); Thyroid Hormones; Thyrotropin; Thyroxine

OBJECTIVE: A DTIC literature search was conducted under DTIC request numbers W6L23A/W6L24A dated 20 October 1989. The objectives of this work are to assess postburn alterations in thyroid function and develop treatment to improve survival of patients with thermal injury. Knowledge of the mechanisms of host hormonal and metabolic responses to injury will ultimately allow more effective interventions and greater salvage of patients with thermal injury.

APPROACH: Development of methods capable of disclosing thyroid axis control in burn injury.

PROGRESS: 9110-9209. Further evaluation of the immunoprecipitation (IMPT) method for quantitating tracer levels of T_4 and T_3 has been necessary. It was found that neither tracer iodide nor iodoalbumin, important tracer metabolites in kinetic studies, has any detectable crossreactivity in the method. Protocols are being developed to employ this technique in in vivo kinetic studies in controls and burns. Also in preparation for these protocols, a chemiluminometric technology capable of third-generation assay of human TSH in serum has been acquired and evaluated. This system is capable of satisfying our requirement to measure TSH well below the normal range, where TSH predicts nonsurvival. A reproducible depression of serum TSH occurs within 30 to 60 min of beginning infusion of a low-dose dopamine in normal subjects and burn patients. Combining these methods (IMPT, chemiluminometric TSH) with the new RIA-assisted direct dialysis technique for determination of endogenous nontracer FT_4, a capability to assess the role of iodothyronine secretion versus clearance in the

thyroid aberrations associated with burn injury and the mechanisms generating them has been developed. For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report* for fiscal years 1979 through 1992.

ABSTRACT

PROJECT NUMBER: 3M161102BS14-304, Research

PROJECT TITLE: ROLE OF THYROID HORMONES IN BURN PATHOPHYSIOLOGY: Acute Thyrotropin (TSH) Response to Low-Dose Dopamine Infusion in Burn Patients

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

PERIOD COVERED IN THIS REPORT: 1 October 1991 - 30 September 1992

INVESTIGATORS: George M. Vaughan, MD, Colonel, MC
Theresa A. Graves, MD, Major, MC
William G. Cioffi, Jr., MD, Major, MC

It is commonly accepted that subjects receiving a dopamine infusion may have suppressed function of the thyroid axis as a result of dopamine-induced depression of pituitary TSH secretion. However, little is known of the acute time course of serum TSH concentration when a "renal" or "low" dose infusion of dopamine is given to ill ICU patients. The effect of dopamine on TSH has not been described in burn patients.

Before and during an infusion of dopamine (3 μg/kg/min), serum TSH was measured with a third-generation (chemiluminometric) double epitope immunoassay. In addition, serum FT_4 concentrations were measured by direct RIA of the 37°C equilibrium dialysate from undiluted samples dialyzed against a buffer specially designed to mimic human serum but without iodothyronine binding proteins.

In the short time of the dopamine infusion (3 h), there was no change in FT_4. The presence of burn injury did not appear to diminish the acute TSH-depressive effect of low-dose dopamine infusion. The rapid response (within 30 to 60 min) was of similar time course and magnitude as in the normal control subjects. This indicates a grossly normal responsiveness of pituitary thyrotrophs to dopamine and suggests that endogenous dopamine could play a role in TSH control in burn patients.

ACUTE THYROTROPIN (TSH) RESPONSE TO LOW-DOSE DOPAMINE INFUSION IN BURN PATIENTS

It is commonly accepted that subjects receiving a dopamine infusion may have suppressed function of the thyroid axis as a result of dopamine-induced depression of pituitary TSH secretion (1-7). However, little is known of the acute time course of serum TSH concentration when a "renal" or "low" dose infusion of dopamine (3 μg/kg/min) is given to ill ICU patients. The effect of dopamine on TSH has not been described in burn patients.

MATERIALS AND METHODS

In conjunction with a separately reported study (8) of the renal effects of low-dose dopamine infusion in burn patients, serum TSH was measured with a third-generation (chemiluminometric) double epitope immunoassay having a functional (between-assay precision-dependent) detectability in serum of 0.01 μU/ml (9,10) performed on instrumentation purchased from Nichols Institute (San Juan Capistrano, CA). The normal range is 0.4 to 4.6 μU/ml. Control serum from a patient with hypothalamic destruction, tertiary hypothyroidism, and T_4 replacement therapy revealed a nondetectable value ≤ 0.01 μU/ml, confirming the specificity of the assay in the highly sensitive (low) range. Use of such an assay capable of measuring low values as distinct from "noise" (e.g., down to just above 0.01 μU/ml) is important in assessing potential depressions of TSH concentrations.

In addition, serum FT_4 concentrations were measured by direct RIA of the 37°C equilibrium dialysate from undiluted samples dialyzed against a buffer specially designed to mimic human serum but without iodothyronine binding proteins (11). This procedure was performed with kits obtained from Nichols Institute and has been thought to have enhanced reliability in measuring FT_4 in samples from patients with nonthyroidal illness, with no initial dilution of binding inhibitors. This procedure can detect depressed FT_4 in burn patients when demonstrated to be present by the conventional tracer dialysis technique (12). The least detectable concentration is 0.2 ng/dl and the normal range is 0.8 to 2.7 ng/dl FT_4 by this "direct" dialysis technique.

Five adult male control subjects 19 to 22 yr old without history of preexisting cardiac or renal disease were admitted one day before study. Twelve hours before study, intravenous dextrose in half-normal saline was administered at an infusion rate which stabilized urine flow rate at about 3.8 ml/min/1.73 m^2 body surface area and Na^+ excretion at about 12 mmole/h/1.73 m^2.

Ten thermally injured patients 19 to 53 yr old with burns covering 29% to 84% of the total body surface area were enrolled in the study. All patients underwent uneventful resuscitation and

were studied between the 9th and 26th postburn days. Only two patients were female. At the time of enrollment, all patients were without signs of infection or sepsis. Patients with preexisting renal or cardiac disease were excluded from study. Patients were not studied within 48 h of a surgical procedure. On the night before the study, the patient's intravenous fluid administration rates were adjusted such that the urine flow rate stabilized at about 1.7 ml/min/1.73 m^2 and Na^+ excretion was about 6.5 mmole/h/1.73 m^2. Composition of the intravenous fluids was tailored according to the patient's fluid and electrolyte status and needs. Routine morning burn care was postponed on the day of study until completion of the protocol. Enteral feedings were continued at a constant infusion rate in the patients.

Isotopic tracers for assessment of renal function were then infused, and the results of renal and cardiac function have been reported elsewhere (8). Samples for the present study underwent virtually total decay of radioactivity (^{99}mTc and ^{131}I) before measurements were made. The approximate time of stabilization of plasma isotope levels was considered experimental time zero. Beginning three hours after initiation of the isotope infusion and two hours after stabilization of renal tracer isotopes, dopamine was administered at a rate of 3 μg/kg/min by continuous intravenous infusion into a central vein between 2 and 5 h experimental time (1100–1400 h). After three hours' duration, the dopamine infusion was discontinued, and the isotope infusion and data collection continued for one additional hour in the patients. For the present study, serum samples were collected at hourly intervals from −1 to 5 or 6 h. Subjects were not given drugs known to influence TSH, such as thyroid hormones, corticosteroids, furosemide, heparin, or phenytoin.

Statistical analysis included obtaining a within-subject mean TSH and FT_4 for the four basal samples (−1 to 2 h) taken before the beginning of the dopamine infusion. Between-group comparisons (control vs burn) were assessed on these values by t test. In order to remove between-subject variation for assessment of changes with experimental time (effect of dopamine) within groups, each value was divided by the mean basal value for the subject and multiplied by the mean basal value for the group. Comparisons among times within a group were assessed by one-way ANOVA and Bonferroni-corrected t tests.

RESULTS

In Figure 1, there is no significant difference between burn and control basal serum TSH. However, by 1 h of dopamine infusion, a significant depression of TSH was seen in both groups. In two patients in whom a sample was available after only 30 min of dopamine, TSH appeared already depressed (not shown). In the burn group from whom samples were available 1 h after the end of the dopamine infusion, TSH had returned to baseline. Figure 2 shows

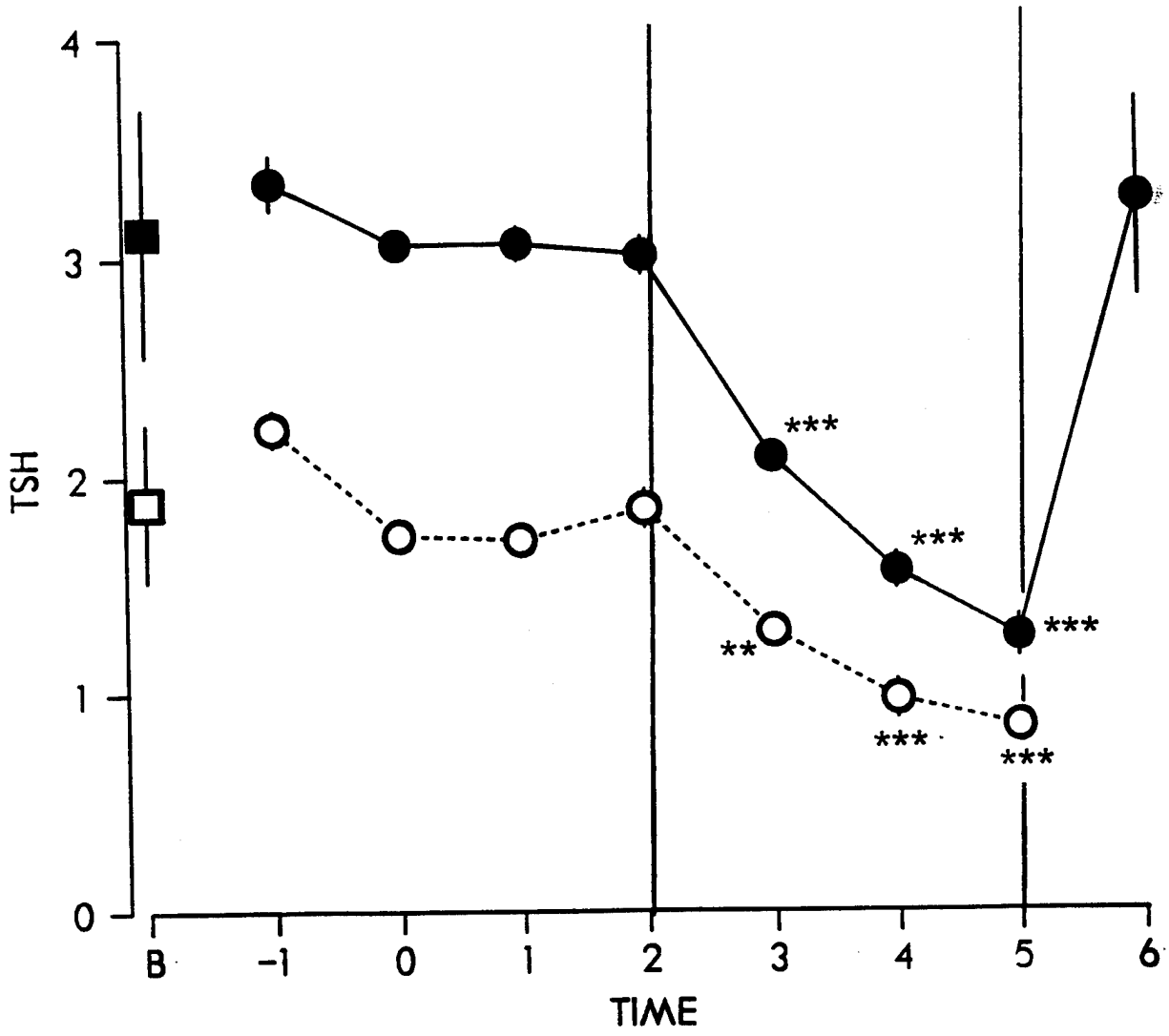


FIGURE 1. Serum thyrotropin (TSH, μU/ml) ± SE sampled in relation to infusion of dopamine (3 μg/kg/min) between time (h) = 2 and time = 5. The square symbols over B on the abscissa have between-subject variation and are derived from within-subject means of basal values from time = −1 to time = 2. The round symbols are means of data calculated as a proportion of the within-patient basal value and multiplied by the group basal mean in order to minimize between-subject variation for assessment of variation between times within-group. Open symbols indicate controls; closed symbols, burn patients. **P < 0.01, ***P < 0.001 vs time = 2.

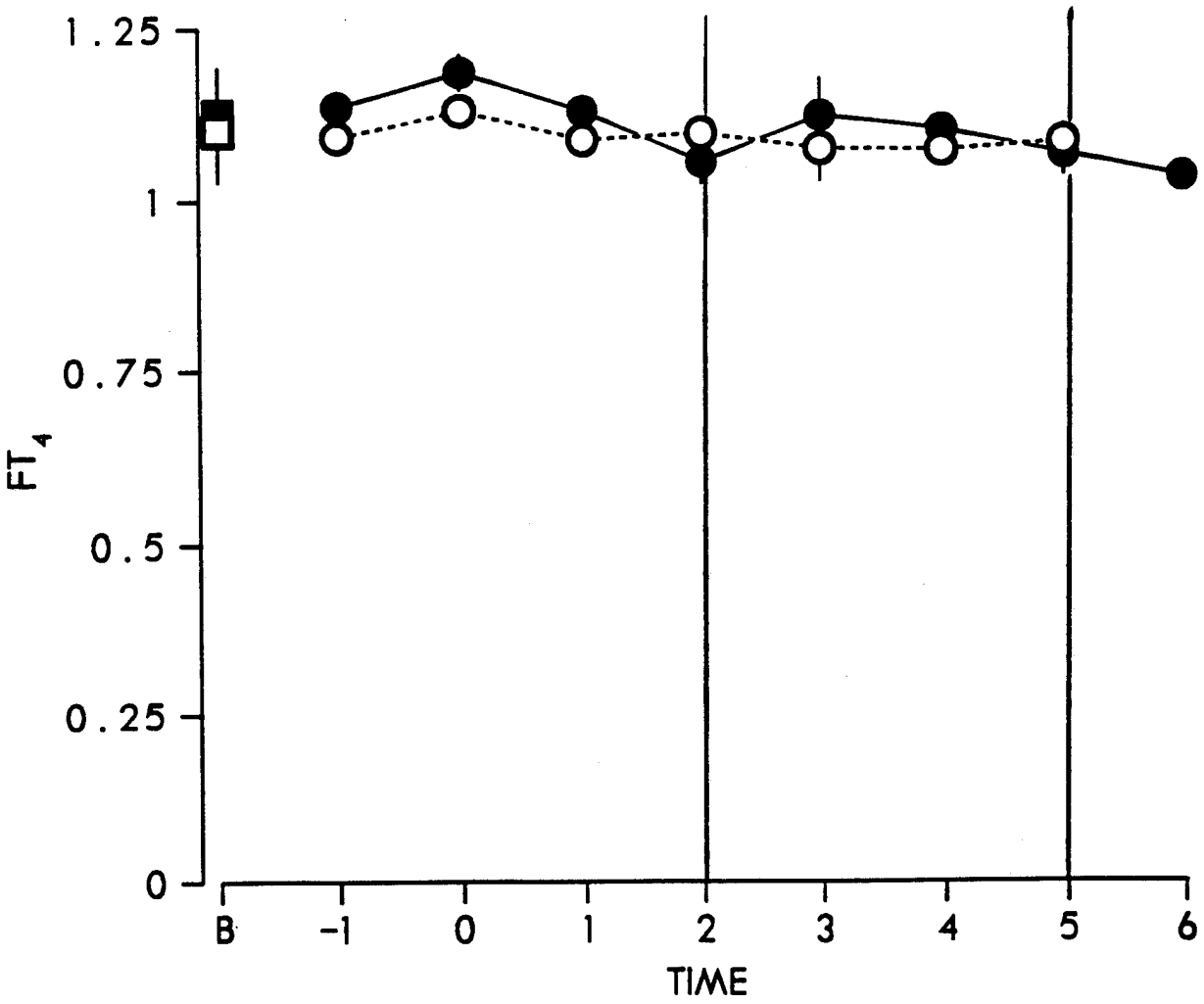


FIGURE 2. Serum free T_4 (FT_4, ng/dl) ± SE sampled in relation to infusion of dopamine (3 µg/kg/min) between time (h) = 2 and time = 5. The square symbols over B on the abscissa have between-subject variation and are derived from within-subject means of basal values from time = −1 to time = 2. The round symbols are means of data calculated as a proportion of the within-patient basal value and multiplied by the group basal mean in order to minimize between-subject variation for assessment of variation between times within-group. Open symbols indicate controls; closed symbols, burn patients. **P < 0.01, ***P < 0.001 vs time = 2.

that these short-term changes in TSH did not produce significant alterations of serum FT_4.

DISCUSSION

The procedure of low-dose dopamine infusion (3 μg/kg/min) commonly used in ICU settings to protect renal function by augmenting renal plasma flow and cardiac output (8) in patients at risk for multiorgan failure dramatically lowers serum TSH within 30 to 60 min in normal subjects and burn patients without multiorgan failure. This effect appears to be reversed quickly at the end of a short infusion and does not influence FT_4 during such an infusion. This latter observation is most likely explained by the relatively long half-life of T_4 (expected to be several days). Even if T_4 secretion were stopped, this would not likely alter FT_4 over the time interval employed. However, longer dopamine-induced depression of TSH does result in depression of T_4 and presumably FT_4 in critically ill patients (2). In that study, the dopamine dose ranged from 2 to 21 μg/kg/min, the infusion time ranged from 1 to 14 days, TSH declined significantly from 2.3 to 1.1 μU/ml over 0.5 to 10 days, and T_4 declined significantly in parallel. Acute hormonal changes at the beginning of the infusion were not assessed. Normal subjects in that study received 6.5 μg/kg/min for 48 h, and acute hormonal changes in the first several hours are not assessable. Though TSH was depressed early in the infusion, T_4 appeared slightly depressed near the end of the infusion.

The pituitary thyrotroph cell is controlled by the hypothalamus by two major substances secreted into the hyothalamo-hypophyseal portal vessels, TSH-releasing hormone (TRH), a peptide which promotes TSH secretion, and dopamine, a catecholamine which inhibits TSH secretion. A third major endogenous influence is circulating thyroid hormones which inhibit TSH secretion. The role of dopamine as a normal mediator of TSH control in this complex system in humans was elucidated by use of acute infusions of dopamine antagonists such as metoclopramide hydrochloride (3,13-16). Such agents have little effect on the depressed TSH of thyrotoxic subjects and raise serum TSH in euthyroid subjects, an effect magnified in hypothyroidism. In normal subjects, the stimulatory effect of dopamine blockade is greatest around midnight at the time of the circadian maximum for basal TSH. The findings support the notions that all three major TSH control factors act at the thyrotroph, the feedback inhibitory action of thyroid hormones is not exerted through dopamine, and the normal nocturnal TSH surge is not a result of diminished dopamine activity but a rise in TRH release.

The role of dopamine in this control system has been little investigated in critically ill patients, although a general TSH lowering effect of dopamine infusion (2) and absence of the nocturnal TSH surge (17,18) have been reported in such patients.

Although daytime serum TSH becomes depressed in nonsurviving burn patients with a complicated course before death and before any dopamine is infused, burn patients without a complicated course

(without dopamine) may exhibit TSH higher than the mean normal level (19) perhaps to maintain T_4 levels in the face of accelerated disposal (20,21). In such patients, resembling the burn patients of the present study, evidence of burn-induced depression of serum TSH might only be deduced from lack of marked elevation of TSH in the face of the low serum T_3 typical of these patients.

The presence of burn injury does not appear to diminish the acute TSH-depressive effect of low-dose dopamine infusion. The rapid response was of similar time course and magnitude as in the normal control subjects. This indicates a grossly normal responsiveness of pituitary thyrotrophs to dopamine and suggests that dopamine could play a role in TSH control in burn patients. Thus, it is reasonable to address the issue of whether an alteration of dopaminergic activity plays a role in the suppression of TSH that can occur in burn injury (20). To do so, it will be necessary to assess patients with more severe injury or with complications who exhibit depressed serum TSH and to employ pharmacologic dopamine blockade.

PRESENTATIONS/PUBLICATIONS

Graves TA, Cioffi WG, Vaughan GM, Pratt L, Heironimous JD, McManus WF, and Pruitt BA Jr: The renal effects of low dose dopamine in thermally injured patients. *J Trauma* 35:97-103, 1993.

REFERENCES

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8. Graves TA, Cioffi WG, Vaughan GM, et al: The renal effects of low-dose dopamine in thermally injured patients. *J Trauma* 35:97-103, 1993.

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19. Vaughan GM: Neuroendocrine and sympathoadrenal response to thermal trauma. In Doleček R, Brizio-Molteni L, Molteni A, Traber D (eds): *Endocrinology of Thermal Trauma - Pathophysiologic Mechanisms and Clinical Interpretation.* Philadelphia: Lea & Febiger, 1990, Chap 14, pp 267-306.

20. Vaughan GM, Pruitt BA Jr: Thyroid function in critical illness and burn injury. *Semin Nephrol* 13:359-70, 1993.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

ACCESSION: DA335692

SUMMARY DATE: 911201 **SUMMARY KIND:** H **PREV DATE:** 911001 **DISTRIBUTION:** CX

PROGRAM #: 61102A **PROJ #:** 30161102BS14 **TASK AREA:** CA **WORK UNIT:** 305

TITLE: Thermal-Dye Double Indicator Technique for Estimating Extravascular Lung Water - A Comparison Study

SUBJ1: 060500 - Medicine and Medical Research

START DATE: 9011 **END DATE:** 9112 **PERFORMANCE METHOD:** C

CONTRACT/GRANT NO:

CONT TOTAL: $
CUM TOTAL: $
TOTAL LAB FUNDS: $

RESOURCES ESTIMATE

FY	WORK YRS	$(Thousands)
91	0.5	$60
92	0.1	$ 5
93	0.0	$ 0

RESPONSIBLE DOD ORGANIZATION: 404885
MRDC INSTITUTE OF SURGICAL RESEARCH USAISR
FT SAM HOUSTON, TX 78234
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PERFORMING ORGANIZATION: 404885
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210-221-8440

ASSOC1: CIOFFI, W G

ASSOC2: BECKER, W K

FOREIGN INTELLIGENCE CONSIDERED: Y **CIVILIAN APPLICABILITY:** M

KEYWORDS: RA II; Lab Animals; Sheep; Burns (Injuries); Lungs; Pulmonary Function; Pulmonary Edema; Gravimetric Analysis

OBJECTIVE: A DTIC literature search was conducted under DTIC request numbers W6048E/W6049C dated 6 November 1990. The objective of this work is to evaluate the efficacy of the thermal-dye indicator method for estimating extravascular lung water as compared with gravimetric analysis.

APPROACH: This technique was evaluated over a range of hemodynamic parameters.

PROGRESS: 9011-9209. Two animals were studied. Preliminary data revealed that the double indicator method was highly influenced by variations in cardiac output and consequently was not an accurate method for determining extravascular lung water under conditions of extremes in hemodynamic performance. For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report* for fiscal years 1991 through 1992.

ABSTRACT

PROJECT NUMBER: 3M161102BS14-305, Research

PROJECT TITLE: Thermal-Dye Double Indicator Technique for Estimating Extravascular Lung Water - A Comparison to Gravimetric Analysis and the Influence of Cardiac Output, Colloid Osmotic Pressure, and Inhalation Injury on Extravascular Lung Water Accumulation

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

PERIOD COVERED IN THIS REPORT: 1 October 1991 - 1 December 1991

INVESTIGATORS: Loring W. Rue, III, Major, MC
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Rey F. Guzman, BS, Sergeant
Jose E. Sanchez, BS, Staff Sergeant
Basil A. Pruitt, Jr., MD, Colonel, MC

Elevation of extravascular lung water occurs in the setting of both cardiogenic and noncardiogenic pulmonary edema, the latter a problem frequently encountered in the thermally injured patient. Validating a clinically feasible method for estimating extravascular lung water and correlating the influence of colloid osmotic pressure and smoke inhalation injury with lung water accumulation may contribute to the overall management of patients with inhalation injury.

Two animals were studied. Preliminary data revealed that the double indicator method was highly influenced by variations in cardiac output and consequently was not an accurate method for determining extravascular lung water under conditions of extremes in hemodynamic performance. Therefore, this project was terminated.

THERMAL-DYE DOUBLE INDICATOR TECHNIQUE FOR ESTIMATING EXTRAVASCULAR LUNG WATER - A COMPARISON TO GRAVIMETRIC ANALYSIS AND THE INFLUENCE OF CARDIAC OUTPUT, COLLOID OSMOTIC PRESSURE, AND INHALATION INJURY ON EXTRAVASCULAR LUNG WATER ACCUMULATION

Starling (1) demonstrated that the flux of water in lung tissue is influenced by both hydrostatic and osmotic forces defined by the equation:

$$Qf = kf[(Pmv - Pi) - \sigma(\pi mv - \pi i)]$$

where Qf = net transvascular flow, Kf = microvascular membrane filtration coefficient, Pmv = microvascular hydrostatic pressure, Pi = interstitial hydrostatic pressure, σ = the reflection coefficient for plasma proteins (usually 0.8, 1.0 being a perfect semipermeable membrane), πmv = microvascular osmotic force, and πi = interstitial osmotic force. Under normal resting circumstances, Qf is always positive; however, this small volume of fluid flux is easily handled by lung lymphatic flow. When the transvascular flow exceeds the transporting capacity of the pulmonary lymphatics, extravascular lung water (EVLW) accumulates and pulmonary edema may develop.

Lowering of interstitial protein concentration in response to increases in microvascular pressure is a protective mechanism preventing edema formation. Under normal circumstances, microvascular oncotic pressure is 20 to 35 cmH_20 in most mammalian species, principally due to the albumin concentration. In certain disease states, this colloid osmotic pressure may decrease, yielding an overall increase in transvascular fluid flux and EVLW.

Other causes of increased EVLW and pulmonary edema include elevated left atrial pressure and/or pulmonary artery pressure, resulting in increased microvascular hydrostatic pressure, an increase in microvascular permeability, which directly influences the osmotic reflection coefficient (σ), and obstruction of the pulmonary lymphatics.

Following inhalation injuries, surfactant depletion occurs, giving rise to ventilation/perfusion abnormalities (2). Pulmonary surfactant is an important contributing factor in the maintenance of normal lung fluid balance. Loss of surfactant gives rise to increased surface tension, resulting in an elevated hydrostatic pressure gradient which favors fluid movement from the interstitium into the alveolus.

Direct measurements of the extent of EVLW accumulation have been proposed as a more specific indicator of the severity of pulmonary derangement. Several methods are available, the gold standard being a postmortem gravimetric estimate of EVLW (3).

Obviously, this approach has little clinical utility and has led investigators to propose alternate in vivo measurements of EVLW. These methods include the inhalation of soluble, inert gases such as helium and dimethyl ether, the so-called Kander and Forester technique, and the intravascular injection of diffusible and nondiffusible tracers, the so-called Chinard technique. Chinard's original approach involved the use of iodinated albumin and tritium (^3H_2O) to assess the vascular and extravascular compartments, respectively, with the difference yielding extravascular fluid volume. Alternatively, a thermal-dye double indicator method has been investigated, which utilizes temperature differences and indocyanine green as the freely distributing and intravascular indicators, respectively (4).

The thermal-dye method for estimating extravascular lung volume involves injecting a fixed volume of chilled indocyanine green via the right atrium and measuring the change in blood temperature by a thermistor-tipped femoral artery catheter and the rate of appearance of indocyanine green by a densitometer. The difference between the two generated curves yields the EVLW. Numerous reports have demonstrated correlation of this method with gravimetric methods of EVLW determination in canine, swine, ovine, and primate models. It has been applied to septic and toxic inhalation models of pulmonary injury (5,6).

Unfortunately, the thermal-dye method has been criticized for either over- or underestimating EVLW in various clinical settings (7,8). Lewis and colleagues (4) reported the results in an ovine model which revealed this technique to overestimate EVLW as compared with gravimetric analysis at normal levels of lung water content (5-10 ml/kg), to be in close agreement at intermediate levels of lung water (10-20 ml/kg), and to underestimate EVLW at high levels of pulmonary EVLW (> 20 ml/kg).

Other investigators have demonstrated either a positive or negative correlation with this technique as compared with gravimetric analysis at varying levels of cardiac output (9). For example, Hill and colleagues (10) demonstrated large increases in cardiac output (60-70%) resulted in a 6-7% underestimate of EVLW using the thermal-dye technique. Carlile et al (11), on the other hand, demonstrated no dependence of lung water estimates on cardiac output, provided the transit times for the thermal and dye indicators were identical. This issue is of particular concern when applying the thermal-dye indicator technique to the burn patient as a consequence of the hyperdynamic state which arises during the flow-phase postinjury.

If this clinically applicable technique for estimating EVLW can be validated and correlated with colloid osmotic pressure and severity of inhalation injury, valuable insight may be gained in assessing the efficacy of various ventilator therapies, the influence of alterations of colloid content of resuscitation

fluids, and the overall management of the acute respiratory distress syndrome postburn. The only inherent risks to the procedure are those associated with a central venous line, a femoral arterial line, and possibly allergic reactions to indocyanine green.

The purpose of this study was to evaluate the efficacy of the thermal-dye indicator method for estimating EVLW as compared with gravimetric analysis. This technique was evaluated over a range of hemodynamic parameters.

MATERIALS AND METHODS

Study Design. Two male sheep weighing 25-45 kg were used to correlate the thermal-dye double indicator technique with gravimetric estimates of EVLW at varying levels of cardiac output. Animals were anesthetized, intubated, and, using the femoral vessels, had a side-to-side arteriovenous fistula constructed so as to increase the cardiac output. The animals were placed in their normal resting positions postoperatively and returned to their cages for a 3-day equilibration period. On postoperative day 4, the animals were again anesthetized, intubated, and instrumented with a Swan-Ganz catheter and a femoral thermistor-tipped arterial line used for lung water analysis. Following instrumentation, the animals were placed in their normal resting upright positions and baseline measurements of hemodynamic indices (pulmonary artery wedge pressure, central venous pressure, and cardiac output), arterial blood gases (PaO_2, PCO_2, pH, and O_2 saturation), and colloid osmotic pressure were made. Three measurements of the thermal-dye double indicator EVLW were then made. This consisted of using indocyanine green dissolved in 5% dextrose and water at a concentration of 0.2 mg/ml and chilled to 0°C. Ten milliliters of this solution was injected into the right atrium and, using a syringe pump, blood was withdrawn at a constant rate of about 20-30 ml/min through a densitometer, and the femoral thermistor-tipped arterial line detected temperature changes to provide estimates of mean transit time for both the thermal and dye indicators. A lung water computer calculated the EVLW. Following completion of these measurements, a partial occluding vascular clamp was placed across the arteriovenous fistula to occlude it and presumably lower cardiac output. Once again, baseline measurements of hemodynamic indices, arterial blood gases, and colloid osmotic pressure were made. Repeat measurements of the thermal-dye double indicator EVLW were then made in triplicate. Following completion of these measurements, the animals were placed on their backs and the thorax opened rapidly with both pulmonary hila clamped, and then euthanized with potassium chloride. The lungs were removed, weighed, homogenized with equal amounts of distilled water and a gravimetric estimate of EVLW, as described by Pearce (3), was performed.

Description of Procedures. Two male sheep weighing 25–45 kg/kg were used for this study. Each animal was housed in a conventional outdoor run and had access to food and water ad libitum. Animals were dewormed 2 weeks before use. On the day of the study, the animals were anesthetized with sodium pentobarbital (35 mg/kg IV, Calbiochem, La Jolla, CA) administered through a 20-ga needle and intubated. They were then instrumented with a peripheral venous catheter. Lactated Ringer's was constantly infused at a rate of 1 ml/kg/h and anesthesia was maintained with sodium pentobarbital. A side-to-side femoral arteriovenous fistula was then constructed using a 6-0 Proline™ suture. The wounds were closed and the animals were placed in the resting upright position and returned to their cages upon awakening. After a 3-day equilibration period, the animals were anesthetized with alpha-chloralose, intubated, and instrumented with a peripheral venous catheter, a balloon-directed thermodilution pulmonary artery catheter (7F, American Edwards Company, Irvine, CA), and a femoral thermistor-tipped arterial line (Model No. 500307, American Edwards Laboratory, Santa Anna, CA) were placed in the femoral artery by cutdown. The previously constructed femoral arteriovenous fistula was dissected out to permit the application of a partial occluding clamp. Anesthesia was maintained with alpha-chloralose and lactated Ringer's was constantly infused at a rate of 1 ml/kg/h. Animals were then paralyzed with pancuronium bromide (0.03–0.04 mg/kg, Pavulon®, Organon Pharmaceuticals, West Orange, NJ).

After instrumentation, the animals were positioned in the resting upright position and conventional ventilation was continued with a volume-limited ventilator (Bear II®, Bear Medical Systems, Inc., Riverside, CA). Ventilator settings were altered to maintain the pH between 7.4 and 7.5. Animals were ventilated with an FIO_2 of 0.21 and a PEEP of 0. Central venous and pulmonary artery pressures were monitored with a Statham P23 Db transducer (Statham Instruments, Oxnard, CA) and systemic arterial pressures with a Hewlett-Packard 1290A Quartz transducer (Hewlett-Packard Company, Waltham, MA). Baseline measurements of heart rate, blood pressure, right atrial pressure, and pulmonary artery occlusion pressure were obtained. Arterial blood gases and mixed-venous blood gases were drawn and immediately analyzed. A serum sample was drawn for an estimate of colloid osmotic pressure. The thermal-dye double indicator estimate of EVLW was performed by dissolving indocyanine green in 5% dextrose and water at a concentration of 0.2 mg/ml chilled to 0°C. Ten milliliters of this solution was injected into the right atrium rapidly and the thermal indicator was detected by the femoral artery thermistor and the indocyanine green by a densitometer with a small cuvette placed external to the catheter and in series with it through which blood was withdrawn at a rate of 30 ml/min (Model No. D402DC410, Water's Instrument Company, Rochester, MN). A lung water computer (Model 930, American Edwards Laboratory) was used to calculate cardiac output and EVLW. Following completion of these measurements in triplicate, a partial occluding clamp was placed on the femoral arteriovenous fistula to

cause an immediate decrease in cardiac output. After a 30-min equilibration period, measurements of heart rate, blood pressure, right atrial pressure, pulmonary artery pressure, and pulmonary artery occlusion pressure were obtained. Blood samples for arterial and mixed-venous blood gases were drawn and immediately analyzed. Again, thermal-dye double indicator estimates of EVLW were performed in triplicate. Following completion of these measurements, the animals were placed on their backs, the chest opened, and vascular clamps placed on the pulmonary hila. The animals were then euthanized with a potassium chloride bolus (20 ml) administered through the central venous catheter and the lungs excised. The lungs were weighed and homogenized in a Waring^{R} blender, to which a measured amount of water was added. An aliquot of the homogenate was centrifuged at 10000 g for 30 min. Aliquots of blood (drawn when the organs were removed), lung homogenate, and homogenate supernatant were weighed before and after drying at 80°C to a constant weight. Standard formulas were then used to calculate EVLW and dry weight.

RESULTS

Two animals were studied. Preliminary data revealed that the double indicator method was highly influenced by variations in cardiac output.

DISCUSSION

This method is not an accurate means for determining EVLW under conditions of extremes in hemodynamic performance, such as may be encountered in burn patients. Therefore, this study was terminated.

PRESENTATIONS/PUBLICATIONS

None.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

ACCESSION: DA318368

SUMMARY DATE: 920930 **SUMMARY KIND:** K **PREV DATE:** 911001 **DISTRIBUTION:** CX

PROGRAM #: 61102A **PROJ #:** 30161102BS14 **TASK AREA:** FA **WORK UNIT:** 307

TITLE: Interleukin-1 (IL1) Activity in the Serum of Burned Rats and Thermally Injured Patients

SUBJ1: 060100 - Biochemistry
SUBJ2: 060500 - Medicine and Medical Research

START DATE: 8905 **END DATE:** 9209 **PERFORMANCE METHOD:** C

CONTRACT/GRANT NO:

CONT TOTAL: $
CUM TOTAL: $
TOTAL LAB FUNDS: $

RESOURCES ESTIMATE

FY	WORK YRS	$(Thousands)
91	1.5	$65
92	1.5	$24
93	0.0	$0

RESPONSIBLE DOD ORGANIZATION: 404885
MRDC INSTITUTE OF SURGICAL RESEARCH USAISR
FT SAM HOUSTON, TX 78234
PRUITT, B A
210-221-2720

PERFORMING ORGANIZATION: 404885
MRDC INSTITUTE OF SURGICAL RESEARCH USAISR
FT SAM HOUSTON, TX 78234
BURLESON, D G
210-221-4858

ASSOC1: DROST, A C

ASSOC2: CIOFFI, W G

FOREIGN INTELLIGENCE CONSIDERED: Y **CIVILIAN APPLICABILITY:** M

KEYWORDS: RA II; Volunteers; Adults; Lab Animals; Rats; Burns (Injuries); Blood; Immunosuppression

OBJECTIVE: A DTIC literature search was conducted under DTIC request numbers W6L37A/W6L37E dated 20 October 1989. The objective of this work is to delineate the roles of IL1, IL6, and TNF in patients with thermal injury, with emphasis on establishing a correlation between serum IL1, IL6, and TNF levels and the degree of burn injury or infection. Improvement of treatment regimens would reduce morbidity and mortality of patients with thermal injury.

APPROACH: The first part of this study developed methodology to detect plasma cytokine activity in patients with thermal injury based on that previously developed for a burned rat model. The second part of the study involved detection of plasma cytokine activity (IL1, IL6, and TNF) in patients with thermal injury. Increases in plasma cytokine levels above that found for normal control subjects was correlated with time postburn, burn size, infection, and other burn-associated manifestations.

PROGRESS: 9110-9209. Twenty-seven burn patients and 17 control subjects were enrolled in this study, 2 burn patients during this reporting period. Plasma IL1, IL6, and TNF were analyzed by ELISA. IL6 and TNF levels were higher in patients who suffered from infections compared to patients who remained infection-free. IL6 levels were higher in nonsurviving patients compared to surviving patients. Only IL1 levels were positively correlated with burn size. All three cytokines were positively correlated with the presence of the others, suggesting interactive control of their secretion. For technical reports, refer to the *US Army Institute of Surgical*

Research Annual Research Progress Report for fiscal years 1989 through 1992.

ABSTRACT

PROJECT NUMBER: 3M161102BS14-307, Research

PROJECT TITLE: Interleukin 1 (IL1) Activity in the Serum of Burned Rats and Thermally Injured Patients

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

PERIOD COVERED IN THIS REPORT: 1 October 1991 - 30 September 1992

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The relationship of plasma cytokine levels to infection, core temperature, and to one another were examined in patients with thermal injury. IL1β, IL6, and TNF$_\alpha$ were measured by ELISA in serial samples of plasma from 27 patients. IL6 and TNF$_\alpha$ were increased in severely infected patients as compared to patients who remained infection-free, and IL6 was higher in nonsurviving infected patients than in survivors. There was no apparent relationship between IL1β levels and infections. IL6 and IL1β were positively correlated with core temperature. The correlations between IL6 and IL1β, between IL6 and TNF$_\alpha$, and between TNF$_\alpha$ and IL1β were significant. These results suggest that IL6 and TNF$_\alpha$ play a role in the response of burn patients to infection.

INTERLEUKIN-1 (IL1) ACTIVITY IN THE SERUM OF BURNED RATS AND THERMALLY INJURED PATIENTS

Thermal injury results in an increased susceptibility to infection that can lead to increased morbidity and mortality. Cytokines such as IL1β, IL6, and TNF_α are involved in the response to infection and have been studied in a number of inflammatory diseases (1-6). We previously reported that IL1β and IL6 levels were significantly elevated in the plasma of patients with thermal injury compared to nonburned controls (7). IL1β correlated with burn size, and IL6 correlated with mortality. Both cytokines were highest during the first week after injury and declined over time. TNF_α was not significantly increased in burn patients, but appeared to be transiently elevated in a subpopulation of patients. In this study, we further examined the previous data and analyzed the relationship of these three cytokines to infection occurring after thermal injury. The study was part of a program to identify modalities useful for the early detection of infection in severely burned patients.

MATERIALS AND METHODS

The 27 patients enrolled in this study had burns ranging from 17.5% to 89% of the total body surface area and an average age of 35.8 yr (21-70 yr). They were normotensive and hemodynamically stable after uneventful resuscitation. Of the 27 patients, 15 had at least one episode of infection during the study period. There were 14 episodes of pneumonia, 1 case of bacteremic wound invasion, 1 case of septicemia, and 6 episodes of tracheobronchitis. Other infections, such as vaginitis, urinary tract infection, and cellulitis were not considered to be severe infections, and treated as noninfected. Blood was drawn from the patients three times weekly between 0500 and 0600 h into EDTA blood collection tubes. Plasma was prepared as previously described (7). IL6 concentration was measured in 419 samples from 27 patients and TNF_α in 409 samples from 27 patients. Sufficient sample volume was present for IL1β measurements in 253 samples from 21 patients.

Cytokines were measured by ELISA. IL1β and TNF_α ELISA kits were purchased from Cistron Biotechnology (Pine Brook, NJ) and IL6 ELISA kits from Genzyme Corporation (Cambridge, MA). The detailed procedure has been previously described (7).

Cytokine concentrations were calculated by comparing sample absorbance with the absorbance of pooled plasma from healthy laboratory employees enriched with increasing amounts of recombinant human cytokine. The pooled plasma did not contain detectable concentrations of endogenous cytokines and was used for the standard curve instead of bovine serum albumin to account for possible interference of plasma factors in the ELISA. Sample measurements were accepted as detectable when their mean absorbance

was ≥ 2 SD that of nonspecific binding. Nonspecific binding consisted of that detected in 2 to 8 aliquots pooled plasma. The mean analytic least detectable of all ELISAs was 2.5 pg/ml for IL1β, 0.03 ng/ml for IL6, and 3.42 pg/ml for TNF_α.

Statistical significance was determined by Chi square test, Mann-Whitney U-test, Duncan multiple range test, or Spearman rank correlation as appropriate (BMDP Statistical Software, Los Angeles, CA).

RESULTS

Plasma cytokine levels in 27 burned patients were examined for their relationship to infection. The patient population was divided into patients who became infected during the study period and patients who remained infection-free. The infected group included all samples from every patient who had at least one infection episode. Table 1 shows that 40.0% (48/120) of samples from 7 infection-free patients had detectable IL1β plasma levels. Of the samples from 14 patients who became infected, 74.4% (99/133) were detectable. Table 2 shows that the corresponding mean IL1β concentrations in detectable samples were not statistically different. Detectable plasma IL6 concentrations were present in 58% (109/188) of samples from 11 infection-free patients and 78.4% (181/231) of those from 16 infected patients (Table 1). The corresponding mean IL6 plasma concentration was significantly higher in detectable samples from infected patients (0.66 ± 0.06 ng/ml) than in those from infection-free patients (0.13 ± 0.03 ng/ml) (Table 2). Only 15.4% (29/188) and 27% (63/233) of plasma samples from 11 infection-free and 16 infected patients, respectively, had positive TNF_α levels (Table 1). The mean TNF_α concentration of detectable samples from infected patients was significantly higher than that from infection-free patients (Table 2).

Plasma cytokine levels from infected surviving patients were compared to those of infected nonsurviving patients. The number of IL1β samples from nonsurviving patients was too small for analysis. IL6 levels were detectable in 76.7% of surviving patients who had infections and in 100% of nonsurviving infected patients. Only 12.5% (2/16) of the samples from nonsurviving patients who became infected had detectable TNF_α levels as compared to 28.1% (61/217) of those from surviving patients who became infected. Table 3 shows that the mean IL6 concentration was significantly higher in nonsurviving infected patients than that in surviving infected patients ($P < 0.01$). Mean TNF_α concentrations were not different. Based on these observations, subsequent analyses included a distinction between surviving and nonsurviving patients.

The temporal relationship of cytokine levels to the diagnosis of infection was evaluated. We assigned an eight-day infection window (INF) to all surviving infected patients, which started one

TABLE 1. Percentage of Detectable Samples in Burn Patients With and Without Infection

Cytokine	With Infection	Without Infection
IL1β	74.4* (99/133)	40.0 (99/133)
IL6	78.4* (181/231)	58.0 (109/188)
TNF_α	27.0** (63/233)	15.4 (29/188)

( ) indicates detectable samples/total samples. Plasma cytokine levels were measured by ELISA in burn patients who had at least one infection during the course of the study and in noninfected patients. *P < 0.00001, **P < 0.05.

TABLE 2. Plasma Cytokine Levels in Burn Patients With and Without Infection (Mean ± SEM)

Cytokine	n	With Infection	n	Without Infection
IL1β (pg/ml)	98	3.61 ± 0.21*	48	3.55 ± 0.2
IL6 (ng/ml)	181	0.66 ± 0.06**	109	0.13 ± 0.03
TNF_α (pg/ml)	29	9.70 ± 0.93**	29	2.52 ± 0.77

Plasma cytokine levels were measured by ELISA in burn patient patients who had at least one infection during the course of the study and in noninfected patients. *Not significant, **P < 0.00001.

TABLE 3. Cytokine Levels in Detectable Samples from Surviving and Nonsurviving Burn Patients with Infection (Mean ± SEM)

Cytokine	n	Survivors	n	Nonsurvivors
IL1β (ng/ml)	165	0.56 ± 0.04	16	1.62 ± 0.45*
TNF_α (pg/ml)	61	9.47 ± 0.91	2	16.70 ± 10.17**

*P < 0.01, **not significant.

day before the infection was diagnosed. The prior eight days were called preinfection (PRE) and the recovery period of eight days following the infection window was defined as postinfection (POST). Diagnosis was based on clinical signs and supporting laboratory data. Clinical treatment of the infection started on the day of diagnosis. Figure 1 compares plasma cytokine levels from patients who remained infection-free to cytokine levels from infected patients before, during, and after the infectious episode. The mean plasma IL6 and TNF_α concentrations in patients who remained infection-free were significantly lower than those of infected patients during any of the three infection periods ($P < 0.01$ and $P < 0.05$, respectively). There was no significant difference in mean IL1β concentrations between the two patient groups or the three time periods. Temporal plasma IL6 profiles of the three nonsurviving infected patients are depicted in Figure 2. These were compared to IL6 levels in infected but surviving patients. Patient 5, who died on postburn day 13, had IL6 levels not very different from those of surviving burned patients. One day after his plasma IL6 concentration peaked on postburn day 5, pneumonia was diagnosed (designated in the figure by an arrow). On postburn day 7, aspergillus wound invasion was diagnosed. Patient 13 expired on postburn day 18. His IL6 level was highest on postburn day 3, one day before he was clinically septic (*Staphylococcus aureus*) (arrow). On postburn day 10, the patient had a second increase in IL6 without signs of infection. Sepsis (*Streptococcus pneumoniae*) was diagnosed in patient 25 on postburn day 3 and this patient died on postburn day 5. Plasma IL6 levels in patients 5 and 13 were lower at the time of death than at the time of diagnosis of infection.

Interrelationship of Plasma Cytokines. Cytokine levels are interrelated, i.e., IL6 may be induced by IL1β or TNF_α. We looked for correlations between plasma cytokine concentrations and found a positive correlation between IL6 and IL1β ($r = 0.5123$, $P < 0.0001$). A weaker positive correlation existed between IL6 and TNF_α ($r = 0.2158$, $P < 0.0001$), and there was a weak negative correlation between TNF_α and IL1β ($r = -0.0761$, $P < 0.0001$).

Plasma Cytokines and Fever. The relationship between plasma cytokine levels and core temperature was examined. Rectal temperatures were taken routinely 3 to 4 h before plasma samples were drawn. Plasma IL1β, IL6, and TNF_α levels were positively correlated with core temperature ($r = 0.2166$, $r = 0.2596$, and $r = 0.0433$, respectively; $P < 0.0001$). These data are consistent with the pyrogenic activities of IL6 and IL1β.

DISCUSSION

The results of this study suggest that plasma IL6 and TNF_α levels are related to severe bacterial infection. IL1β did not appear to be altered by postburn infection.

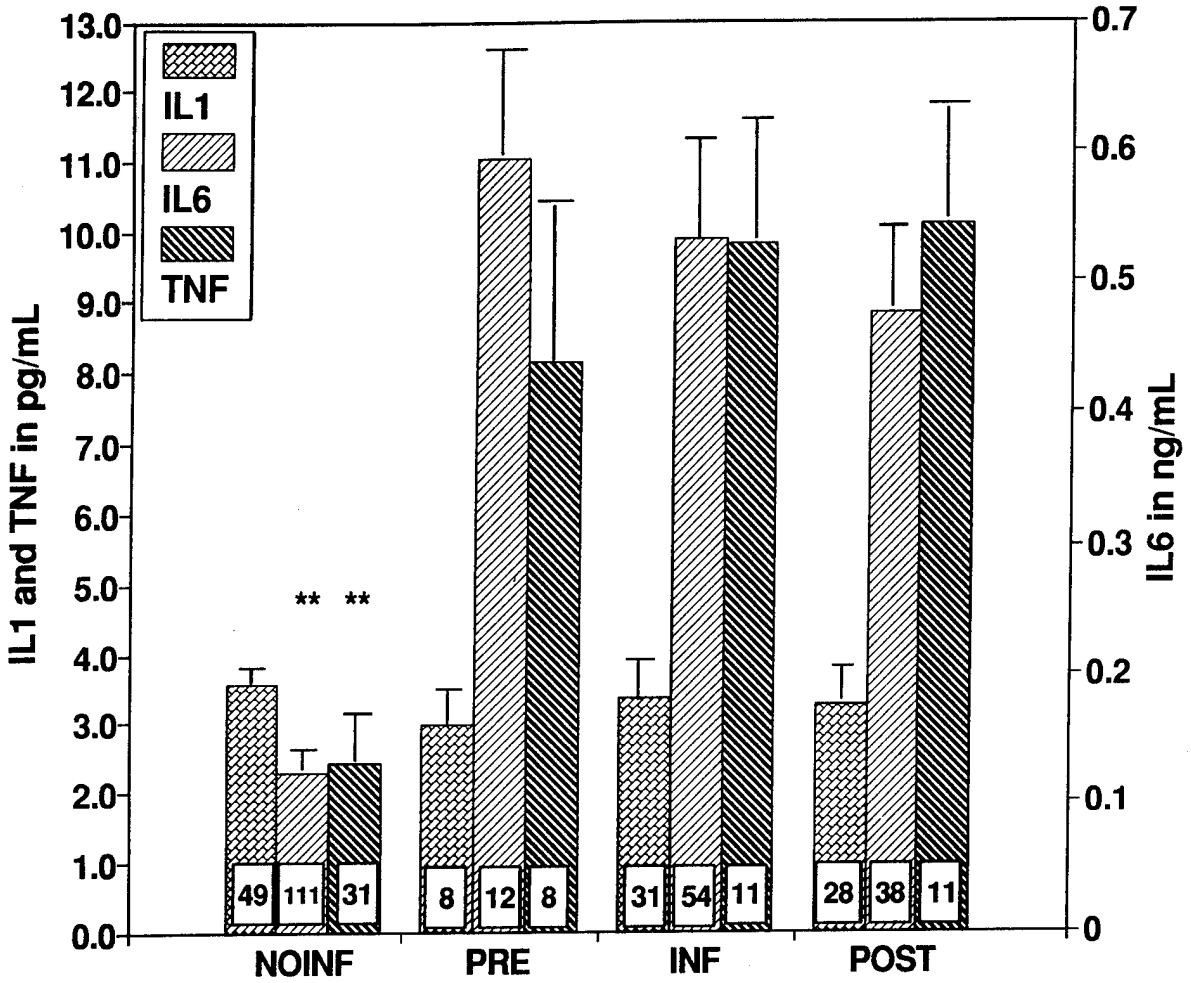


FIGURE 1. Cytokine levels in burn patients with (INF) and without infection (NOINF). NOINF samples were compared to detectable samples from infected patients divided into one of three periods. INF is an eight-day period, with day 1 being one day before infection diagnosis. PRE is the eight-day period before INF and POST reflects an eight-day window after INF. Data are expressed in mean ± SEM. **P < 0.01.

The increase in plasma IL6 levels in patients who subsequently died was more than 10-fold that of surviving patients. Whether these increased IL6 levels in nonsurviving patients were caused by infections or other events associated with terminal illness is not known. We have previously reported that plasma IL6 levels are elevated soon after burn injury, but that severity of the injury is not correlated with plasma IL6 concentration (7).

Our data show that IL6 levels are increased before infection is diagnosed in surviving as well as nonsurviving burned patients. These data are consistent with the findings of Fong et al (8) who

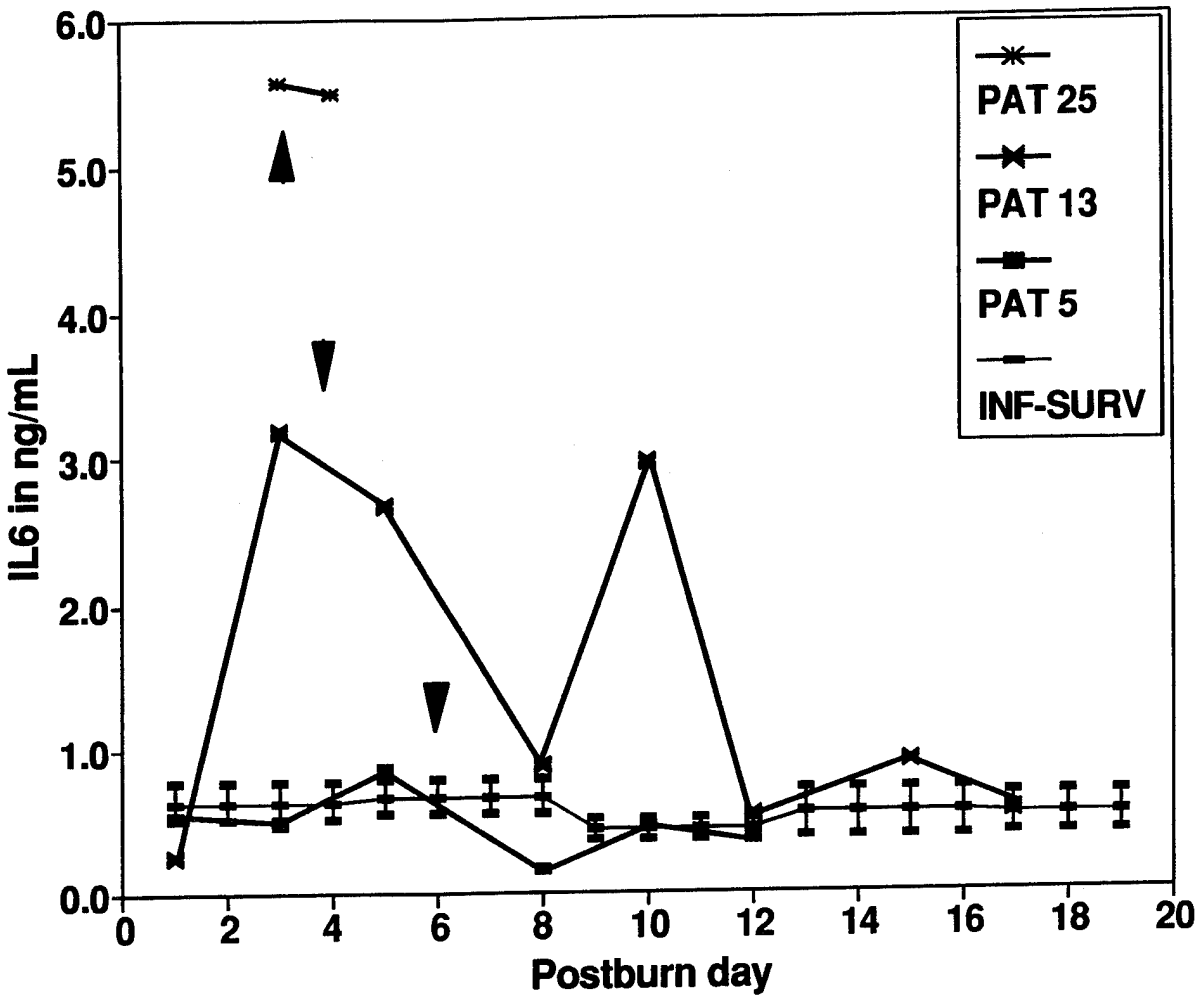


FIGURE 2. Temporal IL6 profiles of three nonsurviving burn patients with infection. Arrows indicate the time of infection diagnosis. IL6 concentrations from surviving burn patients with infection are expressed as the means of four consecutive postburn days ± SEM.

found that plasma IL6 levels peaked 2 to 4 h after injecting healthy volunteers with a single intravenous bolus of endotoxin. These findings suggest that plasma IL6 levels may have some utility in the diagnosis of systemic bacterial infection, in conjunction with other early indications of infection.

Fong et al (9) also reported the measurement of circulating IL1β, IL6, and TNF_{α} levels following intra-aortic infusion of live *Escherichia coli* into baboons. Circulating IL1β was detectable 2 h after infusion and peaked after 3 h. IL6 was detectable 3 h after infusion and rose throughout the 8-h study period. Their findings indicate that IL1β appears early after bacterial infusion and is followed by IL6. Our findings confirm the increase of

plasma IL6 concentrations during infections, but we did not detect an increase in IL1β. Since we measured cytokine levels only three times weekly, it is likely that we missed the early IL1β changes observed by Fong et al (9) which occurred over a few hours.

A number of investigators have reported increased circulating TNF_α following infection, sepsis, septic shock, and even thermal injury (2-4,8). Our results are in agreement with those findings. Marano et al (10) measured TNF_α in the serum of burn patients and found a positive correlation between TNF_α and sepsis. The present study differs in that Marano et al (10) found a correlation between frequency of TNF_α appearance and mortality. Offner et al (4) have reported that serum TNF_α levels in patients with septic shock were higher than those in patients who had infections without clinical signs of sepsis.

The positive correlations between IL1β and IL6 and between TNF_α and IL6 we observed are consistent with the fact that IL6 can be induced by IL1β and TNF_α (11-14). Further studies examining the time course of cytokine appearance after thermal injury will be necessary to confirm the interaction among these three cytokines.

The positive correlation between plasma IL6 and core temperature confirms findings by Nijsten et al (15) who reported a correlation of plasma and serum IL6 with body temperature in 13 patients with severe burns. There was, however, a discrepancy in the absolute levels of IL6 between our studies (3.9 vs 170 pm/1). Nijsten et al (15) used a biological assay, while an immunoassay was used in the present study. The positive correlation of IL1β and core temperature is not surprising; IL1 has been previously described as an endogenous pyrogen (16).

The variation of cytokine levels was not due to transfusions, since there was no significant difference between plasma cytokine levels of samples drawn within 24 h of transfusion and samples collected at other times.

The lack of correlation between IL1β and infections may be due to the presence of relatively low levels of this cytokine in plasma. As the sensitivity of available cytokine-assays increases, the "normal" range for each cytokine also decreases. Future studies with more sensitive assays may show correlations which we were not able to demonstrate.

In conclusion, IL6 and TNF_α appear to play a role in postburn infection, possibly as systemic mediators, whereas the role of free plasma IL1β in postburn infection remains to be defined.

PRESENTATIONS/PUBLICATIONS

None.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

ACCESSION: DA336004

SUMMARY DATE: 920127 **SUMMARY KIND:** A **PREV DATE:** **DISTRIBUTION:** CX

PROGRAM #: 61102A **PROJ #:** 30161102BS14 **TASK AREA:** DA **WORK UNIT:** 308

TITLE: Use of Conjunctival Impression Cytology in Thermally Injured Patients

SUBJ1: 060500 - Medicine and Medical Research

START DATE: 9201 **END DATE:** 9309 **PERFORMANCE METHOD:** C

CONTRACT/GRANT NO:

CONT TOTAL: $
CUM TOTAL: $
TOTAL LAB FUNDS: $

RESOURCES ESTIMATE

FY	WORK YRS	$(Thousands)
91	0.0	$0
92	0.3	$15
93	0.3	$22

RESPONSIBLE DOD ORGANIZATION: 404885
MRDC INSTITUTE OF SURGICAL RESEARCH USAISR
FT SAM HOUSTON, TX 78234
PRUITT, B A
210-221-2720

PERFORMING ORGANIZATION: 404885
MRDC INSTITUTE OF SURGICAL RESEARCH USAISR
FT SAM HOUSTON, TX 78234
MOZINGO, D W
210-221-6690

ASSOC1: CONAWAY, M C

ASSOC2: CIOFFI, W G

FOREIGN INTELLIGENCE CONSIDERED: Y **CIVILIAN APPLICABILITY:** M

KEYWORDS: RA II; Volunteers; Adults; Burns (Injuries); Eye; Eye Disease; Morphology; Cytology

OBJECTIVE: A DTIC literature search was conducted under DTIC request numbers W6O40I/W6O40M dated 27 January 1992. The objective of this work is to characterize the changes in conjunctival cellular morphology in thermally injured patients. If the extent and character of the morphological changes are known, early aggressive treatment for high-risk patients can be instituted to prevent further sequelae of ocular thermal injury.

APPROACH: Samples of surface epithelium will be collected from 50 thermally injured patients upon admission and at 72 h and 1, 2, 3, and 4 weeks. These samples will be analyzed to determine the character and extent of morphologic changes using impression cytology. Using ANOVA statistical analysis, comparisons will be made between thermally injured patients and normal control subjects (under separate study) as well as the changes observed over time (4 weeks) in thermally injured patients.

PROGRESS: 9201-9209. This study was approved by the USAISR Research Council and US Army Institute of Surgical Research Human Use Committee during the first quarter of fiscal year 1992. For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1992.*

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

ACCESSION: DA336004

SUMMARY DATE: 921001 **SUMMARY KIND:** D **PREV DATE:** 920127 **DISTRIBUTION:** CX

PROGRAM #: 61102A **PROJ #:** 30161102BS14 **TASK AREA:** EF **WORK UNIT:** 308

TITLE: Use of Conjunctival Impression Cytology in Thermally Injured Patients

SUBJ1: 060500 - Medicine and Medical Research

START DATE: 9201 **END DATE:** 9309 **PERFORMANCE METHOD:** C

CONTRACT/GRANT NO:

CONT TOTAL: $
CUM TOTAL: $
TOTAL LAB FUNDS: $

RESOURCES ESTIMATE

FY	WORK YRS	$(Thousands)
91	0.0	$0
92	0.3	$15
93	0.3	$22

RESPONSIBLE DOD ORGANIZATION: 404885
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ASSOC1: CONAWAY, M C

ASSOC2: CIOFFI, W G

FOREIGN INTELLIGENCE CONSIDERED: Y **CIVILIAN APPLICABILITY:** M

KEYWORDS: RA II; Volunteers; Adults; Burns (Injuries); Eye; Eye Disease; Morphology; Cytology

OBJECTIVE: A DTIC literature search was conducted under DTIC request numbers W6O40I/W6O40M dated 27 January 1992. The objective of this work is to characterize the changes in conjunctival cellular morphology in thermally injured patients. If the extent and character of the morphological changes are known, early aggressive treatment for high-risk patients can be instituted to prevent further sequelae of ocular thermal injury.

APPROACH: Samples of surface epithelium are collected from 50 thermally injured patients upon admission and at 72 h and 1, 2, 3, and 4 weeks. These samples are analyzed to determine the character and extent of morphologic changes using impression cytology. Using ANOVA statistical analysis, comparisons will be made between thermally injured patients and normal control subjects (under separate study) as well as the changes observed over time (4 weeks) in the thermally injured patients.

PROGRESS: 9201-9209. This study was approved by the USAISR Research Council and US Army Institute of Surgical Research Human Use Committee during the first quarter of fiscal year 1992. Eighteen patients have been enrolled in this study to date. Preliminary cytologic results demonstrate loss of goblet cells, decreased nuclear/cytoplasm ratio, and acute keratinization in the ocular adnexa of thermally injured patients. For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1992.*

ABSTRACT

PROJECT NUMBER: 3M161102BS14-308, Research

PROJECT TITLE: Use of Conjunctival Impression Cytology in Thermally Injured Patients

INSTITUTION: US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas 78234-5012;[1] Department of Ophthalmology[2] and Department of Pathology,[3] Brooke Army Medical Center, Fort Sam Houston, San Antonio, Texas 78234-6200; and Cornea Research Lab, Department of Ophthalmology, McDermott Building, University of Texas Health Science Center, 7703 Floyd Curl Drive, San Antonio, Texas 78229[4]

PERIOD COVERED IN THIS REPORT: 27 January 1992 - 30 September 1992

INVESTIGATORS: David W. Mozingo, MD, Major, MC[1]
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The surface epithelium of the conjunctiva is subject to thermal injury. The extent and character of that injury has not been described. Patients with severe changes in the surface epithelium are at increased risk of developing significant ocular disease as a result of their debilitated state and loss of normal cellular constituents. If the extent and character of the morphological changes are known, early aggressive treatment for high risk patients can be instituted to prevent further sequelae of ocular thermal injury. The objective of this study is to characterize the changes in conjunctival cellular morphology in thermally injured patients.

This study was approved by the USAISR Research Council and US Army Institute of Surgical Research Human Use Committee during the first quarter of Fiscal Year 1992. Eighteen patients have been enrolled in this study to date. Preliminary cytologic results demonstrate loss of goblet cells, decreased nuclear/cytoplasm ratio, and acute keratinization in the ocular adnexa of thermally injured patients.

USE OF CONJUNCTIVAL IMPRESSION CYTOLOGY IN THERMALLY INJURED PATIENTS

Ocular involvement in thermally injured patients is extremely common. Most patients sustaining facial burns have some ocular sequelae that require special attention. Even patients without direct ocular thermal injury are at a greater risk for ocular sequelae due to the hostile environment (immunocompromised, high temperatures and circulating air currents).

It is extremely important that ocular examination be performed on a regular basis to identify any changes that occur to the ocular surface. The most common technique for identifying corneal damage is fluorescein staining. This method identifies corneal epithelial defects that increase the patient's risk for more serious damage, e.g., corneal ulcer. Other important indicators of ocular surface damage include rose bengal corneal staining, conjunctival infection, conjunctival chemosis, and filamentary corneal deposits. No other techniques are used at this time to identify ocular surface disease in the thermally injured patient.

It is well known that if the corneal and conjunctival surface epithelium is exposed to the environment without a protective coating, i.e., lids, tears, lubricants, it will desiccate. This is the rationale for using such generous quantities of lubricants in patients who lack the natural mechanisms for protecting their ocular surface. Some patients require more aggressive surgical intervention to protect their ocular surface. These patients receive suture tarsorrhaphy, lateral tarsorrhaphy, and/or eschar release with grafting. In spite of this, some patients progress to epithelial defects, corneal ulcers, corneal perforations, and, most tragically, enucleation.

Early detection of potential sight-threatening damage to the ocular surface is the secret to prevention. The sooner one knows that there is evidence of epithelial changes consistent with corneal and conjunctival disease, the better prepared one is to intervene with more aggressive treatment.

Although we have a fairly good technique for detecting corneal damage, i.e., fluorescein staining of epithelial defects, there is no method used for evaluating the conjunctival epithelium for evidence of damage.

There are several ocular surface diseases that have been investigated using a safe and simple technique of conjunctival impression cytology for evaluating the morphological changes that occur in those disease processes. The data show that by using epithelial and goblet cell cytologic characteristics, one can stage the conjunctival changes. The higher the stage, the worse the prognosis for ocular health. Conversely, as resolution of the

ocular surface disorder occurs, the conjunctival stage improves. The ocular surface disorders that have been previously investigated are alkali burns, keratoconjunctivitis sicca, glaucoma, ocular pemphigoid, vitamin A deficiency, Stevens-Johnson syndrome, and radiation keratoconjunctivitis.

By applying this safe and simple technique to thermally injured patients, a better understanding of the ocular surface response and a more aggressive treatment plan could be implemented to improve their prognosis. Therefore, the objective of this is to characterize the changes in conjunctival cellular morphology in thermally injured patients.

MATERIALS AND METHODS

Study Design. This prospective study will seek to establish a simple and safe procedure for characterizing the morphologic changes in the conjunctival epithelium of thermally injured patients. The study is designed to collect admission samples of surface epithelium using impression cytology (see Tables 1-3). Patients will be reevaluated at 72 h and 1, 2, 3, and 4 weeks using the same technique. The character and extent of morphologic changes will be compared to normal subjects (under separate study at Brooke Army Medical Center) devoid of ocular surface disease.

Selection of Patients. Fifty thermally injured patients will be enrolled in this study. Properly signed and witnessed DA Forms 5303-R, Volunteer Agreement Affidavits, are obtained from each patient before beginning the study.

Patient Inclusion. Patients meeting all of the following criteria are eligible for enrollment in this study after giving written informed consent:

1. Male or female patients ≥ 18 yr.

2. Patients with facial burns or evidence of ocular injury upon admission to the US Army Institute of Surgical Research.

Patient Exclusion. Patients meeting the following criteria are excluded from participation in the study:

1. Patients < 18 yr.

2. Patients without facial burns or evidence of ocular injury on admission.

Description of Procedures: Fifty thermally injured patients meeting admission criteria will be enrolled in the study after written informed consent is obtained. An initial ocular examination is performed, to include visual acuity, lids, lashes,

TABLE 1. Impression Cytology of Thermally Injured Patients

NAME (Stamp Plate)
AGE
SSN

Etiology of Burn: Scald Flame Chemical Other_____

Total Burn Size (% TBSA):

3° Burn Size (% TBSA):

2° Facial Burn Size (% TBSA):

3° Facial Burn Size (% TBSA):

Eyelid Burns (Y/N) Severity

Associated Injuries:

ADMISSION EXAMINATION

Visual Acuity (If Possible):

Lid:

Lash:

Cornea:

Anterior Chamber:

Bell's Reflex:

TABLE 2. Staging System for Conjunctiva Impression Cytology

NAME (Stamp Plate)						
AGE						
SSN						
TIME	Admit	72 h	1 wk	2 wk	3 wk	4 wk
# goblet cells/hpf						
Nucleus morphology						
Pyknotic (3–5)						
Enucleated (5)						
Nucleus/cytoplasm ratio						
Epithelial cells						
Cyto color						
Green/blue (1–3)						
Pink (3–5)						
Shape						
Epithelial (1–2)						
Squamous (3–5)						
STAGE:						

ADMISSION IMPRESSION CYTOLOGY (GRADE):

LIMBAL	SUPERIOR
	INFERIOR
	MEDIAL
	TEMPORAL
FORNICEAL	INFERIOR

IMPRESSION CYTOLOGY @ 72 HOURS

LIMBAL	SUPERIOR
	INFERIOR
	MEDIAL
	TEMPORAL
FORNICEAL	INFERIOR

IMPRESSION CYTOLOGY @ 1 WEEK

LIMBAL	SUPERIOR
	INFERIOR
	MEDIAL
	TEMPORAL
FORNICEAL	INFERIOR

IMPRESSION CYTOLOGY @ 2 WEEKS

LIMBAL	SUPERIOR
	INFERIOR
	MEDIAL
	TEMPORAL
FORNICEAL	INFERIOR

IMPRESSION CYTOLOGY @ 3 WEEKS

LIMBAL	SUPERIOR
	INFERIOR
	MEDIAL
	TEMPORAL
FORNICEAL	INFERIOR

IMPRESSION CYTOLOGY @ 4 WEEKS

LIMBAL	SUPERIOR
	INFERIOR
	MEDIAL
	TEMPORAL
FORNICEAL	INFERIOR

TABLE 3. Impression Cytology Hospital Course

PBD	LUBES	ABX	CORNEA	LID	CONJ	SURG
1						
2						
3						
4						
5						
6						
7						
8						
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48						
49						
50						
51						

cornea, anterior chamber, conjunctiva, and presence of Bell's reflex. A dilated fundus examination is performed in certain patients who require an extensive fundus examination. Conjunctival impression cytology is performed using the technique described by Tseng (19). One drop of 0.5% proparacaine sterile solution is instilled into each eye. Sheets of cellulose acetate filter paper (millipore, HAWP304) measuring 5 X 5 mm are placed on the following conjunctival locations: superior, inferior, medial, temporal limbal, and inferior forniceal. After gentle pressure with a blunt forcep to ensure close contact of the paper and ocular surface, the paper is removed by grasping the edge and peeling over the desired area. For each sample collection, the paper remains on the patient's eye for 2 to 3 sec. A similar procedure is performed at 72 h and 1, 2, 3, and 4 weeks. Specimens are processed and stained as described by Tseng (19). The filter paper with the detached epithelial/goblet cells is placed in a fixative solution containing glacial acetic acid, 37% formaldehyde, and 70% ethyl alcohol (1:1:20 vol ratio). The staining procedure requires all steps as listed in Table 1. The slides are examined by light microscopy and staged according to the following cytological features:

1. Presence or absence of goblet cells and goblet cell density.

2. Morphological changes of the nucleus.

3. Nucleus-cytoplasm ratios.

4. Metachromatic changes of cytoplasmic color and emergence of keratinization.

Based on these criteria, six stages have been identified.

Determination of Number of Subjects Required. This protocol will involve a study of 50 thermally injured patients. Each patient will have both eyes studied, which will potentially provide 100 thermally injured eyes. It is anticipated that the number of observations made will be sufficient to permit meaningful evaluation of thermally injured patients when compared to a group of normal subjects who visit the Ophthalmology Clinic at Brooke Army Medical Center for complaints other than ocular surface disease (under separate study at Brooke Army Medical Center).

Data Collection. Initial data collection on each patient includes the patient's admission number, age, social security number, total burn size, etiology of burn, eyelid involvement and severity, 3° burn size, 2° facial burn size, and 3° facial burn size is obtained from the patient's medical record. Data from the admission ocular examination is also recorded. Subsequent data is collected on any topical ocular agents used, cornea changes, lid changes, conjunctival changes, and surgery performed during the four-week testing period. Staging of the patients' morphological

conjunctival changes is tabulated based on light microscopy and recorded. Ocular infection, corneal epithelial defects, number of eyelid reconstructions, and other complications are recorded and compared with cytology results to determine if ocular impression cytology is of value in predicting patients who may develop ocular complications of burn injury.

Data Analysis Plan. Using ANOVA statistical analysis, comparisons will be made between the thermally injured patients and normal subjects as well as the changes observed over time (4 weeks) in the thermally injured patients.

RESULTS

This study was approved by the USAISR Research Council and US Army Institute of Surgical Research Human Use Committee during the first quarter of Fiscal Year 1992. Eighteen patients have been enrolled in this study to date. Preliminary cytologic results demonstrate loss of goblet cells, decreased nuclear/cytoplasm ratio, and acute keratinization in the ocular adnexa of thermally injured patients.

DISCUSSION

When the projected total of 50 patients have completed the study, the data will be analyzed and the trends in cytologic changes correlated with clinical outcome.

PRESENTATIONS/PUBLICATIONS

None.

REFERENCES

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

ACCESSION: DA313583

SUMMARY DATE: 920121 **SUMMARY KIND:** H **PREV DATE:** 911001 **DISTRIBUTION:** CX

PROGRAM #: 61102A **PROJ #:** 30161102BS14 **TASK AREA:** CK **WORK UNIT:** 309

TITLE: Caloric Requirements of Thermally Injured Children

SUBJ1: 060500 - Medicine and Medical Research

START DATE: 8710 **END DATE:** 9201 **PERFORMANCE METHOD:** C

CONTRACT/GRANT NO:

CONT TOTAL: $
CUM TOTAL: $
TOTAL LAB FUNDS: $

RESOURCES ESTIMATE

FY	WORK YRS	$(Thousands)
91	0.5	$23
92	0.0	$0
93	0.0	$0

RESPONSIBLE DOD ORGANIZATION: 404885
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ASSOC1: MILNER, E A

ASSOC2: MCMANUS, W F

FOREIGN INTELLIGENCE CONSIDERED: Y **CIVILIAN APPLICABILITY:** M

KEYWORDS: RA II; Volunteers; Children; Burns (Injuries); Nutrition; Metabolism; Therapy

OBJECTIVE: A DTIC literature search was conducted under DTIC request numbers W6L57B/W6M03C dated 20 October 1989. The objective of this work is to define optimal nutritional support for the burned child.

APPROACH: On the fifth postburn day, the REE was to be calculated as well as the RQ. Baseline laboratory data would have been collected and liver function and partial thromboplastin time tests would have been performed. The patient was to begin alimentation that would have been adjusted every 3 days until the caloric need was determined and met for two successive, 3-day cycle measurements as determined by a positive nitrogen balance, a RQ between 0.85 and 1.0, and a caloric intake equal to 1.25 X REE.

PROGRESS: 8710-9201. This study was approved by the USAISR Research Council and US Army Institute of Surgical Research Human Use Committee during the fourth quarter of fiscal year 1987. No suitable pediatric patients were admitted to the Institute. Therefore, this study was terminated. For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report* for fiscal years 1987 through 1992.

ABSTRACT

PROJECT NUMBER: 3M161102BS14-309, Research

PROJECT TITLE: Caloric Requirements of Thermally Injured Children

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

PERIOD COVERED IN THIS REPORT: 1 October 1991 - 30 September 1992

INVESTIGATORS: William G. Cioffi, Jr., MD, Major, MC
Elizabeth A. Milner, RD, Captain, MS
William F. McManus, MD, Colonel, MC
Basil A. Pruitt, Jr., MD, Colonel, MC

This project was approved by the USAISR Research Council and US Army Institute of Surgical Research Human Use Committee during the fourth quarter of Fiscal Year 1987. Since no suitable pediatric patients were admitted to the Institute, this study was terminated.

CALORIC REQUIREMENTS OF THERMALLY INJURED CHILDREN

The optimum nutritional support program for the thermally injured child has not been determined. The caloric requirements of a burned child are only marginally estimated by the existing formulas. The Curreri formulas, the Harris-Benedict equations, and the Wilmore nomograms all differ in their estimation of the caloric requirements for children, e.g., a 2-yr-old girl weighing 12 kg (50th percentile) and measuring 86 cm in length (50th percentile) who has sustained a 40% total body surface area burn will have an estimated daily caloric requirement of 2,120 kcal by the original Curreri formula for children or 2,200 kcal by the Curreri "Junior" formula for 1- to 3-yr-olds, 1,839 kcal by the Harris-Benedict equation, and 1,600 kcal by the Wilmore nomograms. Determination of adequate nutritional support is important since inadequate caloric intake may result in protein wasting and malnutrition, whereas excess caloric intake can result in fatty infiltration of the liver, the fat intoxication syndrome, dehydration secondary to hyperglycemia and glucosuria, and excess carbon dioxide production with subsequent ventilator weaning failure. All of these potential problems could be avoided by the administration of the correct number of calories distributed between protein, fat, and carbohydrate.

Nitrogen requirements in thermally injured patients are increased over those in uninjured people. Numerous studies have demonstrated that injured, hypermetabolic patients demonstrate ineffective utilization of administered protein and have an optimum nitrogen to calorie ratio between 1:135 and 1:200 (grams nitrogen to nonprotein kilocalories). An optimum ratio of 1:150 has been recommended by Goodwin (3). Larger amounts of protein create a progressively more positive nitrogen balance but have not been shown to improve survival (7).

The role of fat as a source of nonprotein calories is dependent upon the extent of injury and the other nutrients administered. When diets lacking in protein are used, carbohydrate is more effective in sparing body protein than fat. However, when a "balanced" alimentation regimen containing protein, fat, and carbohydrate is devised, consideration is given to the administration of sufficient calories as fat not only to prevent essential fatty acid deficiency, but also to supply a large number of calories. Fat administration in excess of 3 g/kg/day in normal infants and 4 g/kg/day in normal adults can produce a fat overload syndrome (4). This has been described as consisting of hyperlipidemia, coagulopathy, fever, cholestatic jaundice, and gastrointestinal distress. This syndrome is believed to occur when the rate of infusion exceeds the maximum rate of peripheral clearance.

Studies comparing the utilization of fat and carbohydrate as energy sources have been undertaken in unburned surgical patients. A controlled study by MacFie et al (6) demonstrated that the administration of as little as 17% of calories as fat can reduce the loss of lean body tissue and the accumulation of body fat which is seen when glucose is used as the sole nonprotein energy source. A positive nitrogen balance has been achieved in postoperative patients with regimens supplying 33% to 38% of nonprotein calories from intravenous fat (9). The fat infusions depressed the RQ and insulin levels and they elevated the serum fatty acid and ketone levels, whereas the glucose infusions elevated the RQ and the pyruvate, lactate, alanine, and insulin levels. A RQ > 1 indicates that lipogenesis is occurring and that some of the administered calories are being utilized to synthesize fat (10).

The amount of glucose which can be effectively utilized by a stressed, injured patient is also unknown. Based on adult burn patients, Burke et al (1) have proposed that a value of 5 mg/kg/min is the maximum rate beyond which physiologically significant increase in protein synthesis and direct oxidation of glucose cannot be expected. At levels above this, there is increased carbon dioxide production and increased fatty infiltration of the liver. Looking at adult surgical patients, Hill and Church (5) have suggested a maximum rate of 7 mg/kg/min. However, neither of these studies addresses the situation of a burned child and the glucose administration ceiling remains unknown in this subpopulation of patients.

In a thermally injured child, these various formulas and recommendations create an impossible situation. Even when the lowest caloric estimate is used, the constraints of a 1:150 gram nitrogen to nonprotein kilocalories ratio, a maximum of 3 g/kg/day fat and a maximum of 5 mg/kg/min glucose are impossible to match. At least one of these recommendations must be ignored. The optimum nitrogen to kilocalorie ratio is well supported in the literature. The fat administration ceiling is well supported in unburned children but no data exist in burned children. The carbohydrate ceiling has also not been determined in burned children. For these reasons, the alimentation regimen which will be used as a starting point in this study will be based on the Wilmore nomograms for determination of the total caloric requirement. A 1:150 nitrogen to kilocalorie ratio will be maintained. The amount of fat will be initially limited to 3 g/kg/day and glucose will supply the remaining calories. It is expected that this glucose infusion rate may be > 5 mg/kg/min. If the patient is unable to tolerate the glucose infusion rate needed to deliver the calculated number of calories based on the initial estimate, the quantity of fat will be increased and the amount of carbohydrate decreased. This will continue until the total number of calories delivered equals that suggested in the initial estimate. The quantity of lipid administered will be kept below that which causes a serum triglyceride level > 150 mg/dl. If it should prove to be

impossible to reach the estimated caloric intake due to severe hyperglycemia and coexisting hyperlipidemia preventing further increase in both glucose and fat infusions, the oxygen consumption and carbon dioxide production will be determined at the maximum infusion rates which the patient will tolerate. These values shall be used as a starting point to calculate a more accurate measure of the caloric need. Further adjustments in the calories administered will follow these measurements and the RQ and resting energy expenditure determinations derived from these two values. The patient's caloric needs will be determined by measurements in the Metabolic Room using the Horizon™ metabolic cart and the nutritional support will be adjusted to administer kilocalories equal to 1.25 X REE (8), maintain the RQ between 0.85 and 1.00, and maintain a positive nitrogen balance. The amount of calories needed to comply with these restraints will be considered the patient's caloric requirement.

MATERIALS AND METHODS

Number of Patients. Twenty patients were authorized for enrollment in this study. Properly signed and witnessed DA Forms 5303-R, Volunteer Agreement Affidavit, were to be obtained for each patient before enrollment in the study.

Patient Inclusion Criteria. Patients meeting the following criteria were eligible for enrollment in the study:

1. Patients admitted to the US Army Institute of Surgical Research with burn injury.

2. Male or female patients < 13 yr.

3. Patients with burn wounds > 30% of the total body surface area.

Patient Exclusion Criteria. Patients meeting any of the following criteria were excluded from enrollment in the study:

1. Patients ≥ 13 yr.

2. Patients with burn wounds < 30% of the total body surface area.

3. Patients with electrical injury.

4. Patients with fractures or major associated injuries.

5. Patients with inhalation injury.

6. Patients who were wards of the state or any other agency, institution, or entity.

Patient Assent. For children from 6–12 yr old, judgment by the primary investigator and the attending surgeon would have been made as to whether the child was capable of assent. In determining whether the child was capable of assent, the primary investigator and the attending surgeon would have taken into account the age, maturity, and psychological state of the child involved. This judgment would have been made for each child. If it was deemed that the child was capable of assent, then the research protocol would have been explained to that child in terms that he/she would have understand. The child would have then been enrolled in the study if his/her assent was given and permission was obtained from the child's parent or legal guardian. If it was deemed that the child was not capable of assent or if the child was ≤ 5 yr of age or younger, then permission would have been obtained from the child's parent or legal guardian only.

Study Procedures. On the fifth postburn day, each patient would have been transported to the Metabolic Room on Ward 14A before the morning dressing change. Oxygen consumption and carbon dioxide production would have been measured using the Horizon™ metabolic cart. The environment temperature and humidity would have been maintained constant throughout each patient's stay in the Metabolic Room. The REE would have been calculated as well as the RQ. Baseline laboratory data would have included serum electrolytes, creatinine, cholesterol, triglycerides, platelet count, prothrombin time, ketone, and insulin values. Liver function and partial thromboplastin time tests would have also been performed. These serum laboratory values would have been repeated at the time of each subsequent trip to the Metabolic Room for further measurements. All measurements in the Metabolic Room were to take place before the morning dressing change. The patient's height and baseline weight were determined upon admission. Weights would have been obtained on a daily basis.

The patient would have then began alimentation using either parenteral hyperalimentation or enteral feeding. If possible, enteral feedings would have been used to supply the patient's nutrition. If the patient's gastrointestinal tract was not capable of tolerating enteral feedings for any reason, intravenous hyperalimentation would have been employed. The total calorie requirement was to be based upon the lowest estimated caloric need as calculated from the Wilmore nomograms, the Curreri formulas, and the Harris-Benedict equations. Nitrogen administration was to be calculated to produce a 1 g nitrogen to 150 nonprotein kilocalorie ratio. Lipids would have been administered at a rate of 3 g/kg/day. Electrolyte composition of the fluids would have been adjusted to the patient's needs. Each patient was to receive standard vitamin and mineral supplements.

Once the patient's intake reached the projected requirements and remained stable for 3 days, the patient would have been transported to the Metabolic Room where oxygen consumption and

carbon dioxide production would have again been measured. A 24-h urine collection would have been obtained on that day as well. From this data, the RQ and REE was to be calculated. The grams of totally metabolized nitrogen, carbohydrate, and fat as well as the nitrogen balance was to be calculated.

Based on the new RQ, REE, and nitrogen balance measurements, the caloric requirements would have been recalculated. If the RQ value was less than 0.85, the total number of calories would have been increased by 10%, maintaining the 1:150 gram of nitrogen to kilocalories ratio and the 3 g/kg/day lipid infusion rate. If the RQ was greater than 1.0, nitrogen, carbohydrate, and fat would have been examined in an effort to determine which component or components (protein, carbohydrate, fat) would be reduced in order to decrease the total number of calories by 10% (2).

After a 3-day stabilization period, these metabolic measurements would have been rechecked and again the caloric intake adjusted to bring the RQ to between 0.85 and 1.0 and to keep the nitrogen balance positive. This 3-day cycle would have been repeated until the caloric need was determined and met for two successive, 3-day cycle measurements. This would have been determined by a positive nitrogen balance, a RQ between 0.85 and 1.0, and a caloric intake equal to 1.25 X REE. Caloric needs would have been redetermined following any operative procedure after a 3-day stabilization period. During these days, alimentation was to be maintained at the preoperative level.

RESULTS

This project was approved by the USAISR Research Council and US Army Institute of Surgical Research Human Use Committee during the first quarter of Fiscal Year 1987. No suitable pediatric patients were admitted to the Institute during this reporting period.

DISCUSSION

Since no suitable pediatric patients were admitted to the Institute, this study was terminated.

PRESENTATIONS/PUBLICATIONS

None.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

ACCESSION: DA314706

SUMMARY DATE: 921001 **SUMMARY KIND:** D **PREV DATE:** 911001 **DISTRIBUTION:** CX

PROGRAM #: 61102A **PROJ #:** 30161102BS14 **TASK AREA:** BG **WORK UNIT:** 310

TITLE: Salt and Water Balance in the Thermally Injured Patient

SUBJ1: 060500 - Medicine and Medical Research

START DATE: 8805 **END DATE:** 9309 **PERFORMANCE METHOD:** C

CONTRACT/GRANT NO:

CONT TOTAL: $
CUM TOTAL: $
TOTAL LAB FUNDS: $

RESOURCES ESTIMATE

FY	WORK YRS	$(Thousands)
91	0.5	$23
92	0.5	$23
93	0.5	$24

RESPONSIBLE DOD ORGANIZATION: 404885
MRDC INSTITUTE OF SURGICAL RESEARCH USAISR
FT SAM HOUSTON, TX 78234
PRUITT, B A
210-221-2720

PERFORMING ORGANIZATION: 404885
MRDC INSTITUTE OF SURGICAL RESEARCH USAISR
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CIOFFI, W G
210-221-8440

ASSOC1: VAUGHAN, G M

ASSOC2: HEIRONIMUS, J D

FOREIGN INTELLIGENCE CONSIDERED: Y **CIVILIAN APPLICABILITY:** M

KEYWORDS: RA II; Volunteers; Adults; Burns (Injuries); Blood Volume; Hormones; Peptides; Renin; Aldosterone; Angiotensin

OBJECTIVE: A DTIC literature search was conducted under DTIC request numbers W6M09B/W6M10A dated 20 October 1989. The objective of this work is to describe the alterations of plasma levels of ADH, atrial natriuretic peptide, and the renin-angiotensin-aldosterone axis following thermal injury as related to plasma volume, osmolality, and tonicity. Better understanding of the hormonal changes which occur following injury will lead to improved resuscitation of patients with thermal injury.

APPROACH: Twenty consecutive patients with thermally injury and 20 control subjects will be enrolled in this study. Intravascular volume measurements will be made utilizing chromium-labeled RBCs to measure red cell volume. The glomerular filtration rate will be measured utilizing inulin and a radiopharmaceutical. Effective renal plasma flow will be measured using a colorimetric hippurate method. The two methods will then be compared.

PROGRESS: 9110-9209. Fourteen burn patients and 10 control subjects have been enrolled in the study to date. Data analysis revealed that despite a consistent hyperdynamic state documented by increased cardiac output and renal plasma flow, patients had a significant reduction in total blood volume with hormone changes appropriate for this condition. An addendum is currently being prepared so that potential treatment options can be defined. For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report* for fiscal years 1988 through 1992.

ABSTRACT

PROJECT NUMBER: 3M161102BS14-310, Research

PROJECT TITLE: Salt and Water Balance in the Thermally Injured Patient

INSTITUTION: US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas 78234-5012,[1] and Nuclear Medicine Department, Brooke Army Medical Center, Fort Sam Houston, San Antonio, Texas 78234-6200[2]

PERIOD COVERED IN THIS REPORT: 1 October 1991 - 30 September 1992

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The relationship between effective blood volume and related hormones in burn patients following resuscitation is not well understood. Previous reports have suggested that hormone secretion is altered by a resetting of neural control mechanisms. We measured serum and urine sodium, plasma renin activity, serum antidiuretic hormone, cardiac index, effective renal plasma flow, and total blood volume in 7 patients with thermal injury. The same values (with the exception of cardiac index and blood volume) were measured in 10 control subjects.

The blood volume of the burn patient was measured by ^{51}Cr RBC labeling and compared to normal predicted values based on total body surface area and sex. Mean serum sodium and osmolality were 138 mM/l and 286 mosm/kg, respectively, in both burn patients and control subjects. Mean ± SEM total blood volume in burn patients was low, 81% ± 4% of predicted values. Cardiac index and renal plasma flow were significantly elevated. Plasma renin activity and antidiuretic hormone levels were elevated and altered in the direction expected from blood volume measurements despite the findings of increased blood flow. Dissociation of organ flow and hormonal response suggests that simultaneous direct blood volume measurements are necessary to elucidate factors other than altered neural control settings to explain hormonal changes in the flow phase of injury. Depressed total blood volume appears to promote elevated antidiuretic hormone levels in burned patients following resuscitation. Whether there is an additional role of altered neural control settings remains to be established.

SALT AND WATER BALANCE IN THE THERMALLY INJURED PATIENT

Factors responsible for sodium and blood volume regulation following injury are not clearly understood. Several authors have interpreted their data to imply that resetting of hormonal control mechanisms occurs following thermal injury, and that this is a stress response which is not sodium- or volume-dependent (1,2). Although various studies have examined one or two factors responsible for sodium and volume regulation following thermal injury, no one has studied this system as a whole.

Antidiuretic hormone (ADH) response following thermal injury has been recently examined (2-4). Morgan et al have concluded that ADH levels were elevated postburn and remained so for 7-10 days. In addition, the increased ADH levels appeared to have little relation to serum osmolality and did not affect urine output. Shirani et al (3) observed elevated plasma ADH levels in association with hyponatremia in burn patients even beyond the first 10 days. Those results were interpreted as being consistent with the diagnosis of the syndrome of inappropriate ADH secretion. However, in those studies blood or plasma volumes were not measured simultaneously with the measurement of ADH.

The renin-angiotensin-aldosterone axis has been examined following thermal injury (1). Shirani et al suggested that the elevated plasma levels of renin activity, angiotensin I, angiotensin II, and aldosterone following thermal injury reflected a resetting of hormonal control and were not dependent upon an effective plasma volume deficit. No volume measurements were made in that study. In that group of patients, combinations of these hormones remained volume-responsive as verified by saline-loading tests.

Atrial natriuretic factor (ANF), a family of potent natriuretic and diuretic peptides, are present in mammalian cardiac atria. Central hypervolemia and increased blood pressure have been postulated as factors which promote ANF secretion (5). An elevation of ANF has been shown to blunt aldosterone response to stimulation by angiotensin II (6). The effect of thermal injury on plasma ANF levels and how it, in turn, affects salt and water balance has not been described.

In an attempt to define more precisely the mechanisms which regulate salt and water balance following thermal injury, we have assessed plasma levels of ADH, ANF, and the renin-angiotensin-aldosterone axis simultaneously with measurement of blood volume and osmolality in burn patients 5 to 16 days postburn.

MATERIALS AND METHODS

Ten healthy control subjects (7 male, 3 female) and 7 patients with thermal injury (6 male, 1 female) were each studied over a 5-h period with blood and urine samples collected hourly for determination of electrolytes and creatinine (see Table 1). Control subjects were allowed nothing by mouth beginning at 0000 h on the day of study while the patients' enteral feedings were continued but oral intake was held. The patients' intravenous fluids were administered at a rate to maintain adequate urine output while allowing for an approximate 10% daily loss of the weight gain from initial resuscitation. The rate of fluid administration in the control subjects was matched to the mean patient hourly intake. Five patients were above preburn weight, while two were below preburn weight on the day of study (Table 1). The mean values during the study period were used as data for each patient.

After a tracer bolus injection of ^{131}I-hippuran (I-HIP) at the second hour (0900 h), additional blood samples were taken to characterize I-HIP decay in the plasma. Just before injection of the I-HIP, a plasma or serum sample was taken for determination of hormone concentrations. Cardiac index (CI) was determined by thermodilution at intervals during the study, and blood volumes were determined with ^{51}Cr-tagged autologous RBCs at the end of the study period. Neither CI nor blood volumes were measured in control subjects.

Sodium and potassium were determined by flame photometry and osmolality by freezing point depression. Urinary excretion rates and clearances were normalized to 1.73 m^2 total body surface area (TBSA): square root of [(height in cm x weight in kg)/3600] (7,8). Preburn weight was used to express variables requiring body weight.

After injection of I-HIP, plasma samples were taken at 5, 10, 15, 20, 40, 50, 60, 70, 80, 100, and 120 min for counting in a gamma scintillation detector and determination of effective renal plasma flow (9). Effective renal plasma flow was calculated as the clearance of I-HIP by fitting the plasma ^{131}I radioactivity to a biexponential expression of time after the dose [$a_1 \exp(b_1 \text{time}) + a_2 \exp(b_2 \text{time})$] and determining the dose of ^{131}I counted in dilution separately [clearance = $-$ dose/$(a_1/b_1 + a_2/b_2)$].

A ^{51}Cr-tagged RBC method using an f cell correction of 0.87 and the peripheral hematocrit was employed to estimate whole blood (Bvol) and plasma (Pvol) volumes from the RBC volume (RBCvol) (10,11). These volumes (measured in milliliters) were compared with those predicted as normal (10) on the basis of sex and body size (males: RBCvol = 1486 TBSA^2 $-$ 4106 TBSA + 4514, Pvol = 995 exp(0.6085 TBSA); females: RBCvol = 1167 TBSA $-$ 479, Pvol = 1278 $\text{TBSA}^{1.289}$; predicted Bvol was the sum of the predicted RBCvol and

TABLE 1. Demographic Variables ± SEM

Group	Age (Yr)	Body Surface Area (M^2)	Total Body Surface Area Burn Size (%)	Postburn Day of Study	% Preburn Weight
Control subjects	23.8 ± 1.4	1.89 ± 0.08	–	–	–
Burn patients	32.4 ± 5.8 (18–24)	1.88 ± 0.05	56.1 ± 5.3 (30–77.5)	8.7 ± 1.5 (5–16)	103.4 ± 3.14 (90.5–112.3)

( ) indicates range.

Pvol). If the mean observed/predicted ratio ± the 95% or 99% confidence interval of the mean for the patients did not overlap 1, the respective (0.05 or 0.01) significance level was determined. Using formulas (12) for direct prediction of expected normal Bvol based on sex and body size gave values very close to the sum of predicted RBCvol + Pvol and did not alter the results.

Cortisol, aldosterone, ADH, and ANF were determined by RIA at the Nichols Institute (San Juan Capistrano, CA), where plasma renin activity (PRA, RIA of generated angiotensin I) and corticotrophin (ACTH, two-site immunoradiometry) were also determined. Hormone values were above the detectable limits, except for ADH in 5 control subjects, in whom the ADH value was recorded as 1 pg/ml, the least detectable value.

Data were analyzed using the BMDP software (13) on a Vax-3500 computer. The nonrectilinear regression (P3R) program was used to determine the parameters of the fit of plasma ^{131}I to time after injection of I-HIP. The P7D program was used to compare variables between burn patients and control subjects with the t test.

RESULTS

As expected, several hemodynamic variables differed significantly between the two groups in a manner consistent with the hyperdynamic response to injury (Table 2). The patients were tachycardic, with a widened pulse pressure. Flow variables (effective renal plasma flow and cardiac index) were significantly increased in patients (Table 2).

The patients' RBC volumes measured by ^{51}Cr-labeled RBCs were significantly less than predicted. Plasma volumes were 100% of the predicted values based upon TBSA, while total blood volumes were 81% of the mean predicted values (fig 1). Because the observed/predicted total blood volumes had a wide variance (95% confidence limits of this value range from 70-92% for this patient population), patient values were also compared to the laboratory reference normal range for blood volumes based upon body weight. Three patients had total blood volume measurements which were within the reference normal range, while 4 patients (two measured twice) had total blood volumes which were below the lower value of the normal range (Table 3).

Mean plasma sodium and osmolality were identical between the two groups (Table 4). Urine flow was significantly higher in control subjects, while urine osmolality was significantly higher in the burn patients despite similar intravenous fluid administration rates. Free water clearance was 2.43 ml/min/1.73 m^2 for control subjects and -1.65 ml/min/1.73 m^2 for burn patients. Elevated urinary potassium concentrations and K+/Na+ ratios were noted in the patients in association with slightly lower serum potassium levels and nondepressed urinary sodium values (Table 4).

TABLE 2. Hemodynamic Variables (Mean ± SEM)

Group	Heart Rate (Beats/Min)	Mean Blood Pressure (mmHg)	Pulse Pressure (mmHg)	Effective Renal Plasma Flow (ml/min/1.73 m^2)	Cardiac Index (l/min/m^2)
Control subjects	62 ± 6	83 ± 3	45 ± 3	525 ± 26	2.3–4.1*
Burn patients	119 ± 3†	77 ± 3	58 ± 4‡	774 ± 96	7.78 ± 0.52§

*Normal range, †$P < 0.05$, ‡$P < 0.01$, §$P < 0.001$.

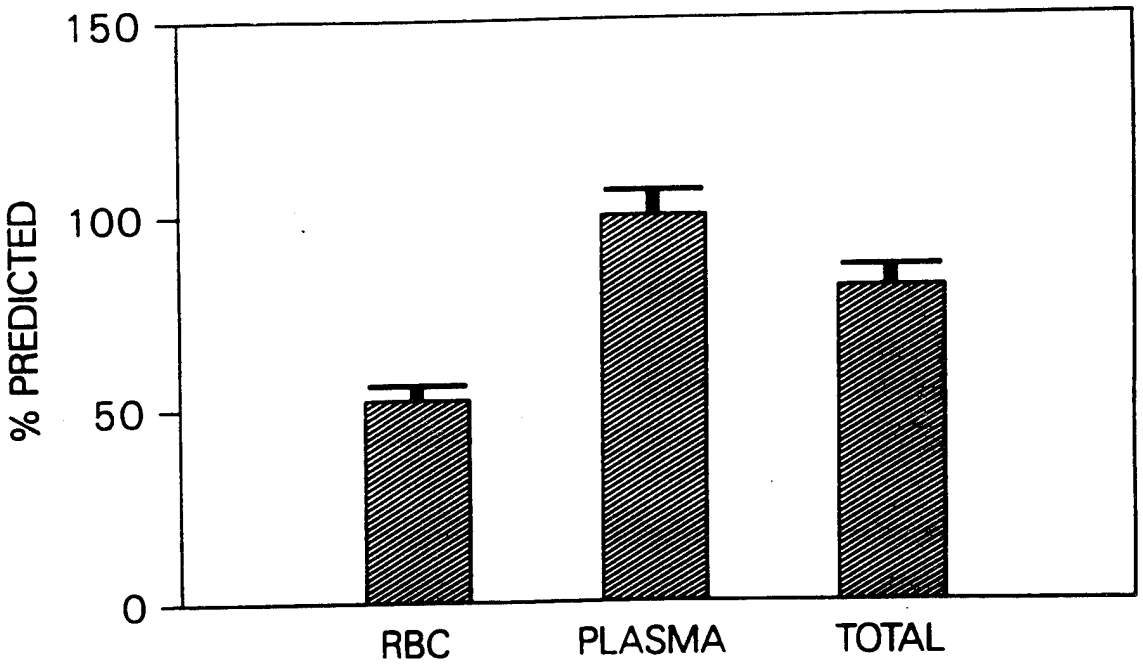


FIGURE 1. The percentage of predicted RBC volume, plasma volume, and total blood volume for all patients is depicted. RBC and total blood volumes differ significantly from predicted values ($P < 0.01$) while calculated plasma volume was normal.

Hormone values for burn patients and control subjects are tabulated in Table 5. Morning cortisol levels were significantly higher in patients as expected, while patient ACTH levels were not elevated. PRA, ADH, and ANF peptide levels were significantly higher in patients. Plasma aldosterone levels tended to be greater in patients but not significantly so when compared to the control population. ADH levels were normal for the 3 patients with normal blood volumes and elevated for the 4 patients with decreased blood volumes ($P = 0.05$) (Table 6).

DISCUSSION

Altered neural setpoints controlling the release of ADH and the renin-angiotensin-aldosterone axis have been invoked as an explanation for the elevated levels of these hormones which are characteristic of the postresuscitative phase of burn care. Previous reports by Soroff et al (14), Collentine et al (15), Dolocek (16), and Shirani et al (1) have all assumed that the findings of significant sodium excretion and urine flow, hyponatremia in some patients, low or normal plasma osmolality, and elevated blood flow as indexed by increased glomerular filtration rates and cardiac output are an indication of normal or increased blood volume. These findings, in combination with less than maximally dilute urine and elevated plasma levels of ADH, have led

TABLE 3. Total Blood Volume

Patient Number	Postburn Day of Measurement	Total Blood Volume (ml/kg)
1	5	51.14
1**	12	55.64
2	7	56.90
3	10	68.34
4	6	46.00
4**	10	53.37
5	16	61.16
6	11	47.11
7	6	63.97

*Normal range for males is 60–80 ml/kg; for females, 55–75 ml/kg.

**The second measurement in these patients was not included in the data for Figure 1.

to the diagnosis of syndrome of inappropriate ADH secretion. The apparent dissociation of blood volume and flow indices documented in our patients indicates that the finding of increased flow may not support the assumption that a volume factor is absent in burn-induced syndrome of inappropriate ADH secretion, especially when plasma ADH and urine tonicity are high, even in the setting of a low plasma tonicity. Thus it is possible that if free water delivery is high enough, low blood volume-induced ADH secretion may promote hyponatremia, as seen in many burn patients. Though we did not test this hypothesis in our patients, the mean 20% decrement in total blood volume would suggest that the elevated levels of plasma ADH are an appropriate response in an attempt to restore blood volume.

Although hypotension and increased serum osmolality, by stimulation of stretch- and osmoreceptors, respectively, are the most potent stimulators of ADH release, modest decrements in blood volume may also cause appreciable pituitary release of this hormone. A 10% decrease in blood volume has been previously shown to result in a 2- to 3-fold increase in plasma ADH levels (17). In addition, blood volume deficits on the order of 10–15% are known to decrease the osmotic threshold for the release of ADH although the linear relationship between plasma osmolality and plasma ADH levels is maintained. In our patients, a 19% blood volume deficit

TABLE 4. Serum and Urine Levels (Mean ± SEM)

	Control Subjects	Burn Patients
Sodium (mmol/l)		
Plasma	137.5 ± 0.8	137.7 ± 1.5
Urine	39.1 ± 4.5	70.0 ± 22.4
Sodium excretion (meq/h/1.73 m2)	11.5 ± 1.0	7.8 ± 3.5
Potassium (mmol/l)		
Plasma	4.52 ± 0.09	3.96 ± 0.12*
Urine	8.9 ± 0.9	52.3 ± 6.3**
Urine potassium/sodium ratio	0.24 ± 0.03	14.3 ± 9.2***
Osmolality (mosm/kg)		
Plasma	286 ± 1	286 ± 6
Urine	160 ± 12	656 ± 45**
Urine output (ml/h/1.73 m2)	303 ± 19	87 ± 15**
Free water clearance (ml/min/1.73 m2)	2.43 ± 0.3	−1.65 ± 0.15**
Intake (ml/h)	250	284 ± 32

*P < 0.05, **P < 0.01, ***P < 0.001.

TABLE 5. Hormone Values

Group	ACTH (pg/ml)	Cortisol (g/dl)	PRA (ng/ml/h)	Aldosterone (ng/dl)	Antidiuretic Hormone (pg/ml)	Atrial Natriuretic Factor (pg/ml)
Control subjects	27.6 ± 5.7	10.8 ± 1.1	1.3 ± 0.3	4.2 ± 1.4	1.2 ± 0.1	78 ± 6
Burn patients	13.0 ± 2.2	22.6 ± 2.8*	28 ± 8*	11.7 ± 5.6	5.6 ± 2.5**	167 ± 34***

*P < 0.05, **P < 0.01, ***P < 0.001.

TABLE 6. Comparison of Antidiuretic Hormone Levels for Patients with Low and Normal Blood Values

	Low (n=6)	Normal (n=3)
Predicted total blood volume (%)	75.8 ± 2.8	91.8 ± 2.7*
ADH level (pg/ml)	7.72 ± 2.5	1.97 ± 0.26**

*P = 0.05, **P < 0.01.

resulted in a 4.5-fold increase in plasma ADH levels. The significantly decreased free water clearance and increased urine osmolality seen in our patients, as compared to the control population, document the expected influence of elevated ADH levels on the kidney.

Elevated plasma renin activity and aldosterone levels have been noted in burned patients during the postresuscitative phase (1). As in the previously referenced ADH studies, blood volumes were not measured and the findings of elevated creatinine clearance and normal plasma tonicity were used as evidence for at least normal blood volume at the time of study. These findings, in concert with a normal plasma aldosterone decline following a mild volume stimulus, were interpreted to mean that the renin-angiotensin-aldosterone system remained volume-responsive in burned patients, but that the elevated level of function occurred because of resetting of control mechanisms. The excess renin release was attributed at least in part to excess sympathetic activity which occurs following burn injury and is also known to increase renin release (18). In our patients, plasma renin activity was significantly elevated compared to the control population. Plasma aldosterone levels, although elevated, were not statistically different from normal. The elevation in PRA in our patients is consistent with the anticipated increase in sympathetic activity in burned patients. It is possible that the one time measurement of fluctuating plasma aldosterone may not have been sufficient to disclose an elevated integral aldosterone level which may have been present in light of the elevated urinary K+/Na+ ratio. Nevertheless, the relatively small aldosterone response to PRA has been commonly described in critically ill patients (19). Our concurrent finding of increased ANF, which is known to decrease aldosterone synthesis, may partially explain this finding (6,20-22). Elevated urinary potassium concentrations and K+/Na+ ratios, despite slightly lower serum potassium and nondepressed urinary sodium in the burned patients, suggest elevated aldosterone effect more likely on the basis of a volume deficit than because of sodium unavailability.

The finding of elevated ANF levels does not fit entirely with our understanding of the normal stimuli for this cardiac hormone's release. Typically, elevations in blood pressure and atrial distension secondary to blood volume excess are the stimuli which result in an increase in ANF release (5). Neither of these mechanisms were operative in our patients. Recently, it has been reported that, in vitro, elevated levels of ADH and angiotensin added to the media with freshly excised rat atria resulted in a significant increase in ANF release (23). Thus, the elevated ADH levels documented in our patients may result in an increased release of ANF. Elevated levels of ANF have been reported following thermal injury and resuscitation (24). Other investigators have reported a correlation between heart rate and ANF release which is independent of volume (25). The tachycardia

documented in our patients may thus be partially responsible for the elevated levels of ANF although this mechanism has recently been disputed (26). This in turn may have a negative feedback effect on aldosterone secretion. ANF appears to decrease aldosterone levels by inhibiting synthesis of this hormone in the adrenal glomerulosa cells (20).

Except for the absence of hyponatremia, our patients are remarkably similar to those previously reported. Shirani et al studied 9 patients with thermal injury in whom elevated ADH levels were documented (1). Those patients had hypertonic urine with a urine output of 2.7 l/day, a mean urine osmolality of 500 mosm/kg, and a mean urine sodium of 80 mM/l. Those values are quite comparable to those of the present study patients, who had a mean urine flow of 2.3 l/day, a urine osmolality of 656 mosm/kg, and a mean urine sodium of 70 mM/l. The mean plasma ADH level of 6.8 pg/ml reported previously was very close to the levels documented in our patients (5.6 pg/ml). The only differences between the two patient populations were the serum sodium and osmolality; the former was 138 mM/l in our patients compared to 130 mM in the earlier study. The mean plasma osmolality of the patients in the earlier study was lower than in the patients in the present study (276 vs 286 mosm/kg). The serum sodium of 130 mM/l was interpreted as being consistent with a free water excess indicative of increased blood volume. However, those laboratory findings are also consistent with an intravascular volume deficit in association with a large intravascular sodium deficit which may occur with excessive third-space fluid losses and an osmotic diuresis prompted by urea, both circumstances which occur following thermal injury.

It appears that the central problem in our cohort of patients involves mobilization of the edema fluid to the intravascular space. Five of the 7 patients were markedly above preburn weight on the day of study. In addition, since the total 24-h intake surrounding the study period exceeded the estimated wound evaporative loss by at least maintenance fluid requirements in each patient, they were all considered to have received adequate replacement of ongoing fluid losses. Fluid administration rates were dictated by the patient's urinary output and serum sodium. In their management, decreasing urine outputs and increasing serum sodium levels were interpreted to be consistent with a contracting blood volume and resulted in an increase in the rate of relatively hypotonic intrevenous fluid administration. Failure to adjust the rate was normally followed by an increase in serum BUN and creatinine levels and other signs of prerenal azotemia. After resuscitation, shifts of water from the interstitial to the intravascular space normally occur secondary to differences in oncotic pressure which normally favors water movement from the interstitial to intravascular space. Following burn injury and resuscitation, the intravascular colloid osmotic pressure is low, thus decreasing the net force that determines water movement. Whether artificially increasing plasma colloid osmotic pressure

during this time period would improve edema mobilization cannot be answered from these data.

Findings of increased blood flow associated with a hyperdynamic circulation (elevated cardiac output) and increased organ flow (increased effective renal plasma flow, increased wound blood flow) have previously been interpreted to indicate a normal or supranormal blood volume. Our findings of increased flow in conjunction with modest decrements in total blood volume appear to be paradoxical. The reasons for the dissociation of flow and volume may lie in the neural peripheral vascular response to injury. The effects of the markedly elevated beta adrenergic activity (18), which occurs following injury in association with what amounts to an effective arterial-venous shunt in the wound, may be enough to counterbalance the measured decrements in blood volume. Thus, the decrease in peripheral vascular resistance, typical of the flow phase of injury, may serve to decrease afterload and raise the effective arterial capacity which is underfilled at sites of hormone control.

The interpretation of blood volume measurements is complicated by various factors. First, there is a difficulty in obtaining simultaneous measurements of RBC volume and plasma volume. The use of ^{51}Cr-labeled RBCs represents a well-standardized and accepted method for the measurement of RBC volume (27,28). To obtain plasma volume estimates, one may either measure the space directly by the use of radiolabeled albumin or estimate it by the use of RBC volume and hematocrit. Use of labeled albumin in critically ill patients significantly overestimates plasma volumes due to the expanded volume of distribution for this molecule. Because of this, plasma and total blood volumes in burned patients are usually estimated from RBC volume and hematocrit measurements. Normal total blood volume has been reported to range from 60-80 ml/kg for adult males and 55-75 ml/kg for adult females. However, for comparison purposes, we first expressed our measured patient values each as a percentage of the expected normal volume for that patient estimated from the TBSA according to previously reported regressions. As a group, our patients had a total blood volume which was 81% of predicted. We then compared each individual patient to the reference range. Three patients had blood volumes within the normal reference range, while the remaining 4 (two measured twice) had blood volumes which were below the expected range. The 3 patients with normal blood volumes had ADH levels which were not different from the normal control population. The patients with low blood volumes had elevated levels of ADH, the expected response based upon blood volume.

It appears from our data that cardiac output and renal perfusion in burned patients may not reflect effective blood volume status as registered at hormonal control sites. The dissociation of organ flow and hormonal response necessitates simultaneous direct measurements of blood volume to elucidate factors other than

resetting of central neural control mechanisms to explain hormonal changes in the flow phase of injury. Further, these data indicate that in burn patients increased blood flow does not depend upon increased blood volume.

PRESENTATIONS/PUBLICATIONS

Graves TA: The renal effects of low dose dopamine in thermally injured patients. Presented at the 53rd Annual Meeting of the American Association for the Surgery of Trauma, Louisville, Kentucky, 17 September 1992.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

ACCESSION: DA315349

SUMMARY DATE: 921001 **SUMMARY KIND:** D **PREV DATE:** 911001 **DISTRIBUTION:** CX

PROGRAM #: 61102A **PROJ #:** 30161102BS14 **TASK AREA:** ED **WORK UNIT:** 311

TITLE: Effect of Growth Factors on the Healing of Partial-Thickness Scald Wounds in the Guinea Pig

SUBJ1: 060400 - Anatomy and Physiology
SUBJ2: 060500 - Medicine and Medical Research

START DATE: 8701 **END DATE:** 9309 **PERFORMANCE METHOD:** C

CONTRACT/GRANT NO:

CONT TOTAL: $
CUM TOTAL: $
TOTAL LAB FUNDS: $

RESOURCES ESTIMATE

FY	WORK YRS	$(Thousands)
91	0.5	$32
92	0.5	$23
93	0.5	$24

RESPONSIBLE DOD ORGANIZATION: 404885
MRDC INSTITUTE OF SURGICAL RESEARCH USAISR
FT SAM HOUSTON, TX 78234
PRUITT, B A
210-221-2720

PERFORMING ORGANIZATION: 404885
MRDC INSTITUTE OF SURGICAL RESEARCH USAISR
FT SAM HOUSTON, TX 78234
CIOFFI, W G
210-221-8440

ASSOC1:

ASSOC2:

FOREIGN INTELLIGENCE CONSIDERED: Y **CIVILIAN APPLICABILITY:** M

KEYWORDS: RA II; Lab Animals; Guinea Pigs; Burns (Injuries); Contraction; Healing; Therapy; Histology

OBJECTIVE: A DTIC literature search was conducted under DTIC request numbers W6M16F/W6M17E dated 20 October 1989. The objective of this work is to determine whether infusion of a recently described copolymer will enhance burn epithelization in a deep partial-thickness burn wound in the guinea pig. If compounds can be identified which hasten healing of deep partial-thickness burn wounds, they can then be applied to accelerate healing in patients with thermal injury. Continued work from DA312335.

APPROACH: After receiving deep partial-thickness burns, male guinea pigs will receive either saline or the copolymer. In the first phase, guinea pigs will be sacrificed at 72 h and the effects of the copolymer on the zone-of-stasis studied histologically. In the second phase, guinea pigs were sacrificed at 5, 10, and 20 days following injury and the effects of the copolymer on reepithelization and hair follicle survival was measured. The extent of healing by contraction was assessed by planimetry and the extent of reepithelization was assessed histologically.

PROGRESS: 9110-9209. The second phase of this study revealed no benefit of copolymer on the rate of reepithelization or wound contracture when guinea pigs with deep partial-thickness wounds were followed for 21 days. For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report* for fiscal years 1987 through 1992.

ABSTRACT

PROJECT NUMBER: 3M161102BS14-311, Research

PROJECT TITLE: Effect of Growth Factors on the Healing of Partial-Thickness Scald Wounds in the Guinea Pig

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

PERIOD COVERED IN THIS REPORT: 1 October 1991 - 30 September 1992

INVESTIGATORS: William G. Cioffi, Jr., MD, Major, MC
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The ability of various growth factors to enhance wound healing has received recent interest with the advent of recombinant DNA techniques. Utilizing this technology, increased quantities of various factors previously available only in extremely small amounts are now being used for study. The purpose of this study is to determine whether epidermal, fibroblast, or platelet-derived growth factors can enhance burn epithelization in a partial-thickness burn wound in guinea pigs. However, a suitable source for the procurement of growth factors has not been found.

EFFECT OF GROWTH FACTORS ON THE HEALING OF PARTIAL-THICKNESS SCALD WOUNDS IN THE GUINEA PIG

The ability of various growth factors to enhance wound healing has received recent interest with the advent of recombinant DNA techniques. Utilizing this technology, increased quantities of various factors previously available only in extremely small amounts are now being used for study. Epidermal growth factor (EGF), initially isolated from the submaxillary gland of mice (1) and subsequently identified in human urine (2), has been shown to increase the rate of endothelial and epithelial proliferation (3). The mitogenic effects of EGF have been documented in several models (4-5), although its effectiveness in stimulating epithelization in burn wounds has not been documented (6-7). Fibroblast growth factor (FGF), originally noted for its mitogenic effect on fibroblast, has recently been found to have potent angiogenic properties. Platelet-derived growth factor (PDGF) appears to have a variety of properties, one of which is stimulation of epithelization.

The purpose of this study is to determine whether these three growth factors can enhance burn epithelization in a partial-thickness burn wound in guinea pigs. If growth factors can favorably alter the course of burn wound healing in this model, it will form the scientific basis for further investigations of tissue growth factors.

MATERIALS AND METHODS

Study Design. Male guinea pigs weighing 400-500 g will be anesthetized with sodium pentobarbital (35 mg/kg IP). The dorsal surface will be shaved and a 20% partial-thickness scald injury produced. Animals will be secured to specially constructed template devices and the exposed dorsal surfaces exposed to a 90°F water bath for 5 sec to actuate a deep partial-thickness burn (8). Upon completion of burn injury, the burn wound edges will be tattooed and the animals will be allowed to recover from anesthesia. They will then be housed in individual cages and fed food and water ad libitum throughout the study period. Four groups of 40 animals each will be studied. Group I will serve as the control group, Group II will receive EGF, Group III will receive FGF, and Group IV will receive PDGF. Group I animals will receive 0.5 cc lanolin cream (Squibb-Novo, Inc., Princeton, NJ) applied to the burn wound twice daily. Group II will receive 0.5 cc EGF in a lanolin base (10 μg/ml) twice daily. Groups III and IV will receive FGF and PDGF, respectively, prepared in a similar manner. Wounds will be measured daily for assessment of contraction. This will be accomplished by measuring the burn wound area utilizing the tattoo mark placed at the time of burning. On postburn days 5 and 10, 5 animals in each group will be sacrificed and 15 animals in each group will be sacrificed on postburn days 20 and 30.

Histological Evaluation. At the time of sacrifice, the extent of healing by contraction will be assessed utilizing a planimeter and the extent of reepithelization will be assessed histologically. Tissues will be taken for evaluation of the general health of the animal and evaluation of concurrent disease. Full-thickness skin sections will be taken at the burn margin (to include burned and nonburned skin) to evaluate the healing process. Electron microscopy will be performed as indicated. All tissues will be preserved, processed, and cut using standard methods.

Statistical Analysis. Data will be analyzed by ANOVA.

RESULTS

This project was approved by the US Army Institute of Surgical Research Animal Care and Use Committee on 14 January 1987. However, a suitable source for the procurement of growth factors has not been found.

DISCUSSION

When a suitable source for the procurement of growth factors has been identified, this study will continue.

PRESENTATIONS/PUBLICATIONS

None.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

ACCESSION: DA315350

SUMMARY DATE: 921001 **SUMMARY KIND:** D **PREV DATE:** 911001 **DISTRIBUTION:** CX

PROGRAM #: 61102A **PROJ #:** 30161102BS14 **TASK AREA:** BA **WORK UNIT:** 312

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

SUBJ1: 060100 - Biochemistry
SUBJ2: 060500 - Medicine and Medical Research

START DATE: 8605 **END DATE:** 9309 **PERFORMANCE METHOD:** C

CONTRACT/GRANT NO:

CONT TOTAL: $
CUM TOTAL: $
TOTAL LAB FUNDS: $

RESOURCES ESTIMATE

FY	WORK YRS	$(Thousands)
91	0.5	$42
92	0.5	$124
93	0.5	$140

RESPONSIBLE DOD ORGANIZATION: 404885
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PERFORMING ORGANIZATION: 404885
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ASSOC1: MASON, A D

ASSOC2:

FOREIGN INTELLIGENCE CONSIDERED: Y **CIVILIAN APPLICABILITY:** M

KEYWORDS: RA II; Volunteers; Adults; Burns (Injuries); Lymphocytes; Immunosuppression; Morbidity

OBJECTIVE: A DTIC literature search was conducted under DTIC request numbers W6M25D/W6M27E dated 20 October 1989. The objectives of this work are to analyze the complex leukocyte mixtures seen in the blood of burn patients, quantitate the changes that occur, and correlate those changes with changes in cell function as well as clinical outcome. Improvement of treatment regimens will reduce morbidity and mortality of thermally injured patients. Continued work from DA 311488.

APPROACH: The immune status of burn patients will be assessed in terms of lymphocyte subpopulation composition and function using flow cytometry to differentiate the populations and quantify functional changes. Data will be correlated with patient mortality and morbidity and compared to data from nonburned control subjects.

PROGRESS: 9110-9209. Forty-six burn patients and 31 control subjects have been enrolled in this study to date, 14 burn patients during this reporting period. A new technique for functional assessment of granulocytes was developed. A fluorescein derivative of phalloidin, a naturally occurring toxin that binds to polymerized actin, was used to quantitate the extent of actin polymerization occurring in peripheral blood PMN neutrophils after stimulation with N-formyl-met-leu-phe. The cells were fixed in such a manner that the actin was exposed to binding by the added phalloidin. The amount of phallodin binding was quantified by flow cytometry. The kinetics of the response to the activator were also determined. For technical reports, refer to the *US Army Institute*

of Surgical Research Annual Research Progress Report for fiscal years 1986 through 1992.

ABSTRACT

PROJECT NUMBER: 3M161102BS14-312, Research

PROJECT TITLE: Cellular Host Defense Function after Thermal Injury: Assessment by Flow Cytometry of Peripheral Blood Cells

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

PERIOD COVERED IN THIS REPORT: 1 October 1991 - 30 September 1992

INVESTIGATORS: David G. Burleson, PhD, Colonel, MS
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Following thermal injury, granulocytes exhibit defective chemotaxis and migration. Both are functions of interaction between chemotactic peptide-receptor complexes and the granulocyte cytoskeleton and depend on the polymerization of G-actin into F-actin. We have serially measured F-actin levels in granulocytes from 14 burned patients (mean age 42.1 ± 17.7 yr, mean total body surface area burn size 39.8% ± 22.2%) during the first five weeks postinjury. Peripheral blood was drawn into heparinized tubes and erythrocytes were hypotonically lysed. To estimate polymerization, granulocytes were either mixed with diluent (UNSTIM) or stimulated with N-formyl-met-leu-phe (fMLP) at 37°C (POL). The cells were permeabilized with lysolecithin, stained for F-actin with fluorescein phalloidin, and concomitantly fixed with paraformaldehyde. Fluorescence was measured by flow cytometry and compared to that of granulocytes from 11 healthy control subjects (mean age 33.8 ± 7.4 yrs). To measure F-actin depolymerization, granulocytes were stimulated with fMLP at 37°C for 10 sec and brought to 0°C before adding the stain-fixative (DEPOL). Baseline levels (BASE) were measured by adding stain-fixative to unstimulated granulocytes at 0°C.

Significant ($P < 0.001$) elevations of mean F-actin were found in granulocytes from burned patients under each condition, i.e., granulocytes from burn patients contained more filamentous actin than granulocytes from controls under BASE, UNSTIM, POL, and DEPOL conditions. Induced polymerization (POL minus UNSTIM) was significantly less in burn granulocytes. On depolymerization, mean F-actin returned to baseline in normal granulocytes, but only to the UNSTIM level in cells from burned patients.

The increased actin polymerization observed in unstimulated granulocytes from these patients supports the hypothesis that burn injury induces granulocyte activation. Such cells do not, however, fully depolymerize F-actin, and this impairment may affect migration and chemotaxis.

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

Granulocyte dysfunctions after thermal injury have been described extensively. They include reduced chemotaxis and increased cytosolic oxidase activity (1). Defects in chemotaxis have been attributed to in vivo granulocyte activation by endotoxin, cytokines, or bacterial products, which are augmented after thermal injury (2,3). The interaction between chemotactic peptides and granulocyte receptors are believed to induce cytoskeleton alterations which are dependent on the polymerization of G-actin into F-actin. This granulocyte activation is most likely regulated by G-protein (4).

This study was designed to examine whether granulocyte activation after thermal injury alters cytoskeleton polymerization, thus affecting motility and chemotactic function.

MATERIALS AND METHODS

Study Participant Data. Data are based on 14 patients with thermal injury and 11 healthy control subjects enrolled in this study. Patients with thermal injury had burn sizes ranging from 12.5% to 91.5% of the total body surface area and an average age of 42 yr. They were normotensive and hemodynamically stable after uneventful resuscitation. Heparinized blood samples (14 cc) were drawn between 0700 and 0800 h three times weekly for the first three weeks and then once weekly for the next three weeks. Samples were immediately transported to the laboratory. Mean age of the control subjects was 34 yr.

F-actin Staining Procedure. The cells were washed, hypotonically lysed, and resuspended to 2×10^6 cells per milliliter in phosphate-buffered saline (Sigma, St. Louis, MO), making the assumption that 75% of leukocytes were granulocytes. The cells were stored on ice in 0.5-ml aliquots until assay. A 2×10^{-3} molar stock solution of N-formyl-met-leu-phe (fMLP, Sigma, St. Louis, MO) was resuspended to 2×10^{-8} molar in phosphate-buffered saline and 0.5-ml aliquots were stored on ice until assay. The stain/fixative was prepared by mixing 7 ml lysolecithin (at 1 mg/ml in ethanol) (Sigma, St. Louis, MO) with 15.12 ml 10% paraformaldehyde, 1.5 ml fluorescein phalloidin (Molecular Probes, Inc., Eugene, OR), and 12.72 ml phosphate-buffered saline. One-milliliter aliquots were stored in the dark until processing.

To estimate polymerization, the cells and fMLP were brought to 37°C in a water bath, granulocytes were stimulated with fMLP for various times, and stain/fixative was added. To measure depolymerization, the cells were stimulated with fMLP at 37°C for 10 sec and brought to 0°C before adding the stain/fixative.

Baseline F-actin levels were measured by adding stain/fixative to unstimulated cells at 0°C.

Fluorescence was measured on a FACScan (Becton Dickinson, San Jose, CA) with an argon laser at 540-nm emission after calibration with Cal-brite™ beads (Becton-Dickinson, San Jose, CA). Fluorescein-phalloidin staining was expressed as mean channel fluorescence on a semilogarithmic scale after analyzing 10000 granulocytes. Flow cytometry data were analyzed with software developed by our laboratory.

Statistical Analysis. Statistical significance was determined by Mann-Whitney U-test or Duncan Rank test as appropriate (BMDP Statistical Software, Los Angeles, CA).

RESULTS

Granulocytes were stimulated with diluent (0) or with fMLP for 10, 60, or 240 sec and F-actin polymerization was measured as described. Figure 1 shows that under each condition, granulocytes from burn patients contained more F-actin than those from control subjects. Maximum polymerization occurred 10 sec after addition of fMLP in control cells, whereas the maximum for burn granulocytes was present at both the 10- and 60-sec time periods. Relative fMLP-induced F-actin polymerization (POL minus UNSTIM) is depicted in Figure 2. After 10 sec of fMLP stimulation, burn granulocytes contained significantly less polymerized actin than control granulocytes, but there were no differences in the amount of relative polymerization at 60 sec after addition of fMLP.

Cells were stimulated for 10 sec with fMLP and brought to 0°C to depolymerize before adding stain/fixative. The results are shown in Figure 3. Granulocytes from burned patients were incompletely depolymerized (DEPOL) compared to control granulocytes which approached baseline depolymerization levels (BASE). F-actin levels of cells stimulated at 37°C for 10 sec with fMLP (POL) before the addition of stain/fixative are included in Figure 3 for reference.

The burn size of all patients included in the study ranged from 12.5% to 91.5% of the total body surface area, with a mean of 36.97%. The changes in any of the stages of granulocyte F-actin polymerization (BASE, UNSTIM, POL, DEPOL) did not correlate with burn size. Similarly, there was no apparent relationship with time postburn in the changes in granulocyte F-actin polymerization.

Figure 4 depicts the relationship of F-actin levels in thermally injured patients with and without infection. F-actin levels of controls are included for reference. Granulocytes from infected patients and patients without infections behaved similarly whether the cells were at baseline, unstimulated or polymerized conditions. Depolymerization, in contrast, was significantly less

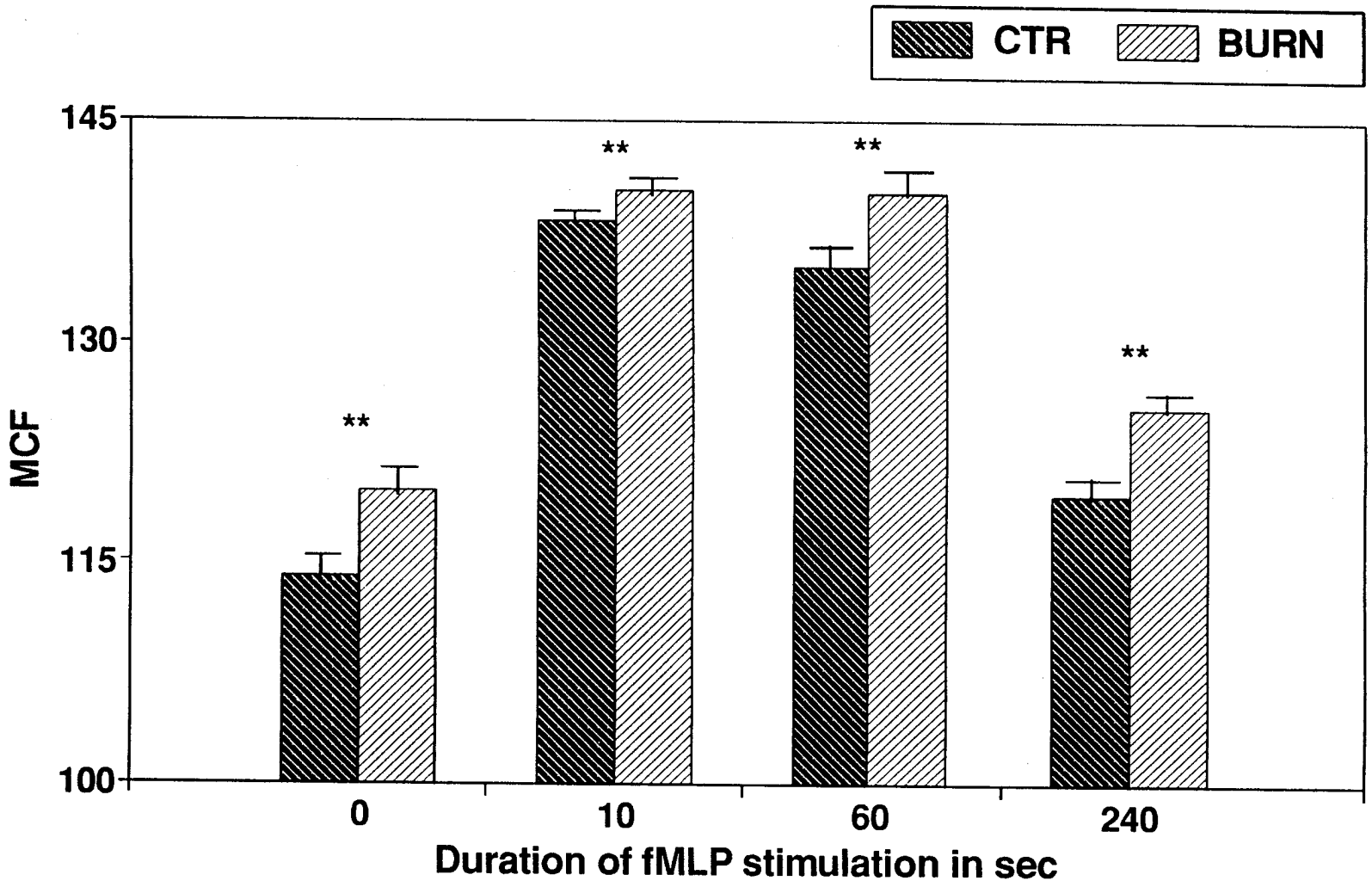


FIGURE 1. F-actin levels were measured in granulocytes from patients with thermal injury (BURN) and control subjects (CTR). Cells were stimulated with diluent (0) or with N-formyl-met-leu-phe (fMLP) for 10, 60, or 240 sec before stain/fixative was added. Data are displayed as mean channel fluorescence (MCF) ± SEM. **$P < 0.01$.

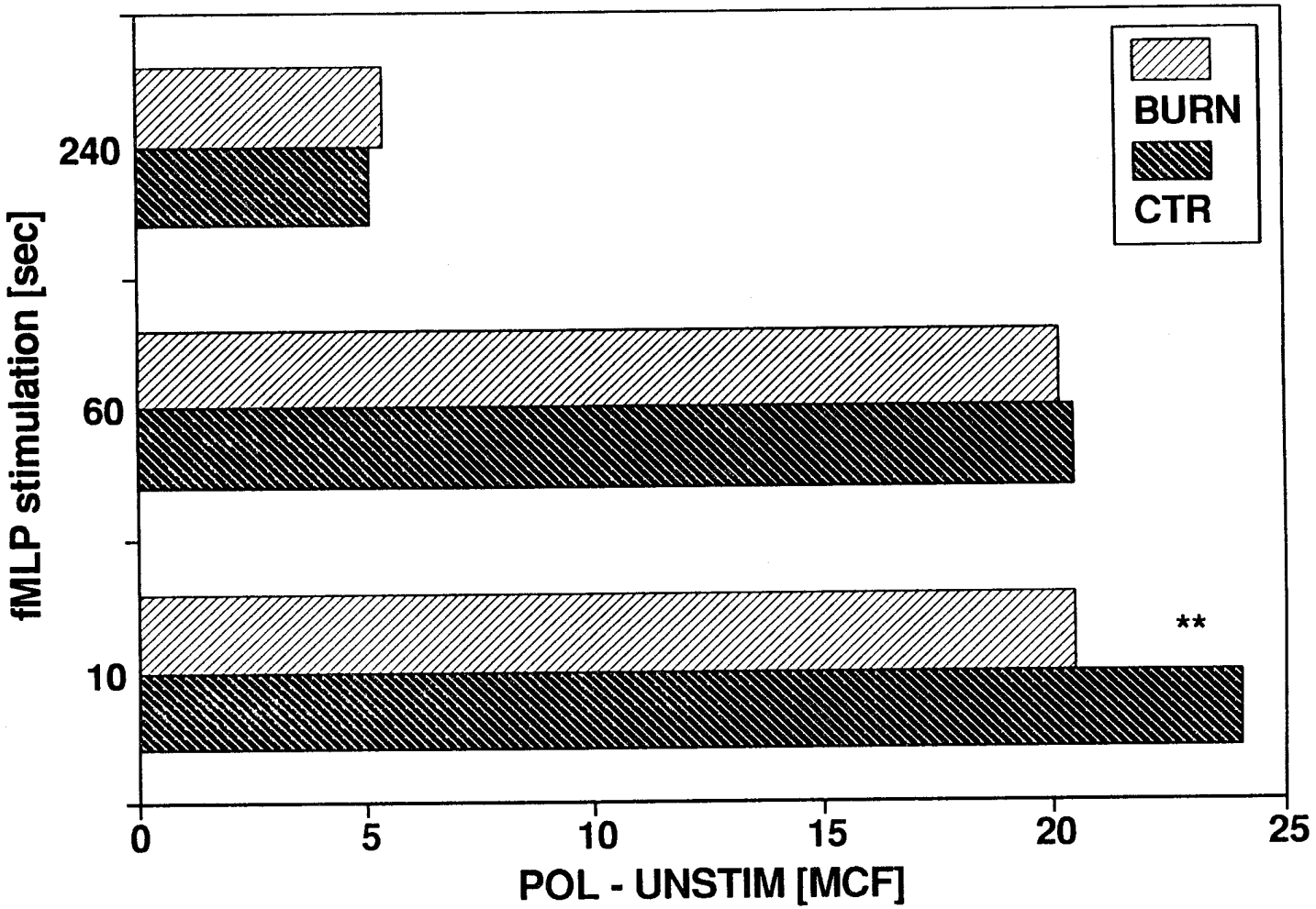


FIGURE 2. F-actin levels were measured in granulocytes from patients with thermal injury (BURN) and control subjects (CTR) and is displayed as the difference of F-actin levels (mean channel fluorescence) in polymerized (POL) and unstimulated (UNSTIM) cells. **P < 0.01.

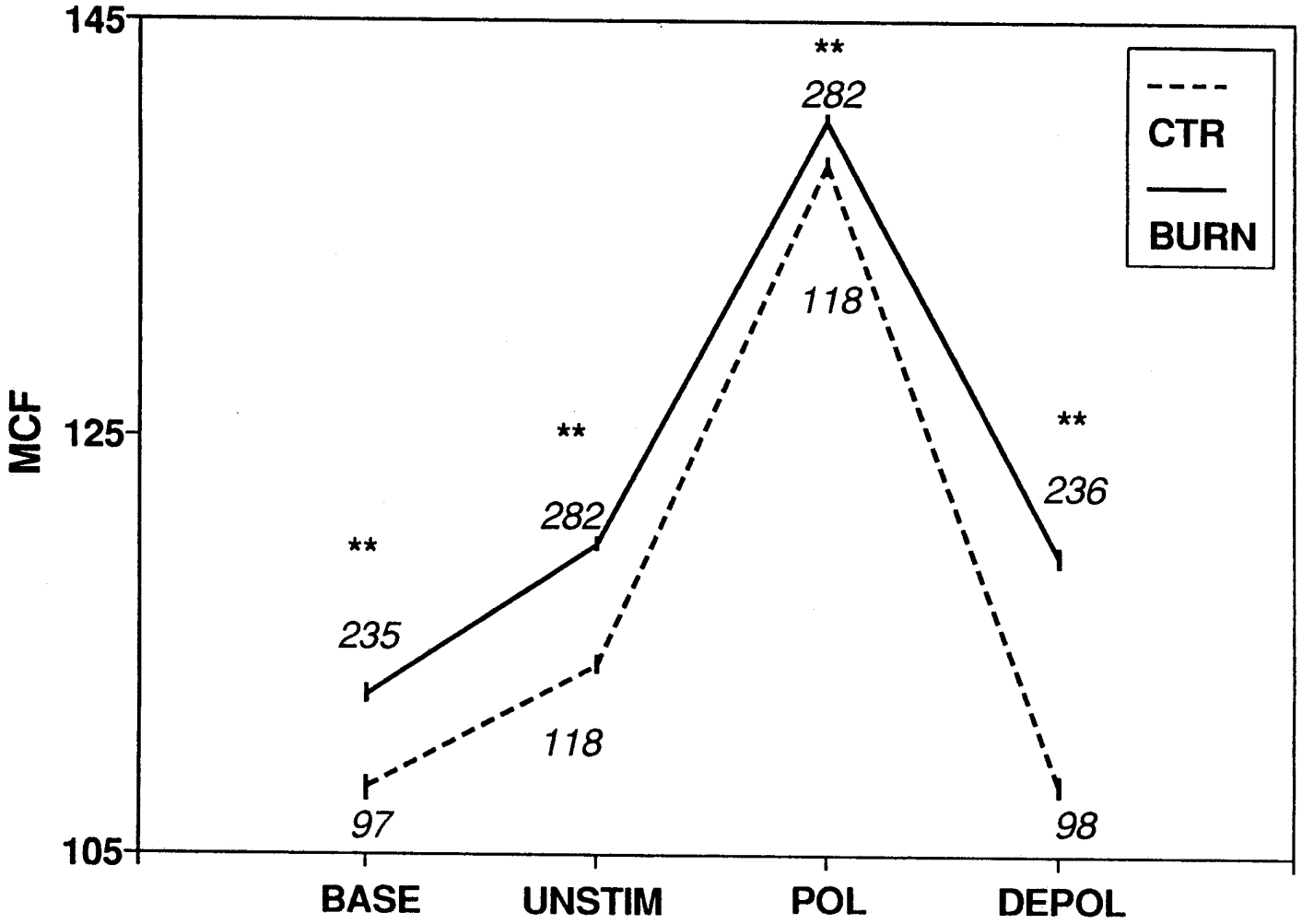


FIGURE 3. F-actin levels in granulocytes from patients with thermal injury (BURN) and control subjects (CTR) stimulated with diluent at 0°C (BASE), with diluent at 37°C (UNSTIM), or with fMLP for 10 sec at 37°C (POL) before the addition of stain/fixative are displayed. DEPOL represents cells which were stimulated with fMLP for 10 sec at 37°C and then brought to 0°C before the addition of stain/fixative. Data is displayed as mean channel fluorescence (MCF) ± SEM. **P < 0.01.

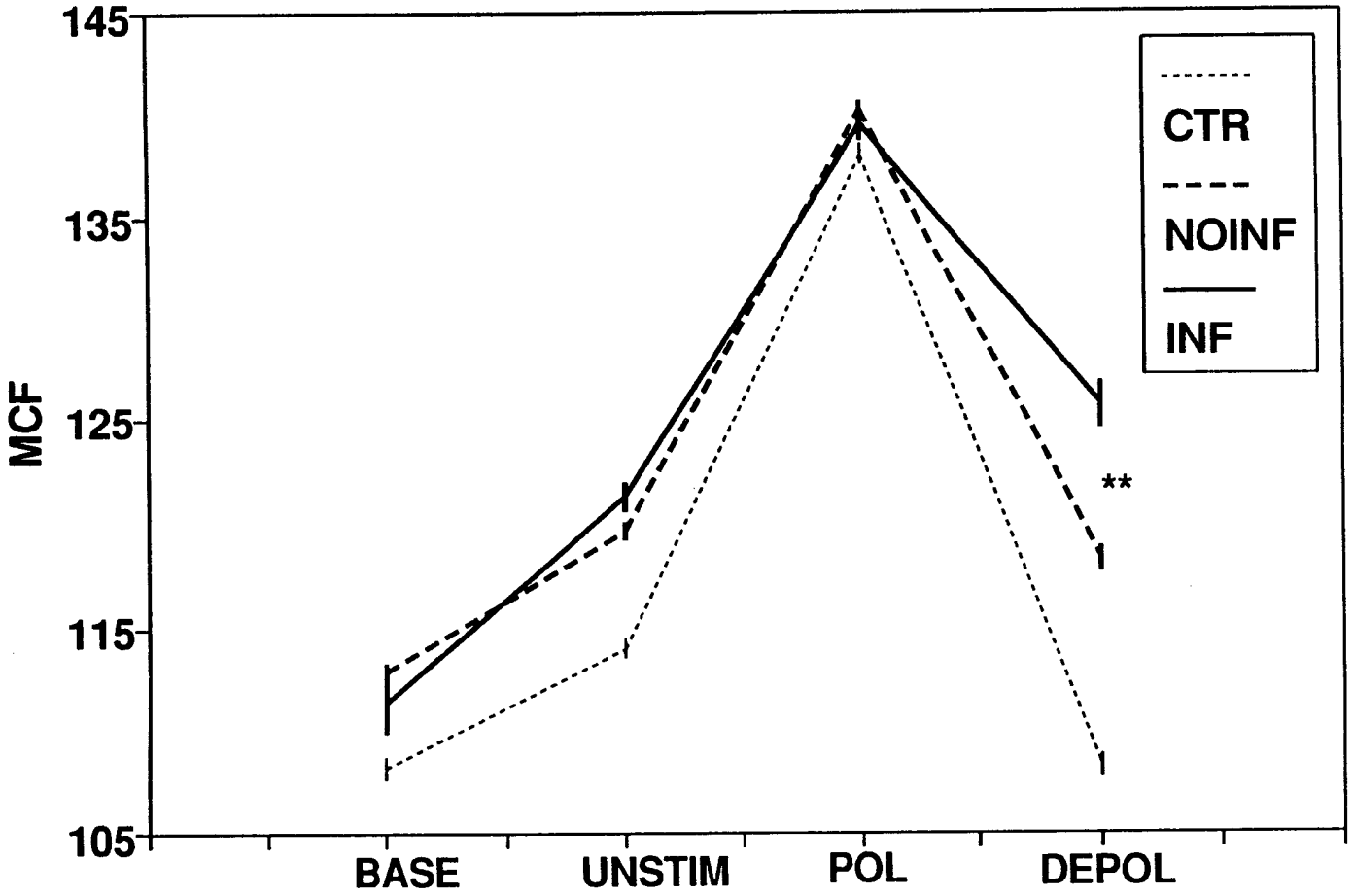


FIGURE 4. F-actin levels in granulocytes from thermally injured patients with (INF) and without (NOINF) infection stimulated with diluent at 0°C (BASE), with diluent at 37°C (UNSTIM), or with fMLP for 10 sec at 37°C (POL) before the addition of stain/fixative are displayed. DEPOL represents cells which were stimulated with fMLP for 10 sec at 37°C and then brought to 0°C before the addition of stain/fixative. Cell data from control subjects (CTR) are included for reference. Data is displayed as mean channel fluorescence (MCF) ± SEM. **$P < 0.01$.

the cells were at baseline, unstimulated or polymerized conditions. Depolymerization, in contrast, was significantly less in granulocytes from infected patients than in those from infection free patients (DEPOL).

We also studied the effects on granulocyte F-actin levels of clinical events, such as packed RBC administration, albumin administration, surgery, inhalation injury, intubation, and mortality. Patients who received packed RBCs, albumin, and/or underwent surgery within 24 h before the blood draw had lower F-actin levels than all other patients under baseline, unstimulated, and polymerized conditions (figs 5-7). There was no difference in depolymerization of the granulocytes from these groups of patients.

Patients with inhalation injury had lower granulocyte F-actin levels in the baseline and polymerized condition than all other patients, but their F-actin levels in the unstimulated or depolymerized condition were similar (fig 8). The same was true for patients who were intubated or patients who did not survive (figs 9,10).

DISCUSSION

These results suggest that burn injury-induced granulocyte activation in vivo. Burn granulocytes could be further stimulated with fMLP to F-actin levels greater than that in healthy subjects.

Depolymerization of filamentous actin was decreased after thermal injury. Whether depolymerization in burn granulocytes simply took longer or whether some cytosolic F-actin was irreversibly polymerized remains unclear, but it seems likely that this anomaly affects granulocyte motility and mobility, thereby altering chemotaxis.

The presence of infection appeared to further decrease the ability of the granulocyte to depolymerize actin, without affecting polymerization. Granulocytes from infected patients contained more filamentous actin after 30 min at 0°C than those from noninfected patients, suggesting that actin polymerization was less reversible in those cells.

These data indicate that thermal injury induces changes in the actin polymerization-depolymerization cascade. Further studies are necessary to examine whether the changes in actin depolymerization are reversible by therapeutic intervention and whether granulocyte functions such as chemotaxis can be restored by restoring the actin cascade.

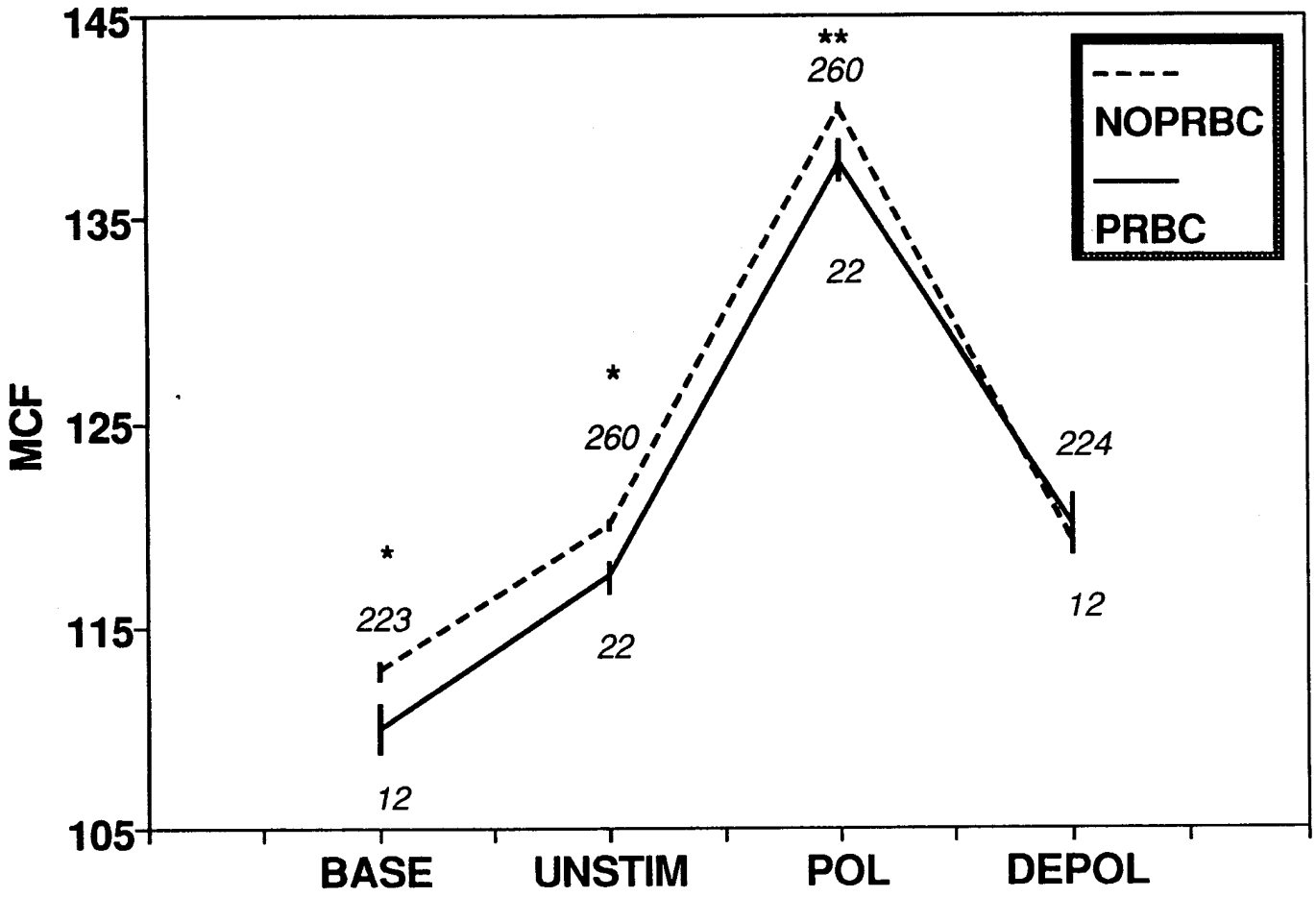


FIGURE 5. F-actin levels in granulocytes from thermally injured patients with (PRBC) and without (NOPRBC) packed RBC administration stimulated with diluent at 0°C (BASE), with diluent at 37°C (UNSTIM), or with fMLP for 10 sec at 37°C (POL) before the addition of stain/fixative are displayed. DEPOL represents cells which were stimulated with fMLP for 10 sec at 37°C and then brought to 0°C before the addition of stain/fixative. Data is displayed as mean channel fluorescence (MCF) ± SEM. **P < 0.01.

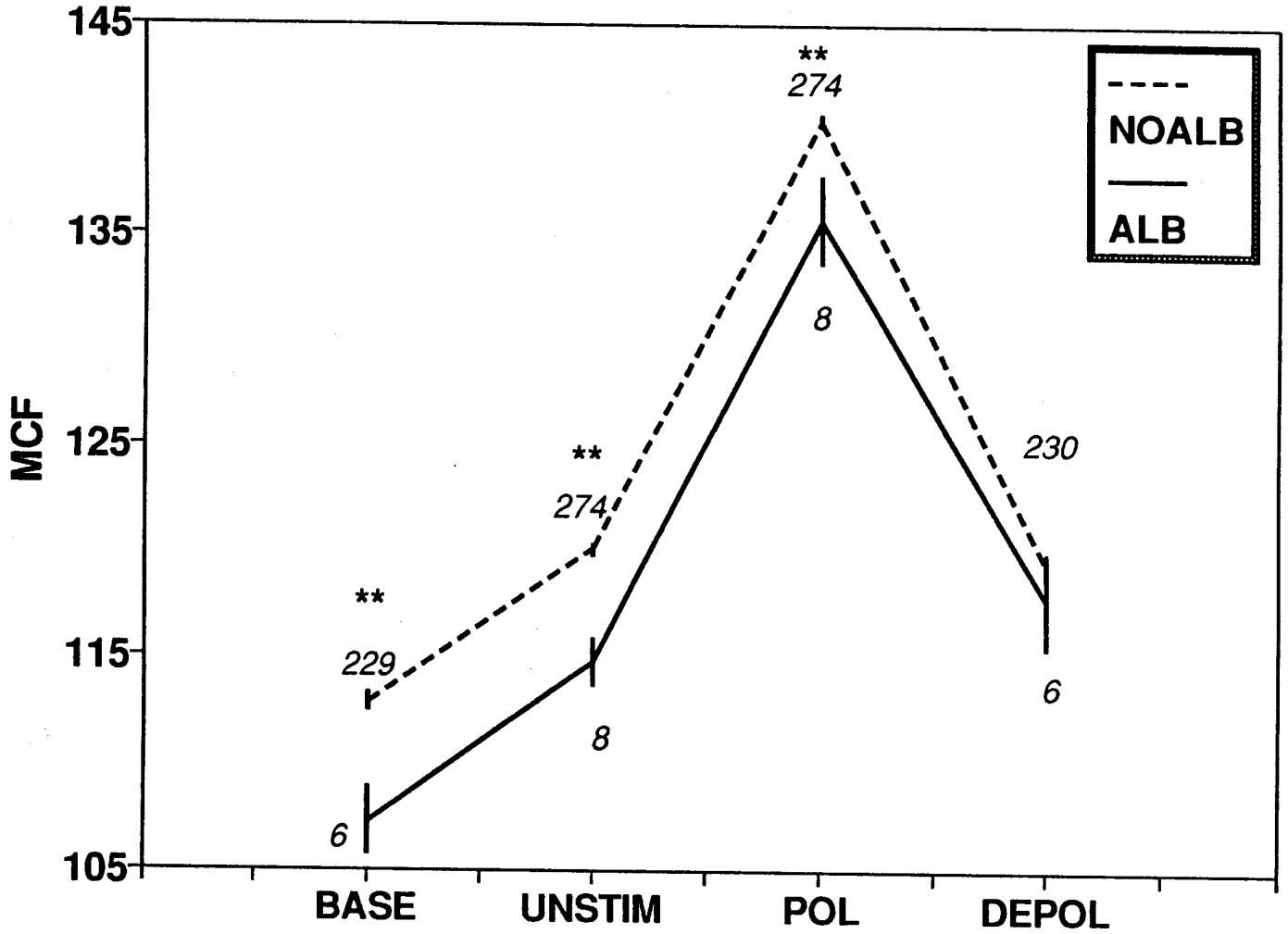


FIGURE 6. F-actin levels in granulocytes from thermally injured patients with (ALB) and without (NOALB) albumin administration stimulated with diluent at 0°C (BASE), with diluent at 37°C (UNSTIM), or with fMLP for 10 sec at 37°C (POL) before the addition of stain/fixative are displayed. DEPOL represents cells which were stimulated with fMLP for 10 sec at 37°C and then brought to 0°C before the addition of stain/fixative. Data is displayed as mean channel fluorescence (MCF) ± SEM. **P < 0.01.

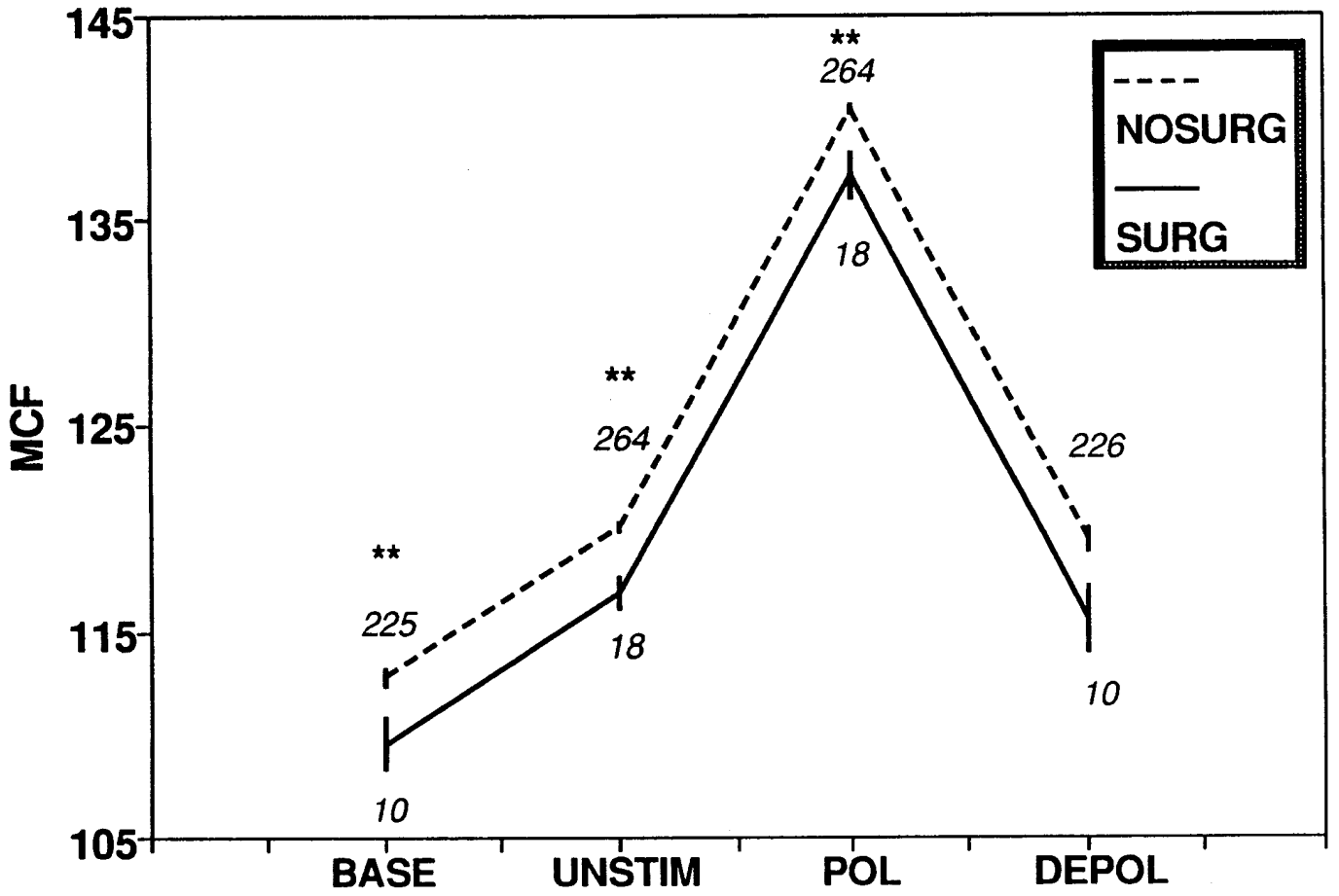


FIGURE 7. F-actin levels in granulocytes from thermally injured patients with surgery (SURG) and without surgery (NOSURG) within the previous 24-h period stimulated with diluent at 0°C (BASE), with diluent at 37°C (UNSTIM), or with fMLP for 10 sec at 37°C (POL) before the addition of stain/fixative are displayed. DEPOL represents cells which were stimulated with fMLP for 10 sec at 37°C and then brought to 0°C before the addition of stain/fixative. Data is displayed as mean channel fluorescence (MCF) ± SEM. **P < 0.01.

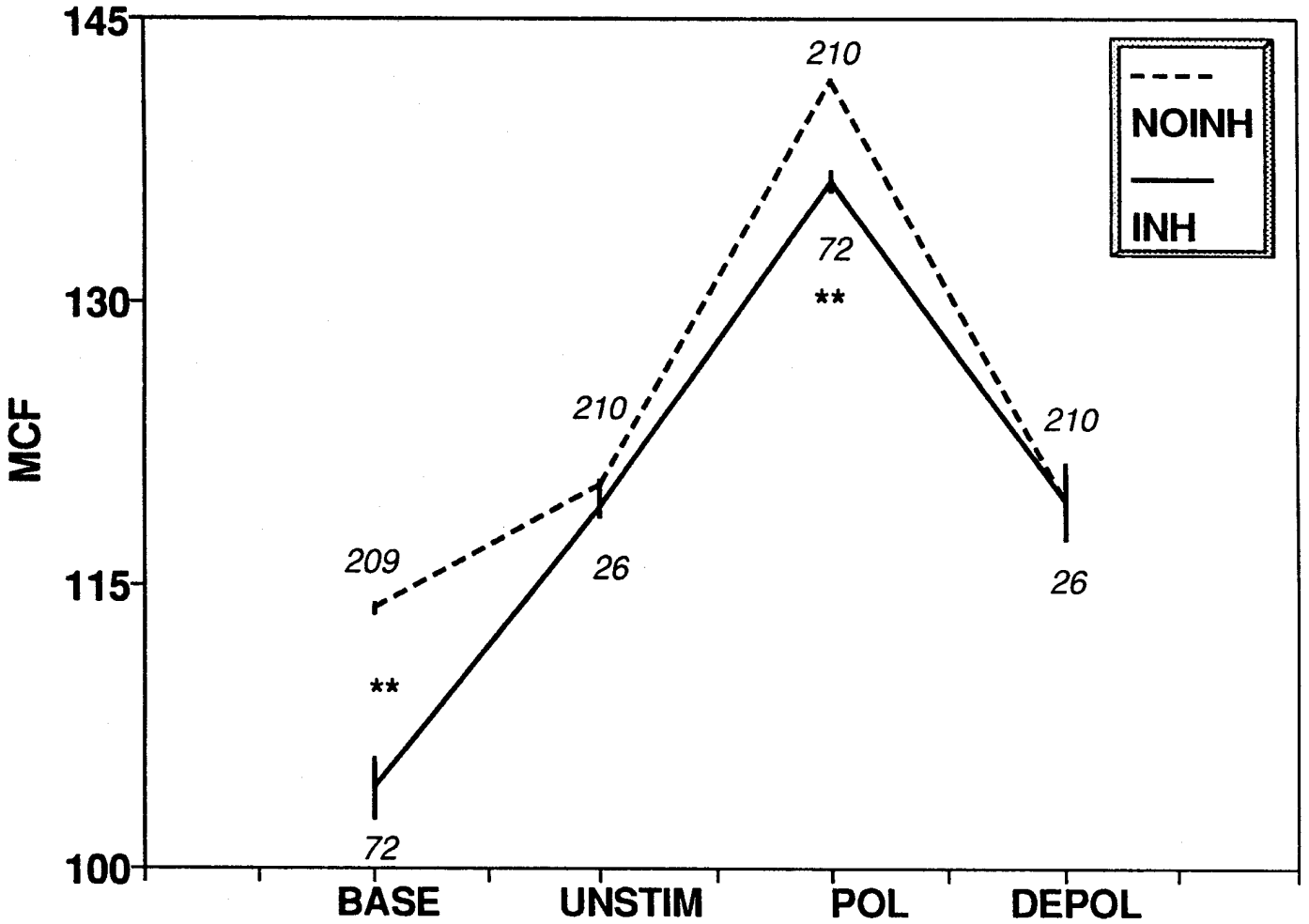


FIGURE 8. F-actin levels in granulocytes from thermally injured patients with (INH) and without (NOINH) inhalation injury stimulated with diluent at 0°C (BASE), with diluent at 37°C (UNSTIM), or with fMLP for 10 sec at 37°C (POL) before the addition of stain/fixative are displayed. DEPOL represents cells which were stimulated with fMLP for 10 sec at 37°C and then brought to 0°C before the addition of stain/fixative. Data is displayed as mean channel fluorescence (MCF) ± SEM. **P < 0.01.

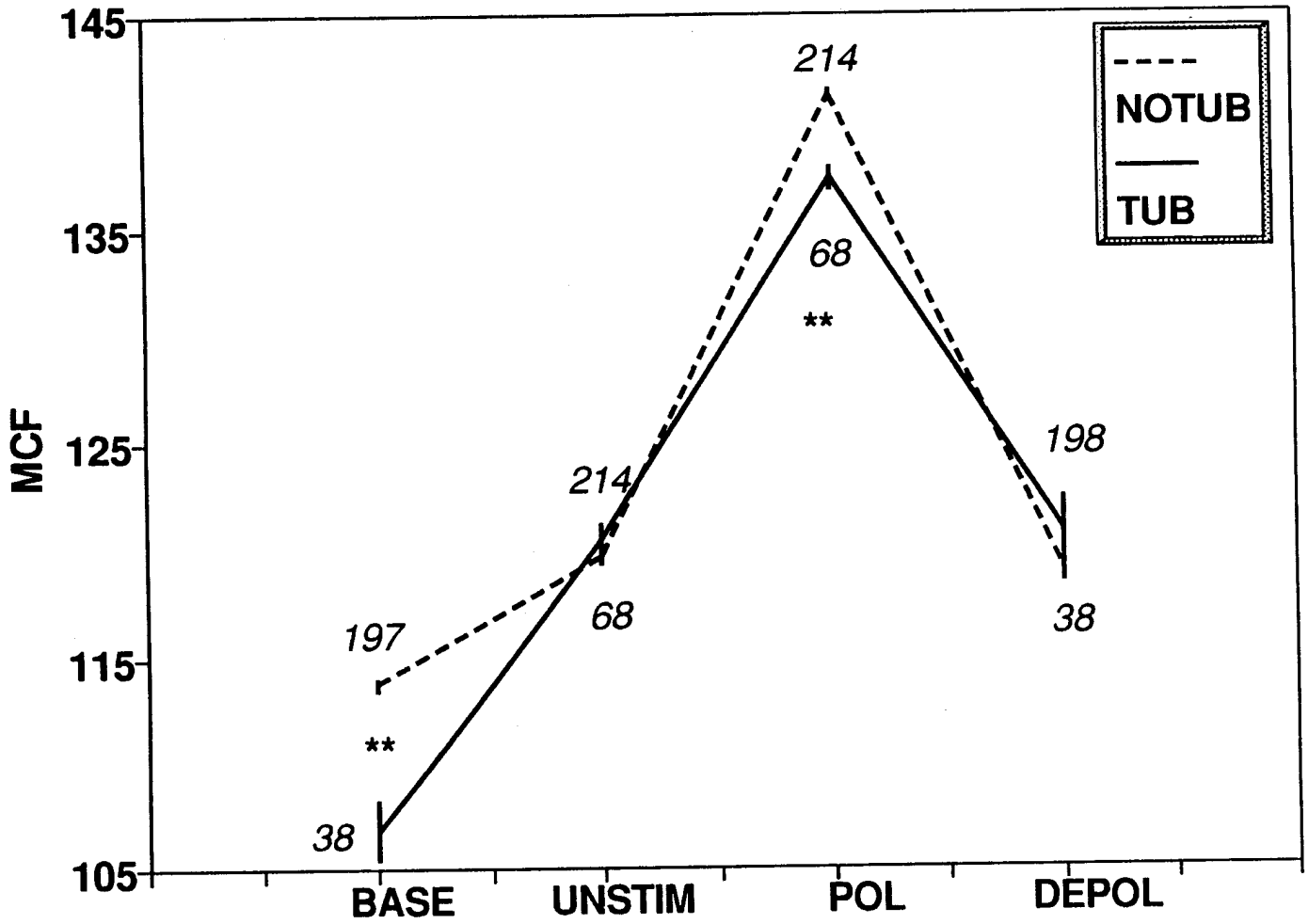


FIGURE 9. F-actin levels in granulocytes from intubated (TUB) and nonintubated (NOTUB) thermally injured patients stimulated with diluent at 0°C (BASE), with diluent at 37°C (UNSTIM), or with fMLP for 10 sec at 37°C (POL) before the addition of stain/fixative are displayed. DEPOL represents cells which were stimulated with fMLP for 10 sec at 37°C and then brought to 0°C before the addition of stain/fixative. Data is displayed as mean channel fluorescence (MCF) ± SEM. **$P < 0.01$.

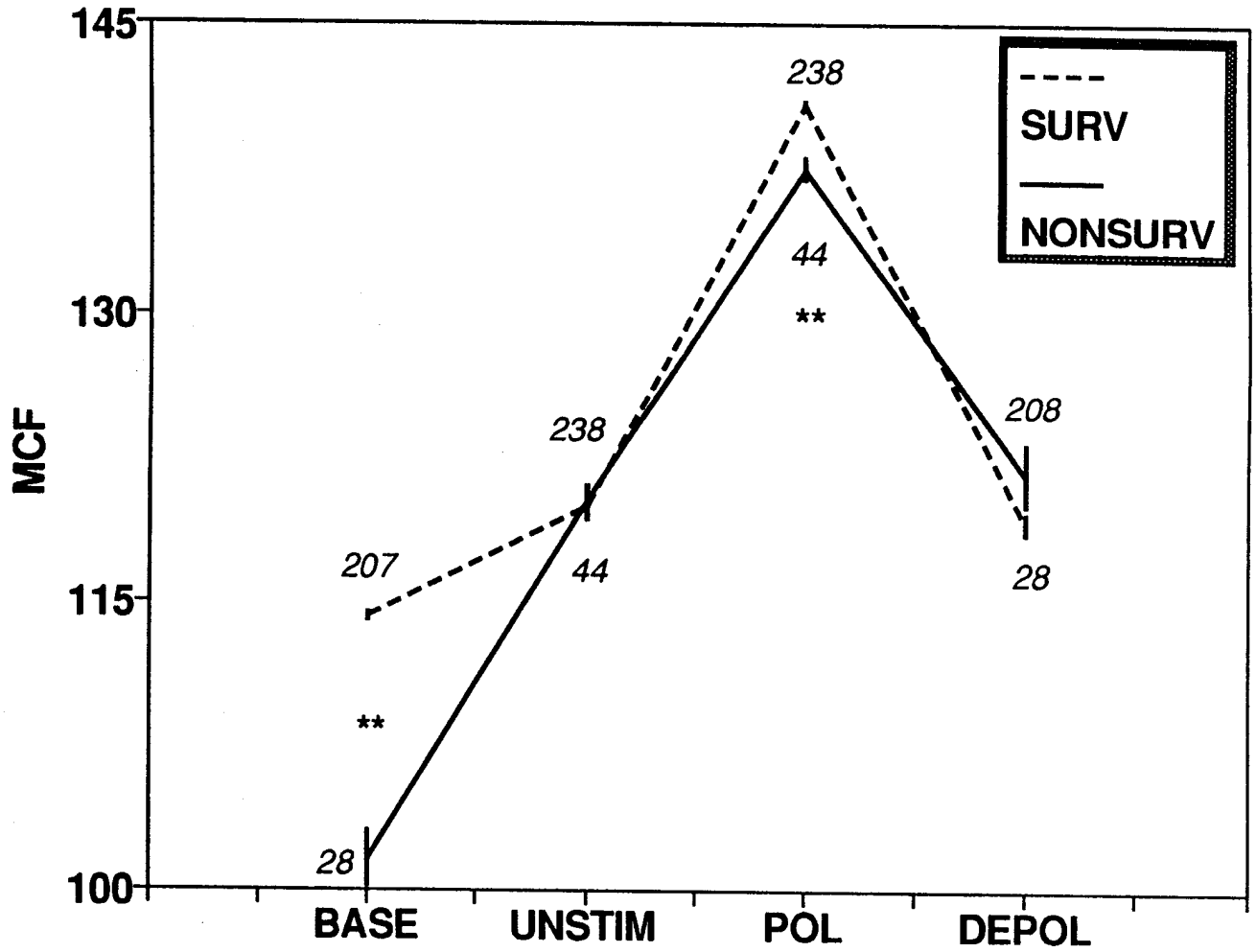


FIGURE 10. F-actin levels in granulocytes from surviving (SURV) and nonsurviving (NONSURV) thermally injured patients stimulated with diluent at 0°C (BASE), with diluent at 37°C (UNSTIM), or with fMLP for 10 sec at 37°C (POL) before the addition of stain/fixative are displayed. DEPOL represents cells which were stimulated with fMLP for 10 sec at 37°C and then brought to 0°C before the addition of stain/fixative. Data is displayed as mean channel fluorescence (MCF) ± SEM. **P < 0.01.

PRESENTATIONS/PUBLICATIONS

Burleson DG, Wolcott KM, Mason AD, and Pruitt BA: Use of leukogate in the analysis of lymphocytes from burned patients. Presented at the 9th International Congress of Histochemistry and Cytochemistry, Maastricht, The Netherlands, 31 August 1992.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

ACCESSION: DA315351

SUMMARY DATE: 921001 **SUMMARY KIND:** D **PREV DATE:** 911001 **DISTRIBUTION:** CX

PROGRAM #: 61102A **PROJ #:** 30161102BS14 **TASK AREA:** ED **WORK UNIT:** 313

TITLE: A Study of Biochemical Changes in the Cellular Environment of Tissue of the in vivo Partial-Thickness Rat Burn Wound

SUBJ1: 060100 - Anatomy and Physiology
SUBJ2: 060500 - Medicine and Medical Research

START DATE: 8609 **END DATE:** 9309 **PERFORMANCE METHOD:** C

CONTRACT/GRANT NO:

CONT TOTAL:	$
CUM TOTAL:	$
TOTAL LAB FUNDS:	$

RESOURCES ESTIMATE

FY	WORK YRS	$(Thousands)
91	1.5	$110
92	1.5	$113
93	1.5	$125

RESPONSIBLE DOD ORGANIZATION: 404885
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ASSOC1: MASON, A D

ASSOC2:

FOREIGN INTELLIGENCE CONSIDERED: Y **CIVILIAN APPLICABILITY:** M

KEYWORDS: RA II; Lab Animals; Rats; Burns (Injuries); Wounds and Injuries; Metabolism; Metabolites

OBJECTIVE: A DTIC literature search was conducted under DTIC request numbers W6M37B/W6N22D dated 20 October 1989. The objectives of this work are to determine the biochemical and metabolic changes that occur in vivo during the early postburn period in partial-thickness burn wounds in the rat and to identify criteria of reversibility of injury. Such data may identify means to block or reverse local metabolic changes and limit progression of cellular death in the wound, reducing the extent and severity of injury in burned soldiers.

APPROACH: Microelectrodes will be used to measure changes in extracellular potassium ion content and in pH and/or PCO_2 at various sites in the burn wound in vivo. Samples will be taken from sites adjacent to the microelectrodes to measure selected metabolites using enzymatic methods. Cells and subcellular organelles will be isolated for measurement of changes in function with time postburn.

PROGRESS: 9110-9209. A method for measurement of intracellular pH using the fluorescent dye BCECF has been adapted for use in cell suspensions isolated by enzymatic treatment of tissue from rat burn and sham-burn wounds. Intracellular pH of cells from sham wounds ranged from 7.05 to 7.20. We are currently using the test to determine changes with time in the intracellular pH of cells from rat burn wounds. These results will be used in conjunction with baseline morphological studies to determine the efficacy of treatments given to reduce the severity of injury of the burn

wound. For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report* for fiscal years 1986 through 1992.

ABSTRACT

PROJECT NUMBER: 3M161102BS14-313, Research

PROJECT TITLE: A Study of Biochemical Changes in the Cellular Environment of Tissue of the in vivo Partial-Thickness Rat Burn Wound

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

PERIOD COVERED IN THIS REPORT: 1 October 1991 - 30 September 1992

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

Extracellular pH was measured in situ in 20% total body surface partial-thickness and sham burn wounds using microelectrodes. Intracellular pH of sham and burn wound cells separated by enzymatic treatment of wound tissue was determined by measuring the ratio of the fluorescent intensity at two excitation wavelengths (490 and 440 nm) of the fluorescent probe BCECF.

The extracellular pH of the burn wound was 0.2 U lower than sham wound at 1 h postburn and 0.3 U lower than sham from 3 to 12 h postburn. Extracellular pH of the burn wound increased slowly from 18 to 72 h postburn and was essentially equal to the sham value at 168 h postburn. Intracellular pH of cells isolated from rat burn wound was approximately 0.2 U less than that of sham wound cells at 3 and 6 h postburn and 0.15 to 0.2 U less from 12 to 48 h postburn. At 72 and 168 h postburn, the pH of cells from sham and burn wound were almost equal.

The time course of these extracellular and intracellular pH changes parallels that of changes in ATP and lactate content of the burn wound that we previously reported and are due, at least in part, to the large quantity of protons generated by the hydrolysis of ATP and the accumulation of lactate in the burn wound.

A STUDY OF BIOCHEMICAL CHANGES IN THE CELLULAR ENVIRONMENT OF TISSUE OF THE IN VIVO PARTIAL-THICKNESS RAT BURN WOUND

The objective of this study is to determine the in vivo biochemical and metabolic changes which occur in partial-thickness rat burn wounds during the early postburn period in order to identify those changes which either foster or impede recovery of cellular function in such wounds.

In previous reports, we have described the changes that occur in the content of ATP and lactate in blood and in wound tissue and preliminary results of measurements of changes in the extracellular pH of the burn wound (1,2). In this report, the changes in the extracellular pH are compared with changes in the intracellular pH of cells isolated from the burn wound at various times during the postburn period.

MATERIALS AND METHODS

Male Sprague-Dawley rats weighing 180 to 200 g were anesthetized with alpha-chloralose (5.5 mg/100 g IP). The hair on the dorsum was clipped and the animals were placed in a protective mold which exposed 20% of the total body surface area. The mold was immersed in water to produce partial-thickness scald burns (80°C for 10 sec) or sham burns (37°C). The margins of the burn wound area were marked in permanent ink. The burn wounds were covered with a layer of 1/4-in foam padding held in place by tubular elastic netting to protect the wounds from mechanical trauma. The animals were then housed in individual cages and allowed access to food and water ad libitum. No parenteral fluids were given. At selected times postburn, the rats were reanesthetized with alpha-chloralose (5.5 mg/100 g IP) before in vivo testing or collection of tissue samples. Upon completion of testing and collection of samples, the animals were sacrificed with alpha-chloralose (50 mg/100 g IP) while under anesthesia.

Extracellular wound pH was measured using a needle pH microelectrode with a micro glass barrel reference electrode (Microelectrodes, Inc, Londonderry, NH) inserted into the wound tissue (1). The microelectrode pairs were connected through a electrode switchbox (Model 607, Orion Research, Inc., Boston, MA) to a microprocessor-controlled IonAnalyzer (Model EA 940, Orion Research, Inc.) equipped with a printer (Model GLP, Orion Research, Inc.).

The procedures used for isolating cells from sham and burn wound tissue for intracellular pH measurements were similar to those described in the previous report (2), except that the tissue incubation solutions were made in Hepes-buffered balanced salt solution (HEPES, pH 7.4) instead of culture media or Hank's nominally bicarbonate-free balanced salt solution (HBSS, pH 7.4).

Briefly, the tissue from the entire wound area was excised and as much muscle tissue as possible removed from the underside. The tissue was cut into 4- to 6-mm squares, weighed, and approximately half of each sample was placed in each of two flasks. The tissue was incubated at 37°C in 15 ml HEPES for 15 min in an orbital shaking water bath (150 rpm) to remove blood from the tissue. The solution was decanted and the tissue was rinsed three times with portions of cold HBSS. These solutions were discarded.

Twenty ml of a solution containing 1.25 mg/ml collagenase CLS4 (Worthington Biochemical Corporation, Freehold, NJ), 5.0 U/ml hyaluronidase (Wyeth Laboratories, Philadelphia, PA), and 4 mg/ml bovine serum albumin (BSA, Sigma Chemical Company, St. Louis, MO) in HEPES was added to the tissue fragments which were then incubated for 45 min at 37°C. The preparation was filtered through a nylon filter cloth and the fragments were rinsed three times with 5 ml cold HBSS. The wash solution was combined with the filtrate and stored on ice. The tissue fragments from each sample were placed back in the flasks and incubated at 37°C for 60 min in 10 ml of a solution containing 12.5 mg/ml Pronase E from *Streptomyces griseus* (Sigma Chemical Company) in HEPES buffer containing 10 mM Mg++. The flasks were then placed in an ice bath, 20 ml fetal bovine serum (FBS, Sigma Chemical Company) was added to each flask, mixed well, and allowed to stand for 20 min to inhibit further activity of the Pronase E. The preparation was then filtered through nylon filtering cloth and the residue rinsed with 5 ml cold HBSS three times. The rinse solution was combined with the filtrate.

All cell suspensions were centrifuged for 2 min at 800 g at 4°C. Cold HBSS (10 ml) containing approximately 5 µg/ml DNase was added to each tube, mixed well, and allowed to stand in an ice bath for 10 min before centrifugation. All supernates from the washes were aspirated and discarded. RBCs in the pellets were lysed by the addition of 2 ml distilled water with mixing, followed immediately by the addition of 2 ml of 0.3 M phosphate-buffered saline. The cell suspensions obtained from the collagenase treatment and those from the Pronase treatment of each wound were combined. HBSS-Dnase solution (10 ml) was added to each tube and mixed well before centrifugation. The resulting cell pellet was washed two more times in 10 ml HBSS before the pellet was finally suspended in 4 ml of 10% FBS in HBSS. The cell suspensions were kept at 2°C to 4°C except when noted otherwise.

An aliquot of each cell suspension was mixed with trypan blue solution and the cells were counted on a hemacytometer. Permeability of cells to trypan blue was an indicator of nonviability. Typically, less than 5% of the cells were permeable to the dye.

An aliquot of each cell suspension containing approximately 1×10^6 cells was added to each of two tubes containing 4 ml of 10%

FBS in HBSS. One tube of each pair was set aside to be used for the measurement of autofluorescence. Fifty micrograms of the cell permeant nonfluorescent dye, 2',7'-biscarboxyethyl-5-carboxyfluorescein, acetoxymethyl ester (BCECF-AM), was dissolved in 25 µl of a 20% (w/w) solution of Pluronic F-127 in DMSO and 187.5 µl FBS. Eighty-five microliters of the dye solution was added to the cells in the second of each pair of tubes which were then incubated at room temperature for 7 min (3). The sample was centrifuged and the pellet washed two times with 10 ml HBSS. The pellet was resuspended in 4 ml of 10% FBS in HBSS and incubated at 37°C for 15 min, during which time cellular esterases converted the dye to the free acid form which is highly fluorescent and is not cell permeant. The test samples and the autofluorescence samples were then centrifuged, washed two times, and finally resuspended in 2 ml each of high K+ Ringer's buffer (pH 7.05) in which all but 10 mM of the NaCl had been replaced with potassium gluconate (4). The samples were transferred to 10-mm^2 fluorescence cells which were placed in the water thermostatted cell holder of a luminescence spectrometer (LS-50, Perkin-Elmer, Ltd, UK). The suspensions were magnetically stirred approximately 5 min before readings were made. Software drove the monochromator back and forth between 440 and 490 nm excitation wavelengths and recorded the value of the fluorescent intensity at 440 and 490 nm every 1.6 sec in an ASCII format file. The intensity of the fluorescence with excitation at 440 nm shows little change with change in pH whereas the intensity with excitation at 490 nm increases in proportion to the increase in pH.

A calibration curve was prepared using the free acid of BCECF in high K^+ Ringer's at pH 6.6 to 7.8, the range over which the plot of the 490/440 fluorescence ratio versus pH was linear. The pH of the samples was calculated using the values obtained for the slope and intercept of the linear regression equation.

RESULTS

The results of the measurements of extracellular and intracellular pH are shown at Table 1. The extracellular pH of the burn wounds was 0.2 U lower than sham wounds at 1 h postburn and 0.3 U lower from 3 to 12 h postburn. The extracellular pH of the wound increased slowly from 18 to 72 h postburn and was essentially equal to the sham value at 168 h postburn. Intracellular pH of cells isolated from the burn wounds was approximately 0.2 U less than that of sham wound cells at 3 and 6 h postburn and 0.15 to 0.2 U less from 12 to 48 h postburn. At 72 and 168 h postburn, the pH of cells from sham and burn wounds was almost equal.

DISCUSSION

The time course of pH changes shown here parallels that of the decrease in ATP content and the increase in lactate in the tissue of the burn wound shown in our previous report (1). These changes are similar to those reported to occur on a much shorter time scale

TABLE 1. pH Content of 20% Total Body Surface Area Partial-Thickness Sham and Burn Wounds Versus Time Postburn in a Rat Model (Mean ± SEM)

Time Postburn (h)	Extracellular	Intracellular
Sham	7.12 ± 0.01*	7.08 ± 0.01
1	6.96 ± 0.07	ND
3	6.81 ± 0.02	6.88 ± 0.02
6	6.79 ± 0.06	6.87 ± 0.01
9	6.83 ± 0.09	ND
12	6.82 ± 0.05	6.93 ± 0.01
18	6.93 ± 0.04	6.93 ± 0.02
24	6.98 ± 0.02	6.96 ± 0.01
48	7.01 ± 0.05	6.90 ± 0.02
72	7.03 ± 0.02	7.02 ± 0.06
168	7.08 ± 0.02	7.01 ± 0.02

ND indicates no data.

in ischemic myocardium (4). In contrast, microelectrode measurements of rat burn wounds (data not shown) showed a decrease in PCO_2 rather than the large increase that was reported for ischemic heart (5).

In normal tissue, intracellular pH is maintained by the cell's intrinsic buffering power, by acid extrusion, and by conversion of acidic metabolites and protons to neutral compounds, e.g., lactic acid to glucose. The persistence of the decrease in intracellular pH of cells from the burn wound indicates that these mechanisms are unable to compensate for the rapid increase in acid equivalents that accumulate in the wound beginning immediately after injury. Moreover, extracellular acidosis has been shown to slow recovery of intracellular pH from an imposed acid load (6). It will be necessary to stimulate the metabolic processes involved in restoring the tissue pH to normal as a step toward improvement of cellular function.

PRESENTATIONS/PUBLICATIONS

None.

REFERENCES

1. Brown WL, Mason AD Jr, Pruitt BA Jr: A study of biochemical changes in the cellular environment of tissue of the in vivo partial-thickness rat burn wound. In *US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1990*, pp 433-42.

2. Brown WL, Mason AD Jr, Pruitt BA Jr: A study of biochemical changes in the cellular environment of tissue of the in vivo partial-thickness rat burn wound. In *US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1991*, pp 492-9.

3. Owen CS: Comparison of spectrum-shifting intracellular pH probes 5'(and 6')-carboxy-10-dimethylamino-3-hydroxyspiro[7H-benzo[c]xanthene-7, 1'(3'H)-isobenzofuran]-3'-one, and 2',7'-biscarboxyethyl-5(and 6)-carboxyfluorescein. *Anal Biochem* 204:65-71, 1992.

4. Ichihara K, Abiko Y: Effect of diltiazem, a calcium antagonist, on myocardial pH in ischemic canine heart. *J Phamacol Exp Ther* 222:720-5, 1982.

5. Vanheel B, de Hemptinne A, Leusen I: Influence of surface pH on intracellular pH regulation in cardiac and skeletal muscle. *Am J Physiol* 250:C748-60, 1986.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

ACCESSION: DA336002

SUMMARY DATE: 920127 **SUMMARY KIND:** A **PREV DATE:** **DISTRIBUTION:** CX

PROGRAM #: 61102A **PROJ #:** 30161102BS14 **TASK AREA:** CK **WORK UNIT:** 314

TITLE: Effect of Wound Closure on Resting Energy Expenditure (REE) and Nitrogen Balance

SUBJ1: 060500 - Medicine and Medical Research

START DATE: 9201 **END DATE:** 9309 **PERFORMANCE METHOD:** C

CONTRACT/GRANT NO:

CONT TOTAL: $
CUM TOTAL: $
TOTAL LAB FUNDS: $

RESOURCES ESTIMATE

FY	WORK YRS	$(Thousands)
91	0.0	$0
92	0.5	$19
93	0.5	$21

RESPONSIBLE DOD ORGANIZATION: 404885
MRDC INSTITUTE OF SURGICAL RESEARCH USAISR
FT SAM HOUSTON, TX 78234
PRUITT, B A
512-221-2720

PERFORMING ORGANIZATION: 404885
MRDC INSTITUTE OF SURGICAL RESEARCH USAISR
FT SAM HOUSTON, TX 78234
MILNER, E A
512-221-6532

ASSOC1: CIOFFI, W G

ASSOC2: ANDRON, L A

FOREIGN INTELLIGENCE CONSIDERED: Y **CIVILIAN APPLICABILITY:** M

KEYWORDS: RA II; Volunteers; Adults; Burns (Injuries); Metabolism; Nutrition; Calorimetry; Oxygen Consumption

OBJECTIVE: A DTIC literature search was conducted under DTIC request numbers W6Q13C/W6Q27F dated 8 November 1991. The objective of this work is to correlate chnages in measured REE with changes in nitrogen balance and body weight during the hospital course as the burn wound closes and healing progresses. An improvement in the understanding of the nutritional needs after thermal injury may improve the outcome of patients with thermal injury.

APPROACH: Fifty patients will undergo indirect calorimetry and urine studies every 7 days until wound closure. REE will be correlated with percent open wound and postburn day. REE and nitrogen balance will be correlated with each other and percent open wound, postburn day, and infection using linear regression analysis.

PROGRESS: 9201-9209. This study was approved by the USAISR Research Council and US Army Institute of Surgical Research Human Use Commitee during the first quarter of Fiscal Year 1992. For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1992.*

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

ACCESSION: DA336002

SUMMARY DATE: 921001 **SUMMARY KIND:** D **PREV DATE:** 911001 **DISTRIBUTION:** CX

PROGRAM #: 61102A **PROJ #:** 30161102BS14 **TASK AREA:** BN **WORK UNIT:** 314

TITLE: Effect of Wound Closure on Resting Energy Expenditure (REE) and Nitrogen Balance

SUBJ1: 060500 - Medicine and Medical Research

START DATE: 9201 **END DATE:** 9309 **PERFORMANCE METHOD:** C

CONTRACT/GRANT NO:

CONT TOTAL: $
CUM TOTAL: $
TOTAL LAB FUNDS: $

RESOURCES ESTIMATE

FY	WORK YRS	$(Thousands)
91	0.0	$0
92	0.5	$19
93	0.5	$26

RESPONSIBLE DOD ORGANIZATION: 404885
MRDC INSTITUTE OF SURGICAL RESEARCH USAISR
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210-221-2720

PERFORMING ORGANIZATION: 404885
MRDC INSTITUTE OF SURGICAL RESEARCH USAISR
FT SAM HOUSTON, TX 78234
MILNER, E A
210-221-6532

ASSOC1: CIOFFI, W G

ASSOC2: ANDRON, L A

FOREIGN INTELLIGENCE CONSIDERED: Y **CIVILIAN APPLICABILITY:** M

KEYWORDS: RA II; Volunteers; Adults; Burns (Injuries); Metabolism; Nutrition; Calorimetry; Oxygen Consumption

OBJECTIVE: A DTIC literature search was conducted under DTIC request numbers W6Q13C/W6Q27F dated 8 November 1991. The objective of this work is to correlate chnages in measured REE with changes in nitrogen balance and body weight during the hospital course as the burn wound closes and healing progresses. An improvement in the understanding of the nutritional needs after thermal injury may improve the outcome of patients with thermal injury.

APPROACH: Fifty patients will undergo indirect calorimetry and urine studies every 7 days until wound closure. REE will be correlated with percent open wound and postburn day. REE and nitrogen balance will be correlated with each other and percent open wound, postburn day, and infection using linear regression analysis.

PROGRESS: 9201-9209. This study was approved by the USAISR Research Council and US Army Institute of Surgical Research Human Use Commitee during the first quarter of Fiscal Year 1992. Seventeen patients have been enrolled in this study to date, 14 having completed the study. Upon completion of patient enrollment and data collection, the data will be analyzed and the results submitted for publication. For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1992*.

ABSTRACT

PROJECT NUMBER: 3M161102BS14-312, Research

PROJECT TITLE: Effect of Wound Closure on Resting Energy Expenditure (REE) and Nitrogen Balance

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

PERIOD COVERED IN THIS REPORT: 27 January 1992 - 30 September 1992

INVESTIGATORS: Elizabeth A. Milner, RD, Captain, SP
William G. Cioffi, Jr., MD, Major, MC
Arthur D. Mason, Jr., MD
William F. McManus, MD, Colonel, MC
Basil A. Pruitt, Jr., MD, Colonel, MC

We have recently developed a burn-specific formula that satisfactorily estimates initial caloric requirements for thermally injured patients. These estimates have been compared with REEs measured weekly by indirect calorimetry in 17 patients thus far. These preliminary measurements (n=117) show the ratio of measured REE to the initial estimated REE (REE ratio) to correlate in a linear manner with postburn day ($r = -0.729$, $P < 0.001$). The correlation between the REE ratio and postburn day for the first 30 days is weak and not statistically significant ($r = -0.301$, $P = 0.056$). Measured and estimated requirements are similar so far during this early interval (2557 ± 537 kcal/day vs 2365 ± 418 kcal/day). The REE ratio is weakly correlated with percent open wound ($r = 0.348$, $P < 0.001$), and more strongly correlated with UUN excretion ($r = 0.615$, $P < 0.001$). Multiple regression analysis indicates a relationship between REE and initial burn size, postburn day, and body surface area in these patients ($r^2 = 0.78$).

Thus far, the decline in metabolic need appears more closely related to postburn day rather than to wound closure. Initial estimates of caloric need based on total body surface and burn size appear adaequate for at least 30 days after injury. Beyond this time, patient variation seems to dictate a need for indirect calorimetry for accurate estimations of caloric need. The remainder of the data will be analyzed after 50 patients have completed the study.

EFFECT OF WOUND CLOSURE ON RESTING ENERGY EXPENDITURE (REE) AND NITROGEN BALANCE

We have recently revised the formula used to predict energy requirements in our population of burned patients (1). In that study, 62 patients with burn sizes ranging from 12% to 91% of the total body surface area (TBSA) were studied using indirect calorimetry between the 5th and 19th day postburn when metabolic demands were at their peak. There have been few longitudinal studies to examine at what point postburn metabolic requirements decrease as the burn wound closes. Cunningham et al (2) retrospectively analyzed 87 patients at various times during recovery, although few patients were studied beyond postburn day 40. These investigators reported a "general trend" in reduction of energy requirements of 15% to 20% per month for 30% to 75% TBSA burns and 10% per month for 76% to 90% TBSA burns. Saffle et al (3) studied 29 patients and found REE to rise for 10-20 days postburn, after which it declined until discharge. Twenty-one of these patients were studied at discharge and found to have a mean REE 24% higher than the predicted BEE. Matsuda et al (4) studied 29 patients with a mean burn size of 29% (range 8%-58%) to determine the relationship of measured REE with the current open wound size. When the wound size was ≤ 10% of the TBSA, measured REE was found to be 27% greater than the predicted BEE. Ireton-Jones et al (5) studied 20 patients with a mean burn size of 50% (range 31%-74%) to determine if wound closure affects energy expenditure and UUN excretion. These investigators reported that measured energy expenditure did not decrease with wound closure and UUN excretion did not correlate with either measured energy expenditure or wound closure.

The objective of this study is to correlate changes in measured REE with changes in nitrogen balance and body weight during the hospital course as the burn wound closes and healing progresses.

MATERIALS AND METHODS

Study Design. Fifty patients will undergo indirect calorimetry and urine studies every 7 days until burn wound closure.

Patient Criteria. Fifty patients will be enrolled in this study. Properly signed and witnessed DA Forms 5303-R, Volunteer Agreement Affidavit, are obtained from each patient before enrollment in the study.

Patient Inclusion. Patients meeting all of the following criteria are enrolled in the study upon giving written informed consent:

1. Male or female patients ≥ 18 yr.

2. Patients admitted to the US Army Institute of Surgical Research within 7 days postburn.

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

Patient Exclusion. Patients meeting any of the following criteria are excluded from participation in the study:

1. Patients < 18 y.

2. Patients not admitted to the US Army Institute of Surgical Research within 7 days postburn.

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

4. Patients requiring ventilatory support on the day of the study.

5. Patients with toxic epidermal necrolysis (TEN).

Description of Procedures. The nutritional support of each patient based on 100% of predicted requirements is initially calculated from the Institute's standard formula. Nutritional support is then tailored to meet the needs of the patient based on REE and RQ measurements from indirect calorimetry studies. Nutritional intake will be monitored for total calories, protein, carbohydrate, and vitamin/mineral intake. Enteral and intravenous feedings will be continued, but oral intake will be withheld for 8 h before the study. All measurements will be performed no less than 48 h after a surgical procedure.

Beginning on or about postburn day 7 at 0600 h, the patient's O_2 consumption and CO_2 production are measured using the Delta-Trac™ metabolic cart. Extubated patients requiring no supplemental O_2 are studied with a canopy over their head for approximately 1 h. All measurements take place before routine nursing care and the morning dressing change while the patient is in a resting state. Metabolic measurements are made at the bedside. Room temperature and humidity are not controlled in order to examine the determinants of REE under the usual clinical conditions. The REE is calculated as well as the RQ. In addition, all urine is collected from the previous 24-h period for the measurement of total urea nitrogen. Nitrogen balance is calculated from the 24-h total urea nitrogen. The patient's temperature at the time of the study is noted. Upon completion of the metabolic measurements, a nude weight is obtained. These studies are performed weekly until the patient's burn wounds are closed.

Determination of Number of Subjects Required. Fifty patients will be required for this study. This is the minimum number needed in order to stratify for the effects of age, burn size, and inhalation injury on metabolic needs.

Data Collection. The postburn day, postoperative day, percent open wound, percent closed wound, glucose load, and protein and calorie intakes are obtained from the patient's medical record. The presence and postburn day of occurrence of all infections will be recorded. O_2 consumption, CO_2 output, REE, RQ, nitrogen balance, total urea nitrogen, vitamin and mineral intakes, and body weight are recorded.

Data Analysis Plan. REE will be correlated with percent open wound and postburn day. REE and nitrogen balance will be correlated with each other and percent open wound, postburn day, and infection using linear regression.

RESULTS

These estimates have been compared with REEs measured weekly by indirect calorimetry in 17 patients thus far. These preliminary measurements (n=117) show the ratio of measured REE to the initial estimated REE (REE ratio) to correlate in a linear manner with postburn day ($r = -0.729$, $P < 0.001$). The correlation between the REE ratio and postburn day for the first 30 days is weak and not statistically significant ($r = -0.301$, $P = 0.056$). Measured and estimated requirements are similar so far during this early interval (2557 ± 537 kcal/day vs 2365 ± 418 kcal/day). The REE ratio is weakly correlated with percent open wound ($r = 0.348$, $P < 0.001$), and more strongly correlated with UUN excretion ($r = 0.615$, $P < 0.001$). Multiple regression analysis indicates a relationship between REE and initial burn size, postburn day, and body surface area in these patients ($r^2 = 0.78$).

DISCUSSION

Thus far, the decline in metabolic need appears more closely related to postburn day than to wound closure. Initial estimates of caloric need based on total body surface and burn size appear adaequate for at least 30 days after injury. Beyond this time, patient variation seems to dictate a need for indirect calorimetry for accurate estimation of caloric need. The remainder of the data will be analyzed after 50 patients have completed the study.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

ACCESSION: DA336003

SUMMARY DATE: 921001 **SUMMARY KIND:** D **PREV DATE:** 920129 **DISTRIBUTION:** CX

PROGRAM #: 61102A **PROJ #:** 30161102BS14 **TASK AREA:** ED **WORK UNIT:** 315

TITLE: Cell Surface Proteoglycans as Markers of Wound Repair Following Thermal Injury - A Pilot Study

SUBJ1: 060500 - Medicine and Medical Research

START DATE: 9201 **END DATE:** 9309 **PERFORMANCE METHOD:** C

CONTRACT/GRANT NO:

CONT TOTAL: $
CUM TOTAL: $
TOTAL LAB FUNDS: $

RESOURCES ESTIMATE

FY	WORK YRS	$(Thousands)
91	0.0	$0
92	0.5	$19
93	0.5	$26

RESPONSIBLE DOD ORGANIZATION: 404885
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PERFORMING ORGANIZATION: 404885
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ASSOC1: OKERBERG, C V

ASSOC2: ELENIUS, K

FOREIGN INTELLIGENCE CONSIDERED: Y **CIVILIAN APPLICABILITY:** M

KEYWORDS: RA II; Lab Animals: Mice; Burns (Injuries); Wounds and Injuries; Epithelium; Cell Structure; Histopathology

OBJECTIVE: A DTIC literature search was conducted under DTIC request numbers W6052J/W6053I dated 27 January 1992. The objective of this work is to determine the utility of immunohistochemical staining of the cell surface proteoglycan, syndecan, as a marker of wound repair after thermal injury. The development of a reliable test to assist in determining the depth of thermal injury and the potential for wound repair may assist in the decision-making process to choose the most appropriate and expeditious therapy for the burn wound.

APPROACH: Eighty mice were anesthetized, the backs of the animals were shaved, and thermal injury was produced by the application of heated brass blocks. Sham-burned animals underwent identical anesthetic and shaving procedures but had the outline of the brass block drawn on their backs. At selected times postburn, the animals were sacrificed. The skin from the back was removed from the animals and submitted for evaluation.

PROGRESS: 9201-9209. This study was approved by the USAISR Research Council and US Army Institute of Surgical Research Animal Care and Use Committee during the first quarter of fiscal year 1992. Animal studies have been completed. Upon completion of the immunohistochemical analyses, the data will be analyzed and the results submitted for publication. For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1992.*

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

ACCESSION: DA336003

SUMMARY DATE: 920129 **SUMMARY KIND:** A **PREV DATE:** **DISTRIBUTION:** CX

PROGRAM #: 61102A **PROJ #:** 30161102BS14 **TASK AREA:** EA **WORK UNIT:** 315

TITLE: Cell Surface Proteoglycans as Markers of Wound Repair Following Thermal Injury - A Pilot Study

SUBJ1: 060500 - Medicine and Medical Research

START DATE: 9201 **END DATE:** 9309 **PERFORMANCE METHOD:** C

CONTRACT/GRANT NO:

CONT TOTAL: $
CUM TOTAL: $
TOTAL LAB FUNDS: $

RESOURCES ESTIMATE

FY	WORK YRS	$(Thousands)
91	0.0	$0
92	0.5	$25
93	0.5	$27

RESPONSIBLE DOD ORGANIZATION: 404885
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ASSOC1: OKERBERG, C V

ASSOC2: ELENIUS, K

FOREIGN INTELLIGENCE CONSIDERED: Y **CIVILIAN APPLICABILITY:** M

KEYWORDS: RA II; Lab Animals: Mice; Burns (Injuries); Wounds and Injuries; Epithelium; Cell Structure; Histopathology

OBJECTIVE: A DTIC literature search was conducted under DTIC request numbers W6052J/W6053I dated 27 January 1992. The objective of this work is to determine the utility of immunohistochemical staining of the cell surface proteoglycan, syndecan, as a marker of wound repair after thermal injury. The development of a reliable test to assist in determining the depth of thermal injury and the potential for wound repair may assist in the decision-making process to choose the most appropriate and expeditious therapy for the burn wound.

APPROACH: Eighty mice will be anesthetized, the backs of the animals will be shaved, and thermal injury will be produced by the application of heated brass blocks. Sham-burned animals will undergo identical anesthetic and shaving procedures but will have the outline of the brass block drawn on their backs. At selected times postburn, the animals will be sacrificed. The skin from the back will be removed from the animals and submitted for evaluation.

PROGRESS: 9201-9209. This study was approved by the USAISR Research Council and US Army Institute of Surgical Research Animal Care and Use Committee during the first quarter of fiscal year 1992.

ABSTRACT

PROJECT NUMBER: 3M161102BS14-315, Research

PROJECT TITLE: Cell Surface Proteoglycans as Markers of Wound Repair Following Thermal Injury - A Pilot Study

INSTITUTION: US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas 78234-5012[1], and Department of Medical Biochemistry, University of Turku, Turku, Finland[2]

PERIOD COVERED IN THIS REPORT: 29 January 1992 - 30 September 1992

INVESTIGATORS: William K. Becker, MD, Lieutenant Colonel, MC[1]
Carlin V. Okerberg, DVM, PhD, Lieutenant Colonel, VC[1]
Klaus Elenius[2]
Markku Jalkanen[2]

The distinction between partial- and full-thickness thermal injury can frequently be made by clinical observation of the burn wound. There are, however, times when this distinction is difficult to make by clinical observation alone. The development of a reliable test to assist in determining the depth of thermal injury and the potential for wound repair may assist in choosing the most appropriate and expeditious therapy for the burn wound. Therefore, the objective of this study is to determine the utility of immunohistochemical staining of the cell surface proteoglycan, syndecan, as a marker of wound repair following thermal injury. If cell surface markers can aid in the distinction between thermal injuries that have the potential for spontaneous healing and those that require surgical management, their measurement may assist in the management and care of patients with thermal injury. Also, these markers may also prove useful in studying the healing of cultured keratinocytes.

CELL SURFACE PROTEOGLYCANS AS MARKERS OF WOUND REPAIR FOLLOWING THERMAL INJURY - A PILOT STUDY

The distinction between partial- and full-thickness thermal injury can frequently be made by clinical observation of the burn wound. There are, however, times when this distinction is difficult to make by clinical observation alone (1,2). This distinction is clinically relevant because the diagnosis of full-thickness injury implies the need for surgical management of the burn wound, while partial-thickness injury will heal, given sufficient time, in the absence of supervening complications such as burn wound infection. Several methodologies to assist in the diagnosis of the depth of cutaneous thermal injury have been proposed; none have found widespread clinical use (3-7). The development of a reliable test to assist in determining the depth of thermal injury and the potential for wound repair may assist in the decision-making process to choose the most appropriate and expeditious therapy of the burn wound.

Healing of partial-thickness thermal injury involves the proliferation of keratinocytes from the epidermis or adjacent dermal structures such as hair follicles. Cellular proliferation during wound repair is a complex process which involves cell-cell and cell-matrix interactions; these interactions resemble, to a degree, the normal sequence of events during early tissue development and growth. A number of cell surface molecules have been identified as being involved in normal tissue development and in wound repair. One of these cell surface molecules, syndecan, a proteoglycan, is expressed during organ formation and enhanced expression of this molecule has been noted during the proliferation and migration of epidermal cells in healing wounds (8-10). The enhanced expression of syndecan in epidermal cells during wound repair was measured by both immunohistochemical staining using a specific monoclonal antibody to syndecan and by in situ hybridization which demonstrated increased levels of mRNA for this molecule (11). The increased expression of syndecan was noted within 1 day of wounding and persisted for 7-10 days in a mouse model of skin incision and primary wound closure. Other studies support a role for syndecan in cell adhesion, matrix adhesion, and in the binding of growth factors (12,13). The early expression of this molecule by epithelial cells during wound repair and the ability to demonstrate this expression by immunohistochemical techniques suggest the possibility that such techniques may prove useful in distinguishing between wounds that have the capacity to heal and those that do not. Because the molecule is expressed very early following injury, rapid estimation of depth of injury may be possible with this technique. Also, the number of cells expressing the molecule and the quantity of expression of mRNA could potentially provide an estimation of the length of time necessary for wound healing to take place. This study will evaluate the expression of syndecan in tissue specimens following full- and

partial-thickness cutaneous thermal injury in the mouse. At the present time, the only monoclonal antibody available for immunohistochemical staining and the only probe for in situ mRNA hybridization are species-specific for the mouse. Previous work in wound healing using this monoclonal antibody has been successfully performed in the BALB/c mouse. The molecule has been demonstrated in human tissue specimens and appears to be highly conserved in mammals.

MATERIALS AND METHODS

Study Design. Mice were anesthetized, the backs of the animals were shaved, and thermal injury was produced by the application of heated brass plates. Sham-burned animals underwent identical anesthetic and shaving procedures and had the outline of the brass block drawn on their backs. At the selected times postburn, the animals were sacrificed. The skin from the back was removed from the animal and submitted for evaluation.

Description of Procedures. Sixty male 3-month-old BALB/c mice weighing approximately 20 g were anesthetized with sodium pentobarbital (35 mg/kg IP) through a 25-ga needle. The dorsal surface of the animal was shaved. Three areas of burn injury were created. For those animals randomized to the burn injury group, three areas received a full-thickness injury, which was produced by heating a ½- by ½-in brass block to 100°C and applying the block to the back of the mouse for 10 sec, or three areas received a partial-thickness injury, which was produced by heating a ½- by ½-in brass block to 80°C and applying the block to the back of the mouse for 5 sec. These areas of injury were separated by a distance of 1 cm. For those animals randomized to the control group, the brass block was not be heated and was applied to the dorsal surface at room temperature. The area of the sham-burn was marked with an indelible ink. There were 20 animals for each group, i.e., full-thickness, partial-thickness, and sham.

Twenty animals were used in a preliminary phase of this study to confirm the depth of injury by light microscopy and to provide tissue for the development of the immunohistochemical techniques described below. Animals received burn injury in groups of 5 and were sacrificed with sodium pentobarbital (150 mg/kg IP) on postburn day 3. The skin from the back was removed and fixed in formalin and examined by light microscopy to determine that a partial-thickness or full-thickness injury had been achieved in the areas under the brass blocks.

Sixty mice were divided into three groups, i.e., full-thickness burn, partial-thickness burn, and sham burn. After injury, 4 animals from each group were sacrificed at 1 h and 2, 4, 6, and 10 days postburn. The backs of the animals were photographed, the dorsal skin was removed and fixed overnight in 10% buffered formalin, and then dehydrated in ascending concentrations of

ethanol and embedded in paraffin with microtome sections taken at 5 μm. Normal skin was also harvested at a site remote from the burn site. Standard light microscopy was performed. Other sections were prepared as follows for immunohistochemistry. Sections were deparaffinized, rehydrated, and incubated in 3% hydrogen peroxide in methanol for 30 min followed by 30 min incubation in Tris-buffered goat serum. The primary antibody was rat anti-mouse MAb 281-2 incubated overnight at 4°C. Appropriate controls using nonspecific rat antibody and omission of the primary antibody were performed. Avidin-biotin peroxidase complex staining was then performed and the immobilized peroxidase visualized by further incubation and counterstaining.

Paraffin sections were sent to the University of Turku for in situ hybridization of mRNA using a 535-bp SacI-KpnI fragment from the partial cDNA clone for mouse syndecan. Quantification of syndecan in the wounds will be done using a qualitative scale of 0 (no expression) to ++++ (strong expression). Quantification of syndecan mRNA will be done by autoradiography and expressed as grains per cell. The order of antibody addition will be MAb 281-2 and peroxidase.

Determination of Number of Animals Required. This was a pilot study attempting to discern whether the immunohistochemical and in situ hybridization techniques described will produce differences in syndecan expression in areas of full- and partial-thickness injury when compared to normal skin. Four animals at each time point should be sufficient to provide a satisfactory analysis of this question.

Data Analysis Plan. As a pilot histopathologic study, the initial data analysis will be descriptive only.

RESULTS

Animal studies have been completed and samples have been submitted to the University of Turku for in situ hybridization.

DISCUSSION

Upon completion of the immunohistochemical analyses, the data will be analyzed and the results submitted for publication.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

ACCESSION: DA315355

SUMMARY DATE: 300792 **SUMMARY KIND:** K **PREV DATE:** 911001 **DISTRIBUTION:** CX

PROGRAM #: 61102A **PROJ #:** 30161102BS14 **TASK AREA:** CA **WORK UNIT:** 316

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

SUBJ1: 060400 - Anatomy and Physiology
SUBJ2: 060500 - Medicine and Medical Research

START DATE: 8701 **END DATE:** 9207 **PERFORMANCE METHOD:** C

CONTRACT/GRANT NO:

CONT TOTAL: $
CUM TOTAL: $
TOTAL LAB FUNDS: $

RESOURCES ESTIMATE

FY	WORK YRS	$(Thousands)
91	0.5	$39
92	0.1	$21
93	0.0	$0

RESPONSIBLE DOD ORGANIZATION: 404885
MRDC INSTITUTE OF SURGICAL RESEARCH USAISR
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PERFORMING ORGANIZATION: 404885
MRDC INSTITUTE OF SURGICAL RESEARCH
FT SAM HOUSTON, TX 78234
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210-221-8440

ASSOC1:

ASSOC2:

FOREIGN INTELLIGENCE CONSIDERED: Y **CIVILIAN APPLICABILITY:** M

KEYWORDS: RA II; Lab Animals; Sheep; Burns (Injuries); Inhalation; Respirations; Pulmonary Function; Therapy

OBJECTIVE: A DTIC literature search was conducted under DTIC request numbers W6003E/W6006A dated 20 October 1989. The objective of this work is to compare the effects of volumetric diffusive ventilation and conventional ventilation on pulmonary and hemodynamic indices which are altered in an ovine inhalation injury model. Continued work from DA312336.

APPROACH: Inhalation injury will be induced using the standard ovine smoke inhalation model developed at this Institute. Animals will be randomized to treatment with conventional, high-frequency flow interruption, or high-frequency oscillatory ventilation. Changes in V_A/Q as well as other pulmonary and physiologic measurements will be compared between groups.

PROGRESS: 9110-9209. The multiple inert gas elimination technique was revalidated. High-frequency flow interruption was found to be inferior to conventional ventilation when instituted 24 h after smoke inhalation injury in an ovine model. High-frequency ventilation resulted in a further shift of the V_A/Q curve to the left, indicating additional V_A/Q mismatching. Data also indicated that high-frequency oscillatory ventilation employing a percussive ventilator was inadequate for support of sheep with inhalation injury 24 h after injury. For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report* for fiscal years 1987 through 1992.

ABSTRACT

PROJECT NUMBER: 3M161102BS14-316, Research

PROJECT TITLE: The Effect of High-Frequency Ventilation on V_A/Q in Sheep with Inhalation Injury

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

PERIOD COVERED IN THIS REPORT: 1 October 1991 - 30 July 1992

INVESTIGATORS: William G. Cioffi, Jr., MD, Major, MC
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Arthur D. Mason, Jr., MD

Severe inhalation injury has been shown to cause hypoxia, hypercarbia, and a shift of V_A/Q to the left, i.e., increase in segments with $V_A/Q > 0$ but < 1. Attempts to alter these derangements with conventional ventilation using PEEP resulted in an increased dead space ventilation but had no significant effect on shunt or low V_A/Q compartments. This study was designed to investigate the effects of high-frequency percussive ventilation on these changes.

Data indicated that high-frequency oscillatory ventilation employing a percussive ventilator was inadequate for support of sheep with inhalation injury 24 h after injury.

THE EFFECT OF HIGH-FREQUENCY VENTILATION ON V_A/Q IN SHEEP WITH INHALATION INJURY

The effect of inhalation injury on V_A/Q using the multiple inert gas elimination technique (MIGET) and cardiopulmonary parameters has been well described in an ovine model (1). Moderate to severe injury causes hypoxia, hypercarbia, and a shift of V_A/Q to the left, i.e., increase in segments with $V_A/Q > 0$ but < 1. In addition, smoke-exposed animals show increased perfusion to shunt and low V_A/Q segments. Attempts to alter these derangements with conventional ventilation using PEEP resulted in an increased dead space ventilation but had no significant effect on shunt or low V_A/Q compartments (unpublished data).

High-frequency ventilation (HFV) has been proposed as a means of increasing ventilation to low V_A/Q compartments. In a dog model using methacholine hydrochloride to induce low V_A/Q compartments, Kaiser et al (2) was unable to demonstrate a beneficial effect of high-frequency oscillation ventilation. This type of ventilator is relatively inefficient in terms of gas exchange and does not allow adequate ventilation of adult humans. Because of this difficulty and the inability of jet ventilators to adequately clear carbon dioxide, a hybrid type of ventilator has been developed that effects what is termed "volumetric-diffusive ventilation." This type of ventilator superimposes high-frequency subtidal volume breaths on conventional convective breaths. In addition, PEEP is employed in an oscillatory nature. This ventilator is actually a flow interrupter and there is no active expiratory phase as seen in oscillation ventilation. Limited clinical use of this ventilator has demonstrated no adverse effects on cardiac parameters (3). In addition, salvage studies performed on patients with ARDS have suggested that previously unsalvageable patients have had reversal of their pulmonary process. The effect of this type of ventilation on disease processes which result in an increase in the number of low V_A/Q compartments is unknown.

The purpose of this study is to compare volumetric diffusive ventilation with conventional ventilation in effecting changes in the pulmonary and hemodynamic parameters which are altered in an ovine inhalation injury model. If volumetric-diffusive ventilation can favorably affect V_A/Q on inhalation injury, its application to humans with inhalation injury would be advantageous.

MATERIALS AND METHODS

Neutered male sheep weighing 25-45 kg were used. Each sheep was housed in a conventional outdoor run and had access to commercial feed and water ad libitum. Inhalation injury is induced using the standard ovine smoke inhalation model developed at this Institute (1).

Animals are studied 24 h after smoke inhalation. On the day of the study, a peripheral venous catheter, a central venous pressure (CVP) catheter, a balloon-directed thermodilution pulmonary artery catheter (7F, American Edwards Company, Irvine, CA), a lung water catheter (American Edwards Company), a femoral artery catheter, and an esophageal balloon were inserted after induction of general anesthesia with alpha-chloralose (0.05 g/kg) and intubation. Animals were paralyzed with pancuronium bromide (0.03-0.04 mg/kg, Pavulon®, Organon Pharmaceuticals, West Orange, NJ). After placement of all catheters, animals were placed in the prone position and conventional mechanical ventilation was continued with a volume-limited ventilator (Bear II™, Bear Medical Systems, Inc., Riverside, CA). Ventilator settings were altered to maintain a pH between 7.35 and 7.40 and a PO_2 between 80 and 100 mmHg. Lactated Ringer's was constantly infused at a rate of 1 ml/kg/h. CVP and pulmonary artery pressure (PAP) were monitored with Statham P23Db transducers (Statham Instruments, Oxnard, CA) and systemic artery pressures with a Hewlett-Packard 1290A quartz transducer (Hewlett-Packard Company, Waltham, MA). Transpulmonary pressure was monitored by a differential transducer (MP-451, Valadine Engineering Corporation, Northridge, CA). Inspiratory and expiratory gas concentration (N, O_2, and CO_2) were monitored by a medical gas analyzer (MGA-1100, Perkin Elmer). Percutaneous O_2 saturation and PO_2 were continuously monitored.

Heart rate, blood pressure, CVP, PAP, cardiac output, arterial blood gases, tidal volume, flow rates, transpulmonary pressures, and O_2 saturation were measured every 30 min. Once the ventilator settings were maximized yielding a PO_2 between 80 and 100 mmHg and a pH between 7.35 and 7.40, the animal was allowed to stabilize for 2 h. V_A/Q distributions were then measured using the MIGET. After stabilization, the lactated Ringer's infusion was replaced with a lactated Ringer's solution containing 6 inert gases (sulphur hexafluoride, krypton, cyclopropane, halothane, ether, and acetone) which were infused at a rate of 0.1 ml/kg/min. After 30 min, arterial and mixed venous blood were drawn anaerobically into preweighed heparinized syringes (30 ml, matched, glass) simultaneously. Mixed-expired gas was obtained from a temperature-controlled copper coil (OD = 3.49 cm, L = 640 cm) 1 min after obtaining the blood samples. Blood and expired gas samples were analyzed immediately by GC-MS (Model 5985, Hewlett-Packard). Repeat cardiopulmonary parameters were measured at this time. MIGET data was stored and quantified by a software program on the Hewlett-Packard 1000 computer system.

The animals were then disconnected from the conventional ventilator and switched to a high-frequency oscillatory ventilation. Cardiopulmonary parameters were then measured every 30 min after stabilization on the ventilator. The lactated Ringer's infusion containing the inert gases was discontinued and lactated Ringer's (1 cc/kg/h) was infused. After a 2-h

stabilization period, V_A/Q distribution was again measured using the MIGET. The animals were then sacrificed.

Necropsies were performed to document the extent of inhalation injury. A complete set of tissues was fixed in 10% neutral buffered formalin and processed by standard methods. The locations of tissue sample collection sites were midtrachea, tracheal bifurcation, right and left proximal and distal bronchi, apical and diaphragmatic lobes, and any other morphologically significant foci.

Data following the stabilization period were compared using the student's t test.

RESULTS

Data indicated that high-frequency oscillatory ventilation employing a percussive ventilator was inadequate for support of sheep with inhalation injury 24 h after injury.

DISCUSSION

Benchwork using the high-frequency oscillatory ventilator in an attempt to increase volume output will be necessary.

PRESENTATIONS/PUBLICATIONS

None.

REFERENCES

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

ACCESSION: DA315356

SUMMARY DATE: 921001 **SUMMARY KIND:** D **PREV DATE:** 911001 **DISTRIBUTION:** CX

PROGRAM #: 61102A **PROJ #:** 30161102BS14 **TASK AREA:** EE **WORK UNIT:** 317

TITLE: Effects of Replacement Therapy on Hemodynamic Parameters in an Ovine Model of Controlled Pure Plasma Loss

SUBJ1: 060400 - Anatomy and Physiology
SUBJ2: 060500 - Medicine and Medical Research

START DATE: 8701 **END DATE:** 9309 **PERFORMANCE METHOD:** C

CONTRACT/GRANT NO:

CONT TOTAL: $
CUM TOTAL: $
TOTAL LAB FUNDS: $

RESOURCES ESTIMATE

FY	WORK YRS	$(Thousands)
91	0.5	$30
92	0.5	$31
93	0.5	$35

RESPONSIBLE DOD ORGANIZATION: 404885
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PERFORMING ORGANIZATION: 404885
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210-221-3742

ASSOC1: MASON, A D

ASSOC2:

FOREIGN INTELLIGENCE CONSIDERED: Y **CIVILIAN APPLICABILITY:** M

KEYWORDS: RA II; Lab Animals; Sheep; Burns (Injuries); Albumins; Shock (Pathology); Resuscitation

OBJECTIVE: A DTIC literature search was conducted under DTIC request numbers W6O21F/W6O23D dated 20 October 1989. The objective of this work is to determine the hemodynamic consequences of controlled pure plasma loss in sheep using a method to simulate the acute burn.

APPROACH: Previously, a plasmapheresis filter was used to produce intravascular plasma loss similar to that caused by burn injury. This device selectively removes plasma while leaving the formed elements of blood in the vascular system. Future studies will be conducted in the ovine model to relate changes seen with plasmapheresis to those manifested by burn injury.

PROGRESS: 9110-9209. In a large animal, it is difficult to induce a significantly large burn that is practical, reproducible, and consistently of the magnitude desired to produce significant hemodynamic effects. Methodology to control both extent as well as depth of burn in sheep is currently under separate study. Once the model is established, our proposed study will begin. For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report* for fiscal years 1987 through 1992.

ABSTRACT

PROJECT NUMBER: 3M161102BS14-317, Research

PROJECT TITLE: Effects of Replacement Therapy on Hemodynamic Parameters in an Ovine Model of Controlled Pure Plasma Loss

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

PERIOD COVERED IN THIS REPORT: 1 October 1991 - 30 September 1992

INVESTIGATORS: Khan Z. Shirani, MD, Colonel, MC
Carlin V. Okerberg, DVM, PhD, Lieutenant Colonel, VC
Arthur D. Mason, Jr., MD
Basil A. Pruitt, Jr., MD, Colonel, MC

Analyses of the data by the previous investigator indicated that pure plasma volume loss can be replaced with either plasma or crystalloid solutions. The volume of crystalloid fluid required to achieve replacement was greater than the volume of colloid. These changes will now be validated in a 50% burn model.

Future studies will be conducted in the ovine model to relate changes seen with plasmapheresis to those manifested by burn injury. In a large animal, it is difficult to induce a significantly large burn that is practical, reproducible, and consistently of the magnitude desired to produce significant hemodynamic effects. Methodology to control both extent as well as depth of burn in sheep is currently under separate study. Once the model is established, our proposed study will begin.

EFFECTS OF REPLACEMENT THERAPY ON HEMODYNAMIC PARAMETERS IN AN OVINE MODEL OF CONTROLLED PURE PLASMA LOSS

Many models have been employed to explore the physiologic and pathophysiologic sequelae of shock, but most have dealt with loss of formed blood elements along with plasma loss. The question which remains to be answered is to what extent the hemodynamic response in the shock model relates to the loss of plasma per se without RBC loss. A controlled plasma loss designed to simulate the rate of intravascular plasma loss in the acute burn period has been developed by the interposition of a plasmapheresis filter between the arterial and venous circulation of experimental animals. This design will allow the simulation of plasma loss of the acute burn which accounts for hemodynamic instability (1-2). In previous work (unpublished data), this model has shown efficacy as a pure plasma loss shock model, albeit an accelerated representation of the burn state. With the control of plasma flux to more closely represent burn shock in a temporal sense, hemodynamic changes can be better defined. Subsequent fluid replacement therapy can then be effected to form the scientific basis for postburn resuscitation in humans.

MATERIALS AND METHODS

The effects of intravascular loss of plasma on cardiovascular performance will be investigated in 20 one- to two-year-old, random source, nonpregnant female sheep weighing 24-40 kg. During the first stage of the study, the animals are prepared under general anesthesia by cannulation of the right femoral artery for blood sampling, the right jugular vein for hemodynamic monitoring, and the left jugular vein and left carotid artery for ultrafiltration. Aortic, central venous, pulmonary artery, left atrial, and pulmonary capillary wedge pressures are recorded (Model 7754A, Hewlett-Packard, Waltham MA) using calibrated pressure transducers (Model 1290A, Hewlett-Packard). Arterial blood gas and cardiac output by the thermodilution method (Model 9520, American Edwards Laboratory) are also determined. A Foley catheter is introduced for urine output monitoring. The animals are placed in metabolic cages for 2 days and fed ad libitum while recovering from the initial procedure. During the second stage of the study, the animals are heparinized and plasmapheresis is initiated using an Asahi™ plasma separator (Parker Hannifin Corporation, Irvine, CA) after baseline measurements of cardiovascular and respiratory indexes and sampling of blood for electrolyte, blood gas, and coagulation determinations. This system has a cellulose acetate hollow fiber core which allows for passage of plasma, but not cellular elements. The unanesthetized animals are subjected to a selective plasma extraction (fig 1) at a plasma flux designed to simulate the rate of loss in the acute burn period as described by Pruitt et al (3).

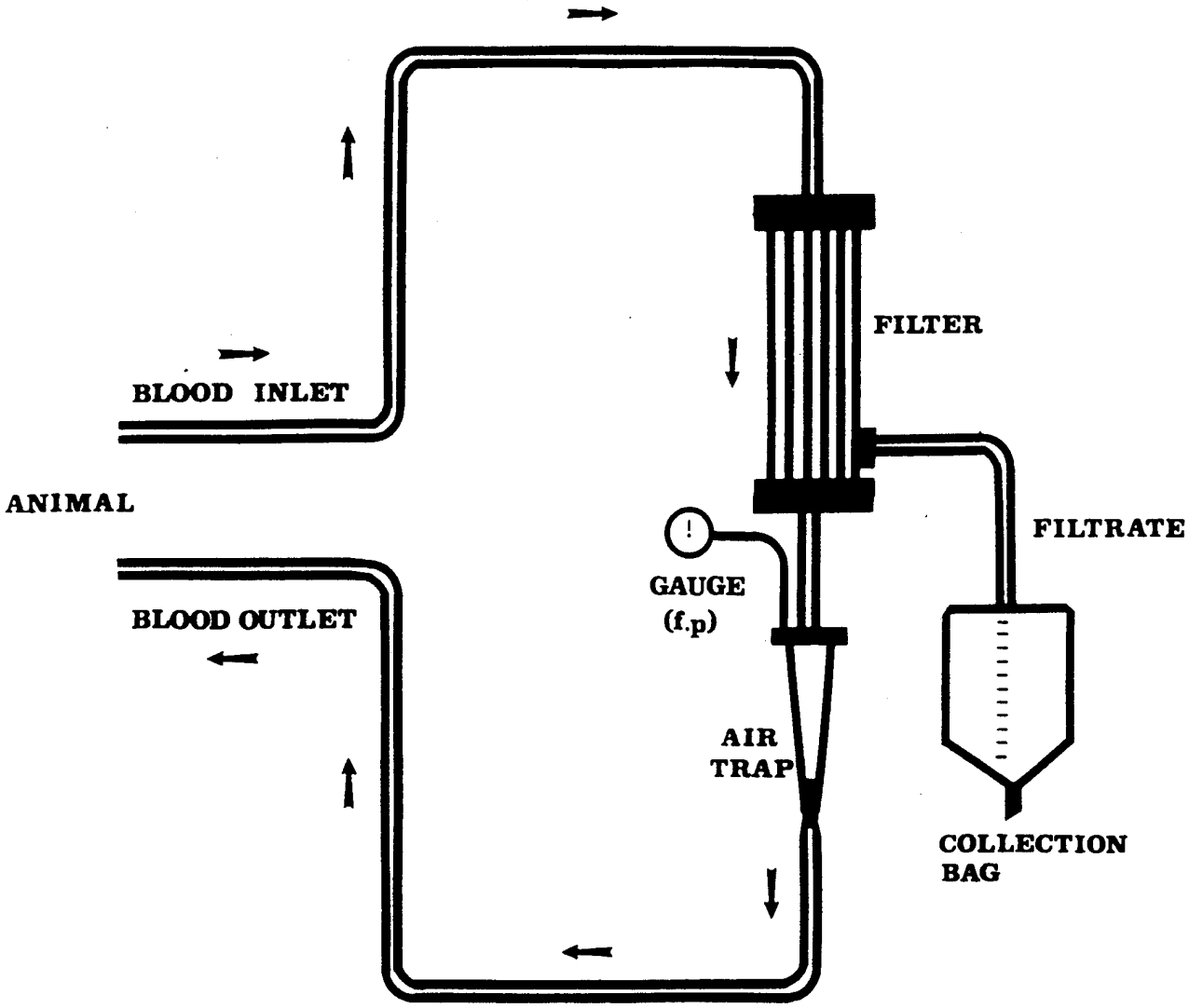


FIGURE 1. Graphic representation of the filtration circuit.

RESULTS

Eight animals have been studied to date and the model has been established in a reliable fashion, with the need for prior splenectomy recognized. Animals subjected to pure plasma loss have been resuscitated with several resuscitation schema, including crystalloid and colloid fluids.

DISCUSSION

Data from 8 animals have been analyzed. A 50% total body surface area burn has been chosen as the initial model for study. Plasma loss will be adjusted on an hourly basis to meet these losses. Ongoing measurements of hemodynamic parameters will be

conducted, to include systolic blood pressure, left atrial pressure, pulmonary capillary wedge pressure, cardiac output, hematocrit, serum chemistries (electrolytes, blood urea nitrogen, creatinine, glucose), serum osmolality, and urine output. After 2 h of plasma loss, fluid resuscitation will begin utilizing several of the most popular burn resuscitation formulae. One group will be resuscitated via the modified Brooke formula, another using the Parkland formula, and another using hypertonic saline. Initial plasma volume will be measured utilizing Evans' blue prior to institution of plasma loss. All animals will be fully heparinized prior to institution of plasmapheresis. An addendum is being written which will validate the model by comparing the hemodynamic changes seen in a 50% burn model with those seen in the pure plasma volume loss model.

Future studies will be conducted in the ovine model to relate changes seen with plasmapheresis to those manifested by burn injury. In a large animal, it is difficult to induce a significantly large burn that is practical, reproducible, and consistently of the magnitude desired to produce significant hemodynamic effects. Methodology to control both extent as well as depth of burn in sheep is currently under separate study. Once the model is established, our proposed study will begin.

PRESENTATIONS/PUBLICATIONS

None.

REFERENCES

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

ACCESSION: DA315357

SUMMARY DATE: 921001 **SUMMARY KIND:** D **PREV DATE:** 911001 **DISTRIBUTION:** CX

PROGRAM #: 61102A **PROJ #:** 30161102BS14 **TASK AREA:** ED **WORK UNIT:** 318

TITLE: Antibacterial and Wound Healing Effects of Silver-Nylon Electrodes with Weak Direct Current

SUBJ1: 060100 - Biochemistry
SUBJ2: 060500 - Medicine and Medical Research

START DATE: 8808 **END DATE:** 9309 **PERFORMANCE METHOD:** C

CONTRACT/GRANT NO:

CONT TOTAL: $
CUM TOTAL: $
TOTAL LAB FUNDS: $

RESOURCES ESTIMATE

FY	WORK YRS	$(Thousands)
91	1.6	$134
92	1.6	$72
93	1.6	$96

RESPONSIBLE DOD ORGANIZATION: 404885
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PERFORMING ORGANIZATION: 404885
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ASSOC1: MC MANUS, A T

ASSOC2: OKERBERG, C V

FOREIGN INTELLIGENCE CONSIDERED: Y **CIVILIAN APPLICABILITY:** M

KEYWORDS: RA II; Lab Animals; Guinea Pigs; Rats; Burns (Injuries); Wounds and Injuries; Healing; Skin Grafts; Direct Current; Therapy

OBJECTIVE: A DTIC literature search was conducted under DTIC request numbers W6035C/W6036B dated 20 October 1989. The objectives of this work are to determine the antimicrobial and wound healing effects of weak direct current and to measure silver concentrations of burn eschars and underlying tissue as a function of time and direct current. Development of an effective hospital and field burn dressing will reduce infection and mortality of patients with thermal injury.

APPROACH: Hartley guinea pigs and Sprague-Dawley rats will be used to study healing in deep 2° burns, 3° burns, donor site wounds, and skin grafts with and without stimulation with weak direct current.

PROGRESS: 9110-9209. Direct current treatment using silver-nylon dressings has been found to enhance the speed and quality of healing of partial-thickness scald burns in guinea pigs and rats. Previously noted reductions in postinjury wound edema have been separated by estimates of protein (Evans blue tag) and water components. Distinct protein and water kinetics are being mapped. At present, the reductions in wound edema appear to be related to increased edema reabsorption rather than prevention of the capillary leak. For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report* for fiscal years 1988 through 1992.

ABSTRACT

PROJECT NUMBER: 3M161102BS14-318, Research

PROJECT TITLE: Antibacterial and Wound Healing Effects of Silver-Nylon Electrodes with Weak Direct Current

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

PERIOD COVERED IN THIS REPORT: 1 October 1991 - 30 September 1992

INVESTIGATORS: Chi-Sing Chu, MD
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Arthur D. Mason, Jr., MD
Carlin V. Okerberg, DVM, PhD, Lieutenant Colonel, VC
Basil A. Pruitt, Jr., MD, Colonel, MC

Direct current treatment using silver-nylon dressings has been found to enhance the speed and quality of healing of partial-thickness scald burns in guinea pigs and rats. Previously noted reductions in postinjury wound edema have been separated by estimates of protein (Evans blue tag) and water components. Distinct protein and water kinetics are being mapped. At present, the reductions in wound edema appear to be related to increased edema reabsorption rather than prevention of the capillary leak.

ANTIBACTERIAL AND WOUND HEALING EFFECTS OF SILVER-NYLON ELECTRODES WITH WEAK DIRECT CURRENT

We have previously shown that silver-nylon dressings (SN) are effective barriers to wound contamination and when used with applied direct current (DC) are also effective in treating invaded burn wounds (1,2). As a consequence of these studies, it was observed that animals that had SN and DC also appeared to have a markedly improved time to wound closure and significantly improved wound contracture when compared to control burned animals. This finding has been verified in grafts, burns, and donor site wounds (3). During the previous reporting period, we reported that SN and DC significantly reduce wound edema when applied within 8 h postinjury for both partial- and full-thickness wounds (4).

In this report, we document the effects of SN with DC on wound healing. We have examined the microcirculation of partial-thickness burns by measurement of wound distribution of intravenously injected India ink (Pelikan™) in partial-thickness scald burns (78°C for 10 sec) of rats and guinea pigs with and without SN and DC. Data are presented in Figures 1 and 2 as the ratio of the percentage of thickness of ischemic wound tissue to the total dermal thickness. The results clearly show that if DC is applied soon after burn injury, the wounds maintain significantly greater perfusion. The effect was most noticeable after 8 h of DC treatment, at which time control animals develop a secondary impairment of microcirculation (zone-of-stasis).

The mechanisms for these improvements in wound perfusion will be the focus during the next reporting period.

PRESENTATIONS/PUBLICATIONS

None.

REFERENCES

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3. Chu C-S, McManus AT, Okerberg CV, et al: Weak direct current accelerates split-thickness graft healing on tangentially

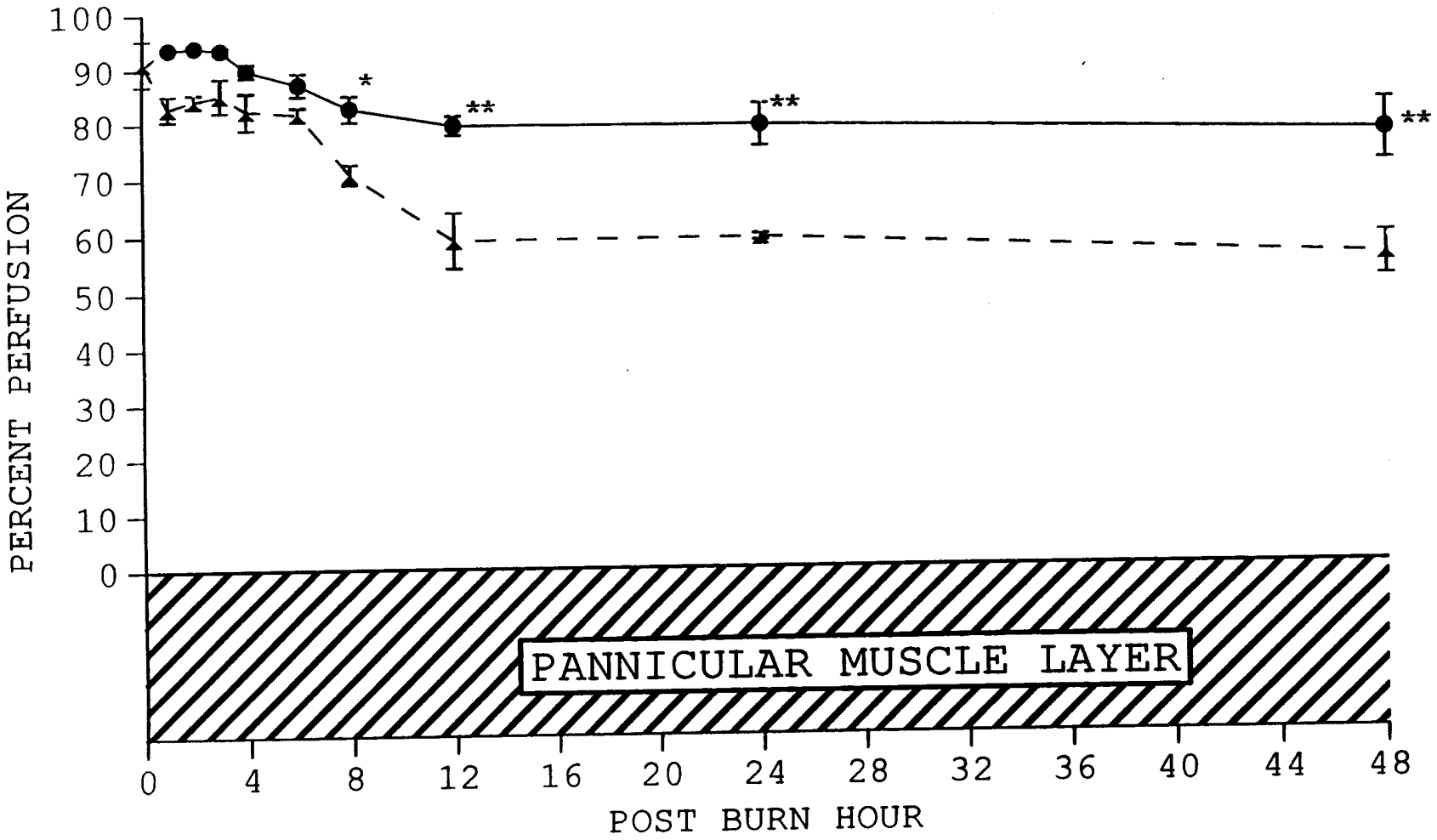


FIGURE 1. Ratio of perfused dermis to total dermis thickness in rats with partial-thickness scald burn injury treated with (•) and without (▲) direct current. *P < 0.05, **P < 0.01.

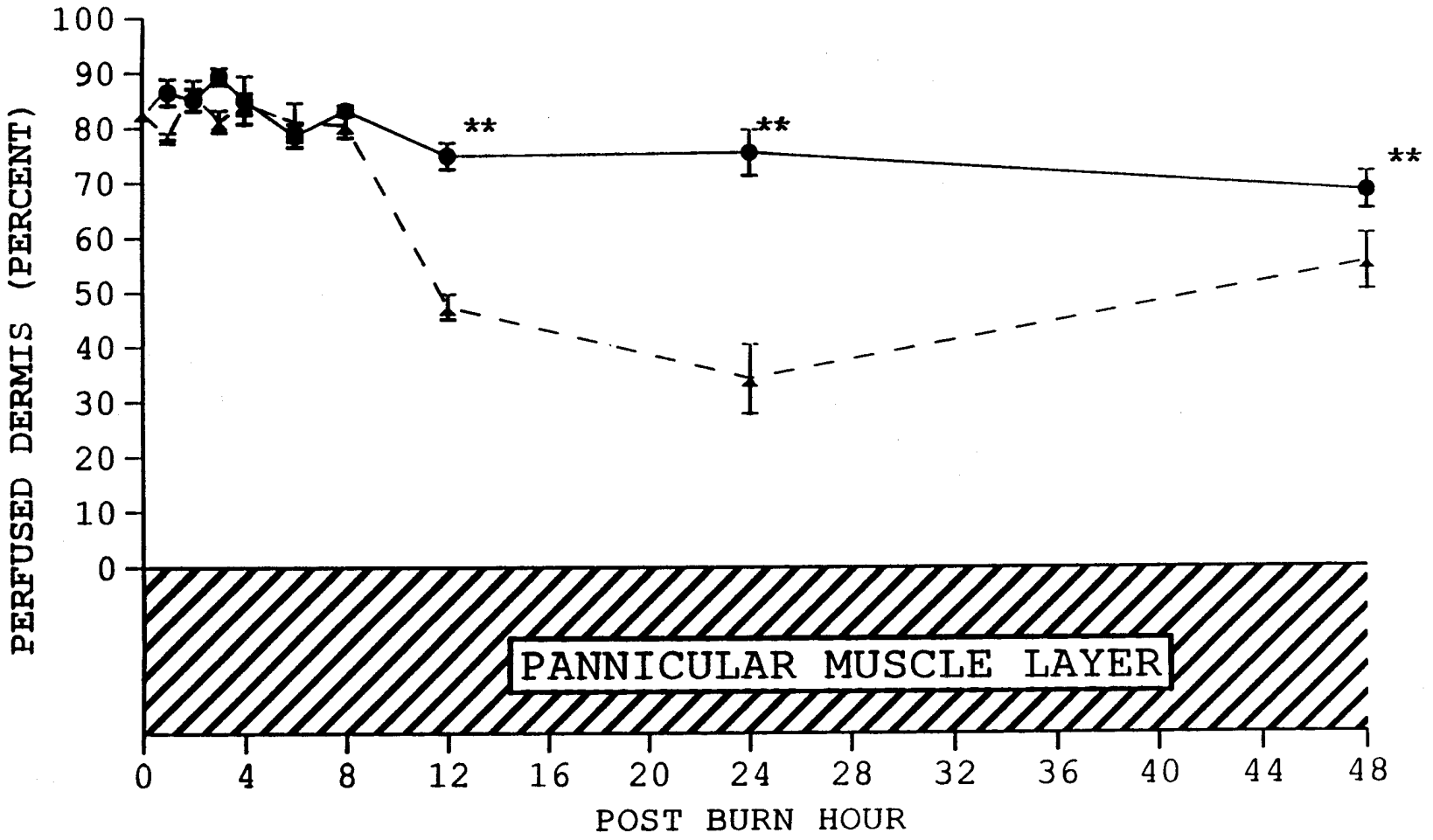


FIGURE 2. Ratio of perfused dermis to total dermis thickness in guinea pigs with partial-thickness scald burn injury with (•) and without (▲) direct current. **P < 0.01.

excised second-degree burns. *J Burn Care Rehabil* 12:285–93, 1991.

4. Chu C-S, McManus AT, Mason AD Jr, et al: Antibacterial and wound healing effects of weak direct current. In US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1991. San Antonio: US Government Printing Office, 1992, pp 521–8.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

ACCESSION: DA336062

SUMMARY DATE: 920930 **SUMMARY KIND:** T **PREV DATE:** 920316 **DISTRIBUTION:** CX

PROGRAM #: 61102A **PROJ #:** 30161102BS14 **TASK AREA:** EF **WORK UNIT:** 319

TITLE: Nitrate Synthesis in Thermally Injured Patients

SUBJ1: 060100 - Biochemistry
SUBJ2: 060500 - Medicine and Medical Research

START DATE: 9203 **END DATE:** 9209 **PERFORMANCE METHOD:** C

CONTRACT/GRANT NO:

CONT TOTAL:	$
CUM TOTAL:	$
TOTAL LAB FUNDS:	$

RESOURCES ESTIMATE

FY	WORK YRS	$(Thousands)
91	0.0	$0
92	0.4	$20
93	0.0	$0

RESPONSIBLE DOD ORGANIZATION: 404885
MRDC INSTITUTE OF SURGICAL RESEARCH USAISR
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ASSOC1: CIOFFI, W G

ASSOC2: ANDRON, L A

FOREIGN INTELLIGENCE CONSIDERED: Y **CIVILIAN APPLICABILITY:** M

KEYWORDS: RA II; Volunteers; Adults; Burns (Injuries); Vasodilation; Metabolism; Urine

OBJECTIVE: A DTIC literature search was conducted under DTIC request numbers W6K35C/W6K36D dated 4 March 1992. The objective of this work is to measure urinary nitrate levels in thermally injured patients. Knowledge of the mechanisms of the metabolism and hemodynamic response to thermal injury may result in new treatment strategies that will improve management of thermally injured soldiers.

APPROACH: Nitrate levels will be performed on 200 24-h urine collections which have been frozen and banked as donated excess clinical specimens from patients with thermal injury admitted to the Institute. Urinary nitrate will be analyzed by reaction with the Griess reagent, following cadmium reduction, with spectrophotometric absorbance at 545 nm or by gas chromatography-nitrogen phosphorous detection of the nitromesitylene derivative of nitrate. Additional data will be collected from each patient's chart, to include age, sex, burn size, and presence/absence of inhalation injury as well as the results of any 24-h nitrogen balance, UUN excretion, and total urinary nitrogen excretion tests. Multiple regression will be used to detect any relationship between the various independent variables from the collected data and dependent variables which are measured.

PROGRESS: 9203-9209. This project was approved by the USAISR Research Council and US Army Institute of Surgical Research Human Use Committee during the second quarter of fiscal year 1992. Laboratory analytical problems could not be resolved. Therefore, this study was terminated at the request of the primary

investigator. For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1992*.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

ACCESSION: DA336062

SUMMARY DATE: 920316 **SUMMARY KIND:** A **PREV DATE:** **DISTRIBUTION:** CX

PROGRAM #: 61102A **PROJ #:** 30161102BS14 **TASK AREA:** DA **WORK UNIT:** 319

TITLE: Nitrate Synthesis in Thermally Injured Patients

SUBJ1: 060100 - Biochemistry
SUBJ2: 060500 - Medicine and Medical Research

START DATE: 9203 **END DATE:** 9309 **PERFORMANCE METHOD:** C

CONTRACT/GRANT NO:

CONT TOTAL: $
CUM TOTAL: $
TOTAL LAB FUNDS: $

RESOURCES ESTIMATE

FY	WORK YRS	$(Thousands)
91	0.0	$0
92	0.5	$20
93	0.5	$22

RESPONSIBLE DOD ORGANIZATION: 404885
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ASSOC1: CIOFFI, W G

ASSOC2: ANDRON, L A

FOREIGN INTELLIGENCE CONSIDERED: Y **CIVILIAN APPLICABILITY:** M

KEYWORDS: RA II; Volunteers; Adults; Burns (Injuries); Vasodilation; Metabolism; Urine

OBJECTIVE: A DTIC literature search was conducted under DTIC request numbers W6K35C/W6K36D dated 4 March 1992. The objective of this work is to measure urinary nitrate levels in thermally injured patients. Knowledge of the mechanisms of the metabolism and hemodynamic response to thermal injury may result in new treatment strategies that will improve management of thermally injured soldiers.

APPROACH: Nitrate levels will be performed on 200 24-h urine collections which have been frozen and banked as donated excess clinical specimens from patients with thermal injury admitted to the Institute. Urinary nitrate will be analyzed by reaction with the Griess reagent, following cadmium reduction, with spectrophotometric absorbance at 545 nm or by gas chromatography-nitrogen phosphorous detection of the nitromesitylene derivative of nitrate. Additional data will be collected from each patient's chart, to include age, sex, burn size, and presence/absence of inhalation injury as well as the results of any 24-h nitrogen balance, UUN excretion, and total urinary nitrogen excretion tests. Multiple regression will be used to detect any relationship between the various independent variables from the collected data and dependent variables which are measured.

PROGRESS: 9203-9209. This project was approved by the USAISR Research Council and US Army Institute of Surgical Research Human Use Committee during the second quarter of fiscal year 1992.

ABSTRACT

PROJECT NUMBER: 3M161102BS14-315, Research

PROJECT TITLE: Nitrate Synthesis in Thermally Injured Patients

INSTITUTION: US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas 78234-5012[1]

PERIOD COVERED IN THIS REPORT: 16 March 1992 - 30 September 1992

INVESTIGATORS: William K. Becker, MD, Lieutenant Colonel, MC[1]
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Leo A. Andron, PhD, Lieutenant Colonel, MS
William F. McManus, MD, Colonel, MC
Avery A. Johnson, BS

Nitric oxide is synthesized from the amino acid L-arginine and functions as a neurotransmitter, vasodilator, and as a possible immunomodulator. The status of nitric oxide synthesis and the excretion of the stable metabolite, nitrate, in thermally injured patients is unknown. Because nitric oxide appears to function in a number of systems which may be clinically relevant in the management of the thermally injured patient, it is desirable to obtain information regarding the rate of synthesis of nitric oxide and nitrate following burn injury. Therefore, the objective of this study is to measure urinary nitrate levels in thermally injured patients.

NITRATE SYNTHESIS IN THERMALLY INJURED PATIENTS

Nitric oxide is synthesized from the guanido-nitrogen of the amino acid, arginine, by both constitutive and inducible enzymes located in the CNS, vascular endothelium, and other tissues (1,2). Physiologic roles for nitric oxide have been established as a neurotransmitter, vasodilator, and possible immunomodulator (3-11). Nitric oxide has a brief half-life and appears to be rapidly oxidized to the stable metabolite, nitrate, which is subsequently excreted in the urine (12). Elevated levels of nitrate have been identified in experimental animals following bacterial challenge, endotoxin, and the administration of the cytokines IL-1 and TNF. The production of nitric oxide in these situations can be blocked, in part, by the administration of specific enzyme inhibitors. Preliminary data from animal studies suggest that nitric oxide production and the subsequent excretion of nitrate are increased following thermal injury. There are no data on the time course of nitric oxide production and nitrate excretion following thermal injury in patients.

The objective of this study is to measure urinary nitrate levels in thermally injured patients.

MATERIALS AND METHODS

Study Design. Nitrate levels will be performed on 24-h urine collections which have been frozen and banked as donated excess clinical specimens from patients with thermal injury admitted to the Institute. Additional data will be collected from each patient's chart. If a burn size-related increase in nitrate excretion is noted, additional studies utilizing stable isotopes will be proposed to evaluate kinetics of nitric oxide production and nitrate excretion.

Description of Procedures. Nitrate levels will be performed on 200 24-h urine samples which have been frozen and banked as donated excess clinical specimens from patients with thermal injury admitted to the Institute. Urinary nitrate will be analyzed by reaction with the Griess reagent, following cadmium reduction, with spectrophotometric absorbance at 545 nm or by gas chromatography-nitrogen phosphorous detection of the nitromesitylene derivative of nitrate (13,14). Urinary nitrate will be expressed as μM of nitrate excreted per day. Additional data will be collected from the patient's chart, to include age, sex, burn size, and presence or absence of inhalation injury as well as the results of any 24-h nitrogen balance, UUN excretion, and total urinary nitrogen excretion tests. For the patient's privacy, the name will not be associated with the extra test and all data will be coded.

Patient Inclusion. Patients meeting the following criteria may be enrolled in the study upon giving written informed consent:

1. Male or female patients ≥ 18 yr.

2. Patients admitted to the US Army Institute of Surgical Research with thermal injury.

3. Patients who have given written informed consent for the donation of excess clinical specimens.

Patient Exclusion. Patients meeting the following criteria will be excluded from participation in the study:

1. Patients < 18 yr.

2. Patients admitted to the US Army Institute of Surgical Research for conditions other than thermal injury.

3. Patients who did not give written informed consent for the donation of excess clinical specimens.

Determination of Number of Subjects Required. Nitrate levels will be analyzed from 200 24-h urine collections. No baseline data on nitrate excretion is available for thermally injured patients. The number of samples analyzed should provide a reasonable estimation of nitrate excretion in thermally injured patients.

Data Collection. Data collection will include the patient's age, sex, burn size, postburn day, episodes of infection/sepsis, associated injuries, and presence/absence of inhalation injury as well as the results of the urinary nitrate test and any 24-h nitrogen balance, UUN excretion, and total urinary nitrogen excretion tests. For the patient's privacy, the name will not be associated with the extra test and all data will be coded.

Data Analysis Plan. Multiple regression will be used to detect any relationship between the various independent variables from the collected data and dependent variables which are measured.

REFERENCES

1. Granger DL, Hibbs JB Jr, Broadnax LM: Urinary nitrate excretion in relation to murine macrophage activation: influence of dietary L-arginine and oral NG-monomethyl-L-arginine. *J Immunol* 146:1294-302, 1991.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

ACCESSION: DA336064

SUMMARY DATE: 920930 **SUMMARY KIND:** K **PREV DATE:** 920408 **DISTRIBUTION:** CX

PROGRAM #: 61102A **PROJ #:** 30161102BS14 **TASK AREA:** BN **WORK UNIT:** 320

TITLE: Control of Urea Synthesis Following Thermal Injury and Burn Wound Infection in a Rat Model

SUBJ1: 060100 - Biochemistry
SUBJ2: 060500 - Medicine and Medical Research

START DATE: 9204 **END DATE:** 9209 **PERFORMANCE METHOD:** C

CONTRACT/GRANT NO:

CONT TOTAL: $
CUM TOTAL: $
TOTAL LAB FUNDS: $

RESOURCES ESTIMATE

FY	WORK YRS	$(Thousands)
91	0.0	$0
92	0.3	$15
93	0.0	$0

RESPONSIBLE DOD ORGANIZATION: 404885
MRDC INSTITUTE OF SURGICAL RESEARCH USAISR
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PERFORMING ORGANIZATION: 404885
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ASSOC1: ANDRON, L A

ASSOC2: MCMANUS, A T

FOREIGN INTELLIGENCE CONSIDERED: Y **CIVILIAN APPLICABILITY:** M

KEYWORDS: RA II; Lab Animals: Rats; Burns (Injuries); Urea; Nitrogen; Metabolism; Catabolism

OBJECTIVE: A DTIC literature search was conducted under DTIC request numbers W6K39D/W6K40B dated 4 March 1992. The objectives of this work are to determine the factors that control urea synthesis and identify and quantify the changes that occur in urea synthesis and excretion after thermal injury and burn wound infection. This study will provide additional information regarding the specific mechanisms of control of urea synthesis after injury and may provide a better understanding of the mechanisms of nitrogen loss after injury and give baseline data by which interventional therapies can be judged.

APPROACH: Sprague-Dawley rats were acclimated to the facility and to a defined amino acid diet for four days before use. Urine output, water intake, and food intake were recorded for the 24-h period before use. Animals were weighed and then randomized to one of three groups, i.e, burn, burn-infection, or sham burn. After burn injury, animals were returned to their cages and allowed to awaken. Urine output, water intake, food intake, and weight were recorded daily. On postburn days 2 and 6, animals from each group were anesthetized and exsanguinated. Samples of plasma and liver and renal tissues were obtained from each animal for biochemical and tissue analyses. For the comparison of multiple means, ANOVA was used. If a significant F-statistic for the group as a whole was generated, further post-hoc intergroup comparisons were performed. Linear regression was performed between levels of urea-cycle enzymes and substrates and n-acetyl-glutamate levels.

PROGRESS: 9204-9209. This study was approved by the USAISR Research Council and US Army Institute of Surgical Research Animal Care and Use Committee during the second and third quarters of fiscal year 1992. Burn injury increased urea output. No change in the activity of urea-cycle enzymes was noted. However, an increase in hepatic n-acetyl-glutamate was observed. For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1992.*

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

ACCESSION: DA336064

SUMMARY DATE: 920408 **SUMMARY KIND:** A **PREV DATE:** **DISTRIBUTION:** CX

PROGRAM #: 61102A **PROJ #:** 30161102BS14 **TASK AREA:** CK **WORK UNIT:** 320

TITLE: Control of Urea Synthesis Following Thermal Injury and Burn Wound Infection in a Rat Model

SUBJ1: 060100 - Biochemistry
SUBJ2: 060500 - Medicine and Medical Research

START DATE: 9204 **END DATE:** 9309 **PERFORMANCE METHOD:** C

CONTRACT/GRANT NO:

CONT TOTAL: $
CUM TOTAL: $
TOTAL LAB FUNDS: $

RESOURCES ESTIMATE

FY	WORK YRS	$(Thousands)
91	0.0	$0
92	0.3	$15
93	0.3	$5

RESPONSIBLE DOD ORGANIZATION: 404885
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ASSOC1: ANDRON, L A

ASSOC2: MCMANUS, A T

FOREIGN INTELLIGENCE CONSIDERED: Y **CIVILIAN APPLICABILITY:** M

KEYWORDS: RA II; Lab Animals: Rats; Burns (Injuries); Urea; Nitrogen; Metabolism; Catabolism

OBJECTIVE: A DTIC literature search was conducted under DTIC request numbers W6K39D/W6K40B dated 4 March 1992. The objectives of this work are to determine the factors that control urea synthesis and identify and quantify the changes that occur in urea synthesis and excretion after thermal injury and burn wound infection. This study will provide additional information regarding the specific mechanisms of control of urea synthesis after injury and may provide a better understanding of the mechanisms of nitrogen loss after injury and give baseline data by which interventional therapies can be judged.

APPROACH: Sprague-Dawley rats will be acclimated to the facility and to a defined amino acid diet for four days before use. Urine output, water intake, and food intake will be recorded for the 24-h period before use. Animals will be weighed and then randomized to one of three groups, i.e, burn, burn-infection, or sham burn. After burn injury, animals will be returned to their cages and allowed to awaken. Urine output, water intake, food intake, and weight will be recorded daily. On postburn days 2 and 6, animals from each group will be anesthetized and exsanguinated. Samples of plasma and liver and renal tissues will be obtained from each animal for biochemical and tissue analyses. For the comparison of multiple means, ANOVA will be used. If a significant F-statistic for the group as a whole is generated, further post-hoc intergroup comparisons will be performed. Linear regression will be performed between levels of urea-cycle enzymes and substrates and n-acetyl-glutamate levels.

PROGRESS: 9204-9209. This study was approved by the USAISR Research Council and US Army Institute of Surgical Research Animal Care and Use Committee during the second and third quarters of fiscal year 1992.

ABSTRACT

PROJECT NUMBER: 3M161102BS14-320, Research

PROJECT TITLE: Control of Urea Synthesis Following Thermal Injury and Burn Wound Infection in a Rat Model

INSTITUTION: US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas 78234-5012[1]

PERIOD COVERED IN THIS REPORT: 8 April 1992 - 30 September 1992

INVESTIGATORS: William K. Becker, MD, Lieutenant Colonel, MC[1]
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Carlin V. Okerberg, DVM, PhD, Lieutenant Colonel, VC
Avery A. Johnson, BS

Factors that control urea synthesis are poorly understood in normal subjects and are unknown following trauma. Injuries such as burns are associated with a marked increase in nitrogen excretion as urea. A number of therapeutic modalities, such as the administration of growth hormone and other growth factors, are being evaluated in an attempt to change the anabolic/catabolic state of the patient. Such interventions could potentially alter urea-cycle function, and if effective, should decrease the amount of urea synthesized and excreted. The objective of this study is to determine the factors that control urea synthesis and to identify and quantify the changes that occur in urea synthesis and excretion following thermal injury and burn wound infection.

CONTROL OF UREA SYNTHESIS FOLLOWING THERMAL INJURY AND BURN WOUND INFECTION IN A RAT MODEL

Urea is the major end product of nitrogen metabolism in mammals. Major thermal injury is associated with an increase in urea excretion, accompanied by an increase in release of amino acids from skeletal muscle, erosion of lean body mass, and negative nitrogen balance (1). Increased amino acid catabolism and urea formation following thermal injury occur despite the provision of exogenous substrates; positive nitrogen balance is difficult to achieve during the acute injury phase, prior to successful wound closure (2). These findings suggest that the mechanisms that control amino acid catabolism and ureagenesis are altered following thermal injury. Sepsis is also associated with increased ureagenesis, and the combination of burn injury and infection can result in massive loss of nitrogen, primarily as urea.

Catecholamines, steroid hormones, and glucagon are all elevated following thermal injury and have been independently associated with changes in ureagenesis and the activity of the urea-cycle enzymes (3-6). The exact mechanisms of the action of these agents are not known. Because of recent interest in treatment modalities that attempt to alter the anabolic/catabolic state of patients with burn injury, it is desirable to better understand the factors that control nitrogen metabolism and urea synthesis, to allow for a more precise analysis of the efficacy of these treatment regimens.

The urea cycle was discovered by Krebs and associates in 1932 and was the first multi-enzyme cycle described. The cycle catalyzes the formation of urea from bicarbonate and ammonia, with multiple intermediates. Five enzymes are involved in this process and, for practical purposes, the complete cycle is found only in the liver. The enzymes are structurally localized to the mitochondria and the cytosol. The enzymes involved are carbamyl phosphate synthetase I (CPS, EC 6.3.4.16)-mitochondria, ornithine transcarbamylase (OTC, EC 2.1.3.3)-mitochondria, argininosuccinate synthetase (AS, EC 6.3.4.5)-cytosol, argininosuccinase (AL, EC 4.3.2.1)-cytosol, and arginase (EC 3.5.3.1)-cytosol.

These enzymes are also found separately, and in groups, in other tissues and are important in amino acid synthesis and creatine synthesis. AS and AL are found in renal tissue and this may be an important site of arginine synthesis (7). Arginase is present in the liver in levels 25 times greater than in any other tissue (8). There are a number of factors that may be involved in the regulation of the urea cycle and urea synthesis, i.e, transcriptional control, translational control, posttranslation modification, co-factors, and substrate control.

Under experimental conditions, the enzyme AS is rate-limiting; under physiologic conditions, the urea cycle may not function at

maximal rates, and it is likely that activators and inhibitors of specific enzymes, and substrate availability, are more important. The best described co-factor in this process is N-acetylglutamate (NAG), which is an obligatory co-factor for CPS; absence of this molecule renders CPS inactive (9). Little is known about the levels of NAG in the liver during normal conditions, or in states of increased urea synthesis. NAG is synthesized by the enzyme NAG-synthetase from glutamate and acetyl-CoA, a Krebs cycle intermediate; and again, little is known about the activity of this enzyme in the control of urea synthesis.

Posttranslational modification of the urea-cycle enzymes (UCE) does not appear to be important in UCE regulation; transcriptional and translational control do, however, appear to be important (10,11). In omnivorous mammals, the activity of UCE is related to the content of protein in the diet; activity is elevated both during periods of starvation and protein excess. The increase in activity associated with a high protein diet is a result of an increase in enzyme mass and an increase in mRNA for the enzymes (12). Hormonal effects on the activity of UCE appear to function at the level of mRNA and it is possible that dietary effects on the UCE are mediated by hormones (13). Although the relationships between trauma, sepsis, and increased ureagenesis have been well described, the mechanisms for these effects are not known. This study will investigate the activity of the UCE, the levels of UCE mRNA in hepatic and renal tissue, and the levels of the co-factor, NAG, and the enzyme, NAG-synthetase, in the livers of sham-burned, burned, and burned-infected rats in an attempt to better understand the factors that control ureagenesis in these conditions. Also, the levels of substrate (ammonia, amino acids) for urea synthesis will be measured both in plasma and hepatic and renal tissue.

MATERIALS AND METHODS

Study Design. Sprague-Dawley rats were acclimated to the facility and to a defined amino acid diet for four days before use. Urine output, water intake, and food intake were recorded for the 24-h period before use. Animals were weighed and then randomized to one of three groups, i.e. burn, burn-infection, or sham-burn (see Table 1). After burn injury, animals were returned to their cages and allowed to awaken. Urine output, water intake, food intake, and weight were recorded daily. On postburn days 2 and 6, animals from each group were anesthetized and exsanguinated (see Table 1). Samples of plasma and liver and renal tissues were obtained from each animal for biochemical and tissue analyses.

Description of Procedures. Forty-eight adult male Sprague-Dawley rats weighing 280-300 g were placed in individual metabolic cages and allowed free access to food and water. Animals were acclimated to the facility and to a defined amino acid diet for four days before use. Urine output, water intake, food intake,

TABLE 1. Number of Animals Per Study Group

Postburn Day of Sacrifice*	Burn	Burn–Infection	Sham Burn
2	8	8	8
6	8	8	8

*These times were chosen, based on data from previous protocols, to maximize the chances of observing a difference in urea synthesis between the study groups.

and weight were recorded for the 24-h period before use. Animals were weighted and then randomized to one of three groups, i.e. burn, burn-infection, or sham-burn.

Group 1. Sixteen animals were anesthetized with sodium pentobarbital (35 mg/kg IP) administered through a 25-ga needle. The dorsal surface was shaved and the animals were placed in a plexiglass mold designed to expose 30% of the total body surface area. A scald burn was inflicted by immersion in 100°C water for 10 sec.

Group 2. Sixteen animals were anesthetized with sodium pentobarbital (35 mg/kg IP) administered through a 25-ga needle. The dorsal surface was shaved and the animals were placed in a plexiglass mold designed to expose 30% of the total body surface area. A scald burn was inflicted by immersion in 100°C water for 10 sec. The burn wound was then painted with *Pseudomonas aeruginosa* (Strain 1244).

Group 3. Sixteen animals were anesthetized with sodium pentobarbital (35 mg/kg IP) administered through a 25-ga needle. The dorsal surface was shaved and the animals were placed in a plexiglass mold designed to expose 30% of the total body surface area. A sham burn was inflicted by immersion in water at room temperature for 10 sec.

Animals were returned to their cages and allowed to awaken. Urine output, water intake, food intake, and weight were recorded daily. On postburn days 2 and 6, 8 animals from each group were anesthetized with sodium pentobarbital (35 mg/kg IP) and exsanguinated. These times were chosen, based on data from previous protocols, to maximize the chances of observing a difference in urea synthesis between the study groups. Animals were weighed immediately before sacrifice. Samples of plasma and liver and renal tissues were obtained from each animal for biochemical and tissue analyses as follows:

Urine. Urinary urea output, orotic acid output (a marker of urea-cycle function), and total urinary nitrogen.

Plasma. Electrolytes, BUN, creatinine, and amino acids.

Hepatic and Renal Tissues. UCE activity (fresh homogenates), albumin, and acute-phase protein mRNA levels (guanidine thiocyanate preserved homogenates), NAG and NAG-synthetase levels (liquid nitrogen snap-frozen specimens), free cytosolic amino acids, and urea and ammonia levels.

Nitrogen balance was calculated based on total urinary nitrogen output and dietary nitrogen intake. mRNA analysis was performed on tissue homogenates by Dr. Sidney Morris of the Department of Molecular Biology at University of Pittsburgh (Pittsburgh PA). NAG and NAG-synthetase levels were performed by Dr. Mendel Tuchman of the Department of Pediatrics located at the University of Minnesota (Minneapolis MN). Amino acid levels were determined by Mr. Frank Konstantinides located at St. Paul Ramsey Medical Center (St. Paul MN).

Determination of Number of Animals Required. Previous work in this area under a separate protocol demonstrated significant differences in urea excretion between sham-burned, burned, and burn-infected animals with 8 animals per group. The differences expected in the other variables were difficult to predict. Because significant differences in urea excretion were noted with 8 animals per group, this appeared to be a reasonable starting point for this study.

Data Analysis Plan. For the comparison of multiple means, ANOVA was used. If a significant f-statistic for the group as a whole was generated, further post-hoc intergroup comparisons were performed. Linear regression was performed between levels of urea-cycle enzymes and substrates and NAG levels.

Safety Considerations for Biohazards. No organisms with higher than containment level two requirements were used. No biocontainment was required for surface inoculation.

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5. Gebhardt R, Mecke D: Permissive effect of dexamethasone on glucagon induction of urea-cycle enzymes in perifused primary monolayer cultures of rat hepatocytes. *Eur J Biochem* 97:29-35, 1979.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

ACCESSION: DA318371

SUMMARY DATE: 920930 **SUMMARY KIND:** K **PREV DATE:** 911001 **DISTRIBUTION:** CX

PROGRAM #: 61102A **PROJ #:** 30161102BB14 **TASK AREA:** BF **WORK UNIT:** 321

TITLE: Effect of Resuscitation Fluid on Hepatic Blood Flow and Hepatic High-Energy Phosphate Production in a Swine Model of Hemorrhagic Shock

SUBJ1: 060500 - Medicine and Medical Research

START DATE: 8905 **END DATE:** 9209 **PERFORMANCE METHOD:** C

CONTRACT/GRANT NO:

CONT TOTAL: $
CUM TOTAL: $
TOTAL LAB FUNDS: $

RESOURCES ESTIMATE

FY	WORK YRS	$(Thousands)
91	0.5	$36
92	0.5	$23
93	0.0	$0

RESPONSIBLE DOD ORGANIZATION: 404885
MRDC INSTITUTE OF SURGICAL RESEARCH USAISR
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PERFORMING ORGANIZATION: 404885
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FT SAM HOUSTON, TX 78234
BECKER, W K
512-221-3349

ASSOC1: MASON, A D

ASSOC2:

FOREIGN INTELLIGENCE CONSIDERED: Y **CIVILIAN APPLICABILITY:** M

KEYWORDS: RA II; Lab Animals: Swine; Burns (Injuries); Hemorrhage; Hemorrhagic Shock; Resuscitation; Liver; Blood; Colloids

OBJECTIVE: A DTIC literature search was conducted under DTIC request numbers W6055C/W6056F dated 20 October 1989. The objective of this work is to evaluate the effects of various resuscitation fluids on support of visceral blood flow and metabolic activity after hemorrhage in an animal model. Improved resuscitation regimens will increase survival and decrease mortality of patients with thermal injury and hemorrhagic shock.

APPROACH: Liver high-energy phosphate levels, hepatic blood flow, and oxygen delivery were measured in swine at baseline, after 25% and 50% hemorrhage/5% and 10% dehydration, and after administration of various resuscitative fluids.

PROGRESS: 9110-9209. Studies were completed in 62 animals. Results indicate that hypertonic saline-dextran administration after hemorrhage results in a period of organ support that is comparable to Ringer's lactate resuscitation. The protocol was amended to investigate the usefulness of hypertonic saline after dehydration. Results from the amended portion of the project indicated that hypertonic saline is effective for early resuscitation after hemorrhage in animals with 5% isotonic dehydration. For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report* for fiscal years 1989 through 1992.

ABSTRACT

PROJECT NUMBER: 3M161102BS14-321, Research

PROJECT TITLE: Effect of Resuscitation Fluid on Hepatic Blood Flow and Hepatic High-Energy Phosphate Production in a Swine Model of Hemorrhagic Shock

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

PERIOD COVERED IN THIS REPORT: 1 October 1991 - 30 September 1992

INVESTIGATORS: William K. Becker, MD, Lieutenant Colonel, MC
William G. Cioffi, Jr., MD, Major, MC
Arthur D. Mason, Jr., MD
Basil A. Pruitt, Jr., MD, Colonel, MC

The role of small volume hypertonic saline-dextran resuscitation after hemorrhage is unclear. Improvement in hemodynamic parameters may be at the expense of cellular function due to shift of intracellular water. Following a 35% hemorrhage, the ability of small volume hypertonic saline-dextran to restore hemodynamic indices and hepatic ATP was compared to Ringer's lactate and no resuscitation in immature swine fitted with arterial and venous catheters and hepatic artery and portal venous ultrasonic flow probes. Resuscitation began 30 min after a 35% hemorrhage which decreased cardiac output (2.28 ± 0.56 to 1.26 ± 0.4 l/min, $P < 0.001$), hepatic blood flow (345 ± 134 to 229 ± 108 ml/min, $P < 0.02$), oxygen delivery (278 ± 64 to 130 ± 40 ml/min, $P < 0.001$), hepatic ATP (3.7 ± 1.9 to 1.5 ± 0.4 µmole/g, $P < 0.001$), and mean blood pressure, (76 ± 23 to 39 ± 8 mmHg, $P < 0.001$).

While hypertronic saline-dextran was less effective than Ringer's lactate in restoring cardiac output, it was equally effective in restoring visceral blood flow, oxygen delivery, and hepatic ATP production. Hypertonic saline-dextran may provide a brief period of organ support when standard resuscitation measures are impractical.

EFFECT OF RESUSCITATION FLUID ON HEPATIC BLOOD FLOW AND HEPATIC HIGH-ENERGY PHOSPHATE PRODUCTION IN A SWINE MODEL OF HEMORRHAGIC SHOCK

Hemorrhagic shock following rapid exsanguination from blunt and penetrating trauma is a frequent cause of death. There is considerable controversy as to the timing of resuscitation, specifically whether resuscitation should begin in the field or after delivery to the site of definite care. Also, the best fluid for acute resuscitation is somewhat unclear. Fluids used have included whole blood, colloids such albumin or hetastarch, and various crystalloid solutions, of which Ringer's lactate is currently the most popular (1-3). In addition, there has been recent interest in the use of hypertonic salt solutions in the early resuscitation from hemorrhagic shock (4). In military applications, the small volumes necessary for resuscitation using hypertonic saline are attractive since the volume of fluid available close to the battlefield is likely to be severely restricted. A significant complication of prolonged hemorrhagic shock is the subsequent development of multisystem organ failure, of which hepatic failure is a leading component and often the final terminal event. Although hepatic failure may develop at a time remote from the initial injury, it is possible that the adequacy of volume resuscitation immediately following hemorrhage may determine the subsequent development of the sequential progression of multisystem organ failure. The adequacy of the resuscitation regimen to support hepatic blood flow, hepatic oxygen delivery, and the formation of hepatic high-energy phosphate compounds such as ATP may be important in preventing the subsequent development of hepatic failure. It is unclear whether the various forms of resuscitation have any significant impact on hepatic blood flow, hepatic oxygen delivery, and the formation of hepatic high-energy phosphate compounds.

The role of small volume resuscitation fluids such as hypertonic saline or hypertonic saline-dextran in the clinical setting following acute hemorrhage is unclear. Potential benefits associated with this form of therapy include decreased tissue edema and a reduction in pulmonary complications related to administration of large volumes of resuscitation fluid. Small volume resuscitation fluids are easier to store and administer compared to standard solutions such as Ringer's lactate. Because hypertonic saline solution must "borrow" water from the extravascular and intracellular spaces to achieve restoration of effective circulating volume, it is possible that the intracellular dehydration caused by the use of these fluids may be detrimental to organ function. Little is actually known about the effect of these fluids on organ function following resuscitation from hemorrhagic shock. The following studies were performed to develop a model in which to explore the effects of resuscitation fluids on hepatic

blood flow and hepatic high-energy phosphate levels following shock and resuscitation.

MATERIALS AND METHODS

Experimental Design. Immature swine were hemorrhaged of 35% of the total blood volume. Animals were then randomized to receive no resuscitation, lactated Ringer's solution, or hypertonic saline-dextran. Pilot studies determined that a 40% total blood volume hemorrhage had an LD_{80} at 24 h and 45% hemorrhage resulted in an LD_{100} at 24 h, with many animals dying before resuscitation could begin. Therefore, a 35% hemorrhage was chosen for this model.

Description of Procedures. Eighteen immature male or female Yorkshire swine weighing 20-25 kg were anesthetized with methohexital sodium (1 cc/3 kg), intubated, and placed on a volume-cycled ventilator, followed by 0.51% halothane gas anesthesia. A thermodilution pulmonary catheter was placed percutaneously in the right jugular vein and an intravenous catheter for volume infusion was placed in the left jugular vein. An arterial cannula for blood pressure measurement and hemorrhage was placed in the right femoral artery. EKG leads for heart monitoring were applied to shaven skin. An upper abdominal flap incision was performed through the which the hepatic artery and portal vein were mobilized. Hepatic artery branches outside the liver were ligated and the liver was freed of its ligamentous attachments. This dissection and preparation was similar to that of harvesting the liver for hepatic transplantation. The goal was to insure that no collateral circulation to the liver was maintained and that all blood flow to the liver entered through the hepatic artery or portal vein. A catheter for blood sampling was placed through a side branch of the portal vein. Ultrasonic flow probes (Transonics Systems, Inc., Ithaca, NY) were placed on the hepatic artery and portal vein in the porta hepatis. After obtaining baseline cardiac output, heart rate, blood pressure, total hepatic blood flow, and oxygen delivery, a liver Truecut™ needle biopsy was performed.

Animals underwent hemorrhage of 35% of the blood volume over a 30-min period. Animals randomized to the control group were hemorrhaged but not resuscitated. After 30 min, resuscitation fluids were given to animals assigned to the treatment groups, i.e., lactated Ringer's solution (3 ml per milliliter shed blood) or hypertonic saline-dextran (7.5% NaCl, 4 ml/kg) over a 25-min period. Hemodynamic data and blood samples were collected 15 min after hemorrhage, immediately after resuscitation, and at 60 min after resuscitation. When this was completed, the catheters were removed and the incisions were closed. The animals returned to their cages and observed for 24 h. Each animal was given food and water ad libitum following completion of all surgical procedures and allowed unrestricted activity inside the cages. Wounds were

treated with dry gauze dressings. Buprenorphine (0.005–0.01 mg/kg IM) was administered every 12 h for pain. All surviving animals were sacrificed at the end of the 24-h period with sodium pentobarbital (60 mg/kg IV) and exsanguinated. A section of liver was obtained from each animal after sacrifice for histologic analysis to determine liver architecture 24 h posthemorrhage and resuscitation.

Determination of Number of Animals Required. Previous studies using this swine model of hemorrhage have used 5 animals in each study group.

Data Analysis Plan. Hepatic blood flow, oxygen delivery, and formation of hepatic high-energy compounds as well as hemodynamic data will be compared between the control groups and each of the hemorrhage groups and will be analyzed for statistical differences.

RESULTS

Based on the results of this portion of the study (see Table 1), it appears that hypertonic saline-dextran is a relatively effective resuscitation fluid for acute hemorrhagic shock. Hypertonic saline-dextran was as effective as Ringer's lactate in restoring hepatic blood flow and hepatic ATP, although less effective than Ringer's lactate in restoring cardiac output.

TABLE 1. Parameters at 1 h Postresuscitation

	No Resuscitation (n=6)	Ringer's Lactate (n=6)	Small Volume Hypertonic Saline-Dextran (n=6)
Cardiac output (l/min)	1.2 ± 6	2.7 ± 0.8	1.9 ± 0.5
Hepatic blood flow (ml/min)	189 ± 103	331 ± 182	362 ± 101
Oxygen delivery (ml/min)	134 ± 55	247 ± 80	184 ± 69
ATP (μmole/g)	1.3 ± 0.3	2.5 ± 0.6*	3.5 ± 1.2*
Blood pressure (mmHg)	42 ± 13	60 ± 13	58 ± 5

*$P < 0.05$ vs no resuscitation.

DISCUSSION

While hypertonic saline-dextran was less effective than Ringer's lactate in restoring cardiac output and visceral blood flow, it was equally effective in restoring oxygen delivery and

hepatic ATP production. Hypertonic saline-dextran may provide a brief period or organ support when standard resuscitation measures are impractical.

PRESENTATIONS/PUBLICATIONS

None.

REFERENCES

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

ACCESSION: DA346176

SUMMARY DATE: 920214 **SUMMARY KIND:** K **PREV DATE:** 911001 **DISTRIBUTION:** CX

PROGRAM #: 61102A **PROJ #:** 30161102BS14 **TASK AREA:** CK **WORK UNIT:** 323

TITLE: Correlation of Plasma Amino Acid and Pyridoxal-5'-Phosphate (PLP) Levels in Thermally Injured Patients

SUBJ1: 060100 - Biochemistry
SUBJ2: 060500 - Medicine and Medical Research

START DATE: 8906 **END DATE:** 9202 **PERFORMANCE METHOD:** C

CONTRACT/GRANT NO:

CONT TOTAL: $
CUM TOTAL: $
TOTAL LAB FUNDS: $

RESOURCES ESTIMATE

FY	WORK YRS	$(Thousands)
91	0.1	$51
92	0.0	$0
93	0.0	$0

RESPONSIBLE DOD ORGANIZATION: 404885
MRDC INSTITUTE OF SURGICAL RESEARCH USAISR
USAISR
FT SAM HOUSTON, TX 78234
PRUITT, B A
210-221-2720

PERFORMING ORGANIZATION: 404885
MRDC INSTITUTE OF SURGICAL RESEARCH

FT SAM HOUSTON, TX 78234
BECKER, W K
210-221-3349

ASSOC1: N/A

ASSOC2: N/A

FOREIGN INTELLIGENCE CONSIDERED: Y **CIVILIAN APPLICABILITY:** M

KEYWORDS: RA II; Volunteers; Adults; Burns (Injuries); Trauma; Septicemia; Morbidity; Amino Acids; Enzymes; Metabolism;

OBJECTIVE: A DTIC literature search was conducted under DTIC request numbers W6R20M/W6R22N dated 29 May 1990. The objectives of this work are to measure PLP levels in thermally injured patients and correlate those with abnormalities in amino acid metabolism.

APPROACH: Burn patients had plasma PLP and amino acid profiles drawn on admission, weekly, and when indicated by a change in clinical status. Burn size, presence of inhalation, morbidity, mortality, liver function test results, nitrogen balance, calories predicted and received, usage of aminoglycosides, theophylline, and/or digoxin, and the amount of vitamin B-6 supplementation received in tube feedings or hyperalimentation were recorded. Multiple regression was used to detect relationships between the various independent variables which were measured and the dependent variables.

PROGRESS: 9110-9202. Thirty patients were enrolled in this study. Data indicated that there is a significant depression of plasma PLP levels beginning shortly after thermal injury and continuing through the convalescent period. The levels observed are consistent with those seen in severe vitamin B-6 deficiency. A question arose as to whether the depression in PLP levels observed in thermally injured patients represented a true deficiency of the vitamin or alternatively represented dilution and/or redistribution of the vitamin. Therefore, the study was

amended to determine whether this depression represented a true vitamin deficiency. Data from the amended study indicated a persistent depression of vitamin B-6 levels. However, ornithine decarboxylase was technically not able to be performed. Therefore, this study has been completed. For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report* for fiscal years 1989 through 1992.

ABSTRACT

PROJECT NUMBER: 3M161102BS14-323, Research

PROJECT TITLE: Correlation of Plasma Amino Acid and Pyridoxal-5'-Phosphate (PLP) Levels in Thermally Injured Patients

INSTITUTION: US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas 78234-5012,[1] and Department of Clinical Investigation, William Beaumont Army Medical Center, El Paso, Texas 79920[2]

PERIOD COVERED IN THIS REPORT: 1 October 1991 - 14 February 1992

INVESTIGATORS: William K. Becker, MD, Lieutenant Colonel, MC[1]
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Results indicate that there is a significant depression of plasma PLP levels beginning shortly after thermal injury and continuing through the convalescent period. The levels observed are consistent with those seen in severe vitamin B_6 deficiency.

CORRELATION OF PLASMA AMINO ACID AND PYRIDOXAL-5'-PHOSPHATE (PLP) LEVELS IN THERMALLY INJURED PATIENTS

Critically ill patients, including patients with major thermal injuries, are known to have alterations in plasma amino acid levels. Stress and critical illness are associated with hypermetabolism and increased amino acid flux. Amino acids are released from the carcass through catabolism of skeletal muscle and transported to central organs, principally the liver and gut, for use in production of acute-phase proteins, gluconeogenesis, and energy production. In progressive multiorgan failure associated with sepsis, severe burn injury, and multiple trauma, a characteristic picture of plasma amino acids emerges. Aromatic amino acid levels are elevated and levels of branch-chain amino acid levels are depressed. These changes are often associated with progressive hepatic dysfunction, hyperbilirubinemia, coagulation disorders, and subsequent death. Various explanations for this pattern have emerged; however, none are entirely satisfying. PLP is a cofactor form of vitamin B_6 and is required for the normal function of numerous enzymes, including many in amino acid synthesis and degradation.

Deficiency of PLP, until recently, has been thought to be rare and only associated with severe forms of dietary malnutrition. However, recent studies have demonstrated that under certain conditions, especially severe stress associated with a major illness, PLP deficiency may be present (1,2). In critically ill surgical ICU patients, extremely low levels of PLP have been found, with the level of depression correlating with mortality (2). Possible reasons for the depression of PLP in critically ill patients include elevated levels of polyamines such as spermidine and putrescine, which form a Schiff's base with PLP. Also, aminoglycoside antibiotics and theophylline preparations, agents frequently used in ICU patients, may also interact with PLP and depress levels (3). The increase in metabolic activity associated with critical illness may also increase nutritional requirements for PLP.

If depressed PLP levels correlate with abnormalities in amino acid profile that are associated with multiorgan failure, supplementation, either prophylactic or therapeutic, may prevent or decrease the occurrence and consequences of multiorgan failure and therefore enhance survival in critically ill patients. Therefore, the objective of this study is to measure PLP levels in thermally injured patients and correlate this with abnormalities in amino acid metabolism.

MATERIALS AND METHODS

Description of Procedures. Twenty-four patients with thermal injury had plasma PLP and amino acid profiles drawn on admission,

weekly, and when indicated by a change in clinical status. Burn size, presence of inhalation injury, morbidity, mortality, complications, liver function test results, nitrogen balance, calories predicted and received, usage of aminoglycosides, theophylline, and/or digoxin, and the amount of vitamin B6 supplementation received in tube feedings or hyperalimentation were recorded. No additional supplementation of vitamin B_6, beyond that normally present in the diet, enteral, or parenteral feedings, was given to the patients.

Patient Inclusion. Twenty-four patients meeting the following criteria were eligible for enrollment in the study. Properly signed and witnessed DA Forms 5303-R, Volunteer Agreement Affidavits, were obtained from each patient, or his/her legal guardian, before beginning the study.

1. Male or female patients ≥ 18 yr.

2. Patients admitted to the US Army Institute of Surgical Research within 72 h postburn.

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

Patient Exclusion. Patients meeting any of the following criteria were excluded from participation in this study.

1. Patients < 18 yr.

2. Patients not admitted to the US Army Institute of Surgical Research within the first 72 h postburn.

3. Patients with burns < 20% of the total body surface area or toxic epidermal necrolysis.

Amino Acid Analysis. Blood for amino acid analysis was collected in a 7-ml green-top tube (lithium-heparin) and placed directly on ice. The plasma was separated by centrifugation and stored in a plastic cryotube at -80°C. Plasma amino acid analysis was performed on an amino acid analyzer (Beckman 6300). This technique involves lithium-based buffering for HPLC on a 20-cc column with ninhydrin analysis.

Plasma PLP Analysis. Blood for plasma PLP analysis was collected in a 7-ml purple-top (EDTA preservative) tube on ice protected from light. Plasma was separated by centrifugation and stored in a plastic cryotube at -80°C prior. The technique for determining PLP was the undeproteinized tyrosine apodecarboxylase RIA, a technique which appears to correlate better with survival in critically ill patients than bioassays or functional assays.

Nitrogen Balance Studies. The formula by Waxman et al (8) with silver sulfadiazine modification was used as follows:

$$\text{Nitrogen Intake} - \text{Nitrogen Output} = \text{Nitrogen Balance}$$

$$\text{Nitrogen Intake} = \frac{\text{Protein Intake (g)}}{6.25}$$

$$\text{Nitrogen Output by UUN Method} = \text{Urinary Urea Nitrogen} + 4\text{ g} + \text{Wound Loss}$$

$$\text{Nitrogen Output by TUN Method} = \text{Total Urea Nitrogen} + 2\text{ g} + \text{Wound Loss}$$

$$\text{Wound Loss} = 0.1 \times \text{TBSA} \times \%\text{TBSA Burn} \times 0.8$$

Data Collection. Data collection included results of both plasma PLP and amino acid analyses, burn size, presence of inhalation injury, mortality, morbidity, caloric intake, amount of vitamin B_6 supplementation, use of aminoglycoside antibiotics, theophylline drugs, and/or digoxin, and nitrogen balance, and results of any routine liver function tests. Data was tabulated for each patient on flow sheets.

Data Analysis Plan. Multiple regression was used to detect any relationship between the various independent variables from the collected data and dependent variables which were measured.

RESULTS

Results indicate that there is a significant depression of plasma PLP levels beginning shortly after thermal injury and continuing through the convalescent period. The levels observed are consistent with those seen in severe vitamin B_6 deficiency.

DISCUSSION

A question arose as to whether the depression in PLP levels observed in thermally injured patients represented a true deficiency of the vitamin or alternatively represented dilution and/or redistribution of the vitamin. Future investigations will be necessary to answer this question.

PRESENTATIONS/PUBLICATIONS

None.

REFERENCES

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In *Clinical and Physiological Applications of Vitamin B-6.* Alan R. Liss, Inc., pp 425-433, 1988.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

ACCESSION: DA346164

SUMMARY DATE: 920930 **SUMMARY KIND:** D **PREV DATE:** 911001 **DISTRIBUTION:** CX

PROGRAM #: 61102A **PROJ #:** 30161102BS14 **TASK AREA:** BN **WORK UNIT:** 324

TITLE: Effect of Arginine Deprivation on the Response to Thermal Injury and Burn Wound Infection in the Rat - A Pilot Study

SUBJ1: 060500 - Medicine and Medical Research

START DATE: 9002 **END DATE:** 9209 **PERFORMANCE METHOD:** C

CONTRACT/GRANT NO:

CONT TOTAL: $
CUM TOTAL: $
TOTAL LAB FUNDS: $

RESOURCES ESTIMATE

FY	WORK YRS	$(Thousands)
91	1.0	$65
92	1.0	$69
93	0.0	$0

RESPONSIBLE DOD ORGANIZATION: 404885
MRDC INSTITUTE OF SURGICAL RESEARCH USAISR
FT SAM HOUSTON, TX 78234
PRUITT, B A
210-221-2720

PERFORMING ORGANIZATION: 404885
MRDC INSTITUTE OF SURGICAL RESEARCH USAISR
FT SAM HOUSTON, TX 78234
BECKER, W K
210-221-3349

ASSOC1: CIOFFI, W G

ASSOC2: MC MANUS, A T

FOREIGN INTELLIGENCE CONSIDERED: Y **CIVILIAN APPLICABILITY:** M

KEYWORDS: RA II; Lab Animals; Rats; Burns (Injuries); Nutrition; Metabolism; Therapy

OBJECTIVE: A DTIC literature search was conducted under DTIC request numbers W6N22E/W6M25A dated 8 January 1990. The objective of this work is to determine the effect of a diet deficient in arginine on the response to thermal injury and burn wound infection. If it can be demonstrated that supplying sufficient arginine after burn injury improves outcome or better supports certain metabolic processes after burn injury, the ability to treat patients with thermal injury may be improved.

APPROACH: During the first phase of this study, rats were fed regular, arginine-deficient, or arginine-sufficient diets for five weeks. Weekly weight gain was recorded and urinary orotic acid and polyamine excretions were measured. At the end of five weeks, the animals were administered 30% total body surface area full-thickness scald or sham burns. One-half these animals were inoculated with Pseudomonas. Blood and urine samples were obtained from any animal not surviving to 15 days and all surviving animals sacrificed at 15 days for plasma amino acid analyses, BUN determination, and urine orotic acid and polyamine excretion. Mortality was compared by life table analysis. Differences in biochemical parameters were studied by ANOVA and, where warranted, intergroup significance was determined by the Scheffee' technique. As the result of an addendum, rats were equally divided into five groups. Group I (n=30) was fed regular rat chow, Group II (n=30) was fed an amino acid control diet, Group III (n=30) was fed an isonitrogenous diet identical to the amino acid control diet but without the amino acid arginine, Group IV (n=30) was fed a diet identical to the amino acid control diet

except arginine was replaced with ornithine on an isonitrogenous basis, and Group V (n=30) was fed a diet identical to the amino acid control diet except that arginine was replaced with citrulline on an isonitrogenous basis. Ten animals from each group were followed for 15 days. Blood and urine samples were obtained from any animal not surviving to 15 days and all surviving animals sacrificed at 15 days. Twenty animals from each group were exposed to 30% total body surface area full-thickness scald (n=10) or sham (n=10) burns. Five animals from each subgroup were inoculated with Pseudomonas. These animals were followed for 5 days. Blood and urine samples were obtained from any animal not surviving to 5 days and all surviving animals sacrificed at 5 days. Peripheral blood mononuclear cells and splenocytes were isolated and ConA and phytohemagglutinin studies were performed and MLR and IL2 marker expressions were measured. Samples of hepatic tissue were also obtained at the time of sacrifice and assays for five urea-cycle enzymes were performed.

PROGRESS: 9110-9209. Initial results indicated that arginine deprivation results in alterations in growth, T-cell function, and orotic acid excretion. The protocol was amended to include other urea-cycle amino acids in the study diets. Survival studies did not yield a significant difference with any of the diets following burn or burn wound infection. Additional studies in rats using the modified urea-cycle diets demonstrated differences in plasma amino acid levels and growth curves, both before and after thermal injury. For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report* for fiscal years 1990 through 1992.

ABSTRACT

PROJECT NUMBER: 3M161102BS14-324, Research

PROJECT TITLE: Effect of Arginine Deprivation on the Response to Thermal Injury and Burn Wound Infection in the Rat - A Pilot Study

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

PERIOD COVERED IN THIS REPORT: 1 October 1991 - 30 September 1992

INVESTIGATORS: William K. Becker, MD, Lieutenant Colonel, MC
William G. Cioffi, Jr., MD, Major, MC
Albert T. McManus, PhD
Carlin V. Okerberg, DVM, PhD, Lieutenant Colonel, VC
Basil A. Pruitt, Jr., MD, Colonel, MC

Thermal injury induces a hypermetabolic response, with increases in energy expenditure, protein turnover, and amino acid flux. Nutritional support of the thermally injured patient is generally considered important in minimizing negative nitrogen balance and erosion of lean body mass. The exact composition of the nutritional support regimen to best accomplish these goals is unclear. This study was undertaken to help elucidate the role of arginine and the other urea cycle amino acids, ornithine and citrulline, in normal animals and following experimental thermal injury and burn wound infection.

The studies presented demonstrated alterations in weight gain and plasma amino patterns following the alteration of dietary urea-cycle amino acids. In addition, increases in urea synthesis after burn injury and burn wound infection were not accompanied by changes in urea-cycle enzyme activity. A significant and prolonged increase in nitrogen oxide synthesis from the guanido-nitrogen of the amino acid arginine was demonstrated. The kinetics of nitrate excretion after burn injury and the conversion of arginine to nitrate were also demonstrated. Future studies will be needed to elaborate on the findings reported here. Of particular interest are the mechanisms that control the increase in urea synthesis noted after traumatic injury. There are under clinical investigation a number of hormones and other modulators that attempt to improve the anabolic/catabolic state of the critically ill patient. It is quite likely that these mediators may function, in part, at the level of urea synthesis or at the level of interaction between the urea cycle and oxidation pathways. Specific knowledge of how urea synthesis is controlled in vivo after traumatic injury may allow for a more exact understanding of how mediators such as growth hormone act after a traumatic injury.

Future proposed studies will look at the role of N-acetylglutamate synthetase and N-acetylglutamate in the control of urea synthesis after traumatic injury and how the oxidative metabolic pathways and the urea cycle interact at this step. In addition, the physiologic significance of the increase in nitric oxide production and nitrate synthesis after traumatic injury will require investigation. Specifically, it remains to be determined whether the increase in nitric oxide production directly affects peripheral vascular tone after traumatic injury and whether effects on macrophages, leukocytes, and the hepatocyte can be documented in vivo.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

ACCESSION: DA346177

SUMMARY DATE: 921001 **SUMMARY KIND:** D **PREV DATE:** 911001 **DISTRIBUTION:** CX

PROGRAM #: 61102A **PROJ #:** 30161102BS14 **TASK AREA:** EC **WORK UNIT:** 326

TITLE: Effect of Surfactant Replacement on V_A/Q in Sheep with Inhalation Injury

SUBJ1: 060500 - Medicine and Medical Research

START DATE: 9002 **END DATE:** 9309 **PERFORMANCE METHOD:** C

CONTRACT/GRANT NO:

CONT TOTAL: $
CUM TOTAL: $
TOTAL LAB FUNDS: $

RESOURCES ESTIMATE

FY	WORK YRS	$(Thousands)
91	0.5	$47
92	0.2	$23
93	0.2	$29

RESPONSIBLE DOD ORGANIZATION: 404885
MRDC INSTITUTE OF SURGICAL RESEARCH USAISR
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PRUITT, B A
210-221-2720

PERFORMING ORGANIZATION: 404885
MRDC INSTITUTE OF SURGICAL RESEARCH USAISR
FT SAM HOUSTON, TX 78234
CIOFFI, W G
210-221-8440

ASSOC1: JORDAN, B S

ASSOC2: MASON, A D

FOREIGN INTELLIGENCE CONSIDERED: Y **CIVILIAN APPLICABILITY:** M

KEYWORDS: RA II; Lab Animals; Sheep; Burns (Injuries); Inhalation; Therapy; Morbidity

OBJECTIVE: A DTIC literature search was conducted under DTIC request numbers W6015A/W6015D dated 30 May 1990. The objective of this work is to examine the effect of exogenously administered surfactant on V_A/Q changes seen following inhalation injury in an ovine model. If exogenous surfactant administration can favorably affect V_A/Q after inhalation injury, its application to patients with inhalation injury would be advantageous.

APPROACH: In a preliminary phase, 42 sheep were randomized to one of three groups. Group I was exposed to a moderate smoke injury and administered surfactant 24 h after injury. Groups II and III were exposed to a severe smoke injury. Group II was administered surfactant or saline immediately after injury and every 12 h for 24 h. Group III was administered surfactant or saline continuously during the first 24 h after injury. Group I data obtained following surfactant replacement was indexed to pretreatment data and compared by the student's t test. Group II and III data were compiled and, after analysis of mean and standard deviations, compared utilizing the student's t test and ANOVA. As the result of an addendum to the study, 20 sheep, in pairs, will be exposed to a moderate smoke injury and administered either aerosolized EXOSURF® or EXOSURF® vehicle using the Visan™ intermittent signal, actuated nebulizer with ventilator interface.

PROGRESS: 9110-9209. Despite difficulty with the delivery vehicle for surfactant, there was a near significant improvement in pulmonary function associated with surfactant use. The delivery vehicle was redesigned by the manufacturer and an addendum was written and

approved for its use. Refinement of the model is currently in progress. For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report* for fiscal years 1990 through 1992.

ABSTRACT

PROJECT NUMBER: 3M161102BS14-326, Research

PROJECT TITLE: Effect of Surfactant Replacement on V_A/Q in Sheep with Inhalation Injury

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

PERIOD COVERED IN THIS REPORT: 1 October 1991 - 30 September 1992

INVESTIGATORS: William G. Cioffi, Jr., MD, Major, MC
Bryan S. Jordan, RN, MSN
Arthur D. Mason, Jr., MD
Carlin V. Okerberg, DVM, PhD, Lieutenant Colonel, VC
Basil A. Pruitt, Jr., MD, Colonel, MC

The loss of surfactant after smoke exposure may partially explain the atelectasis and marked instability of alveolar walls seen after injury. The recent availability of synthetic surfactant has led to the suggestion that surfactant replacement may have a therapeutic effect in ARDS. The purpose of this study is to examine the effect of exogenously administered surfactant on V_A/Q changes seen after inhalation injury in an ovine model.

A moderate smoke inhalation injury was induced in the ovine model developed at this Institute. One hour postinjury, the animals were randomized to receive either aerosolized surfactant or surfactant vehicle while remaining intubated and on mechanical ventilatory support. Animals were followed for 48 h and standard physiologic measurements were made.

Multiple problems encountered during the study greatly limit the validity of findings. Despite these problems, the suggestion of benefit of surfactant administration after smoke exposure in these animals indicates that further studies to define the utility of surfactant replacement are necessary. Before performing these studies, a more refined and constant smoke exposure protocol will be necessary, a project which is already underway. In addition, animals with abnormal calculated shunt before smoke exposure should either be eliminated from the study or observed for a longer period of time until the venous admixture returns to normal before smoke exposure. In order to prove the benefit of surfactant replacement, it is mandatory that the two groups be as homogeneous as possible before smoke exposure and before the initiation of therapy at 1 h after smoke exposure.

EFFECT OF SURFACTANT REPLACEMENT ON V_A/Q IN SHEEP WITH INHALATION INJURY

The effect of inhalation injury on V_A/Q utilizing the multiple inert gas elimination technique (MIGET) and cardiopulmonary parameters has been well described in an ovine model (1). Moderate to severe injury causes hypoxia, hypercarbia, and a shift of V_A/Q to the left, i.e., increase in lung segments with $V_A/Q > 0$ but < 1. In addition, smoke-exposed animals show increased perfusion to shunt and low V_A/Q lung segments. Attempts to alter these derangements with conventional ventilation utilizing PEEP resulted in an increased dead space ventilation but had no effect on shunt or low V_A/Q compartments (unpublished data).

It has been previously shown in a canine model that inhalation injury is associated with a marked increase in minimum surface tension from bronchoalveolar lavage samples. This indicates that surfactant is no longer active or that less surfactant is available (2). Additionally, repeated exposure of rats to cigarette smoke is followed by a significant reduction in the recovery of pulmonary surfactant. The degree of reduction in surfactant recovery was dose-dependent (3).

This loss of surfactant after smoke exposure may partially explain the atelectasis and marked instability of alveolar walls seen after injury. The recent availability of synthetic surfactant has led to the suggestion that surfactant replacement may have a therapeutic effect in respiratory distress syndromes, including that caused by inhalation injury. Severe respiratory insufficiency induced by repeated lung lavage in the guinea pig model was significantly reversed by the administration of exogenous surfactant (4). Nonrandomized trials in infants with neonatal respiratory distress syndrome have shown increased compliance and improved blood gases after surfactant administration (5). In a recently completed randomized controlled trial, human surfactant was administered endotracheally at birth to very premature infants. The surfactant-treated group had significantly fewer deaths than the control group, fewer cases of bronchopulmonary interstitial emphysema and pneumothoraxes (6). Prophylactic treatment with surfactant also substantially reduced the period of neonatal intensive care. These authors concluded that treatment with human surfactant offered promise for improving the survival of very premature infants and for reducing the pulmonary sequelae of the respiratory distress syndrome.

The purpose of this study is to examine the effect of exogenously administered surfactant on VA/Q changes seen after inhalation injury in an ovine model.

MATERIALS AND METHODS

Study Design. Twenty neutered male sheep weighing 25–45 kg will be utilized throughout this study. Inhalation injury will be induced using a standard ovine smoke inhalation model developed at this Institute. The animals, in pairs, will be anesthetized, intubated and instrumented prior to the smoke inhalation procedure. Animals will be allowed to recover from anesthesia, during which time, heart rate, blood pressure, central venous pressure, pulmonary artery pressure, cardiac output, arterial blood gases, tidal volume, flow rates, transpulmonary pressures, and static and dynamic compliance will be measured until the ventilator settings are maximized. The animals will then be exposed to a moderate smoke injury. During a 1-h recovery period, ventilator settings will be established and maintained throughout the study. At the conclusion of the 1-h recovery period, heart rate, blood pressure, central venous pressure, pulmonary artery pressure, cardiac output, arterial blood gases, tidal volume, airflow rates, transpulmonary pressure, and static and dynamic compliance measurements will be repeated. At the conclusion of these measurements, each animal will receive either aerosolized EXOSURF® or EXOSURF® vehicle in the amount of 1X concentration via the tracheostomy for 48 h. Each animal's heart rate, blood pressure, central venous pressure, pulmonary artery pressure, cardiac output, arterial blood gases, tidal volume airflow rates, transpulmonary pressures, and static and dynamic compliance will be measured every 4 h for 48 h. At the conclusion of 48 h, V_A/Q distributions will be measured in each animal utilizing the multiple inert gas elimination technique.

Description of Procedures. Twenty neutered male sheep weighing 25–45 kg will be utilized throughout this study. Each animal will be housed in a conventional outdoor run and have access to food and water ad libitum. Sheep will be dewormed with injectable ivermectin (IvomecR, Merck and Co, Rahway, NJ 07065) 2 weeks prior to use. Inhalation injury will be induced using the standard ovine smoke inhalation model developed at this Institute.

On the day of the study, two animals will be selected and a body weight obtained for each. The animals will be anesthetized with pentobarbital sodium (20–30 mg/kg IV). When fully anesthetized, each animal will be placed in the supine position and restrained. Mechanical ventilation during the surgical procedure will be provided by a Bear II™ volume-limited ventilator (Bear Medical Systems, Inc., Riverside, CA). Mechanical ventilation during the remainder of the study will be provided by a Servo 900-C volume-limited ventilator (Siemens-Elema AB, Life Support Systems Division, Sona, Sweden). Femoral venous and arterial catheters and a jugular catheter introducer sheath (8.5 F, Baxter Healthcare Corp, Edwards Critical-Care Division, Santa Ana, CA) with a balloon-directed thermodilution pulmonary artery catheter (7.5 F, Baxter Healthcare Corp) will be inserted through respective cutdowns. A tracheotomy will be performed and a tracheostomy tube

(9.0 mm, Shiley, Inc., Irvine, CA) inserted. Arterial blood pressure and central venous and pulmonary venous pressures will be monitored with disposable monitoring kits (Trantec®, No. 53, DTS-260 and No. 53-1X2-260-DTS, respectively, Baxter Healthcare Corp). Cardiac output will be measured with the Co-Set II™ closed injectate delivery system (Model 93-500, Baxter Healthcare Corp). Cardiac monitoring will be accomplished with a Hewlett-Packard 78353C patient monitor (Hewlett-Packard Company, Medical Products Group, 3000 Minuteman Road, Andover, MA). Transpulmonary pressures will be monitored with a Bicore C-100 pulmonary monitor (Bicore Monitoring Systems, Irvine, CA). Ringer's lactate solution will be infused at a rate of 0.1 ml/kg/min upon initiation of venous access.

Upon completion of the surgical interventions, the animals will be placed in individual metabolic cages in the prone position and allowed to recover. At the completion of recovery as denoted by the animal's willingness to stand, the animal's heart rate, blood pressure, central venous pressure, pulmonary artery pressure, cardiac output, arterial blood gases, tidal volume, airflow rates, transpulmonary pressure, and static and dynamic compliance will be measured. The animals will then be exposed to a moderate smoke inhalation injury using the standard ovine smoke inhalation model developed at this Institute. After a recovery time of 1 h, the animals' heart rate, blood pressure, central venous pressure, pulmonary artery pressure, cardiac output, arterial blood gases, tidal volume, airflow rates, transpulmonary pressures, and static and dynamic compliance will be measured. At the conclusion of these measurements, each animal will begin receiving aerosolized EXOSURF® (Burroughs Wellcome Company, Research Triangle Park, NC) or EXOSURF® vehicle by a Visan-9™ and TriNEB™ canister aerosolizer (Vortran Medical Technology, Inc.) continuously for the next 48 h.

At the conclusion of 48 h, V_A/Q distributions will be measured utilizing the multiple inert gas elimination technique. The lactated Ringer's infusion will be replaced with a lactated Ringer's solution containing 6 inert gases (sulphur hexafluoride, ethane, cyclopropane, halothane, ether, and acetone) which will be infused at a rate of 0.1 ml/kg/min. After 1.0 h, arterial and mixed venous blood will be drawn anaerobically into preweighed heparinized syringes (30 ml, matched, glass) simultaneously. Mixed expired gas will be obtained from a temperature-controlled copper coil (outside diameter = 3.49 cm, length = 640 cm) 1 min after obtaining the blood samples. Blood and expired gas samples will be analyzed immediately by a GC-MS (Model 5890, Series 2, Hewlett-Packard Company). Repeat cardiopulmonary parameters and blood and expired gas samples will be obtained after 10 minutes.

Upon completion of measurements, each animal will be euthanized by pentobarbital sodium (60 mg/kg IV) and necropsied. One lung will be removed for measurement of extravascular lung water by the

gravimetric method and the second lung will be removed along with selective tissues for histologic study.

RESULTS

A preliminary study using the Visan™ intermittent signal, actuated nebulizer with ventilator interface (V1-4, Vortran Medical Technology, Inc., Sacramento, CA) demonstrated that the equipment was difficult to control and cumbersome to maintain (7). Further development of the aerosolizer by its manufacturer has effected substantial improvements in the unit to permit delivery of precise dosages of EXOSURF® or EXOSURF® vehicle during the course of the study. Specifically, the redesign loads the inspiratory limb of the ventilator circuit with aerosol during the exhalation phase for delivery of the medication to the patient with the next ventilator-assisted breath. Additionally, the redesign significantly enhances monitoring capability for increased airway pressures, employs improved in-line filters, and provides a heat source for more effective medication delivery. Despite the problems encountered with the Vortran device, initial animal trial suggested a significant effect of surfactant replacement in smoke-injured animals compared to saline-treated controls. Therefore, an addendum was developed during this reporting period to determine the effects of surfactant replacement using the redesigned aerosolizer.

DISCUSSION

In a preliminary study (7), animals in both groups demonstrated the predictable pulmonary and hemodynamic responses to smoke inhalation injury. The pulmonary artery hypertension and the decline in pulmonary function manifested by declining arterial oxygenation and increasing calculated shunt was evident in both groups. In addition, CO_2 clearance tended to decrease, necessitating increases in minute ventilation in an attempt to maintain the same arterial PCO_2. The data suggested, but did not prove, a potential benefit in the surfactant-treated animals in terms of arterial oxygenation and venous admixture. However, multiple problems encountered during the study greatly limited the validity of these findings. First, although all 13 animals were exposed to seven units of smoke in an identical fashion, carboxyhemoglobin levels varied significantly between the animals. The carboxyhemoglobin levels varied between 40% and 98%. Although animals with carboxyhemoglobin levels greater than 90% were excluded from final analysis, the great variability in initial carboxyhemoglobin levels indicated a lack of constancy in the smoke exposure, and thus, the degree of insult. In addition, the finding of a slight but significant increase in calculated shunt in the saline-treated group compared to the surfactant-treated group before smoke exposure was another parameter indicating marked heterogeneity in the groups. Despite these problems, the suggestion of benefit of surfactant administration after smoke

exposure in these animals indicated that further studies to define the utility of surfactant replacement was necessary. Before performing these studies, a more refined and constant smoke exposure protocol was necessary, a project which was initiated during this reporting period. In addition, animals with abnormal calculated shunt before smoke exposure will be eliminated from the study or observed for a longer period of time until the venous admixture returns to normal before smoke exposure. In order to prove the benefit of surfactant replacement, it will also be mandatory that the two groups be as homogeneous as possible before smoke exposure and before the initiation of therapy at 1 h after smoke exposure.

Upon completion of data collection for animals studied under the addendum, the data will be analyzed in an attempt to further define the efficacy of surfactant replacement after smoke exposure.

PRESENTATIONS/PUBLICATIONS

None.

REFERENCES

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Annual Research Progress Report for Fiscal Year 1991. San Antonio: Fort Sam Houston, pp 560–76, 1992.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

ACCESSION: DA346160

SUMMARY DATE: 921001 **SUMMARY KIND:** D **PREV DATE:** 911001 **DISTRIBUTION:** CX

PROGRAM #: 61102A **PROJ #:** 30161102BS14 **TASK AREA:** BC **WORK UNIT:** 327

TITLE: Intestinal Permeability Following Thermal Injury

SUBJ1: 060500 - Medicine and Medical Research

START DATE: 9003 **END DATE:** 9309 **PERFORMANCE METHOD:** C

CONTRACT/GRANT NO:

CONT TOTAL: $
CUM TOTAL: $
TOTAL LAB FUNDS: $

RESOURCES ESTIMATE

FY	WORK YRS	$(Thousands)
91	0.5	$33
92	0.5	$24
93	0.5	$35

RESPONSIBLE DOD ORGANIZATION: 404885
MRDC INSTITUTE OF SURGICAL RESEARCH USAISR
FT SAM HOUSTON, TX 78234
PRUITT, B A
210-221-2720

PERFORMING ORGANIZATION: 404885
MRDC INSTITUTE OF SURGICAL RESEARCH USAISR
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210-221-8440

ASSOC1: MCMANUS, W F

ASSOC2:

FOREIGN INTELLIGENCE CONSIDERED: Y **CIVILIAN APPLICABILITY:** M

KEYWORDS: RA II; Volunteers; Adults; Burns (Injuries); Septicemia; Intestines; Bacteria; Mannitol

OBJECTIVE: A DTIC literature search was conducted under DTIC request numbers W6L38M/W6L39L dated 9 January 1990. The objective of this work is to assess alterations in intestinal permeability in the acute phase of thermal injury. If alterations in intestinal permeability are identified, treatments designed to decrease intestinal permeability may improve the outcome of patients with thermal injury.

APPROACH: Twenty consecutive patients had intestinal permeability measured on postburn days 2, 4, 6, 8, 10, and 12; 10 healthy volunteers were individually studied for 2 consecutive days. Intestinal permeability was measured by administering lactulose and mannitol, two different molecular weight sugars which are absorbed via different mechanisms in the gastrointestinal tract. Lactulose/mannitol ratios were subjected to repeated measures ANOVA and multiple regression analysis to determine variation of lactulose excretion with burn size and postburn day. As the result of an addendum, 50 consecutive patients will have intestinal permeability measured each day for the first 5 days postburn. After postburn day 5, intestinal permeability will be measured every fourth day until postburn day 21. After postburn day 21, intestinal permeability will be measured once a week until discharge. Plasma samples will be obtained for determination of IL6 and endotoxin levels upon admission and every 5 h for the first 48 h after injury. After 48 h, samples will be obtained daily until postburn day 14, at which time weekly samples to coincide with the day of the permeability study will be obtained. Detailed data concerning the resuscitation regimen will be compiled. Diagnosis of infection will be made according to

standard criteria. For comparison purposes, 10 healthy volunteers will be individually studied for 2 consecutive days. A 3-ml blood samples will be drawn each day for endotoxin level determination.

PROGRESS: 9110-9209. Fifteen burn patients and 10 control subjects have been enrolled in this study to date, 7 burn patients during this reporting period. Patients who developed infection had a significant increase in intestinal permeability compared to other patients and normal controls, occurring well before the episode of infection. For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report* for fiscal years 1990 through 1992.

ABSTRACT

PROJECT NUMBER: 3M161102BS14-327, Research

PROJECT TITLE: Intestinal Permeability Following Thermal Injury

INSTITUTION: US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas 78234-5012,[1] and the US Army Medical Research Institute of Infectious Diseases, Fort Detrick, Frederick, Maryland 21702-5011[2]

PERIOD COVERED IN THIS REPORT: 1 October 1990 - 30 September 1991

INVESTIGATORS: William G. Cioffi, Jr., MD, Major, MC[1]
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Alterations in intestinal permeability have been postulated to occur after thermal injury. We evaluated the status of intestinal permeability during the first 2 weeks postburn in 15 patients with thermal injury by measuring the differential excretion of enterally administered lactulose and mannitol. The mean age and burn size of these patients were 32.7 ± 3.6 yr and 53.3% ± 5.1% of the total body surface area, respectively. Ten healthy volunteers were also studied. The lactulose-mannitol excretion ratio was 0.159 ± 0.017 for burn patients and 0.017 ± 0.003 for control subjects. The increased ratio did not correlate with burn size or postburn day. Patients who developed significant clinical infections during the first 2 weeks postburn had lactulose-mannitol ratios on postburn day 2 that were significantly higher than those of control subjects and patients who did not develop infections. This suggests a relationship between susceptibility to infection and early alterations in intestinal permeability.

Whether there is a causal relationship between early changes in intestinal permeability and infection cannot be ascertained from this preliminary study. In order to define this relationship more clearly, an addendum was written and approved by USAISR Research Council and US Army Institute of Surgical Research Human Use Committee during the fourth quarter of Fiscal Year 1991. When 50 patients authorized by the addendum have completed the study, the data will be analyzed.

INTESTINAL PERMEABILITY FOLLOWING THERMAL INJURY

Sepsis and multiple organ failure contribute significantly to morbidity and mortality after thermal injury. In recent years, the gastrointestinal tract has been implicated in the development of multiple organ failure. It has been proposed that enteric organisms, or their toxins, translocate across the intestinal mucosa, enter the systemic circulation, and contribute either directly or indirectly to the development of the hypermetabolic and catabolic responses associated with injury and sepsis.

Berg (1) has defined bacterial translocation as the passage of viable bacteria from the gastrointestinal tract to the mesenteric lymph nodes and other organs. Conditions which promote translocation include alteration of the indigenous gastrointestinal microflora leading to bacterial overgrowth, impaired host-immune defenses, and physical disruption of the gut mucosal barrier (2). Thermally injured patients may manifest all of these conditions during the course of their illness. Maejima et al (3) documented bacterial translocation within 2 days after injury in a rat model of thermal injury. Ziegler et al (4), using the lactulose/mannitol clearance assay, reported a threefold increase in intestinal permeability to lactulose in infected thermally injured patients but no alteration in permeability in uninfected burned patients. Patients in this study were examined on the average of 15–18 days after injury. However, those authors could not define whether the increased intestinal permeability was secondary to an infection superimposed upon the thermal injury or whether the infection was a result of altered intestinal permeability in the early postburn period.

MATERIALS AND METHODS

Study Population. Fifteen patients with burns > 20% of the total body surface area admitted to the US Army Institute of Surgical Research within 24 h of injury during the period 8 April 1990 through 23 August 1990 were enrolled in this study. These patients had no evidence of preexisting renal dysfunction, gastrointestinal disease, chronic alcohol abuse, or diabetes mellitus. Ten healthy volunteers served as normal controls. These control subjects were healthy, without any history of recent or remote gastrointestinal, cardiovascular, or renal disease, and none were taking medication at the time of study.

Burn patients and control subjects were studied beginning at 0800 hours. Patients were studied on postburn days 2, 4, 6, 8, 10, and 12. Control subjects were studied on 2 consecutive days. Control subjects and patients who were on an enteral oral diet were fasted for 6 h before study. Continuous small-bowel enteral feedings were continued in those patients in whom this therapy was

employed. Before administration of the test solution, the urinary bladder was emptied in all subjects.

Study Design. Intestinal permeability was assessed on postburn days 2, 4, 6, 8, 10, and 12 by simultaneous enteral administration of two different molecular weight sugars, mannitol and lactulose. On the day of study, a test solution consisting of 10 g lactulose and 5 g mannitol mixed in 60 ml distilled water was instilled via the patient's nasogastric tube or, in the case of control subjects and patients without nasogastric tubes, was orally administered. All urine was then collected for a 6-h period and refrigerated. At the completion of collection, the urine was divided into aliquots and frozen at -20°C before analysis.

Lactulose and Mannitol Assay. Urinary lactulose and mannitol concentration were simultaneously determined using GLC. This analytic method was employed because urine from thermally injured patients contains multiple fluorescent substances which interfere with the enzymatic assays usually employed for mannitol and lactulose measurement. We have previously reported the application of GLC for the measurement of these simple sugars (5). In brief, small diluted aliquots of urine were dried under nitrogen in a heating block at 75°C. After cooling, 100 µl of an oxime solution was added to convert the sugars to oximes before analysis. After 30 min incubation at 75°C, the samples were allowed to cool and 100 µl trimethylsilyl imidazole derivatizing reagent was added, followed by a 15-min incubation period at 75°C. Two microliters of the prepared sample were then injected into a DB-5 capillary column (ID = 15 M X 0.53 MM) installed in a GLC (Model 5859, Series II, Hewlett-Packard, Atlanta, GA). Injection temperature was set at 220°C with a detection temperature of 300°C and a flow rate of 9.7-9.9 ml/min.

Chromatographs of standard solutions containing mannitol and lactulose were run daily. Linearity of the standard curves for mannitol and lactulose was demonstrated. The minimum detectable concentrations for mannitol and lactulose in the urine were 5 nmol/l and 1 nmol/l, respectively. Urine samples were routinely spiked with mannitol and lactulose and recovery data indicated the accuracy of the assay. The test results were then expressed as milligrams of mannitol and lactulose excreted per 6-h period.

The amount of each sugar excreted in the urine over 6 h was then converted to a percentage of the amount of the sugar that had been enterally administered. The fraction of lactulose excreted was then indexed to mannitol excretion by dividing the lactulose excretion fraction by the mannitol excretion fraction yielding a permeability index (L/M ratio).

Statistical Analysis. Statistical analysis was carried out using the BMDP Statistical Program package. Unpaired t tests, ANOVA, and linear regression analyses were used as indicated.

Differences were considered significant at $P < 0.05$. All values are expressed as the mean ± SEM unless otherwise specified.

RESULTS

A preliminary study of 15 burn patients and 10 normal volunteers showed a significant increase in intestinal permeability to lactulose during the first two weeks postburn in patients who developed early infection. Patients who did not develop early infection had permeability indices which were not different from control subjects. In addition, these differences were present on postburn day 2, before development of clinical infection in any patient. Whether there is a causal relationship between early changes in intestinal permeability and infection could not be ascertained from this preliminary study. Therefore, an addendum was written to define more clearly this relationship in a larger group of patients.

In order to define this relationship more clearly in a larger group of patients, an addendum was submitted and approved by the USAISR Research Council and US Army Institute of Surgical Research Human Use Committee during the fourth quarter of Fiscal Year 1991. Five patients were enrolled in the amended study during this reporting period. There were 3 males and 2 females, with a mean age of 29.5 ± 2.5 yr and mean total body surface area burn size of 50.6 ± 6.4%.

DISCUSSION

Altered intestinal permeability has been documented in many clinical states to include celiac disease, Crohn's disease, and other intestinal mucosal disorders (6-7). Recently, it has been proposed that alterations in intestinal permeability may contribute to the hypermetabolic response after injury and to subsequent development of infection. To assess whether intestinal permeability is altered after thermal injury, we used two unmetabolized low molecular weight sugars; lactulose, a disaccharide with a molecular weight of 342, and mannitol, a monosaccharide with a molecular weight of 182. Normally, lactulose is poorly absorbed enterally. When absorbed, these sugars passively cross the gut, enter the circulation, remain unmetabolized, and are excreted by the kidney. Mannitol is reported to be absorbed via a transcellular pathway through aqueous pores in the cell membrane. Normally, 10-20% of an enteral load is absorbed. Lactulose, on the other hand, is larger and its absorption occurs via pericellular pathways across damaged tight junctions. Normally, lactulose is poorly absorbed enterally. Mucosal damage leading to altered intestinal permeability has a greater effect upon lactulose absorption and subsequent renal excretion than on mannitol. Factors other than permeability may influence the absorption and subsequent excretion of both sugars. These include gastric emptying, intestinal transit time, mucosal

surface area, cardiac output, and renal function. Since these factors affect each sugar equally, indexing excretion of one to the other controls for these factors unrelated to intestinal permeability.

We documented a 10-fold increase in intestinal permeability as measured by the L/M ratio in thermally injured patients during the first 2 weeks postburn. The increased ratio did not correlate with postburn day or burn size. Deitch (8) recently reported a threefold increase in intestinal permeability in thermally injured patients during the first 24 h postinjury using the L/M ratio. The significantly larger increase in intestinal permeability documented in our patients may be explained by the methods used for analysis of urinary lactulose and mannitol concentrations. We employed GLC to measure urinary lactulose and mannitol concentrations simultaneously. Previous work used the enzymatic method of Behrens et al (9) to measure lactulose content. Our laboratory, as well as others, have found that urine from patients with severe burns frequently contains multiple fluorescent substances which interfere with enzyme assays of lactulose content.

Only one previous study has addressed the relationship between altered intestinal permeability and infection in thermally injured patients. Ziegler et al (4), who studied patients 2 weeks after injury using the L/M ratio noted that only infected burn patients had altered permeability. However, due to the design of the study, no data were available for the first week postburn or for the time period immediately preceding the infection episode. Our data showed a significant increase in permeability occurring on postburn day 2, before infection, in those patients who ultimately became infected. On postburn day 2, uninfected patients had a permeability index which was not different from control subjects. Although in the subsequent time period, the L/M ratio increased in this population, it still was not statistically different from the control subjects. Whether this represents a Type II error cannot be discerned from our data, but the mean L/M ratio of the group remained statistically less than the infected group. Our finding of increased intestinal permeability before the episode of infection suggest, but do not prove, a causal relationship. Six of the 13 infections were caused by enteric organisms, 4 by Klebsiella species and 2 by Enterobacter species. The remaining infections were caused by Gram-positive organisms and other nonenteric Gram-negatives such as Serratia and Pseudomonas species.

The cause of the altered intestinal permeability in our patients who ultimately became infected remains unclear. Several hypotheses exist which may help explain this finding. It has been well documented in a canine model that intestinal mucosal blood flow is markedly decreased after thermal injury. At 1 h postinjury, Asch et al (10), using microsphere techniques, reported that mucosal flow was 40% of preinjury levels and returned to 70% of preinjury levels by 4 h after injury and fluid resuscitation.

Thus, the early increase in intestinal permeability documented by Deitch (8) and this study may be explained on the basis of an ischemia-reperfusion injury. Why only some of the patients developed this abnormality may be related to their resuscitation. Eight patients, all of whom developed an infection, were admitted approximately 24 h after injury. Detailed records of the resuscitation were not available for 3 patients, but 4 had a 4-h delay in resuscitation. The one remaining patient who developed an infection was transferred to our unit 12 h after injury and underwent a relatively uneventful resuscitation.

Winchurch et al (11) have noted a temporal relationship between systemic endotoxin levels and postburn day, with peak endotoxin levels measured on postburn days 3 and 4. Although there is a positive correlation between burn size and endotoxemia, not every burn patient develops endotoxemia during the postburn course. It is possible that a periresuscitation ischemia/reperfusion injury may result in endotoxemia only in those patients who develop significant alterations in intestinal permeability. Endotoxin has been shown to alter intestinal permeability in healthy laboratory controls and laboratory animals (12,13). The possibility exists that the sustained elevation in intestinal permeability may be secondary to the effects of endotoxin. Navaratnam et al (14) have evaluated the hemodynamic effects of endotoxin on the gastrointestinal tract. Using an ovine model, they reported that endotoxin increased mesenteric vascular resistance, resulting in a more than 50% reduction in mesenteric blood flow. Sheep receiving endotoxin had an 100% incidence of bacterial translocation to mesenteric lymph nodes, while only 15% of sheep not receiving endotoxin had similar findings. The exact mechanism by which endotoxin promoted increased intestinal permeability in these animals has not been elucidated. It appears, however, that ischemia/reperfusion injury may play a central role. Endotoxin-treated mice have been shown to have increased intestinal mucosal activities of xanthine oxidase and dehydrogenase after exposure. Inhibition of xanthine oxidase with allopurinol and inactivation with a tungsten diet has been shown to inhibit endotoxin-induced bacterial translocation (15,16).

We clearly demonstrated an increase in intestinal permeability to lactulose in a subset of thermally injured patients who subsequently developed an infection during their first 2 weeks postburn. It was not possible, from these data, to confirm a causal relationship, although these data suggest one. Recently Deitch et al (17) have reported that bacterial translocation from the gut results in significant impairment of systemic immunity. This impaired systemic immunity was characterized by a decreased mitogenic response of lymphocytes isolated from mice who were monoassociated with the *Escherichia coli* C25. In addition, the monoassociated mice were less able to control a local injection of *Staphylococcus aureus*, suggesting that the changes in mitogen responsiveness may be of biologic significance. Thus, the

increased permeability seen in our patients early in the postburn course before an episode of infection appears to be associated with the later occurrence of infection and may even be a contributing factor to the increased susceptibility to infection in burn patients.

After enrollment of an additional 50 patients authorized by the addendum, the data will be analyzed to more clearly define the relationship between early changes in intestinal permeability and infection.

PRESENTATIONS/PUBLICATIONS

LeVoyer T, Cioffi WG Jr, Pratt L, Shippee R, McManus WF, Mason AD Jr, and Pruitt BA Jr: Alterations in intestinal permeability after thermal injury. *Arch Surg* 127(1):26-30, January 1992.

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17. Deitch EA, Xu DZ, Qi L, Berg RD. Bacterial translocation from the gut impairs systemic immunity. *Surgery* 109:269-76, 1991.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

ACCESSION: DA346161

SUMMARY DATE: 920323 **SUMMARY KIND:** K **PREV DATE:** 911001 **DISTRIBUTION:** CX

PROGRAM #: 61102A **PROJ #:** 30161102BS14 **TASK AREA:** CK **WORK UNIT:** 328

TITLE: Mineral Absorption and Metabolism in a Burned Rat Model Using the Everted Gut Sacs Technique

SUBJ1: 060100 - Biochemistry
SUBJ2: 060500 - Medicine and Medical Research

START DATE: 9004 **END DATE:** 9203 **PERFORMANCE METHOD:** C

CONTRACT/GRANT NO:

CONT TOTAL: $
CUM TOTAL: $
TOTAL LAB FUNDS: $

RESOURCES ESTIMATE

FY	WORK YRS	$(Thousands)
91	0.5	$36
92	0.1	$14
93	0.0	$0

RESPONSIBLE DOD ORGANIZATION: 404885
MRDC INSTITUTE OF SURGICAL RESEARCH USAISR
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PERFORMING ORGANIZATION: 404885
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210-221-4858

ASSOC1: YOUNG, E

ASSOC2: OKERBERG, C V

FOREIGN INTELLIGENCE CONSIDERED: Y **CIVILIAN APPLICABILITY:** M

KEYWORDS: RA II; Lab Animals; Rats; Burns (Injuries); Metabolism; Nutrition; Zinc; Isotopes

OBJECTIVE: A DTIC literature search was conducted under DTIC request numbers W6R28L/W6R30I dated 9 January 1990. The objective of this work is to use the everted gut sac method to investigate the effect of burn injury on mineral absorption in a burned rat model. Results from this study will contribute to a better understanding of mineral metabolism during recovery from burn injury that will lend support to a better rationale for mineral supplementation in burned soldiers.

APPROACH: Lewis rats were administered either a 30% full-thickness scald or sham burn. At either 24 h or 5 days after burn injury, the rats were sacrificed and the small intestine was removed. The absorption of four radioactive-labeled nutrients was then assessed.

PROGRESS: 9004-9203. The gut sac technique was successfully developed in the rat model using radioactive zinc to study zinc absorption after burn injury. The technique demonstrated a decrease in zinc absorption 4 days postburn; however, by postburn day 8, the absorption was higher than that obesrved for nonburned rats. These findings agree wtih previous studies using different techniques to investigate the metabolism of zinc after burn injury. For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report* for fiscal years 1990 through 1992.

ABSTRACT

PROJECT NUMBER: 3M161102BS14, Research

PROJECT TITLE: Mineral Absorption and Metabolism in a Burned Rat Model Using the Everted Gut Sacs Technique

INSTITUTION: US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas 78234-5012,[1] and Department of Gastroenterology and Nutrition, University of Texas Health Science Center, 7703 Floyd Curl Drive, San Antonio, Texas 78284[2]

PERIOD COVERED IN THIS REPORT: 1 October 1990 - 30 September 1991

INVESTIGATORS: Ronald L. Shippee, PhD, Major, MS[1]
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Considering the hypermetabolic state of patients with severe burns, it would appear that above normal supplementation of essential minerals is warranted. However, little information is available to support a rationale for aggressive mineral supplementation. The present study used the everted gut sacs technique to determine the effect of burn injury on zinc absorption in a rat model. Although burn injury caused decreased absorption on days 1 and 4 postinjury, absorption reached normal levels by day 8. Data are consistent with previous results and the hypothesis that during burn injury, absorption of zinc is unobstructed and liver zinc stores are increased to insure adequate availability of this important trace element.

MINERAL ABSORPTION AND METABOLISM IN A BURNED RAT MODEL USING THE EVERTED GUT SACS TECHNIQUE

Considering the hypermetabolic state of patients with severe burns, it would appear that above normal supplementation of essential minerals is warranted. However, little objective information is available to support the rationale for this nutritional supplementation.

Some mix of parenteral and enteral feeding modalities is usually needed to meet the increased caloric requirements of patients with major burn injuries. The goal is usually to taper the parenteral nutrition as soon as practical, with a concomitant increase in enteral alimentation. There is a paucity of research concerning the effect of burn injury on gut absorption of essential minerals.

Mochizuki et al (1) found that burn injury had a detrimental effect on mucosal integrity in guinea pigs. They were able to prevent the depletion in mucosal integrity by immediate postburn enteral feeding. Carter et al (2) have used the everted gut sac transport technique to show the detrimental effect of burn injury on calcium, glucose, and leucine transport 24 h after burn injury in a rat model.

This report describes the results of initial studies involving the use of the everted gut sacs technique to study zinc absorption during recovery from a burn injury in a rat model.

MATERIALS AND METHODS

Study Design. Sprague-Dawley rats were maintained for 2 weeks on semipurified ration (Ziegler, Inc., PO Box 95, Gardners, PA 17324) designed to meet all known nutrient requirements of the adult rat. After the 2-week equilibration period, the animals received either a 30% full-thickness scald or sham burn. On day 1, 4, or 8 after burn injury, the animals were sacrificed and the small intestine was removed.

Description of Procedures.

Phase I. This phase was designed to determine the area of maximum zinc absorption along the small intestine. Four Lewis rats weighing ± 250 g were anesthetized and sacrificed by exsanguination. The small intestine was removed and placed in a cold buffer (125 mM NaCl, 10 mM fructose, 30 mM TRIS (ph 7.4 at 37°C), 0.5 mM $CaCl_2$, 1.2 mM MgCl). Starting at the pylorus, four lengths of small intestine measuring 10 cm were excised. Each segment was everted by inserting a small crochet hook through the segment and pulling a suture through the segment. One end was tied off and the segment everted by pulling the tied end gently through

the segment. The sac was filled with 1 ml buffer. A piece of polyethylene tubing with an inside diameter approximately the size a 25-ga needle was inserted into the sac and immobilized with a suture. The prepared gut sac was then inserted into a 25-ml Erlenmeyer flask with 8 ml of buffer containing 1 mM zinc and 0.05 μCi ^{65}Zn per milliliter. The flask was incubated at 37°C for 90 min in a shaking water bath under 100% oxygen. After incubation, contents of the sac were removed by aspiration through the plastic tube with a tuberculin syringe. The volume removed was recorded and expelled into a plastic test tube and placed in a gamma counter. The sac was placed on a preweighed plastic boat and placed in vacuum drying oven at 78°C for 4 h to determine dry tissue weight.

Phase II. After it was determined which 10-cm segment gave maximum zinc absorption, 18 male Sprague-Dawley rats were divided equally between burn and sham-burn groups. The dorsal surface was shaved and a 30% total body surface area scald burn (90°C for 5 sec) or sham burn (37°C) was administered. On postburn days 1, 4, and 8, 3 animals from each group were anesthetized and sacrificed by exsanguination. Sections of the small intestine measuring 10 cm were removed and prepared as described above.

Safety Considerations for Biohazards. Standard safety procedures as outlined in the Brooke Army Medical Center Radiation Safety SOP were followed. All biological tissues contaminated with radioactivity were placed in appropriate containers for disposal.

RESULTS

Phase I. Figure 1 demonstrates zinc absorption from individual gut segments. Maximum absorption occurred in the second 10-cm segment. This is in agreement with similar studies in rats reported by Kowarski et al (3). Maximum zinc absorption occurs in the upper portion of the small intestine.

Phase II. Table 1 demonstrates zinc absorption on postburn days 1, 4, and 8. Although burn injury inhibited zinc absorption on postburn days 1 and 4, by postburn day 8, absorption was comparable to control animals.

DISCUSSION

This is in agreement with our earlier reports concerning zinc metabolism in the burned rat model (4). We analyzed the protein bound zinc in mucosal intestinal and hepatic cytosol fractions, using gel column chromatography. On postburn day 10, there was a dramatic increase in zinc bound to the zinc storage protein, metallothioneine, in liver tissue. However, no increase in zinc bound to metallothioneine was seen in cytosol preparations from mucosal tissue.

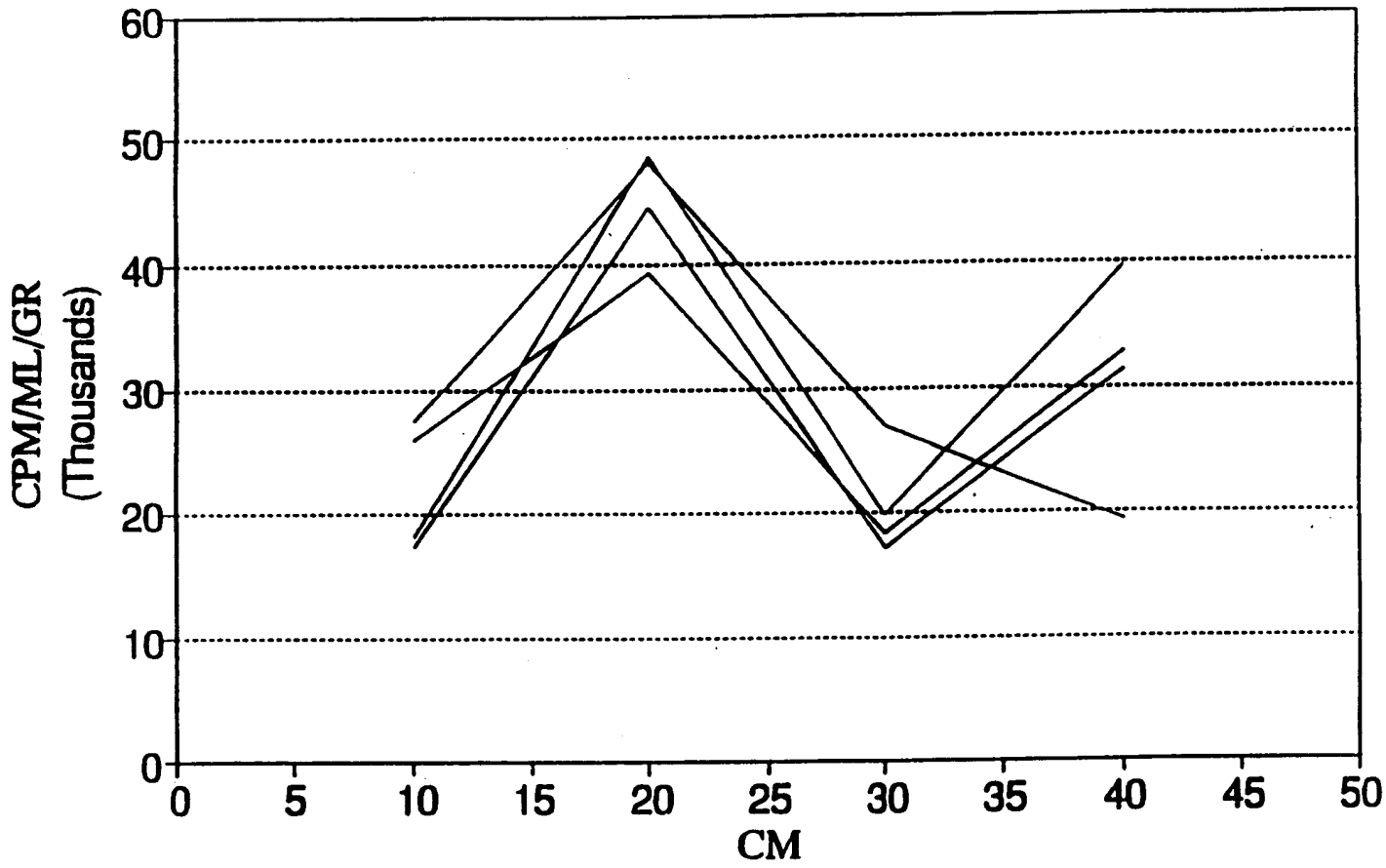


FIGURE 1. ^{65}Zinc absorption of 10-cm segments of small intestine from 4 sham-burn rats.

TABLE 1. Zinc absorption (cpm/ml per gram dry tissue) on postburn days 1, 4, and 8

Postburn Day	Control Group				Burn Group			
	Animal 1	Animal 2	Animal 3	Mean ± SEM	Animal 4	Animal 5	Animal 6	Mean ± SEM
1	13353	9423	36207	19661 ± 6818	5765	14569	17209	12514 ± 2825
4	15851	10882	17194	14642 ± 1567	9977	4860	8890	7909 ± 1271
8	16097	17367	28491	20652 ± 3214	17097	36062	23678	26882 ± 4373

Cousins (5) has proposed a role for intestinal metallothioneine in the excretion of zinc that is in excess of metabolic requirements. Intestinal metallothioneine is induced in response to zinc loading, binds excess zinc, and accumulates in the mucosal cells. Subsequently, the zinc bound to metallothioneine is lost when cells are sloughed into the lumen, thereby increasing fecal endogenous zinc excretion. Consistent with this suggested role of intestinal metallothioneine, it could be hypothesized that the lack of an increase in metallothioneine binding of zinc in the intestinal cytosol of the burned animals would ensure unobstructed zinc absorption and decrease obligatory loss of fecal zinc. Our earlier results (4) support this hypothesis in that total endogenous fecal excretion for 10 days postburn did not differ significantly between the burned and sham-burned rats. The present study showing a return to normal absorption by postburn day 8 gives further support to the hypothesis.

Our burned rat model is the first reported instance of a differential induction of zinc binding to metallothioneine in liver and mucosal tissue. Models using excess zinc or cadmium always cause increased metallothioneine induction in both tissue types. Early studies by Pekarek and Evans (6) showed that intraperitoneal injection of a crude preparation of heat-inactivated leukocytic endogenous mediator in rats caused increased absorption of ^{65}Zn in liver tissue. Leukocytic endogenous mediator has been purified and well characterized during the past few years and is now known to be the cytokine, interleukin 1. Interleukin 1 has been shown to alter metallothioneine gene expression in liver tissue and affect zinc metabolism (7). Burn injury is known to cause elevated interleukin 1 levels in both humans and animals. Hempe et al (8) recently reported that rat intestinal tissue was completely refractory to interleukin 1 induction of metallothioneine.

PRESENTATIONS/PUBLICATIONS

None.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

ACCESSION: DA335424

SUMMARY DATE: 911201 **SUMMARY KIND:** H **PREV DATE:** 911001 **DISTRIBUTION:** CX

PROGRAM #: 61102A **PROJ #:** 30161102BS14 **TASK AREA:** CH **WORK UNIT:** 330

TITLE: Donor-Specific Bone Marrow and Antithymocyte Preparations for Establishment of Selective Tolerance to Allografted Skin

SUBJ1: 060400 - Anatomy and Physiology
SUBJ2: 060500 - Medicine and Medical Research

START DATE: 9007 **END DATE:** 9112 **PERFORMANCE METHOD:** C

CONTRACT/GRANT NO:

CONT TOTAL: $
CUM TOTAL: $
TOTAL LAB FUNDS: $

RESOURCES ESTIMATE

FY	WORK YRS	$(Thousands)
91	0.5	$42
92	0.0	$0
93	0.0	$0

RESPONSIBLE DOD ORGANIZATION: 404885
MRDC INSTITUTE OF SURGICAL RESEARCH USAISR
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PERFORMING ORGANIZATION: 404885
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ASSOC1: CIOFFI, W G

ASSOC2: BECKER, W K

FOREIGN INTELLIGENCE CONSIDERED: Y **CIVILIAN APPLICABILITY:** M

KEYWORDS: RA II; Lab Animals; Rat; Burns (Injuries); Bone Marrow; Skin Grafts; Immunosuppression; Lymphocytes

OBJECTIVE: A DTIC literature search was conducted under DTIC request numbers W6L46K/W6L47I dated 31 May 1990. The objectives of this work were to demonstrate selective unresponsiveness to skin allografts in a rat model of thermal injury using antithymocyte preparations and donor-specific bone marrow, demonstrate the time course of restoration of the MLR to third-party cells following induction of tolerance in a rat burn model, and investigate the need for antithymocyte preparations in tolerance induction in the rat burn model.

APPROACH: Groups I and II were subjected to 30% total body surface area scald burns. Groups I underwent skin grafting from Brown Norway rat donors on the second postburn day. Group II was administered antithymocyte serum on the first postburn day, underwent skin grafting from a Brown Norway rat donor on the second postburn day, administered antithymocyte serum on the second postoperative day, and administered the bone marrow preparation from the Brown Norway rat donor on the seventh postoperative day. Each of these two groups underwent mixed-lymphocyte reaction surveillance at the time of burn injury, 1 week postinjury, and monthly thereafter.

PROGRESS: 9007-9112. Data for the control group (n=30) demonstrated a mean allograft acceptance time of 10.3 days. Animals in the treatment group receiving both antithymocyte globulin and bone marrow had an allograft acceptance time of 14.2 days. Though this shows a statistically significant increase in allograft acceptance time, the length of allograft prolongation is far less than would be

expected. For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report* for fiscal years 1990 through 1992.

ABSTRACT

PROJECT NUMBER: 3M161102BS14-330, Research

PROJECT TITLE: Use of Donor-Specific Bone Marrow and Antithymocyte Preparations for the Establishment of Selective Tolerance to Allografted Skin in a Rat Burn Model

INSTITUTION: US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas 78234-5012,[1] and Section of Transplantation, University of Alabama, Birmingham, Alabama 35294[2]

PERIOD COVERED IN THIS REPORT: 1 October 1991 - 1 December 1991

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Early excision and grafting of full-thickness burns has been shown to diminish the length of hospital stay, the incidence of infectious complications, and the cost of hospitalization. Definitive closure of thermal injuries greater than 40% of the total body surface area is limited by the availability of skin graft donor sites, necessitating multiple operative procedures and prolonging the hypermetabolic and immunosuppressive stresses faced by the thermally injured patient.

The ability of immunosuppression to prolong the take of skin allografts has been demonstrated in rats and humans. Unfortunately, this creates a state of nonspecific immunosuppression which increases the risks of infectious complications. There has been some success in inducing long-term and even permanent survival of skin allografts in mice with the use of antilymphocyte preparations and donor-specific bone marrow, essentially inducing a specific state of immune tolerance to allografted skin but ultimately restoring the organism's ability to respond to third-party allogeneic stimulation.

The objectives of this study were to demonstrate selective unresponsiveness to skin allografts in a rat model of thermal injury using antithymocyte preparations and donor-specific bone marrow, demonstrate the time course of restoration of the MLR to

third-party cells following induction of tolerance in a rat burn model, and investigate the need for antithymocyte preparations in tolerance induction in the rat burn model.

This study was completed during this reporting period. Data for the control group demonstrated a mean allograft acceptance time of 10.3 days. Animals in the treatment group receiving both antithymocyte globulin and bone marrow had an allograft acceptance time of 14.2 days. Though this shows a statistically significant increase in allograft acceptance time, the length of allograft prolongation was far less than expected.

USE OF DONOR-SPECIFIC BONE MARROW AND ANTITHYMOCYTE PREPARATIONS FOR THE ESTABLISHMENT OF SELECTIVE TOLERANCE TO ALLOGRAFTED SKIN IN A RAT BURN MODEL

Sir Peter Medawar introduced the concept of actively acquired immune tolerance in 1953. In a series of murine studies, he demonstrated that prolonged tolerance of skin allografts could be achieved through the introduction of allograft lymphoid preparations to the developing recipient fetus. He demonstrated that animals never develop, or do so to a limited degree, the ability to immunologically react to foreign antigens to which they have been exposed during fetal life. This involved a modulation of the host immune system, not antigen adaptation. Furthermore, strict paring of donor and recipients was shown to be essential in heterogenous strains of animals.

Subsequent murine studies by Monaco et al (1) demonstrated that nonspecific immunosuppressive states could be converted to specific states of unresponsiveness by the use of antilymphocyte preparations and donor lymphoid cells in adult murine recipients. Lance and Medawar (2) postulated that the antilymphocyte preparations created an environment of generalized unresponsiveness which potentiated the induction of specific immune tolerance to donor antigen when donor-specific lymphoid cells were administered.

Concerns of inducing graft versus host responses have been unwarranted. Multiple studies have examined the ability of various cell preparations to induce tolerance. Wood et al (3,4) demonstrated that renal, hepatic, and epidermal cells had no effect on augmenting allograft survival. However, bone marrow, lymphoid, and spleen cells all significantly augmented allograft survival when administered in combination with antilymphocyte preparations. Donor-specific bone marrow appears to be the most effective donor cell preparation for tolerance induction, probably as a consequence of the presence of both Class I and Class II major histocompatibility antigens in donor bone marrow. Wood and Monaco (5) further established that the timing of donor cell introduction was critical in achieving the optimal tolerogenic effect. Specifically, they demonstrated that effective specific unresponsiveness was achieved only when the donor cells preparations were given after treatments with antilymphocyte preparations and allografting. Earlier administration had little or no effect on improving graft survival.

Multiple investigators have studied the cellular element in bone marrow responsible for inducing the tolerance phenomenon. These studies have all implicated the T suppressor cell as being the responsible element for tolerance induction (6). It has been proposed that T suppressor cell clones are generated from antigen challenge presented by the allograft in the milieu of generalized immune unresponsiveness induced by antilymphocyte preparations.

Further administration of the donor-specific bone marrow creates a second set of T suppressor cell clones which contribute to prolonged allograft survival.

Wood and colleagues (7) also established that cyclosporine, when administered after administration of donor-specific bone marrow, had a synergistic effect with the antilymphocyte preparations in inducing specific unresponsiveness. Interest in applying these principles of immune tolerance in the human cadaveric transplant setting led investigators to evaluate the possibility of freezing the donor-specific bone marrow for subsequent administration. DeFazio and colleagues (8) demonstrated that freeze/thawed bone marrow was actually more effective than fresh bone marrow in inducing immune tolerance. It is thought that the freezing may bring about a partial fractionization of the bone marrow and that the lower temperatures increase the fragility of the polymorphonuclear cells as compared with the mononuclear cells. Previous studies had clearly demonstrated that the graft prolonging cells were recovered from the mononuclear-rich fraction of the bone marrow. These observations of immune tolerance have been extended to larger animal models. Caridis and colleagues (9) used mongrel dogs in a similar protocol of administering antilymphocyte preparations a week prior and a week following renal transplantation and subsequently administering donor-specific bone marrow several days following the transplant procedure. It was found that a combination of high-dose antilymphocyte serum and donor-specific bone marrow given on postoperative days 10 and 20 significantly improved survival time of renal allografts. Again, no graft versus host phenomenon was demonstrated. Further studies by Hartner and colleagues (10) demonstrated significant prolongation of renal allografts with this approach. Additionally, they demonstrated that the MLR to donor and third-party cells was uniformly depressed at 30-45 days following transplantation. However, by 60 days posttransplantation, the MLR to third-party cells was restored to normal, with the response to donor cells persistently depressed.

A primate study was undertaken by Thomas and colleagues (11) which was felt to be a logical step between the animal models previously presented and application in the clinical arena. Primates, being genetically similar to man and having similar major histocompatibility antigens and specificity of lymphocyte subset differentiation antigens were subjected to transplantation and subsequently administered donor-specific bone marrow on the 12th postoperative day. Again, this study demonstrated marked improvement in mean survival time with the antithymocyte preparation and marrow administration and again demonstrated a consistently negative MLR to donor cells but restoration of normal activity by 8 months posttransplant to indifferent allogeneic cells. A follow-up study (12) further supported the evidence that adjunctive immunosuppressive drugs improve allograft survival with

this protocol. The use of cyclosporine A and low-dose steroids increase mean survival time by 50% in a similar primate model.

Human application of these principles has been confined to the renal transplantation population. Monaco et al (13,14) used a donor-specific bone marrow protocol in five living related donor renal transplants who were all one haplotype high mixed-lymphocyte culture reactive. In this study, no graft versus host phenomenon was noted. Two of the patients demonstrated no rejection episodes and maintained serum creatinines between 1.5 and 2.0 with normal mixed-lymphocyte culture reactivity to third-party cells at 6 months but consistently suppressed mixed-lymphocyte culture reactivity to the specific donor. The third patient had excellent renal function for 6 weeks but, due to noncompliance on the immunosuppressive regimen, rejected his kidney. A fourth patient developed a positive cross-match and sustained a violent rejection episode on the 7th postoperative day prior to marrow administration. A fifth patient, who was removed from the study as a consequence of a cardiac arrhythmia, demonstrated normal mixed-lymphocyte culture reactivity to both third-party and specific donor cells by 6 months posttransplantation. The most recent and comprehensive application of these principles has been undertaken by Barber and colleagues (15). Twenty patients were entered in a donor-specific bone marrow protocol, 19 of whom were discharged with functioning grafts and 8 of whom were completely off steroids at 3-6 months following transplantation. The other patients in this protocol were on significantly lower doses of steroids as compared to the 20 patients receiving the contralateral kidney and conventional immunosuppressive regimens. A personal communication with this investigator has indicated that approximately 50 patients have been enrolled in the study, all being cadaveric transplant recipients. Current graft survival approaches 90% with this protocol versus a 78% 1-yr graft survival rate with conventional immunosuppressive regimens which include cyclosporine. Again, a significant decrease in immunosuppressive medications has been enjoyed.

Extensions of these observations in a burn model may provide a means to effect the early definitive and long-term closure of large thermal injuries and reduce infectious complications and the length of hospital stay (16). Evaluation of the ability to selectively establish unresponsiveness to skin allografts in a thermal injury model is of initial interest. Additionally, the time course for restoring immune responsiveness to third-party cells following induction of tolerance in the burn model may also have implications with respect to infectious complications. Further, the ability to utilize freeze/thawed preparations of donor bone marrow and skin for inducing immune tolerance has direction implication with respect to its potential clinical applications.

The objectives of this study were to demonstrate selective unresponsiveness to skin allografts in a rat model of thermal

injury using antithymocyte preparations and donor-specific bone marrow, demonstrate the time course of restoration of the MLR to third-party cells following induction of tolerance in a rat burn model, and investigate the need for antithymocyte preparations in tolerance induction in the rat burn model.

MATERIALS AND METHODS

Study Design. This study involved two groups. Group I was subjected to a 30% total body surface area burn and underwent skin grafting from Brown-Norway rat donors on the second postburn day. Group II was subjected to a 30% total body surface area burn, administered antithymocyte serum on the first postburn day, underwent skin grafting from a Brown-Norway rat donor on the second postburn day, administered antithymocyte serum on the second postoperative day, and administered the bone marrow preparation from the Brown-Norway rat donor on the seventh postoperative day.

Description of Procedures. In Group I (n=40), 20 Lewis rats were anesthetized with sodium pentobarbital (35 mg/kg IP), the dorsal surface was shaved, and a 30% total body surface area scald burn (90°C for 5 sec) was administered using the Walker-Mason method. Two days later, they underwent excision of the burn wound to fascia and skin grafting from a group of Brown-Norway rat donors. Nonadherent gauze was applied to the skin graft site and secured with self-adherent elastic wrap. On postoperative day 7, the dressings were removed and the skin grafts assessed on a daily basis by tactile and visual inspection.

In Group II (n=40), 20 Lewis rats were anesthetized with sodium pentobarbital (35 mg/kg IP), the dorsal surface was shaved, and a 30% total body surface area scald burn (90°C for 5 sec) was administered using the Walker-Mason method. They were then administered antithymocyte serum (2 cc IP) on the first postburn day. On the second postburn day, the wounds were excised to fascia and skin grafts from Brown Norway rat donors were applied as described for Group I. Two days after application of the graft, another dose of antithymocyte serum (2 cc IP) was administered and a standard bone marrow preparation of 5×10^7 viable nucleated cells from the Brown Norway rat donor were administered intravenously with a 25-ga needle through the dorsal penile vein on postoperative day 6. Wounds were examined on postoperative day 7 and then daily by tactile and visual inspection for rejection of the graft. When < 10% of the graft remained, it was considered total rejection.

Data Analysis Plan. Descriptive statistics and life-table analyses of survival data were performed.

RESULTS

The study was completed during this reporting period. In the early phase of this study, problems were encountered with the administration of the antithymocyte globulin. Specifically, death occurred after administration and necropsy revealed this to be the consequence of massive hemolysis. This necessitated the incubation of the antithymocyte globulin with rat RBCs to precipitate the offending antibodies and then subsequent purification of the antithymocyte globulin. Using this approach, no toxic reactions were subsequently noted with the administration of the antithymocyte globulin.

This study revealed the mean graft survival time in animals receiving only the allogeneic skin graft was 10.3 ± 0.41 days. Group II animals receiving antithymocyte preparations and donor-specific bone marrow had a mean graft survival time of 14.2 ± 0.37 days. The P value less than 0.001 on pool t testing. A life-table analysis of the two groups was performed and the graphic presentation of this data is presented in Figure 1.

DISCUSSION

Though the data demonstrates a statistically significant increase in allograft acceptance time, the length of allograft prolongation was far less than expected.

PRESENTATIONS/PUBLICATIONS

None.

REFERENCES

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5. Wood ML, Monaco AP: The effect of timing of skin grafts on subsequent survival in ALS-treated, marrow-infused mice. *Transplantation* 23:78-86, 1977.

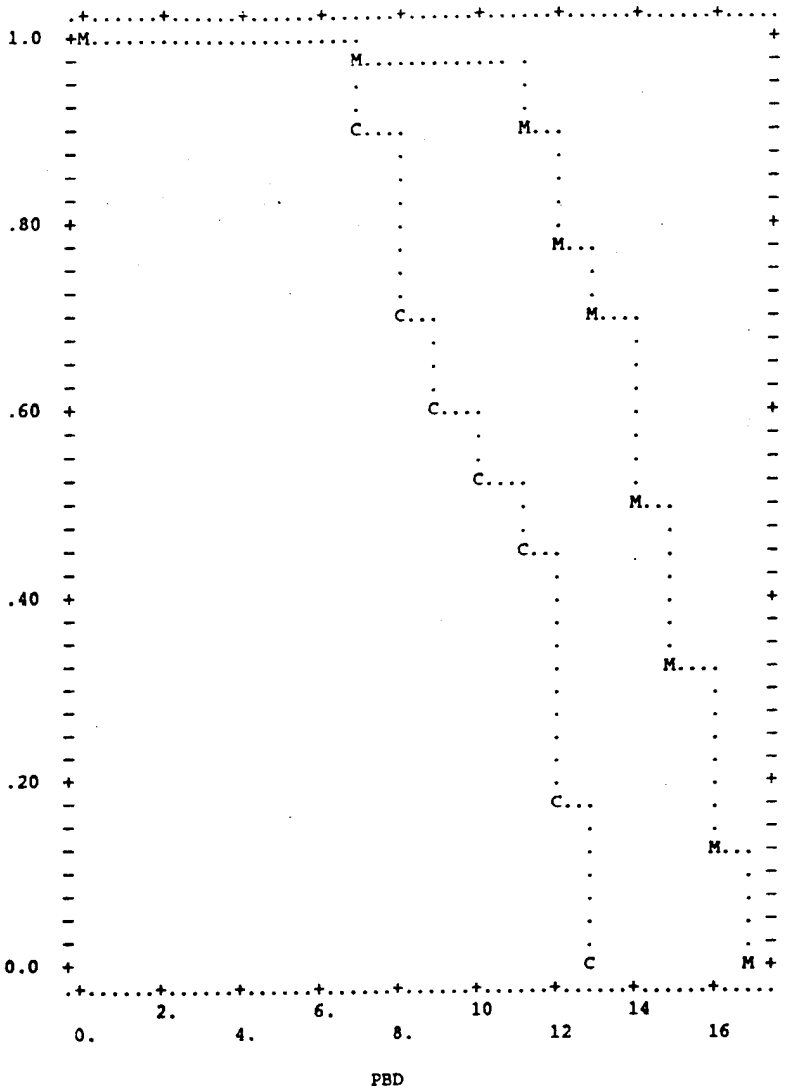


FIGURE 1. Cumulative proportion surviving. C indicates group receiving allogeneic skin grafts only; M, group receiving antithymocyte preparations and donor-specific bone marrow.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

ACCESSION: DA335423

SUMMARY DATE: 921001 **SUMMARY KIND:** D **PREV DATE:** 911001 **DISTRIBUTION:** CX

PROGRAM #: 61102A **PROJ #:** 30161101A91C **TASK AREA:** EC **WORK UNIT:** 075

TITLE: Development of a Rat Model of Inhalation Injury - A Pilot Study

SUBJ1: 060500 - Medicine and Medical Research

START DATE: 9006 **END DATE:** 9309 **PERFORMANCE METHOD:** C

CONTRACT/GRANT NO:

CONT TOTAL: $
CUM TOTAL: $
TOTAL LAB FUNDS: $

RESOURCES ESTIMATE

FY	WORK YRS	$(Thousands)
91	0.5	$48
92	0.5	$31
93	0.5	$33

RESPONSIBLE DOD ORGANIZATION: 404885
MRDC INSTITUTE OF SURGICAL RESEARCH USAISR
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210-221-2720

PERFORMING ORGANIZATION: 404885
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ASSOC1: CIOFFI, W G

ASSOC2: MC MANUS, A T

FOREIGN INTELLIGENCE CONSIDERED: Y **CIVILIAN APPLICABILITY:** M

KEYWORDS: ILIR; Lab Animals; Rats; Burns (Injuries); Inhalation; Lung; Carbon Monoxide; Hydrochloric Acid; Mortality

OBJECTIVE: A DTIC literature search was conducted under DTIC request numbers W6R05I/W6R06K dated 29 May 1990. The objective of this work is to develop a reliable small animal model of smoke inhalation injury. This model will be used to study interventions and treatments of smoke inhalation injury.

APPROACH: A smoke delivery system was developed for the In-Tox™ small animal exposure device, to include a furnace, an air delivery pump, an in-line carbon monoxide monitor, a mixing and cooling chamber, and a temperature monitor. Initial studies will be performed to ensure uniform smoke and hydrochloric acid exposures. Animal studies will include a time-dose mortality curve and measurement of blood carbon monoxide levels. At various times after exposure, histopathological examination of the lungs will be performed.

PROGRESS: 9110-9209. The smoke-generating apparatus was totally redesigned during the last reporting period to decrease the early mortality associated with carbon monoxide poisoning noted with the original exposure device. The use of this new device has resulted in the development of a reproducible murine model of smoke inhalation injury. Current studies are detailing the extent of injury using histopathology, electron microscopy, and biochemical analyses. For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report* for fiscal years 1990 through 1992.

ABSTRACT

PROJECT NUMBER: 3A161101A91C-075, In-House Laboratory Independent Research

PROJECT TITLE: Development of a Rat Model of Inhalation Injury - A Pilot Study

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

PERIOD COVERED IN THIS REPORT: 1 October 1991 - 30 September 1992

INVESTIGATORS: William K. Becker, MD, Lieutenant Colonel, MC
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Loring W. Rue, III, MD, Major, MC
Albert T. McManus, PhD
Basil A. Pruitt, Jr., MD, Colonel, MC

The objective of this project is to develop a small animal (rodent) model of smoke inhalation injury. The model should provide a reproducible pathologic and physiologic response to smoke exposure and permit exposure of sufficient numbers of animals to the same, or similar, smoke conditions to yield statistically meaningful results. The model is intended as a vehicle for the study of interventions or treatments of smoke inhalation injury.

DEVELOPMENT OF A RAT MODEL OF INHALATION INJURY - A PILOT STUDY

Up to one-third of patients admitted to a burn center will have sustained inhalation injury. Such injury may increase the mortality expected based on age and burn size alone by up to 20% (1). In addition, inhalation injury predisposes a thermally injured patient to pneumonia and pulmonary failure. At present, treatment for inhalation injury is primarily supportive; treatment regimens commonly include humidified air, supplemental oxygen, endotracheal intubation, and mechanical ventilation (2). Endobronchial secretions are monitored for signs of infection and chest radiographs are routinely taken to determine the presence or absence of pneumonia. There are at present no clinically proven treatment methodologies to eliminate or ameliorate the pathophysiologic response to inhalation injury.

The pathophysiology of smoke inhalation injury is under active investigation in several laboratories (3,4). Experimental animals commonly used in these investigations include the dog, sheep, and goat (5-7). Research in this area has almost uniformly been confined to large animals. Several early attempts to develop small animal models of smoke injury appear to have been abandoned (8). Difficulties encountered in utilizing large animals to study such injury include cost, lack of suitable resources, and difficulty in carrying out sufficient numbers of studies to yield statistically valid results. This Institute was instrumental in developing and investigating small animal models of cutaneous thermal injury and burn wound infection. These models have permitted economical, efficient investigation of burn treatment modalities that have subsequently proved clinically useful. It is desirable to develop a similar small animal model of inhalation injury. Such a model can be combined with the well developed models of cutaneous thermal injury to permit efficient, economical exploration of treatment modalities addressing the morbidity and mortality associated with smoke inhalation.

As previously noted, experimental models of smoke inhalation injury have been largely confined to large animals. Small animal (rodent) models of smoke injury have been hampered by several factors. Among these are the large, efficient rodent upper airway filtration system, which impedes the delivery of smoke particulates to the major airways, and what is termed "huddling" behavior, exhibited by rodents during group exposure, in which the animals appear to use the fur of other animals as a respiratory filter. In addition, the small relative airway size limits distal deposition of particulates, as does the rodents' capacity for prolonged breath-holding when exposed to toxic materials in the atmosphere. During short exposures, this latter ability permits the animals to avoid significant pulmonary exposure to toxic materials. This investigation attempts to overcome these obstacles and produce a reliable, efficient rodent model of inhalation injury.

MATERIALS AND METHODS

The initial approach to this project utilized two parallel tracks. The first involved direct instillation of smoke into the trachea of Sprague-Dawley rats. Rats weighing approximately 300 g were anesthetized with sodium pentobarbital (35 mg/kg IP) and underwent direct endotracheal intubation using 21-ga catheters. Smoke was generated by burning a complex fiber and plastic pad in a galvanized steel container, and was directed to a large stainless steel respirometer, where it was stored and used within 10 min of generation. The anesthetized and intubated animals were exposed to the smoke for 1-2 min. The smoke was delivered to the endotracheal tubes by use of a 2-l anesthesia bag with a suitable fitting for the endotracheal tube. Animals were followed for 24-48 h following exposure, surviving animals were sacrificed and the lung was removed, fixed in formalin, and examined by light microscopy. Blood carboxyhemoglobin levels were also measured in selected animals.

The second track involved exposure of multiple groups of Sprague-Dawley or Lewis rats in a nose-only exposure manifold which was constructed of aluminum and provided the capability to expose 24 animals to a single source of smoke at one time. The aluminum manifold had intake and exhaust ports for smoke delivery and nose-only exposure ports for 24 animals. Animals were confined in plexiglass tubes, their noses protruding into a brass nose cone which was fitted to the exposure port of the manifold. In these studies, smoke was also generated in a galvanized steel container by the combustion of fiber and plastic pads, collected in a large stainless steel respirometer, and used within 20 min of generation. Animals were loaded into the plexiglass exposure tubes, placed in the exposure manifold, and allowed a period of time for equilibration prior to smoke exposure. Smoke stored in the respirometer was then delivered into the intake port of the manifold and timed exposures were performed. Surviving rats were observed for 24-48 h. Histopathologic examination of the lungs was performed. In addition, carboxyhemoglobin levels were measured in selected animals.

Results from the first two tracks of this investigation indicated a substantial problem with early mortality due to carbon monoxide poisoning. Because the mortality from carbon monoxide poisoning limited exposure time, it was necessary to modify the exposure apparatus to minimize carbon monoxide toxicity. In addition, it was also noted that the quality of smoke generated under various atmospheric conditions appeared somewhat variable. Because one of the goals of this research project is a reproducible and uniform injury, it was elected to further modify the smoke-generating apparatus to eliminate variables associated with the lack of control of combustion. To accomplish this, the entire smoke-generating apparatus and the exposure devices were redesigned. The smoke-generating apparatus was changed to a

microprocessor-controlled retort furnace. The microprocessor programs the rate of temperature rise and can hold the furnace at a preset final temperature. The furnace itself can contain up to 2 l of combustible material and has a maximum temperature of 1200°C. Air flow into the retort is produced by a pump capable of delivering flows of 0–50 l/min. Air is dried by passage through calcium carbonate crystals prior to entry into the retort. The combustion or pyrolysis products are delivered from the retort to the manifold intake port. The manifold has been placed inside a glove box with exhaust from the manifold directed through a filter. These last changes were undertaken to minimize exposure of the investigators to combustion products and to minimize the release of combustion products to the environment. In order to evaluate the toxicity of various combustion products, cotton fabric, polystyrene, polytetrafluoroethylene, polyvinyl chloride, and cedar wood chips have been evaluated. Flow settings through the retort have varied between 10 and 20 l/min. In addition, oxygen can be delivered to the manifold at rates of up to 15 l/min. It is felt that by delivering oxygen during the exposure period, the risk of early morbidity from carbon monoxide poisoning can be minimized. Timed exposures of small groups of Lewis rats have been performed.

RESULTS

The animals that were anesthetized, intubated, and exposed to smoke demonstrated a mild parenchymal injury when examined by light microscopy 48 h after smoke exposure. Smoke exposure lasted 1 min and histopathologic examination revealed tracheal erosion in 62% (10 of 16), atelectasis and congestion in 88% (14 of 16), and pneumonia in 37% (6 of 16). There was a linear relationship between exposure time and the level of carboxyhemoglobin in the blood. At 0.5 min postexposure, the carboxyhemoglobin level was 21% ± 8.6%, at 1 min, it was 38.3% ± 10.8%, and at 1.5 min, it was 55% ± 11.1%. In this model, it was noted that early mortality due to carbon monoxide poisoning developed after approximately 2.5 min of smoke exposure. In addition, the technique of endotracheal intubation unavoidably introduces oropharyngeal bacteria into the trachea. One of the goals of this model is to evaluate subsequent development of pneumonia in these animals, and such unavoidable contamination may compromise analysis of this process. Because of the limiting features of carbon monoxide toxicity and the introduction of oropharyngeal bacteria into the trachea, further pursuit of this line of investigation has been abandoned.

In rats exposed to smoke in the nose-only exposure tubes attached to the aluminum exposure manifold, exposure times > 25 min were associated with unacceptable carbon monoxide toxicity. There was, however, a linear relationship between exposure time and the level of blood carboxyhemoglobin. Lungs from surviving animals were subjected to histopathologic examination at 24 and 48 h. Minimal tracheal and parenchymal injury was noted. The limiting

factor in this phase was exposure time, which was limited to 20–25 min by the occurrence of carbon monoxide poisoning and death.

The new exposure device with the microprocessor-controlled furnace and smoke delivery system has been tested in 65 animals in 15 exposure experiments. Complete histopathologic data are now available for 20 animals. Using cotton fabric and polystyrene as the pyrolysis compounds and a furnace temperature of 300°C, mild to moderate pulmonary injury was noted in 60% of animals exposed for 30 min. Polyfluorotetraethylene, pyrolyzed at 560° caused injury in 80% of exposed animals.

As noted, a mild to moderate histopathologic injury has been observed in many of the animals. This injury, however, has not been lethal. Carboxyhemoglobin levels were measured in selected animals; none exceeded 45% despite exposures of up to 45 min. It appears that the new smoke-generating device and exposure apparatus minimize the risk of carbon monoxide poisoning and permit mild to moderate histopathologic pulmonary parenchymal injury. With further refinement of the exposure technique and other combustion and pyrolysis products, we anticipate a more significant histopathologic injury that will result in a measurable level of mortality.

Work performed under the most recent addendum to this protocol indicates the following exposure regimen produces a parenchymal pulmonary injury. The compound for pyrolysis is PTFE (1.2 g). The furnace temperature is 600°C, the airflow through the retort is 20 l/min, and oxygen flow at the manifold is 8 l/min. In small group animal exposures using this regimen at 30 min of exposure time, a mild to moderate parenchymal injury is produced with a mortality rate of approximately 25%. At an exposure time of 40 min, a moderate to severe parenchymal injury is produced, with mortality rates ranging from 50% to 100%. All mortality occurred at time points greater than 30 min postexposure. Early mortality due to carbon monoxide poisoning has been completely eliminated by the introduction of higher manifold flows and oxygen to the manifold. Carboxyhemoglobin levels in the animals immediately postexposure have ranged from 10% to 15%. PTFE and related perfluoropolymers such as tetrafluoroethylene or hexafluoropropylene copolymer have previously been demonstrated to produce pulmonary toxicity after pyrolysis exposure (9,10). These compounds are widely used in synthetic fabrics and as insulators in electrical wiring and computer devices (11). Pyrolysis of this compound results in particle sizes that are very small (< 1 μm). The small particle size appears to eliminate the problem seen in exposing rodents to other types of smoke, where many of the larger particles appear to be filtered in the upper airway. Previous histopathological studies after exposure to fumes from PTFE have been characterized by the development of alveolar and interstitial edema, intraalveolar hemorrhage, fibrin deposition, and an inflammatory infiltrate.

Pulmonary injury after exposure to PTFE fumes is limited to the alveoli in the present rat inhalation injury model. The injury appears as acute damage of alveolar epithelial cells secondary possibly due to exposure to hydrogen fluoride. Airway injury as observed in patients with smoke inhalation does not occur in this model. Particles are major components of smoke which carry noxious chemicals to the airways. Synthetic smoke generated from a mixture of chemical and carbon particles was reported to induce airway injury in a rat model (13). The size of the particles is one important factor which determines the extent and site of inhalation injury. Since the size of PTFE fume is less than 0.1 μm and is delivered to the alveoli, larger particles may cause more proximal airway injury. A mixture of PTFE fumes and carbon particles (5 μm) may be suitable to induce both airway and pulmonary injury. Therefore, an addendum was developed to allow modification of the rat model of smoke inhalation injury in order to produce airway injury.

As a result of the addendum, 100 male Sprague-Dawley rats weighing 350 to 380 g were studied. Smoke was a mixture of two different streams. One stream consisted of a constant flow of PTFE fumes and the other, compressed air through an inverted flask filled with carbon particles (X 5 μm, DARCO G-60, Fisher Scientific Company). These two streams were mixed before entering the exposure chamber. Animals were exposed at the conditions described above and then randomized to one of four groups. These groups were observed for 1, 2, 3, or 7 days after exposure and mortality was recorded. Animals surviving to the end of the assigned observation period were sacrificed as described in the parent protocol and histologic evaluation of airway and lung parenchyma were conducted by light microscopy. Measurements for wet-to-dry lung weight ratio and conjugated diene levels in plasma and lung homogenate as well as analyses for WBC, PMN counts, and total protein content in bronchoalveolar lavage fluid were used to evaluate the severity of inhalation injury.

DISCUSSION

Preliminary results failed to demonstrate evidence of gross or histopathologic airway or parenchymal injury in rats receiving 5-μm carbon particles or PTFE smoke combined with 5-μm carbon particles. Possible explanations for this lack of injury include the inactivation or pyrolysis-generated toxins by carbon binding and the efficient nasal filtration of ≥ 5 μm carbon particles.

The immediate goal of this project is evaluation of a wide variety of exposure regimens and combustion products to identify those which produce moderate to severe parenchymal injury and measurable mortality. Once this has been accomplished, it is our intention to define the nature of the injury by measuring lung water gravimetrically and to refine the histopathologic assessment of injury using morphometry and electron microscopy. Once a

well-defined injury has been identified, we plan to combine smoke exposure with cutaneous thermal injury and to expose smoke-injured animals to aerosols of bacteria to identify the role that smoke exposure plays in the development of pneumonia.

PRESENTATIONS/PUBLICATIONS

None.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

ACCESSION: DA335691

SUMMARY DATE: 920323 **SUMMARY KIND:** K **PREV DATE:** 911001 **DISTRIBUTION:** CX

PROGRAM #: 61102A **PROJ #:** 30161101A91C **TASK AREA:** CK **WORK UNIT:** 076

TITLE: Effect of Silver Sulfadiazine on Copper Status in Rats with Thermal Injury

SUBJ1: 060100 - Biochemistry
SUBJ2: 060500 - Medicine and Medical Research

START DATE: 9011 **END DATE:** 9203 **PERFORMANCE METHOD:** C

CONTRACT/GRANT NO:

CONT TOTAL: $
CUM TOTAL: $
TOTAL LAB FUNDS: $

RESOURCES ESTIMATE

FY	WORK YRS	$(Thousands)
91	0.4	$21
92	0.1	$14
93	0.0	$0

RESPONSIBLE DOD ORGANIZATION: 404885
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ASSOC1: BOOSALIS, M G

ASSOC2: MC CLAIN, C J

FOREIGN INTELLIGENCE CONSIDERED: Y **CIVILIAN APPLICABILITY:** M

KEYWORDS: ILIR; Lab Animals; Rats; Burns (Injuries); Sulfadiazine; Copper; Tissue Extracts

OBJECTIVE: A DTIC literature search was conducted under DTIC request numbers W6R04J/W6R06J dated 9 October 1990. The objective of this work is to determine the effect of topical silver sulfadiazine on tissue copper concentration in a rat burn model.

APPROACH: Male Sprague-Dawley rats received either 30% full-thickness scald or sham burns. Animals randomized to the silver sulfadiazine groups had silver sulfadiazine cream applied over the dorsal area daily. At 7 days postburn, serum samples were collected and analyzed for copper, zinc, silver, and ceruloplasmin concentrations. The liver, femur, testes, and kidneys were excised and processed for determination of copper, zinc, and silver concentrations. Data were analyzed using a 2-by-2 factorial ANOVA.

PROGRESS: 9011-9203. The initial study showed that silver was absorbed through the skin as shown by elevated serum silver concentrations. Both burned and sham-burned animals receiving the silver sulfadizine cream had significantly lower serum copper and ceruloplasmin concentrations. Because high levels of zinc are known to interfere with copper metabolism and zinc supplementation is often included in burn patient TPN and diet regimen, a 2 X 2 X 2 factorial study was designed to investigate the interaction fo burn injury, topical silver sulfadiazine, and high or normal zinc intake on serum copper and selenium status. Although the sample size of this study was not high enough to determine statistical significance, there did not appear to be an

effect of the high zinc level. The effect of silver sulfadiazine on copper metabolism was confirmed and an additional finding that the silver sulfadiazine affected RBC selenium and RBC selenium-dependent glutathione peroxidase activity was shown. For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report* for fiscal years 1991 and 1992.

ABSTRACT

PROJECT NUMBER: 3A161101A91C-076, In-House Laboratory Independent Research

PROJECT TITLE: Effect of Silver Sulfadiazine on Copper Status in Rats with Thermal Injury

INSTITUTION: US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas 78234-5012;[1] General Clinical Research Center, University of Southern California School of Medicine, Los Angeles, California 90033;[2] and Department of Medicine, University of Kentucky, Lexington, Kentucky 40536[3]

PERIOD COVERED IN THIS REPORT: 1 October 1991 - 23 March 1992

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The objective of this study was to determine the effect of topical AgSD on copper metabolism after thermal injury in a rat model. After 7 days of topical silver sulfadiazine treatment, plasma silver levels were significantly elevated in both burn and control animals. Treatment caused lower plasma copper and ceruloplasmin levels but had no significant effect on plasma zinc levels. These findings are of possible clinical significance. A survey of burn units in the United States by this Institute showed that many units supplement patients having large burns with high levels of zinc. Given the fact that zinc lowers copper absorption, some caution may be advisable if copper status is affected by silver sulfadiazine as suggested by this animal model.

EFFECT OF SILVER SULFADIAZINE ON COPPER STATUS IN RATS WITH THERMAL INJURY

Considering the increased protein and caloric needs accompanying thermal injury, aggressive trace mineral supplementation may be warranted. Very little experimental evidence to support such a regimen is available, however, and contradictions exist between the results of clinical and animal research.

Copper status may be of particular concern during recovery from thermal injury. The cuproenzyme, lysyl oxidase, is required for the oxidation of peptidyl lysine, a step necessary for the cross-linking of collagen, and may be of significance in wound healing in the burn patient. Ceruloplasmin, the major protein carrier of copper in the blood, has ferroxidase activity that is required for the normal utilization of iron and subsequent synthesis of hemoglobin.

Cohn et al (1) measured copper levels in 16 thermally injured patients and found that 15 had serum copper levels within normal limits. Most of these determinations, however, were performed 1–6 months postburn and the percent burn was relatively low. Shakespeare (2) observed normal serum copper levels in 11 patients with 8–60% total body surface area (TBSA) burns. Boosalis et al (3) and Sanchez-Agreda et al (4) reported lower than normal serum copper levels in the early stages of burn recovery in patients with 20–40% TBSA burns. In both studies, serum levels returned to normal 2–3 weeks postburn. Sanchez-Agreda et al (4) also report that patients with > 60% TBSA burns had subnormal serum copper levels 2 weeks postburn. Boosalis et al (3) found subnormal serum copper and ceruloplasmin concentrations in patients with large burns (> 60% TBSA burns) up to 5 weeks postburn. Urinary excretion of copper was significantly higher ($P < 0.01$) 3 weeks postburn in patients with > 60% TBSA burns compared to those patients with < 40% TBSA burns.

An additional concern that may be unique to the burn patient is the known antagonistic relationship between silver and ceruloplasmin synthesis (5–7). Silver sulfadiazine is an effective topical antimicrobial agent used to reduce infectious complications in patients with burn wounds. Boosalis et al (8) found that thermally injured patients treated with topical silver sulfadiazine had elevated serum silver levels. Those patients with > 60% TBSA burns had the greatest elevation in serum silver levels as well as the greatest and most prolonged depression of ceruloplasmin levels. In addition, in human and animal studies, increased tissue concentrations of silver from silver sulfadiazine applied to burn wounds have been reported (9–11). However, the effect of increased silver absorption on copper metabolism during recovery from thermal injury remains to be investigated. The objective of this study was

to determine the effect of topical silver sulfadiazine on tissue copper concentration in the burned rat.

MATERIALS AND METHODS

Study Design. Male Sprague-Dawley rats received either 30% full-thickness total body surface area scald burns (n=12) or sham burns (n=12). Six animals from each group had silver sulfadiazine cream applied over the dorsal area daily. Seven days postburn, the animals were sacrificed and serum samples were analyzed for copper, zinc, silver, and ceruloplasmin concentrations. The liver, femur, testes, and kidneys were excised and processed for determination of copper, zinc, and silver concentrations.

Description of Procedures. Twenty-four male Sprague-Dawley rats (Harlan Sprague-Dawley, Inc., Houston, TX) weighing 200-250 g were housed in individual stainless steel cages and maintained on Purina Chow™ and deionized water ad libitum. The animals were maintained on a 12:12 light:dark schedule. On the day of the study, the animals were anesthetized with sodium pentobarbital (35 mg/kg IP) administered through a 25-ga needle. The dorsal area was shaved. Animals were placed in a plexiglass mold designed to expose 30% of the total body surface area. Animals in the 30% burn groups (n=12) were exposed to 100°C water for 10 sec while animals in the sham-burn groups (n=12) were exposed to water at room temperature. Animals randomized to the silver sulfadiazine groups had approximately 3 g of silver sulfadiazine cream (Silvadene® Cream 1%, Marion Merrell Dow Inc., Kansas City, MO) applied over the dorsal area daily. Seven days postburn, the animals were again anesthetized with sodium pentobarbital (35 mg/kg IP). A ventral laparotomy was performed and the animals were exsanguinated via the caudal vena cava.

Zinc and copper were determined in plasma diluted 1:3 with 20% TCA. Tubes were heated at 85°C for 1 h, centrifuged, and the supernatant aspirated into an atomic absorption spectrophotometer. Silver and selenium were determined in plasma diluted 1:10 and 1:100, respectively, with 0.1N nitric acid and aspirated into a graphite furnace atomic absorption spectrophotometer.

Ceruloplasmin was determined as described by Curzon and Vallet (12). One milliliter of plasma was diluted with 1 ml H_2O and 2 ml 0.2 M sodium acetate (pH 5.5). One milliliter of N-dimethyl-p-phenylenediamine was added to each tube and change in absorbance monitored at E_{550} nm. One unit of activity was defined as that quantity of ceruloplasmin which gave a change in E_{550} nm of 0.01/min.

Determination of Number of Animals Required. Sample size was determined as described by Sokal and Rohlf (13). An estimated ceruloplasmin activity was obtained from a published study of copper deficiency in rats (14). Given a standard deviation of 63

U/l, the smallest detectable difference of 52, significance level of 0.05, and the desired probability that a difference would be found to be significant at 0.2, n was estimated to be 6 rats per group.

Data Analysis Plan. Data were analyzed using a 2 X 2 factorial ANOVA (Program 2V, BMDP Statistical Software, Berkeley, CA).

RESULTS

Data for plasma silver, copper, zinc, and ceruloplasmin concentrations are shown in Table 1. AgSD markedly elevated plasma silver concentrations in both the burned and sham-burned animals. There was a significant ($P < 0.01$) effect on plasma copper and ceruloplasmin concentrations due to AgSD treatment but not due to burn injury. Neither AgSD nor burn injury affected plasma zinc or selenium concentrations.

TABLE 1. Plasma Silver, Copper, Zinc, and Ceruloplasmin Concentrations in Burned and Sham-Burned Rats (Mean ± SEM)

	Burn Group		Sham-Burn Group	
	+ AgSD	− AgSD	+ AgSD	−AgSD
Silver (μg/dl)	54 ± 10	< 2	54 ± 14	< 2
Copper (μg/dl)	48 ± 5	113 ± 18	45 ± 8	105 ± 17
Zinc (μg/dl)	117 ± 11	123 ± 10	131 ± 5	105 ± 10
Ceruloplasmin (μ/l)	3.2	27	6.3	19.3

DISCUSSION

The biological effects of silver toxicity have recently been reviewed (15). Although the risk of silver toxicity is low for the human population as a whole, the antagonistic effects on selenium and copper metabolism cause concern for individuals having low dietary intakes of these nutrients. A recent report (9) that silver ion is absorbed through the burn wound in severely burned patients treated with AgSD justifies concern about copper and selenium metabolism in these patients.

There is a paucity of animal research concerning the absorption of silver and possible antagonistic effects of such absorption on copper and selenium metabolism after burn injury. Harrison (16) studied the penetration of silver in burned rats using AgSD labeled

with tracer amounts of Ag^{110}. Although the silver was tenaciously attached to the burn wound after exhaustive wet-sponge treatment to dislodge the cream, blood levels of Ag^{110} were not elevated above background levels at 7, 14, and 26 days postburn. This contradiction with the present study may reflect the fact that the detection method used in the older study was not sensitive enough for the low specific activity of the AgSD in the cream. This contrasts with our present method of detecting total silver concentration using the graphite furnace atomic absorption spectrophotometer. Furthermore, Harrison (16) did not report burn size.

Sano et al (17) found negligible silver in the blood of normal and burned rats treated with AgSD and sacrificed 5 days postburn. It is difficult to compare the results of that study with the present study because of the difference in burn size and depth. Sano's study used an 80°C burn that covered only 4% of the total body surface and probably was not full-thickness.

Boosalis et al (8) have reported depressed serum copper and ceruloplasmin concentrations in patients with thermal injury. These findings, coupled with increased serum silver concentrations in patients with thermal injury treated with AgSD (9) and the present study showing the occurrence of increased plasma silver concentrations and impaired copper metabolism in a burned rat model, raise a number of questions concerning the nutritional supplementation of patients with thermal injury.

PRESENTATIONS/PUBLICATIONS

None.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

ACCESSION: DA335815

SUMMARY DATE: 920930 **SUMMARY KIND:** K **PREV DATE:** 911001 **DISTRIBUTION:** CX

PROGRAM #: 61102A **PROJ #:** 30161102A91C **TASK AREA:** CA **WORK UNIT:** 077

TITLE: Effects of Pressure-Controlled Inverse Ratio Ventilation (PcIRV) on Smoke Inhalation Injury in an Ovine Model

SUBJ1: 060500 - Medicine and Medical Research

START DATE: 9102 **END DATE:** 9209 **PERFORMANCE METHOD:** C

CONTRACT/GRANT NO:

CONT TOTAL: $
CUM TOTAL: $
TOTAL LAB FUNDS: $

RESOURCES ESTIMATE

FY	WORK YRS	$(Thousands)
91	1.0	$27
92	1.5	$42
93	0.0	$12

RESPONSIBLE DOD ORGANIZATION: 404885
MRDC INSTITUTE OF SURGICAL RESEARCH USAISR
FT SAM HOUSTON, TX 78234
PRUITT, B A
210-221-2720

PERFORMING ORGANIZATION: 404885
MRDC INSTITUTE OF SURGICAL RESEARCH USAISR
FT SAM HOUSTON, TX 78234
OGURA, H
210-221-3349

ASSOC1: CIOFFI, W G

ASSOC2: JOHNSON, A A

FOREIGN INTELLIGENCE CONSIDERED: Y **CIVILIAN APPLICABILITY:** M

KEYWORDS: ILIR; Lab Animals; Sheep; Burns (Injuries); Lungs; Pulmonary Function; Pulmonary Insufficiency

OBJECTIVE: A DTIC literature search was conducted under DTIC request numbers W6M48B/W6M50E dated 30 January 1991. The objective of this work is to determine the effects of PcIRV on smoke inhalation injury in an ovine model as compared with conventional ventilation. If PcIRV can favorably affect V_A/Q_C and oxygenation in inhalation injury, its application to patients with inhalation injury may be advantageous.

APPROACH: Eighteen sheep were anesthetized, orally intubated, mechanically ventilation, and catheterized. Smoke inhalation injury was produced by smoke insufflation at a dose that produced a carboxyhemoglobin level of 50% to 60% (moderate injury). After smoke exposure, animals were extubated and observed for 24 h. At the end of 24 h, the animals were anesthetized, orally intubated, and mechanically ventilated as follows: first hour, volume-controlled ventilation with I/E = 1:2; second hour, PcIRV with I/E = 2:1; third hour, PcIRV with I/E = 3:1; fourth hour, PcIRV with I/E = 4:1; and fifth hour, volume-controlled ventilation with I/E = 1:2. During mechanical ventilation, the tidal volume was set at 15 ml/kg and the respiratory rate was controlled to maintain a $PaCO_2$ between 30 and 35 mmHg and an arterial pH between 7.35 and 7.40. FIO_2 was kept at 0.21 and PEEP was 5 cmH_2O throughout the study period. Cardiopulmonary variables and blood gases were measured every 30 min during mechanical ventilation. Measurement of V_A/Q_C using the multiple inert gas elimination technique was performed every hour during mechanical ventilation. Blood and expired gas samples were

immediately analyzed by GC. ANOVA for a mixed factorial design and multivariate analysis (regression) was used.

PROGRESS: 9110-9209. This project was approved by the USAISR Research Council and US Army Institute of Surgical Research Animal Care and Use Committee during the second quarter of Fiscal Year 1991. All modes of PcIRV produced significantly greater mean airway pressure and lower peak inspiratory pressure and expiratory minute ventilation volume than volume-controlled ventilation. Such ventilation did not improve physiologic shunt or oxygenation. With PcIRV, pulmonary vascular resistance, pulmonary arterial pressure, and pulmonary capillary wedge pressure were greater than with volume-controlled ventilation, but PcIRV had no significant effect on cardiac output or systemic blood pressure. The lower peak inspiratory pressure and expiratory minute ventilation volume observed with PcIRV in this study may prevent barotrauma during mechanical ventilation. Oxygenation, however, was not improved by PcIRV after smoke exposure. For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report* for fiscal years 1991 through 1992.

ABSTRACT

PROJECT NUMBER: 3A161101A91C-077, In-House Laboratory Independent Research

PROJECT TITLE: Effects of Pressure-Controlled Inverse Ratio Ventilation (PcIRV) on Smoke Inhalation Injury in an Ovine Model

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

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The effects of PcIRV were compared with those of conventional ventilation in an ovine model of respiratory failure due to smoke inhalation. Seven sheep were exposed to doses of smoke producing moderate inhalation injury. After 24 h, the animals were intubated and mechanically ventilated as follows:

First Hour = Volume-controlled (Vc) ventilation with an I/E of 1:2.

Second Hour = Pressure-controlled (Pc) ventilation with an I/E of 1:1.

Third Hour = PcIRV with an I/E of 2:1.

Fourth Hour = PcIRV with an I/E of 4:1.

Fifth Hour = Vc ventilation with an I/E of 1:2.

Cardiopulmonary functions were measured every 30 min during the study.

All modes of Pc ventilation produced significantly greater mean airway pressure and lower peak inspiratory pressure and expiratory minute ventilation volume than Vc ventilation. Such ventilation did not improve physiologic shunt or oxygenation. With Pc ventilation, pulmonary vascular resistance, pulmonary artery pressure, and pulmonary capillary wedge pressure were greater than

with Vc ventilation, but Pc ventilation had no significant effect on cardiac output or systemic blood pressure.

The lower peak inspiratory pressure and expiratory minute ventilation volume observed with PcIRV in this study may prevent barotrauma during mechanical ventilation. Oxygenation, however, was not improved by PcIRV after smoke exposure.

EFFECTS OF PRESSURE-CONTROLLED INVERSE RATIO VENTILATION (PcIRV) ON SMOKE INHALATION INJURY IN AN OVINE MODEL

Bronchopulmonary injury due to smoke inhalation is a potentially lethal complication of burn injury. Such inhalation injury causes acute airway inflammation in the early phase, with subsequent pulmonary edema and infection. In the early phase, airway occlusion worsens V_A/Q_C inequality and oxygenation (1). Treatment to recruit alveoli and prevent airway closure has the potential to improve hypoxia following such injury; positive end-expiratory pressure (PEEP) treatment at 12 or 72 h after smoke inhalation did not improve oxygenation in an ovine model (2).

PcIRV has been reported to improve oxygenation and prognosis in patients with ARDS (3-5). In these patients, increased mean airway pressure and prolonged inspiratory time are thought to stabilize and recruit alveoli, and lower peak inspiratory pressures are thought to reduce the likelihood of barotrauma. In other studies, however, PcIRV has not improved oxygenation in some patients with severe ARDS (6,7). The difference between successful and unsuccessful use of PcIRV has not been clarified. The present study was designed to determine the effects of PcIRV on respiratory impairment following smoke inhalation in a sheep model.

MATERIALS AND METHODS

Study Design. Sheep were exposed to doses of smoke producing moderate inhalation injury. After 24 h, the animals were intubated and mechanically ventilated as follows:

First Hour = Volume-controlled (Vc) ventilation with an I/E of 1:2.

Second Hour = Pressure-controlled (Pc) ventilation with an I/E of 1:1 (Pc Mode 1).

Third Hour = PcIRV with an I/E of 2:1 (Pc Mode 2).

Fourth Hour = PcIRV with an I/E of 4:1 (Pc Mode 3).

Fifth Hour = Vc ventilation with an I/E of 1:2.

Cardiopulmonary functions were measured every 30 min during the study.

Description of Procedures. Seven 1- to 2-yr old neutered male, commercially available, random source sheep weighing 35.2 ± 1.5 kg were studied. The animals were housed in covered outdoor runs, treated for parasites (1% ivermectin, 1 ml/75 lb), and fed commercial chow and water ad libitum. Baseline hematologic data (CBC, total proteins, and blood chemistries) were obtained 3 weeks

before study. All animals were fasted for 24 h before smoke exposure and use. The animals were anesthetized with sodium pentobarbital (25 mg/kg IV, Sigma Chemical Company, St. Louis, MO), orally intubated, mechanically ventilated, placed in the supine position, and catheterized. Two Silastic® medical grade cannulae (30 cm) were inserted, one into a femoral artery and one in a femoral vein. A radiopaque sheath introducer (8.5F) was inserted into an external jugular vein using sterile technique. A Swan-Ganz catheter (7.5F, American Edwards Laboratories, Irvine, CA) was inserted through the sheath into the external jugular vein. After cannulation, the sheep were paralyzed with pancuronium bromide (0.03 mg/kg IM, Astra Pharmaceutical Products, Inc., Westboro, MA) and exposed to smoke to produce a moderate degree of inhalation injury as previously described (8). Immediately after smoke exposure, each animal was extubated and housed in an individual cage in climate-controlled facilities at 74°C to 76°F (24°C to 25°C) with a relative humidity of 40% to 50% and observed while spontaneously breathing in the awake state for 24 h after smoke insufflation.

At the end of 24 h, the animals were pretreated with glycopyrrolate (0.02 mg/kg IM, AH Robins Company, Inc., Richmond, VA) and anesthetized with sodium pentobarbital (25 mg/kg IV). The animals were then paralyzed with pancuronium bromide (0.03 mg/kg IV) and intubated. The animals were positioned prone and mechanical ventilation using a Servo™ 900C ventilator (Siemens-Elema, Solna, Sweden) was used as described above.

During mechanical ventilation, the tidal volume was set at 15 ml/kg and the respiratory rate was controlled to maintain a constant $PaCO_2$. FIO_2 was kept at 0.21 and PEEP at 5 cmH_2O throughout the study period. Pancuronium bromide (0.03 mg/kg, Astra Pharmaceutical Products, Inc.) was given every 1.5 to 2 h to maintain paralysis.

Cardiopulmonary variables and blood gases were measured before smoke exposure, before mechanical ventilation, and every 30 min during mechanical ventilation. Respiratory rate, mean airway pressure, and peak inspiratory pressure were monitored by the digital display of the ventilator. Inspiratory tidal volume and expiratory minute ventilation volume were measured by a Wright™ respirometer (Mercury Medical, Clearwater, FL). Systemic blood pressure, pulmonary artery pressure, central venous pressure, and pulmonary capillary wedge pressure were monitored using a pressure monitor (Model 78354A, Hewlett-Packard Company, Waltham, MA). Cardiac output was measured in triplicate by the thermodilution technique (Cardiac Output Computer, Model 9520A, American Edwards Laboratories).

Gas analyses of arterial and mixed-venous samples were performed using an IL 1303 pH/blood gas analyzer and an IL282 CO-oximeter (Instrumentation Laboratories, Inc., Lexington, MA).

An esophageal balloon was inserted before mechanical ventilation, and intrapleural and transpulmonary pressures were monitored with a differential transducer (MP-451, Validine Engineering Corporation, Northridge, CA). Respiratory flow rates were monitored with a pneumotachograph (Model 17212, Gould, Inc., The Netherlands). These respiratory indices were recorded every hour during the study on a four-channel recorder (Model 7754A, Hewlett-Packard). Auto-PEEP was calculated as the difference between end-expiratory intrapleural pressure and the airway PEEP setting.

Respiratory index (RI) and physiologic shunt (Qs/Qt) were calculated using the following formulae:

$$RI = (PAO_2 - PaO_2)/PaO_2$$

$$Qs/Qt\ (\%) = 100 \times (CcO_2 - CaO_2)/(CcO_2 - CvO_2)$$

where PaO_2 indicates arterial O_2 pressure (mmHg); PAO_2, alveolar O_2 pressure (mmHg); CaO_2, O_2 concentration in arterial blood (Vol%); CvO_2, O_2 concentration in mixed-venous blood (Vol%); and CcO_2, O_2 concentration in pulmonary capillary blood.

Statistical Analysis. All data are shown as mean and standard error of mean. One- and two-way ANOVA programs (VAX BMDP Program 7D) were utilized for comparisons between PC ventilation and Vc ventilation and comparisons among the three different Pc ventilation modes. Among Pc ventilation modes, mode 2 was compared with mode 1, and mode 3 was compared to the mean of modes 1 and 2. Differences were considered significant at $P < 0.05$.

RESULTS

Table 1 depicts the serial changes of respiratory indices during this study. Mean airway pressure was elevated significantly in the Pc ventilation modes as compared with the Vc ventilation modes ($P < 0.0001$). In the three Pc ventilation modes, mean airway pressure increased with increasing relative duration of the inspiratory phase ($P < 0.0001$).

Peak inspiratory pressure was decreased significantly in the Pc ventilation modes as compared with the Vc ventilation modes ($P < 0.0001$). There was no significant difference in peak inspiratory pressure between Pc ventilation modes 1 and 2, but the peak inspiratory pressure was elevated significantly in mode 3 as compared with modes 1 and 2 ($P < 0.05$).

Expiratory minute ventilation volume was also decreased significantly with the Pc ventilation modes as compared with the Vc ventilation modes ($P < 0.0001$). There were no significant differences in expiratory minute ventilation among the three Pc ventilation modes.

TABLE 1. Respiratory Indices (Mean ± SEM)

	Mean Airway Pressure (cmH_2O)	Peak Inspiratory Pressure (cmH_2O)	Expiratory Minute Ventilation Volume (l/min)
Presmoke	8.1 ± 0.3	19.6 ± 0.9	6.76 ± 0.27
Vc1-1	8.1 ± 0.3	20.8 ± 1.8	7.54 ± 0.56
Vc1-2	8.2 ± 0.4	21.0 ± 1.7	6.71 ± 0.54
Pc1-1	11.4 ± 0.6	17.3 ± 1.2	6.04 ± 0.31
Pc1-2	11.4 ± 0.4	16.4 ± 1.1	5.97 ± 0.51
Pc2-1	13.5 ± 0.6	17.3 ± 1.0	6.26 ± 0.35
Pc2-2	13.3 ± 0.5	15.6 ± 1.0	5.81 ± 0.38
Pc3-1	15.3 ± 0.4	18.4 ± 1.4	5.56 ± 0.25
Pc3-2	14.9 ± 0.5	16.6 ± 0.9	5.81 ± 0.42
Vc2-1	8.3 ± 0.3	20.5 ± 1.4	6.40 ± 0.59
Vc2-2	8.3 ± 0.2	20.5 ± 1.1	6.42 ± 0.66

Vc1-1 indicates 30 min after using volume-controlled ventilation; Vc1-2, 1 h after using volume-controlled ventilation; Pc1-1, 30 min after using pressure-controlled ventilation at an I/E ratio of 1:1 (mode 1); Pc1-2, 1 h after using pressure-controlled ventilation at an I/E ratio of 1:1; Pc2-1, 30 min after using pressure-controlled ventilation at an I/E ratio of 2:1 (mode 2); Pc2-2, 1 h after using pressure-controlled ventilation at an I/E ratio of 2:1; Pc3-1, 30 min after using pressure-controlled ventilation at an I/E ratio of 4:1 (mode 3); Pc3-2, 1 h after using pressure-controlled ventilation at an I/E ratio of 4:1; Vc2-1, 30 min after using volume-controlled ventilation; and Vc2-2, 1 h after using volume-controlled ventilation.

Flow curves indicated that expiratory flow returned to 0 before the beginning of the inspiratory phase in the Vc ventilation modes and Pc ventilation modes 1 and 2. Auto-PEEP was not detected in these modes. In Pc ventilation mode 3, however, expiratory flow continued until interrupted by the inspiratory phase, and auto-PEEP between 0.7 and 1.7 cmH_2O was detected in 5 animals.

Table 2 shows the serial changes of blood gas exchange indices during this study. $PaCO_2$ and PaO_2 values are direct readings from the electrode set at 37°C. $PaCO_2$ was controlled by altering respiratory rates and did not change significantly during the study.

TABLE 2. Blood Gas Exchange Indices (Mean ± SEM)

	$PaCO_2$ (mmHg)	PaO_2 (mmHg)	Respiratory Index	Physiologic Shunt
Presmoke	27.1 ± 1.0	94.1 ± 2.9	0.242 ± 0.030	3.5 ± 1.0
Pretreatment	30.1 ± 1.0	65.4 ± 3.7	0.769 ± 0.133	22.7 ± 5.7
Vc1-1	27.9 ± 1.2	76.4 ± 4.7	0.556 ± 0.128	12.4 ± 4.4
Vc1-2	27.1 ± 1.0	78.3 ± 4.4	0.529 ± 0.121	11.4 ± 3.9
Pc1-1	28.1 ± 1.3	77.6 ± 4.2	0.519 ± 0.106	14.0 ± 4.0
Pc1-2	26.6 ± 0.8	80.4 ± 4.7	0.497 ± 0.119	10.8 ± 3.9
Pc2-1	27.3 ± 1.0	79.4 ± 4.0	0.491 ± 0.093	10.9 ± 3.5
Pc2-2	26.6 ± 0.8	81.1 ± 4.2	0.473 ± 0.097	9.7 ± 3.3
Pc3-1	27.3 ± 0.9	79.6 ± 4.2	0.488 ± 0.092	10.0 ± 3.5
Pc3-2	27.2 ± 0.8	81.0 ± 4.0	0.461 ± 0.089	9.4 ± 3.3
Vc2-1	28.8 ± 1.1	78.3 ± 2.8	0.476 ± 0.067	11.9 ± 3.1
Vc2-2	27.2 ± 0.6	80.6 ± 3.2	0.463 ± 0.078	9.3 ± 2.3

$PaCO_2$ and PaO_2 values are direct readings from the electrode set at 37°C. Vc1-1 indicates 30 min after using volume-controlled ventilation; Vc1-2, 1 h after using volume-controlled ventilation; Pc1-1, 30 min after using pressure-controlled ventilation at an I/E ratio of 1:1 (mode 1); Pc1-2, 1 h after using pressure-controlled ventilation at an I/E ratio of 1:1; Pc2-1, 30 min after using pressure-controlled ventilation at an I/E ratio of 2:1 (mode 2); Pc2-2, 1 h after using pressure-controlled ventilation at an I/E ratio of 2:1; Pc3-1, 30 min after using pressure-controlled ventilation at an I/E ratio of 4:1 (mode 3); Pc3-2, 1 h after using pressure-controlled ventilation at an I/E ratio of 4:1; Vc2-1, 30 min after using volume-controlled ventilation; and Vc2-2, 1 h after using volume-controlled ventilation.

PaO_2 increased during the first hour of conventional ventilation, but did not improve with Pc ventilation. The respiratory index was not improved with Pc as compared with the Vc ventilation modes. There were no significant differences in PaO_2 or respiratory index among the three Pc ventilation modes.

Physiologic shunt decreased significantly with a change from PcIRV mode 1 to 2 ($P < 0.05$). As a whole, it did not decrease significantly with Pc as compared with Vc.

Table 3 shows the hemodynamic parameters measured during this study. Pulmonary vascular resistance, mean pulmonary artery pressure, and pulmonary capillary wedge pressure were significantly

TABLE 3. Hemodynamic Indices (Mean ± SEM)

	Mean Pulmonary Artery Pressure (mmHg)	Pulmonary Vascular Resistance (dyn/sec/cm^{-5})	Pulmonary Capillary Wedge Pressure (mmHg)	Central Venous Pressure (mmHg)	Mean Systemic Pressure (mmHg)	Cardiac Index (l/min/m^2)	Total Peripheral Resistance (dyn/sec/cm^{-5})	Systemic Oxygen Delivery (ml/min)
Presmoke	14.3 ± 0.8	125.4 ± 12.9	8.7 ± 0.5	2.1 ± 0.6	123.6 ± 2.8	4.16 ± 0.26	2757.6 ± 140.0	737.0 ± 49.0
Pretreatment	17.9 ± 1.2	136.6 ± 14.8	10.0 ± 0.8	1.3 ± 0.4	95.0 ± 2.4	5.44 ± 0.38	1640.4 ± 121.7	753.6 ± 49.0
Vc1-1	16.0 ± 0.9	145.9 ± 16.1	8.3 ± 0.8	2.1 ± 0.4	120.0 ± 4.2	4.95 ± 0.28	2244.6 ± 122.1	729.3 ± 49.5
Vc1-2	15.9 ± 0.9	143.0 ± 14.6	8.1 ± 0.6	2.1 ± 0.5	117.4 ± 3.6	5.20 ± 0.51	2193.4 ± 259.8	777.9 ± 86.7
Pc1-1	18.4 ± 1.3	179.7 ± 14.2	8.7 ± 0.8	2.3 ± 0.4	113.6 ± 2.7	5.00 ± 0.52	2173.4 ± 264.5	744.3 ± 88.4
Pc1-2	18.7 ± 0.8	178.0 ± 13.8	8.3 ± 0.7	1.7 ± 0.4	118.7 ± 2.6	5.65 ± 0.63	2077.6 ± 241.5	845.0 ± 103.4
Pc2-1	19.6 ± 0.8	208.6 ± 13.3	8.4 ± 0.7	1.6 ± 0.6	118.7 ± 3.2	5.07 ± 0.37	2209.4 ± 165.6	755.6 ± 61.0
Pc2-2	19.4 ± 0.6	197.9 ± 20.3	8.7 ± 0.8	2.0 ± 0.5	121.4 ± 1.9	5.34 ± 0.61	2220.7 ± 215.9	796.1 ± 90.8
Pc3-1	19.7 ± 0.8	220.3 ± 20.3	9.6 ± 0.7	3.3 ± 0.4	122.3 ± 1.3	4.42 ± 0.35	2583.4 ± 179.3	654.7 ± 49.2
Pc3-2	18.9 ± 0.8	209.4 ± 19.9	9.1 ± 0.8	3.6 ± 0.5	120.3 ± 2.2	4.50 ± 0.39	2521.9 ± 226.7	671.6 ± 59.6
Vc2-1	16.7 ± 0.9	195.0 ± 16.9	7.4 ± 0.9	2.3 ± 0.7	119.3 ± 2.9	4.50 ± 0.33	2493.4 ± 205.9	668.9 ± 55.8
Vc2-2	16.0 ± 0.9	168.0 ± 16.9	8.1 ± 1.0	2.0 ± 0.5	118.1 ± 2.4	4.50 ± 0.36	2462.9 ± 146.5	672.7 ± 59.9

Vc1-1 indicates 30 min after using volume-controlled ventilation; Vc1-2, 1 h after using volume-controlled ventilation; Pc1-1, 30 min after using pressure-controlled ventilation at an I/E ratio of 1:1 (mode 1); Pc1-2, 1 h after using pressure-controlled ventilation at an I/E ratio of 1:1; Pc2-1, 30 min after using pressure-controlled ventilation at an I/E ratio of 2:1 (mode 2); Pc2-2, 1 h after using pressure-controlled ventilation at an I/E ratio of 2:1; Pc3-1, 30 min after using pressure-controlled ventilation at an I/E ratio of 4:1 (mode 3); Pc3-2, 1 h after using pressure-controlled ventilation at an I/E ratio of 4:1; Vc2-1, 30 min after using volume-controlled ventilation; and Vc2-2, 1 h after using volume-controlled ventilation.

elevated in the Pc ventilation modes as compared to the Vc ventilation modes ($P < 0.0001$). In the three Pc ventilation modes, pulmonary vascular resistance increased gradually with increasing I/E ($P < 0.05$). Pulmonary capillary wedge pressure and central venous pressure were also increased significantly with PcIRV mode 3 as compared with modes 1 and 2 ($P < 0.01$).

There were no significant differences in cardiac index, mean systemic blood pressure, total peripheral resistance, or systemic oxygen delivery between Pc and Vc ventilation modes. The cardiac index did, however, decrease significantly with the use of Pc ventilation mode 3 as compared with modes 1 and 2 ($P < 0.01$). In mode 3, mean systemic blood pressure did not change significantly with the increase of total peripheral resistance ($P < 0.01$), but systemic oxygen delivery decreased significantly ($P < 0.01$).

DISCUSSION

Moderate to severe smoke inhalation causes progressive airway inflammation and hypoxia. Soon after exposure, pseudomembranes formed as a result of this inflammatory reaction cause extensive occlusion of small airways. Shimazu et al (1) reported that smoke inhalation results in an increase in shunt and low V_A/Q_C areas, worsening V_A/Q_C inequality in the lung. These changes predispose the burn patient to pulmonary edema and pneumonia, and materially increase mortality. Cioffi et al (9) have reported that prophylactic use of high-frequency percussive ventilation reduces the incidence of pneumonia and mortality in patients with inhalation injury (9). The physiologic mechanisms responsible for this beneficial effect have not been clarified.

Therapy designed to prevent airway closure and recruit alveoli could potentially improve oxygenation in inhalation injury. Abdi et al (10) reported that the use of PEEP 24 h after smoke exposure decreased bronchial blood flow, which is thought to decrease lung lymph flow and pulmonary edema. Shimazu et al (2), however, found that PEEP treatment at 12 or 72 h after smoke exposure had no positive effect on oxygenation in an ovine model. In those studies, PEEP increased dead space and exerted no significant effect on shunt or low V_A/Q_C areas (2).

PcIRV has been reported to improve oxygenation and prognosis in patients with ARDS (3-5). In those patients, PcIRV increased mean airway pressure and decreased peak inspiratory pressure, but had no significant effect on cardiac function. The prolonged inspiratory phase and increased mean airway pressure were thought to stabilize and recruit alveolar units and prevent airway closure. Concerning relationships between mean airway pressure and oxygenation, Boros (11) reported that arterial oxygenation correlated best with mean airway pressure in comparing different I/E ratios and airway pressure waves. Bowe et al (12), however, have reported that mean airway pressure is not the major determinant of oxygenation, and

that the elevation of mean airway pressure has the potential to reduce venous return and cardiac output. The physiologic mechanisms by which PcIRV affects oxygenation in patients with ARDS have not been fully defined.

The inspiratory flow pattern of PcIRV is a rapidly decaying curve, and differs from the square wave flow pattern used in Vc ventilation. This special flow pattern and decreased peak inspiratory pressure are thought to prevent overinflation and to protect the lung from barotrauma (3). In studies by Lachmann et al (13), PcIRV with 80% inspiratory time significantly reduced the morphologic evidence of injury in surfactant-deficient rabbit lungs.

There are conflicting reports concerning the effect of inverse ratio ventilation on cardiopulmonary function. Hubmayr et al (14) have reported that the reduction in peak airway pressure overestimates the decrease in alveolar pressure in inverse ratio ventilation. Duncan et al (15) found that inadequate expiratory time in Vc inverse ratio ventilation may lead to occult positive end-expiratory pressure (auto-PEEP), with possible depression of cardiac output. The studies of Berman et al (16) indicate no beneficial effect of Vc inverse ratio ventilation in a canine model of aspiration. There are also reported studies in which PcIRV did not improve oxygenation in patients with severe ARDS (6,7). The underlying reasons for these differences in the effects of PcIRV are unknown.

In the present study, three modes of Pc ventilation with increasing I/E ratios were used in animals with moderate inhalation injury and compared with conventional Vc ventilation. During Pc, increasing relative inspiratory periods were achieved by plateauing airway pressure as a square wave, which may have contributed to the elevation of mean airway pressure and the decrease of peak inspiratory pressure, as compared with the triangular pressure waves of the Vc ventilation modes. In spite of the elevation of mean arterial pressure in the Pc ventilation modes; however, oxygenation was not improved. This may depend on some difference between airway injury by smoke inhalation and that present in ARDS. Extensive mechanical small airway occlusion and pulmonary surfactant deficiency are thought to make it difficult to recruit alveoli after smoke inhalation, and the inspiratory pressure plateaus used in this study may not have been high enough to open occluded small airways or alveoli. From our results, mean airway pressure is not a reliable determinant of improved oxygenation after smoke inhalation injury.

The hemodynamic changes following the institution of PcIRV ventilation in this model are different from those reported with PcIRV in ARDS patients. In this study, pulmonary artery pressure was elevated with increased pulmonary vascular resistance, and pulmonary capillary wedge pressure was also higher than with Vc.

Elevation of mean airway pressure, with possible air trapping in the lung, can interfere with venous return and pulmonary blood flow. With Pc at an I/E ratio of 4:1, we observed reduced cardiac output and a reduction of systemic oxygen delivery. At this ratio, auto-PEEP was detected and peak inspiratory pressure was significantly greater than with the Pc ventilation modes with lower I/E ratios. The decrease of physiologic shunt effected by changing from Pc ventilation mode 1 to Pc ventilation mode 2 suggests that, in this model, this ratio may approach the optimal mode for further studies of PcIRV in inhalation injury.

A significant disadvantage of PcIRV is that inverse ratio ventilation mandates the use of heavy sedation and paralysis, possibly enhancing the risk of pneumonia (15). A second difficulty is that inspiratory tidal volume is dependent upon pulmonary compliance. Clinical application would demand continuous monitoring and adjustment of pressure settings.

In this study, each Pc ventilation mode was used for 1 h. Since Greaves et al (17) have reported that it may take 2-6 h for the beneficial effects of inverse ratio ventilation to be fully realized, more prolonged tests of PcIRV may be indicated.

In summary, Pc ventilation modes with longer inspiratory phases decreased peak inspiratory pressure and expiratory minute ventilation volume; both may protect against barotrauma during mechanical ventilation. Despite increased mean arterial pressure, oxygenation was not improved by Pc, and Pc with an I/E ratio of 4:1 produced auto-PEEP and depressed cardiac output.

PRESENTATIONS/PUBLICATIONS

None.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

ACCESSION: DA336886

SUMMARY DATE: 921001 **SUMMARY KIND:** D **PREV DATE:** 911001 **DISTRIBUTION:** CX

PROGRAM #: 61102A **PROJ #:** 30161101A91C **TASK AREA:** EC **WORK UNIT:** 078

TITLE: Effects of Pentoxifylline and Protein Kinase C Inhibitor (H-7) on Smoke Inhalation Injury in an Ovine Model

SUBJ1: 060500 - Medicine and Medical Research

START DATE: 9105 **END DATE:** 9309 **PERFORMANCE METHOD:** C

CONTRACT/GRANT NO:

CONT TOTAL: $
CUM TOTAL: $
TOTAL LAB FUNDS: $

RESOURCES ESTIMATE

FY	WORK YRS	$(Thousands)
91	0.1	$23
92	0.1	$50
93	0.1	$53

RESPONSIBLE DOD ORGANIZATION: 404885
MRDC INSTITUTE OF SURGICAL RESEARCH USAISR
FT SAM HOUSTON, TX 78234
PRUITT, B A
210-221-2720

PERFORMING ORGANIZATION: 404885
MRDC INSTITUTE OF SURGICAL RESEARCH USAISR
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ASSOC1: CIOFFI, W G

ASSOC2: JOHNSON, A A

FOREIGN INTELLIGENCE CONSIDERED: Y **CIVILIAN APPLICABILITY:** M

KEYWORDS: ILIR; Lab Animals; Sheep; Burns (Injuries); Lungs; Inhalation; Edema; Therapy

OBJECTIVE: A DTIC literature search was conducted under DTIC request numbers W6M59E/W6N01F dated 30 January 1991. The objective of this work is to determine the physiologic effects of pentoxifylline and H-7 on smoke inhalation injury in an ovine model. If pentoxifylline and/or H-7 can favorably affect the inflammatory process after inhalation injury, the application of these drugs to patients with inhalation injury might be advantageous.

APPROACH: Twenty-four sheep were divided into three groups. Group I (n=8) received smoke inhalation injury without treatment, Group II (n=8) received smoke inhalation injury with continuous infusion of pentoxifylline postinjury, and Group III (n=8) received smoke inhalation injury with continuous infusion of H-7 postinjury. Cardiopulmonary variables and blood gases were measured presmoke and at 1, 4, 8, 12, 16, 20, and 24 h postsmoke. Measurement of V_A/Q_C using the multiple inert gas elimination technique was performed at the end of the 24-h study period. After blood sample collections, bronchoalveolar lavage was performed to obtain samples from the lower lung lobes for measurement of 6-keto-$PGF_{1\alpha}$, thromboxane B_2, and conjugated dienes. Extravascular lung water was determined by a gravimetric method upon sacrifice. ANOVA for a mixed factorial design and multivariate analysis (regression) were utilized.

PROGRESS: 9110-9209. Treatment with pentoxifylline after smoke inhalation injury attenuated pulmonary hypertension, airway inflammation, and pulmonary edema. V_A/Q_C mismatching and oxygenation were significantly improved with pentoxifylline. These findings

suggest that pentoxifylline may be useful in the management of smoke inhalation injury. An addendum is currently being developed to study the use of pentoxifylline in an ovine model of combined smoke inhalation and burn injury. For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report* for fiscal years 1991 through 1992.

ABSTRACT

PROJECT NUMBER: 3A161101A91C-077, In-House Laboratory Independent Research

PROJECT TITLE: Effects of Pentoxifylline and Protein Kinase C Inhibitor (H-7) on Smoke Inhalation Injury in an Ovine Model

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

PERIOD COVERED IN THIS REPORT: 1 October 1991 - 30 September 1992

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Bronchopulmonary injury secondary to smoke inhalation is a significant comorbid factor associated with thermal trauma. The present study evaluated the effect of pentoxifylline (PTX) on pulmonary function in an ovine model of smoke inhalation injury.

After a controlled smoke exposure that produces moderate inhalation injury, 16 one- to two-year-old random-source, awake, nonintubated male sheep were divided into two groups and observed for 48 h. Eight animals received only a maintenance infusion of lactated Ringer's solution. The remaining 8 sheep received a bolus injection of PTX (20 mg/kg) immediately after smoke exposure, followed by continuous infusion of PTX (2 mg/kg/h) in lactated Ringer's solution for 48 h. The arterial COHb levels immediately after smoke exposure did not differ significantly between the two groups. Cardiopulmonary variables and blood gases were serially measured.

Ventilation-perfusion distribution was assessed 48 h after injury using the multiple inert gas elimination technique (MIGET). Briefly, after continuous infusion of six inert gases with different solubilities, retention and excretion of these gases were measured by GC and analyzed using a specific computer program designed to determine V_A/Q ratios for 50 lung compartments (13).

Bronchoalveolar lavage was also performed at 48 h and the fluid assayed for WBC, total protein content, and conjugated diene concentration. The wet-to-dry lung weight ratio was measured after necropsy.

Statistical analyses were performed using student's t test between the two groups at equivalent times and ANOVA (Tukey method) among the treatment groups and a normal, uninjured control group containing four animals.

Continuous treatment with PTX lessened hypoxemia and V_A/Q mismatching in the lung, decreased pulmonary interstitial edema and airway epithelial damage, and attenuated pulmonary arterial hypertension after smoke inhalation. The decrease in conjugated dienes in both blood and bronchoalveolar lavage fluid with PTX treatment suggests that the effects of PTX may be due in part to a reduction in oxidant injury.

EFFECTS OF PENTOXIFYLLINE AND PROTEIN KINASE C INHIBITOR (H-7) ON SMOKE INHALATION INJURY IN AN OVINE MODEL

The physiologic changes after smoke inhalation injury have been observed in a sheep model at this Institute (1). The influence of medical interventions in this model, however, has not been thoroughly investigated. Pentoxifylline (PTX) has been reported to increase RBC and WBC deformability, lower blood viscosity, and cause prostacyclin release. These effects are thought to improve microcirculatory blood flow. Recently, PTX has been shown to decrease production of TNF, activity of IL1, aggregation of platelets, and function of activated PMNs (2). These effects are thought to counter the cytokine-induced inflammatory process (3,4). In some animal endotoxin shock models, PTX improved the survival rate, with reduction of TNF formation and platelet aggregation (5,6). In hemorrhagic shock models, the survival rate using PTX was improved in association with improved tissue oxygenation (7,8). PTX also attenuated lung edema in a TNF-induced lung injury model (9) and a proteolytic enzyme-induced lung injury model (10).

Protein kinase C plays a potentially key role in modulating the oxidative response of neutrophils (11). In the endotoxic shock state, human platelets are also stimulated by the modulation of protein kinase C by endotoxic lipid A (12). Recently, protein kinase C appeared to be a common mediator of endothelial cell activation by LPS, TNF, and IL1 (13). H-7 is thought to inhibit protein kinase C by competing at the ATP-binding site (14). In animal models, H-7 reduced PMA-induced lung edema by reducing albumin leak (15). Both PTX and H-7 have the potential to inhibit the inflammatory process after smoke inhalation injury. Therefore, the objective of this study is to determine the physiologic effects of PTX and protein kinase C inhibitor (H-7) on smoke inhalation injury in an ovine model.

MATERIALS AND METHODS

Study Design. Sixteen 1- to 2-year-old neutered male, commercially available, random source sheep weighing 25-45 kg were be studied. The animals were housed in covered outdoor runs, treated for parasites (1% ivermectin, 1 ml/75 lb), and fed commercial chow and water ad libitum. Baseline hematologic data (CBC, total proteins, and blood chemistries) were obtained three weeks before study. All animals were fasted 24 h before smoke exposure and use. The animals were randomized to one of two groups. Group I (n=8) received smoke inhalation injury without treatment and Group II (n=8) received smoke inhalation injury with continuous infusion of PTX postinjury.

All animals were anesthetized, orally intubated, mechanically ventilated, placed in the supine position, and catheterized. Cannulae were placed in a femoral artery and a femoral vein. A

radiopaque sheath was placed into an external jugular vein and a Swan-Ganz catheter was inserted through the sheath. Smoke inhalation injury was produced by smoke insufflation at a dose that produces a carboxyhemoglobin level of 50-60% (moderate injury). After smoke exposure, the animals were extubated and observed in the awake state for 24 h. Group I did not receive any treatment with PTX and Group II received PTX as a bolus intravenous injection just after smoke exposure and a continuous intravenous infusion for 24 h. At the end of 24 h, the animals were anesthetized with sodium pentobarbital, orally intubated, mechanically ventilated, and placed in the prone position. The animals were then paralyzed with pancuronium bromide, V_A/Q_C measurements were obtained, and bronchoalveolar lavage was performed.

During mechanical ventilation, the tidal volume was set at 15 ml/kg and the respiratory rate was 12/min. PEEP was 5 cmH_20 and FIO_2 was kept at 0.21 throughout the study period.

Cardiopulmonary variables and blood gases were measured presmoke and at 1, 4, 8, 12, 16, 20, and 24 h postsmoke. Cardiopulmonary measurements included systemic blood pressure, heart rate, pulmonary artery pressure, pulmonary capillary wedge pressure, cardiac output, and pulmonary resistance. Arterial and mixed venous blood samples were analyzed for blood gases. Blood samples for the measurement of 6-keto-PGF_{1a}, thromboxane B_2, and conjugated dienes were drawn at the same time.

Measurement of V_A/Q_C using the multiple inert gas elimination technique (MIGET) was performed at the end of the 24-h study period. Lactated Ringer's solution containing six inert gases was infused. After 30 min when equilibrium of gas exchange occurred, arterial and mixed-venous blood samples were obtained. Mixed-expired gas was collected from a temperature-controlled copper coil about 1 min after blood sampling. Blood and expired gas samples were immediately analyzed by GC.

After blood sample collections, bronchoalveolar lavage was performed to obtain samples from the lower lung lobes for measurement of 6-keto-PGF_{1a}, thromboxane B_2, and conjugated dienes. Extravascular lung water, determined by a gravimetric method (16), was determined after sacrifice.

Description of Procedures. Before smoke exposure, two Silastic® medical grade cannulae (30 cm) were inserted into a femoral artery and femoral vein and one radiopaque sheath introducer (8.5F) was inserted into an external jugular vein using sterile technique after general anesthesia (sodium pentobarbital, 35 mg/kg IV). The arterial line was used for obtaining blood samples for blood gas analyses. The venous line was used for infusion of the solution containing the six inert gases (sulfur hexafluoride, krypton, cyclopropane, halothane, diethyl ether, and acetone) to measure V_A/Q_C inequality using the MIGET. A Swan-Ganz

catheter (7F, American Edwards Laboratories, Irvine, CA) was inserted through the sheath in the jugular vein into the pulmonary artery. Smoke insufflation resulting in a moderate inhalation injury was produced by the method developed at this Institute (1). Animals in Group I did not receive any treatment with PTX. Animals in Group II were administered PTX (Hoechst-Roussel Pharmaceuticals, Inc., Sommerville, NJ) as a bolus injection (20 mg/kg IV) immediately after smoke exposure and a continuous infusion (6 mg/kg/h) for a 24-h period. After smoke exposure, each animal will be extubated upon recovering swallowing/gag reflexes and regaining consciousness, housed in an individual cage in climate-controlled facilities at 74-76°F (24-25°C) with a relative humidity of 40-50%, and observed while spontaneously breathing in the awake state for 24 h after smoke insufflation. At the end of 24 h, the animals were again anesthetized with sodium pentobarbital (35 mg/kg IV), orally intubated, paralyzed with pancuronium bromide (0.03-0.04 mg/kg, Pavulon®, Organon Pharmaceuticals, West Orange, NJ), and placed in the prone position.

Cardiopulmonary variables and blood gases were measured presmoke and at 1, 4, 8, 12, 16, 20, and 24 postsmoke. Cardiopulmonary measurements included systemic blood pressure, heart rate, pulmonary artery pressure, pulmonary capillary wedge pressure, cardiac output, and pulmonary resistance. Pulmonary artery pressure was monitored with Statham P23Db transducers (Statham Instruments, Oxnard, CA) and systemic arterial pressure was monitored with a Hewlett-Packard 1290A quartz transducer (Hewlett-Packard Company, Waltham, MA). These pressures were recorded on a Hewlett-Packard four-channel recorder (Model 7754A). Cardiac output was measured in triplicate by the thermodilution technique (Cardiac Output Computer, Model 9520A, American Edwards Laboratories).

Arterial and mixed-venous blood samples were analyzed for blood gases. Blood gas analyses were performed using an IL1303 pH/blood gas analyzer and an IL282 CO-oximeter (Instrumentation Laboratories, Inc., Lexington, MA). Blood samples for measurement of 6-keto-PGF_{1a}, thromboxane B_2, and conjugated dienes were drawn at the same time. After centrifugation, the plasma of these samples were stored at -70°C for possible future testing. Due to the expense of such measurements, the assays were performed only if expected pathophysiological changes are seen. Thromboxane B_2 and 6-keto-PGF_{1a}, the stable derivations of thromboxane A_2 and PGI_2, were measured by RIA (17). Conjugated dienes, products of lipid peroxidation, were determined by techniques described by Ward et al (18). They were read at an optical density of 233 nm in a spectrophotometer.

Measurement of V_A/Q_C using the MIGET was performed at 24 h after smoke exposure (19). Lactated Ringer's solution containing six inert gases (sulfur hexafluoride, ethane, cyclopropane, halothane, diethyl ether, and acetone) was infused at a rate of 0.1

ml/kg/min. After 30 min when equilibrium of gas exchange occurred, arterial and mixed-venous blood (10 cc each) samples were drawn anaerobically into preweighed, heparinized syringes (30 ml, matched, glass, Becton, Dickinson, and Company) simultaneously. Mixed-expired gas was collected from a temperature-controlled copper coil (OD = 3.49 cm, L = 620 cm) about 1 min after blood sampling, compensating for the delay of the mixing chamber. Blood and expired gas samples were immediately analyzed by GC.

After sample collections, bronchoalveolar lavage was performed with a bronchofiberscope (Olympus CLV-10) to obtain samples from the lower lung lobes for measurement of 6-keto-PGF_{1a}, thromboxane B_2, and conjugated dienes. Twenty milliliters of 0.9% sterile saline were infused in the suction port by the use of a syringe on a three-way stopcock. The fluid was immediately pulled back with suction. This process of lavage and suction was repeated five times (total fluid = 100 ml).

Extravascular lung water, determined by a gravimetric method (16), was measured after sacrifice.

Necropsies were performed on all animals dying spontaneously or sacrificed at the end of the study. A complete set of tissues was fixed in 10% neutral buffered formalin, processed by standard methods, and stained with hematoxylin-eosin. Histologic evaluation with light microscopy was performed on the lung tissue of all animals for quality control and to establish the extent of pulmonary injury, edema formation, and neutrophil and platelet aggregation. Tissue was collected for transmission and scanning electron microscopy, fixed in 2.5% glutaraldehyde, and processed as indicated.

Data Analyses. Statistical analyses were performed using student's t test between the two groups at equivalent times and ANOVA (Tukey) among the treatment groups and a normal, noninjured control group containing four animals.

RESULTS

The progressive hypoxemia observed in the lactated Ringer's treatment group was significantly attenuated in the PTX group during the second 24 h after exposure ($P < 0.05$). The elevated mean pulmonary artery pressure in the nontreated control group was significantly diminished in the PTX group during the entire second 24 h. The pulmonary vascular resistance in the nontreated group rose gradually; in the PTX group, pulmonary vascular resistance was relatively stable throughout the study, and the difference between the two groups was significant at 48 h. Throughout the study, there was no significant difference between the two groups with respect to cardiac index or systemic vascular resistance.

MIGET analysis showed significantly increased dispersion of blood flow distribution on the V_A/Q axis in the nontreated group compared to either the PTX group or normal controls. The percentage of blood flow to the shunt and low V_A/Q area ($V_A/Q < 0.1$) was greater in the nontreated group than in the PTX group or normal controls. These results indicated that the V_A/Q mismatching observed in the untreated group was significantly attenuated in the PTX group.

Total WBC and PMN counts in bronchoalveolar lavage fluid were significantly higher in the nontreated group than in the PTX group or normal controls. Elevated plasma conjugated diene levels observed in the nontreated group were attenuated by PTX treatment; the difference between the groups was statistically significant at 48 h. Conjugated diene levels in bronchoalveolar lavage fluid were significantly higher in the nontreated group than in the PTX group or normal controls. The wet-to-dry lung weight ratio and the total protein content in bronchoalveolar lavage fluid were significantly increased in the nontreated group, but not in the PTX-treated group.

Light microscopic evaluation revealed a significant morphologic difference between the two groups at bronchus level. A significant loss of cilia and erosion of bronchoepithelial cells occurred in the nontreated group; these changes were attenuated in the PTX group. On scanning electron micrographic examination, the bronchial epithelium at the level of segmental bronchus showed no cilia in the nontreated group; in the PTX group, cilia still remained on the epithelial cells, but were matted and disoriented.

DISCUSSION

Bronchopulmonary injury secondary to smoke inhalation is a significant comorbid factor associated with thermal trauma. The present study evaluated the effect of PTX on pulmonary function in an ovine model.

PTX, a methylaxanthine derivative, appears to exert hemorrheologic and antithrombotic effects that may improve microcirculatory blood flow and tissue oxygenation (20) as well as an inhibitory effect on cytokine secretion and leukocyte activation that may attenuate inflammatory response (21). Recently, beneficial effects of PTX treatment have been reported in animal models of injury induced by TNF, chymotrypsin, or *Escherichia coli* (9,10,22-27). In animal models of infection, treatment with PTX has been reported to improve survival rate (5,28).

In this study, continuous treatment with PTX lessened hypoxemia and V_A/Q mismatching in the lung, decreased pulmonary interstitial edema and airway epithelial damage, and attenuated pulmonary arterial hypertension after smoke inhalation. The decrease in conjugated dienes in both blood and bronchoalveolar lavage fluid

with PTX treatment suggests that the effects of PTX may be due in part to a reduction in oxidant injury.

PRESENTATIONS/PUBLICATIONS

None.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

ACCESSION: DA335938

SUMMARY DATE: 921001 **SUMMARY KIND:** D **PREV DATE:** 911001 **DISTRIBUTION:** CX

PROGRAM #: 61102A **PROJ #:** 30161101A91C **TASK AREA:** BD **WORK UNIT:** 079

TITLE: Endocrine Responses of the Burned Rat to Infection and Tumor Necrosis Factor (TNF) Challenge

SUBJ1: 060100 - Biochemistry
SUBJ2: 060500 - Medicine and Medical Research

START DATE: 9108 **END DATE:** 9309 **PERFORMANCE METHOD:** C

CONTRACT/GRANT NO:

CONT TOTAL: $
CUM TOTAL: $
TOTAL LAB FUNDS: $

RESOURCES ESTIMATE

FY	WORK YRS	$(Thousands)
91	0.0	$0
92	0.8	$70
93	0.8	$72

RESPONSIBLE DOD ORGANIZATION: 404885
MRDC INSTITUTE OF SURGICAL RESEARCH USAISR
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FOREIGN INTELLIGENCE CONSIDERED: Y **CIVILIAN APPLICABILITY:** M

KEYWORDS: ILIR; Lab Animals; Rats; Burns (Injuries); Metabolism; Hormones; *Pseudomonas aeruginosa*; Mortality

OBJECTIVE: A DTIC literature search was conducted under DTIC request numbers W6L24K/W6L25M dated 5 August 1991. The objectives of this work are to determine if the endocrine and metabolic responses to burn injury are TNF-mediated and to assess whether infection and antibiotic therapy can modify those responses. These studies will expand understanding of some of the responses to injury and infection and lead to improved management of burn injury and increased survival of burned soldiers.

APPROACH: One hundred and sixty will be assigned to one of 16 groups to assess the hormonal and metabolic influences of burn injury, Pseudomonas infection, and TNF infusion. Body weight, mortality, bacteriologic observations, food and water intake, urinary excretion variables, heat exchange and production (VO_2, VCO_2, metabolic rate, RQ), core temperature, heart rate, and other motor activity will be assessed as influenced by burn, infection, infection treatment, and TNF infusion with suitable ANOVA and regressional models. For those variables collected at multiple times in a 24-h period (mainly temperature, heart rate, and motor activity and perhaps metabolic rate), rhythm analyses will be included with standard cosinor models.

PROGRESS: 9110-9209. Equipment for the determination of metabolic rate, body temperature, heart rate, and motor activity has recently arrived. A full-time technician has been trained to use the equipment. The preliminary phase to determine the thermoneutral zone of rats under various conditions of treatment, e.g., after

hair removal, burns, and burns and infection, is currently in progress. For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report* for fiscal years 1991 and 1992.

ABSTRACT

PROJECT NUMBER: 3A161101A91C-079, In-House Laboratory Independent Research

PROJECT TITLE: Endocrine Responses of the Burned Rat to Infection and Tumor Necrosis Factor (TNF) Challenge

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

PERIOD COVERED IN THIS REPORT: 1 October 1991 - 30 September 1992

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Major changes in thyroid, adrenal, and sympathetic function and in metabolism occur in severe injury and illness and are maintained for days or weeks. Currently, very little is known about how these changes come about and how they relate to one another. The objective of this work is to determine if the endocrine and metabolic responses in burns are TNF-mediated and to assess whether infection and antibiotic therapy can modify those responses.

One hundred and sixty rats will be assigned to one of 16 groups to assess the hormonal and metabolic influences of burn injury, Pseudomonas infection, and TNF infusion. Body weight, mortality, bacteriologic observations, food and water intake, urinary excretion variables, oxygen consumption, heat production, metabolic rate, core temperature, heart rate, and motor activity will be assessed as influenced by burn, infection, infection treatment, and TNF infusion with suitable ANOVA and regressional models. For those variables collected at multiple times in a 24-period (mainly temperature, heart rate, and motor activity, and perhaps metabolic rate), rhythm analysis will be included with standard cosinor models.

ENDOCRINE RESPONSES OF THE BURNED RAT TO INFECTION AND TUMOR NECROSIS FACTOR (TNF) CHALLENGE

Both infection and the administration of TNF produce metabolic changes such as fever, increased energy expenditure, negative nitrogen balance, and muscle wasting that are commonly seen in patients with burn injury. It is possible that infection-induced TNF release is involved in mediating the metabolic and hormonal responses associated with thermal injury. Several studies indicate the involvement of TNF in the control of metabolic responses to injury and infection.

A single intravenous injection *Escherichia coli* endotoxin (4 ng/kg) in normal human volunteers resulted in increased TNF production that peaked between 90-180 min and caused leukocytosis, fever, and a rise in ACTH concentrations (1).

An endotoxin bolus (20 U/kg) in humans increased plasma TNF levels within 90 min, resulted in an elevated total body oxygen consumption by 40%, increased splanchnic glucose output, and augmented splanchnic blood flow by 91%. The peripheral output of lactate and free fatty acids and also the glucose uptake by the periphery increased. Arterial cortisol rose within 2 h of endotoxin challenge and remained elevated during the 6-h study period. Arterial epinephrine rose between 1-2 h. Glucagon and insulin did not change (2).

Studies from this Institute in burn patients with bacteremia have shown findings similar to those seen with endotoxin infusion and TNF actions, i.e., enhanced splanchnic blood flow, increased oxygen consumption, increased lactate and amino acid uptake, and glucose output by the visceral bed (3).

Rats receiving sublethal bolus injections of TNF became tolerant after 4 days, their food intake increased, and their nitrogen balance resembled that of saline-treated animals. Conversely, a continuous infusion of the same dosage of TNF in the rat produced anorexia, weight loss, loss of body proteins and lipids, generalized edema, and a 56% mortality over the 8-day infusion period, indicating that the mode of administration determines TNF actions (4).

A single intravenous injection of 3% and 12% of lethal TNF dose (90 μg/100 g) in normal versus adrenalectomized rats caused 50% and 100% mortality, respectively. The increased sensitivity to TNF in the adrenalectomized rat was associated with hypothermia and severe hypoglycemia. The hypoglycemia resulted from the lack of glucocorticoid-mediated glucose homeostasis and was reversible when the rats were treated with dexamethasone or glucose, suggesting the protective role of steroids in acute injury (5).

A continuous TNF infusion over 1–6 days increased adrenal weight and plasma corticotrophin levels in the rat. Corticosterone or TNF, when infused over 6 days, resulted in marked nitrogen loss, but only TNF infusion resulted in increased liver nitrogen content and reduced jejunal mucosal DNA protein, suggesting an anabolic effect of TNF on liver and a catabolic effect on intestine (6).

Studies at this Institute in rats receiving 30% total body surface area burns and seeded with multiple strains of bacteria revealed that oxygen consumption increased in the bacteremic animals by 40–80%, and in the infected but nonbacteremic animals by 21–28%. Further, topical mafenide acetate application limited the rise in oxygen consumption due to gram-negative, but not gram-positive, wound infection (7).

A 10-day continuous infusion of TNF (~100 μg/kg/day) in the rat produced anorexia, hypermetabolism, hyperglycemia, increased BUN, increased brain tryptophan and 5-hydroxyindole-3-acetic acid, and significant loss of muscle mass, and gain in liver, heart, and lung mass with associated increase in organ DNA and protein content. TNF-mediated hypermetabolism produces visceral anabolic effects at the expense of loss of skeletal muscle mass (8).

Short-term incubation (30–60 min) with TNF neither stimulated lipolysis in rat adipocytes nor did it influence glycogenolysis or gluconeogenesis in the hepatocytes. However, when adipocytes were preincubated with TNF, the adrenaline-stimulated fatty acid release was increased (9).

Coinfusion of TNF with IL1 in the rat increased net hepatic anabolism at the expense of skeletal protein breakdown, suggesting that TNF facilitates the metabolic actions of IL1 (10).

A 4-h TNF infusion in the rat, either alone or together with IL1, raised the body temperature by 1.8°C, caused a 40% reduction in serum zinc and iron, and produced 45% neutrophilia. TNF or TNF plus IL1, but not IL1 alone, increased proteolysis (11).

Plasma IL1 and TNF levels were measured by RIA in normal subjects, septic patients, and endotoxin-infused volunteers. With endotoxin infusion, IL1 increased from 35 pg/ml to 69 pg/ml at 3 h and TNF levels rose > 500 pg/ml in 90 min in human volunteers. IL1β was 62 pg/ml in normal subjects and 120 pg/ml in septic patients. TNF concentrations were 73 pg/ml in normal subjects and > 119 pg/ml in septic patients. TNF concentrations were correlated with disease severity in the septic patients, such that higher IL1 levels were associated with patient survival (12).

Several of the metabolic responses to injury can be reproduced by the infusion of hormones (13,14) or cytokines (1,15). The increased whole-body proteolysis measured by plasma leucine flux observed with the earlier preparations of IL1 (16,17) has not been

reproduced in subsequent studies with recombinant IL1, indicating that the catabolic effects seen with crude preparations of IL1 might have been due to contamination of previous IL1 preparations with other macrophage products (18).

Taken together, the above studies indicate that infection, both with gram-positive and gram-negative bacteria, stimulates TNF production, which, in turn, mediates some of the metabolic and hormonal responses to injury through mechanisms that may alter the release or modify the actions of other cytokines and hormones. Most studies thus far have been conducted on a short-term basis and do not permit the assessment of effects of continuous and prolonged exposure of various organ systems to the actions of TNF.

Since continuous and prolonged TNF release might be essential to the development of changes typical of flow-phase of injury, e.g., increased metabolic rate and elevated stress hormones, this study will simulate TNF release during disease by continuously infusing TNF in the rat over a 10-day to one-week period, which will allow assessment of whether or not the prolonged administration of TNF can alter stress hormones, metabolism, serum TNF, and interleukin levels or mortality of the animals. In parallel studies in nontreated and antibiotic-treated animals, the metabolic disturbances produced by experimental burn wound infection will be investigated.

In aggregate, this study in the rodent will help define the role of burn wound infection and TNF in the metabolic responses of an organism to injury.

MATERIALS AND METHODS

Design. One hundred and sixty male Sprague-Dawley rats weighing 180 to 200 g will be assigned to one of 16 groups to assess the hormonal and metabolic influences of burn injury, Pseudomonas infection, and TNF infusion as indicated in Table 1.

Description of Procedures. One hundred and sixty healthy adult male Sprague-Dawley rats weighing 180 to 200 g will be used for this study. Animals will be housed in individual metabolic cages in a 14:10 h light:dark-cycled room at a constant 30°C ambient temperature and fed tap water and standard laboratory chow ad libitum. All animals will be observed for a period of two weeks prior to use to exclude the possibility of any preexisting disease.

After the two-week acclimatization period, a temperature monitoring transmitter (Mini-Mitter, Inc, Sun River, OR) will be placed intraperitoneally in all animals for daily monitoring of body temperature, heart rate, and activity without handling the animals. Animals will be then be allowed to recover for 5 to 7 days.

TABLE 1. Animal Group Designations

Group	Description	n=
C	20% sham burn	10
B	20% TBSA burn	10
BI	20% TBSA burn + infection	10
BP24	20% TBSA burn + infection + piperacillin sodium @ 24 h	10
BP72	20% TBSA burn + infection + piperacillin sodium @ 72 h	10
TNF	Osmotic pump + buffer	10
TNF1	Osmotic pump + 10 µg/kg TNF/day	10
TNF2	Osmotic pump + 30 µg/kg TNF/day	10
TNF3	Osmotic pump + 100 µg/kg TNF/day	10
PF	Pair-fed controls for Groups B, BI, BP24, BP72, TNF1, TNF2, and TNF3	70

Animals in Groups B, BI, BP24, BP72, and C (n=10 each) will be anesthetized with sodium pentobarbital (35 mg/kg IP) administered through a 25-ga needle. The dorsal area will be shaved. Animals will be placed in a plexiglass mold designed to expose 20% of the total body surface area (TBSA). Animals in the 20% TBSA burn groups (designated as B) will be exposed to 100°C water for 10 sec while animals in the 20% TBSA sham burn group (Group C) will be exposed to water at room temperature.

The burn wounds of animals in Groups BI, BP24, and BP72 will be painted with 10^8 cfu *Pseudomonas aeruginosa* (Strain 1244). An aliquot of frozen *Pseudomonas aeruginosa* will be incubated in trypticase soy broth for a period of 18 h at a temperature of 37°C in a shaker bath. The bacterial suspension will then be centrifuged at 3000 rpm for 5 min and the resulting bacterial pellet will be washed three times in normal saline solution. After the final wash, the pellet will be resuspended in sufficient normal saline to achieve a final concentration of 10^8 cfu/ml. This suspension will be applied to the animals' burn wounds at 15 min postburn.

Animals in Groups BP24 and BP72 will be administered piperacillin sodium (50 mg/kg SC) every 12 h beginning at 24 (Group BP24) or 72 h (Group BP72) postinfection for a period of six days. Based on previous experience, we expect infected animals to succumb within two weeks of infection and antibiotic-treated animals to survive for varying lengths of time.

Animals in Groups TNF, TNF1, TNF2, and TNF3 (n=10 each) will be anesthetized with sodium pentobarbital (35 mg/kg IP) administered through a 25-ga needle. A small subcutaneous incision will be made

on the dorsal area of skin and an osmotic pump (Alzet™, Alza Corporation, Palo Alto, CA) will be implanted. The incision will be closed with a monofilament suture. The osmotic pump will allow for a constant rate continuous infusion of buffer or TNF. Animals in Group TNF will be administered diluent buffer, Group TNF1 will be administered recombinant human TNF (Genentech, Inc., South San Francisco, CA) at 10 μg/kg/day, Group TNF2 at 30 μg/kg/day, and Group TNF3 at 100 μg/kg/day.

Food intake of animals assigned to Groups B, BI, BP24, BP72, TNF1, TNF2, and TNF3 will be assessed daily to determine the amount of food that the pair-fed animals in Group PFC will receive the next day.

Housing of animals individually in metabolic cages will allow measurement of food intake, water intake, and urine output as well as urinary excretion of electrolytes, nitrogen, catecholamines, and corticosterone.

Blood samples from the tail vein will be drawn using a 27-ga needle in restrained surviving animals seven days after the time of burn or osmotic pump implantation for determination of thyroid hormones T_4 and T_3, free T_4 by dialysis, T_3U, corticosterone, and recombinant human TNF. A second blood sample will be obtained on day 14 (trunk blood) upon sacrifice of the animals by decapitation for determination of the above variables as well as electrolytes, glucose, BUN, and creatinine. TNF will be measured by ELISA (19,20).

The heart, lungs, liver, spleen, and mesenteric lymph nodes of all animals will be cultured for bacteriologic growth after spontaneous death or sacrifice.

For surviving animals at each time point, gas exchange and REE will be determined on the day prior to and 6 h and 1, 2, 5, and 10 days after burn injury/implantation by means of indirect calorimetry (Columbus Instruments, Columbus, OH). Individual animals will be acclimatized in the metabolic chambers for 30-60 min to achieve a steady state prior to measuring their metabolic rate (MR). For 1 to 3 h, animals (up to 12 at a time in separate chambers) will be sampled for approximately 1 min in sequence with automatic continual rotation. MR will be calculated using the following equation that utilizes Abramson's coefficients (21) and gas exchange measured with special airtight chambers, O_2 (electrochemical) and CO_2 (infrared spectrometric) sensors, and computer interfaces:

$$MR = VO(-4.83x + 0.218y)$$

where VO indicates test chamber ventilation rate; x, the difference in gas fraction of O_2 that appears across the test chamber; and y,

the difference in gas fraction of CO_2 that appears across the test chamber.

Temperature, heart rate, and activity of the animals will be monitored using telemetry devices. Such data can be collected whether the animals are in their "home" metabolic cages or in the gas exchange chambers for MR measurement. Model CTA-F40 small transmitter units implanted intraperitoneally will permit temperature, heart rate, and muscular activity (body movement) measurements when used with CTR86 receivers (Mini-Mitter Co.). Temperature (as fever) is a classical response to injury, infection, and cytokines such as TNF and together with heat production (MR) and nitrogen excretion contributes an important element in assessing the metabolic response to injury, infection, and TNF. Heart rate provides an assessment of sympathetic activity. We will be able to determine whether there is an influence of injury, infection, or TNF on the 24-h pattern of temperature, heart rate, and skeletal motor activity. An additional advantage of activity measurement will be to allow us to identify (for MR measurements) comparable periods of inactivity or activity between groups and to obtain MR values for periods of high (night) and low (day) motor activity. Correlation of MR and temperature with motor activity should allow us to assess the influence of motor activity on MR and temperature in control, burn, infected, and TNF-infused states.

For MR interpretation, not only the total MR but also that not due to motor activity is of importance. If motor activity occurs and influences MR during the latter's measurements in a given session (1-3 h), periods of varying motor activity will have MR varying accordingly to permit regressional assessment of nonmobile MR. This approach will likely also permit assessment of a different effect of exercise on MR among groups. The effect of heat loss is produced through a thermoregulatory reflex designed to defend normal body temperature at ambient temperatures below the thermoneutral. Because heat loss is not measured directly in this system, an interference from heat loss will be minimized in two ways. First, the MR will be measured at an ambient temperature in the thermoneutral range (often 25°C to 30°C for sham-burn rats and 30°C to 34°C for burns; but, this must be determined, as below, for our treatment groups). Second, the intensity of collection (continuous) of core temperature data will allow monitoring of whether this variable is maintained at or above that for respective control rats without burns, infection, or TNF infusion. Such would indicate a resetting of metabolic drive (defending an elevated core temperature) rather than just a failing attempt to defend the control (normal) core temperature.

Measurement of MR and core temperature in a preliminary experiment with the same groups (as outlined in Table 1) at several chamber temperatures will establish the upper and lower critical limits of the thermoneutral range of ambient temperature for each

group. There is no way otherwise to insure that we can predict an ambient chamber temperature that is in the respective thermoneutral range, which has not been determined for the particular kinds of treatments (groups) we require in the main phase of this protocol, as explained at the end of paragraph (i) above. This will be done at three or more times in the first 14 days after treatment. This will require the animals to be out of their "home" cages for much of the time for very extensive MR measurements which will not permit a meaningful plan for sampling of blood and urine during the times of correct ambient temperature as required on a schedule in the main phase of this protocol. This preliminary phase will also allow us to work out ("trouble-shoot") many potential problems in the coordination of the many exacting procedures related to blood and urine sampling, electronic data collection, and operation of the equipment.

Determination of Number of Animals Required. There will be seven experimental groups, two control groups, and seven pair-fed groups for a total of 16 groups. For this pilot study, 10 animals per group will be necessary to achieve statistical significance. Therefore, 320 animals (160 for the preliminary phase plus 160 for the main phase of the study) will be required.

Data Analysis Plan. Body weight, mortality, bacteriologic observations, food and water intake, urinary excretion variables, blood chemical and hormonal variables, variables related to gas exchange and heat production (VO_2, VCO_2, MR, RQ), core temperature, heart rate, and motor activity will be assessed as influenced by burn, infection, infection treatment, and TNF infusion with suitable ANOVA and regressional models. For those variables collected at multiple times in a 24-h period (mainly temperature, heart rate, and motor activity, and perhaps MR), rhythm analysis will be included with standard cosinor models. From these analyses, inferences can likely be drawn concerning the role of several hormonal systems in the metabolic response to burn/infection, and the mediation of the hormonal and metabolic responses by TNF. Then, future studies can be designed (adrenalectomy, thyroidectomy, hormonal replacement) to test specific inferences.

RESULTS

A preliminary phase of this study was conducted to determine the thermoneutral zone of rats under various conditions of treatment, e.g., after hair removal, burns, and burns and infection.

Both fur clipping and burn injury raised the metabolic rate, but the metabolic rate in the burned animals was significantly higher than that of nonclipped and clipped controls. As anticipated, the metabolic rate was temperature-sensitive under all experimental conditions, i.e., in cold environment, the metabolic

rate rose and in warm environment it fell. The burned animals developed fever (mean body temperature 38°C), which was maintained throughout the three-week study period.

DISCUSSION

These results indicate that burn injury in the rat produces a state of hypermetabolism that can be explained neither on the basis of body cooling, since burned animals had an elevated body temperature, nor on a Q10 effect of the fever, since metabolic rate after burns rose considerably more than calculated for a Q10 of 2.3.

From these observations, it was concluded that the thermographic control after burn injury is reset at a higher level and that the metabolic rate in the burned animals, though temperature-sensitive, is not temperature-dependent.

Based on regression analysis, the lower critical temperature values were 29°C, 32°C, and 34°C for normal, clipped, and burned rats, respectively. This rightward shift of the lower critical temperature ($P < 0.05$) resulted in a higher metabolic rate than in controls at a given ambient temperature below the lower critical temperature. As anticipated, the metabolic rate remained ambient temperature-responsive.

The modified linear regression technique developed for use in the present analysis permits direct estimates of the lower critical temperature and thermoneutral metabolic rate for seven or more points of metabolic rate and ambient temperature data for individual rats and for a group of rats studied under a given treatment condition.

During this reporting period, it was discovered that though the Oxymax™ system appears to detect directional changes in the metabolic rate of the animals, the equipment produces unexpected, variable results. Presently, possible sources of error in this equipment, such as individual component malfunction, as leaks, or inadequate mixing of the gases within the test chambers, are being investigated.

PRESENTATIONS/PUBLICATIONS

None.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

ACCESSION: DA336048

SUMMARY DATE: 920311 **SUMMARY KIND:** A **PREV DATE:** **DISTRIBUTION:** CX

PROGRAM #: 61101A **PROJ #:** 30161101A91C **TASK AREA:** EA **WORK UNIT:** 080

TITLE: Studies of Wound Healing in a Rat Model

SUBJ1: 060500 - Medicine and Medical Research

START DATE: 9203 **END DATE:** 9309 **PERFORMANCE METHOD:** C

CONTRACT/GRANT NO:

CONT TOTAL: $
CUM TOTAL: $
TOTAL LAB FUNDS: $

RESOURCES ESTIMATE

FY	WORK YRS	$(Thousands)
91	0.0	$0
92	0.3	$16
93	0.3	$18

RESPONSIBLE DOD ORGANIZATION: 404885
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ASSOC1: MASON, A D

ASSOC2: OKERBERG, C V

FOREIGN INTELLIGENCE CONSIDERED: Y **CIVILIAN APPLICABILITY:** M

KEYWORDS: ILIR; Lab Animals; Rats; Burns (Injuries); Wounds and Injuries; Skin Grafts; Healing; Therapy

OBJECTIVE: A DTIC literature search was conducted under DTIC request numbers W6P08I/W6P09I dated 27 January 1992. The objective of this work is to develop a pragmatic solution to the problem of burn wound closure in patients with large burns. Development of techniques making autogenous skin available in abundance and permitting swift wound closure could improve patient survival.

APPROACH: Sprague-Dawley rats will be randomized to one of four groups, i.e., autologous explant, allogeneic explant, mixed explant, or no explant. After hair removal, a 2-X 2-cm strip will be removed from the dorsal area. A 2 X 0.5-cm strip will be removed from the edge of this skin strip. This smaller skin strip will be diced into 1-mm cubes and used as explants to seed excised wounds. After undermining the wound edges to facilitate closure, the larger skin strips will be stapled in place over the wound. Following separation of the allogeneic skin dressings, the wounds will be photographed at weekly intervals. After sacrifice at the time of complete healing of the excised wounds, pelts will be harvested for histopathologic study.

PROGRESS: 9203-9209. This study was approved by the USAISR Research Council and US Army Institute of Surgical Research Animal Care and Use Committee during the second quarter of Fiscal Year 1992. Equipment and supplies have been ordered and work will be initiated shortly. For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1992.*

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

ACCESSION: DA336048

SUMMARY DATE: 921001 **SUMMARY KIND:** D **PREV DATE:** 920311 **DISTRIBUTION:** CX

PROGRAM #: 61101A **PROJ #:** 30161101A91C **TASK AREA:** EB **WORK UNIT:** 080

TITLE: Studies of Wound Healing in a Rat Model

SUBJ1: 060500 - Medicine and Medical Research

START DATE: 9203 **END DATE:** 9309 **PERFORMANCE METHOD:** C

CONTRACT/GRANT NO:

CONT TOTAL: $
CUM TOTAL: $
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ASSOC1: MASON, A D

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FOREIGN INTELLIGENCE CONSIDERED: Y **CIVILIAN APPLICABILITY:** M

KEYWORDS: ILIR; Lab Animals; Rats; Burns (Injuries); Wounds and Injuries; Skin Grafts; Healing; Therapy

OBJECTIVE: A DTIC literature search was conducted under DTIC request numbers W6P08I/W6P09I dated 27 January 1992. The objective of this work is to develop a pragmatic solution to the problem of burn wound closure in patients with large burns. Development of techniques making autogenous skin available in abundance and permitting swift wound closure could improve patient survival.

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PROGRESS: 9203-9209. This study was approved by the USAISR Research Council and US Army Institute of Surgical Research Animal Care and Use Committee during the second quarter of Fiscal Year 1992. The experimental technique did not permit differentiation between wound healing due to proliferation of skin explants and that due to wound contraction. Future efforts will be directed toward development of a model that will allow assessment of wound closure with skin explants without the influence of wound contraction. For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1992.*

ABSTRACT

PROJECT NUMBER: 3A161101A91C-080, In-House Laboratory Independent Research

PROJECT TITLE: Studies of Wound Healing in a Rat Model

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

PERIOD COVERED IN THIS REPORT: 11 April 1992 - 30 September 19912

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Development of techniques making autogenous skin available in abundance and permitting swift wound closure could improve patient survival. The objective of this study is to develop a pragmatic solution to the problem of burn wound closure in patients with large burns.

Sprague-Dawley rats are randomized to one of four groups, i.e., autologous explant, allogeneic explant, mixed explant, or no explant. After hair removal, a 2-X 2-cm strip is removed from the dorsal area. A 2 X 0.5-cm strip is removed from the edge of this skin strip. This smaller skin strip is diced into 1-mm cubes and used as explants to seed excised wounds. After undermining the wound edges to facilitate closure, the larger skin strips are stapled in place over the wound. Following separation of the allogeneic skin dressings, the wounds are photographed at weekly intervals. After sacrifice at the time of complete healing of the excised wounds, pelts are harvested for histopathologic study.

The experimental technique did not permit differentiation between wound healing due to proliferation of skin explants and that due to wound contraction. Future efforts will be directed toward development of a model that will allow assessment of wound closure with skin explants without the influence of wound contraction.

STUDIES OF WOUND HEALING IN A RAT MODEL

Current methods of burn wound closure are less than ideal and impose technique-specific restrictions. For example, autografting, which remains the ultimate aim of excisional burn wound therapy, is limited by the availability of unburned donor sites in patients with extensive burns. Further, sequential excision with autografting of the burn wound incurs at least a two-week delay before donor sites heal and become available for reharvesting. Moreover, with multiple harvesting, the donor site skin becomes progressively thinner with each harvest, takes longer to reepithelialize and, if a donor site becomes infected, converts to a full-thickness skin defect. Such limitations on the autografting of patients with large burns has stimulated interest in other means of wound closure.

Wound coverage with cultured human epidermal keratinocytes has recently become possible (1-3). With this technique, however, there is a minimum delay of 2-3 weeks from the time of skin sampling to the delivery of cultured cells ready for use. Moreover, the delicate cultured cells, unlike conventional split-thickness skin grafts, require a meticulously prepared wound bed for survival and acceptance, are destroyed in the presence of minimal wound infection, take poorly over chronic granulation tissue, and are easily sheared from the wound bed. Consequently, keratinocyte-grafted wounds develop abnormally fragile skin due to the poor development of anchoring fibrils (4).

Another method of closure of the excised burn wound employs allograft skin in the form of split-thickness graft or as cultured epidermal cells. Reportedly, human allograft, under protective cover of systemic immunosuppressive therapy (5), and allogeneic cultured epidermal cells without immunosuppression (6,7) engraft successfully. The results of these studies, however, have been neither confirmed nor duplicated by other investigators. When cultured allogeneic keratinocytes were examined with DNA hybridization technique in long-term studies in sex-mismatched grafts, the allograft biopsies after the first posttransplant week exhibited the phenotype of the host, indicating that shortly after transplantation, the allogeneic grafts were replaced with regenerated host epithelium (8,9).

A lack of dermis in keratinocyte-grafted wounds may be responsible to some extent for the poor results seen with the cultured cells. To counter this problem, an artificial skin consisting of a collagen-based dermal element and a disposable outer Silastic® membrane, which is replaced later with an autologous split-thickness skin graft, has been studied in humans (10). Evaluation of such artificial skin at this Institute showed no significant advantage over customary skin grafting procedures because of the delay incurred while awaiting dermal analogue

vascularization before the Silastic® covering membrane could be stripped off and replaced with split-thickness graft. Wounds treated with artificial skin took twice as long to heal as the ones grafted in the usual manner. Even when data were pooled from all 11 centers participating in this study, the artificial skin showed no time-saving advantage over ordinary skin grafts (11).

Yet another attempt at mimicking normal skin is the development of a composite graft consisting of allograft skin modified by the removal of its epidermis and seeded with either autologous epidermal cells harvested from blister epithelium (12) or cultured keratinocytes (13). The composite graft reportedly provides durable skin coverage. However, before autologous grafting may be performed, mechanical removal of the epidermal elements of the adherent allograft is necessary. The removal of the allograft epidermis is not only tedious and time-consuming, but can also result in complete dislodgment of the allograft from the recipient wound bed, with consequent delay of wound closure.

In sum, the surgical treatment of a patient with extensive burns is restricted by a paucity of unburned autologous skin. None of the methods currently available for closing such wounds is ideal; all need further refinement. A search continues both for an ideal skin replacement material and for techniques that induce accelerated skin growth. If cultured keratinocytes, which can be grown in quantities sufficient to cover an entire human body, can be qualitatively improved, this will facilitate wound coverage following extensive burns. In order to study the failure of cultured keratinocytes to adhere well to an excised burn wound, we need to develop an animal model in which the normal healing process can be examined in detail.

Normal healing is a complex and dynamic process that involves cell proliferation, cell-to-cell and cell-to-substrate interactions and the physiologic adhesion of the cells. Basement membrane, integrins, intermediate filaments, and some of the better characterized mechanisms of cellular adhesion are summarized below:

The basement membrane, basal lamina, or dermal-epidermal junction anchors the basal epidermal cells to the underlying dermis, is acellular, stains red with periodic acid-Schiff stain, and consists of four distinct layers when visualized by electron microscopy.

Zone I = Composed of the most superficial layer of the basement membrane; contains inner and outer leaflets of the basal cell plasma membrane, tonofilaments and hemidesmosomal pyramids.

Zone II = Composed of the lamina Lucida accommodating two structures:

Subbasal dense plaques which are as long as the hemidesmosomal pyramids.

Anchoring filaments located perpendicular to the dermis and running parallel to each other between the outer leaf of the basal cell plasma membrane above and the lamina densa below and connecting the basal cell plasma membrane to the underlying lamina densa.

Zone III = Composed of lamina densa, which attaches epidermis to the dermis.

Zone IV = Zone of attachment of the lamina densa to the dermal layer of the skin containing three different types of collagen bundles, i.e., anchoring fibrils, microfibrillar bundles, and collagen fibers (14).

Wound healing studies indicate that epithelial cells that migrate to the epidermal-dermal interface and come in contact with collagen fibers form the lamina densa. When trypsin-separated epidermis recombined with freeze-thaw killed and inverted dermis was transplanted into chick chorioallantoic membrane, a new lamina densa formed opposite in location to the hemidesmosomes, which may have some control over the regeneration of lamina densa (15).

Epidermal cells, when cultured in Petri dishes, form no lamina densa, indicating that the inanimate plastic Petri dish lacks the substrate needed to form a lamina densa (16). On the other hand, both hemidesmosomes and a lamina densa are reported to form at the collagen interface when guinea pig epidermal cells are grown on collagen substrate (17). The order of events leading to lamina densa formation in collagen gel proceeds from the formation of hemidesmosomal plaques through the expression of subbasal dense plaques and anchoring filaments (16).

Similarly, when explants of full-thickness human skin were implanted on to inverted freeze-thawed, gamma irradiation-killed pig dermis, the transplanted epidermis grew from the explant and onto the dead pig dermis, but hemidesmosomes and a lamina densa formed only in areas where either the epidermal cells were in contact with a living substrate or with remnants of elastic fibers, specifically the oxytalan fibrils of the dead dermis (5). Cultured human epidermal keratinocytes when transplanted to excised burn wounds in humans form basal lamina only after years of contact with the wound bed.

In summary, the studies cited indicate that basal epidermal cells in contact with living substrate or collagen fibers develop basal lamina that anchor the basal epidermal cells to the connective tissue of the wound.

Aside from the basal lamina described above, other well characterized substances that participate in cellular adhesion are integrins, intermediate filaments, and collagen bundles. Integrins are a family of glycoproteins containing two subunits, α and β, which function as transmembrane extracellular receptors. The presence, for instance, of $\alpha_2\beta_1$ and $\alpha_3\beta_1$ complexes in the lateral and apical surfaces of the basal epidermal cells allows them to adhere to adjacent cells and to the immediately proximal cells, while expression of $\alpha_6\beta_4$ complexes on the hemidesmosomes helps attach polar ends of the basal cells to the connective tissue of the wound (19).

In experimentally induced corneal wounds, integrin $\alpha_6\beta_4$ appears within 24 h along the surface of the epithelial cells that have migrated from the wound edges to the connective tissue of the wound base. At this stage, however, anchoring filaments and laminin are detectable in the cell; hemidesmosomes have not yet formed. At a later stage, when the migrant cells express cytosolic hemidesmosomal components, i.e., bullous pemphigoid antigens and type VII collagen, the integrin $\alpha_6\beta_4$ preferentially concentrates along the basal pole of the epithelial cells in contact with the connective tissue of the wound (20). Both the anchoring filaments, which are composed of a 125 Kd polypeptide and the $\alpha_6\beta_4$ glycoprotein complex, form the extracellular elements of the hemidesmosomes. The bullous pemphigoid antigens and type VII collagen containing anchoring fibrils, which respectively form cytosolic and extra-cytosolic components of the hemidesmosomes, appear between 48 and 72 h after cell migration. Compared to the early appearance of anchoring fibrils in the epithelial explants, the regeneration of those elements in the grafted keratinocytes is reported to be a much lengthier process (21).

The cytoskeleton plays an important role in maintaining cell integrity and is made of intermediate filaments belonging to a heterogenous family of over 30 proteins (22). The intermediate filaments that make up the cytoskeleton of the keratinocyte are known as tonofilaments and are composed of keratin molecules of two different types, i.e., Type I, which is relatively acidic, and Type II, which is relatively basic. Though one acidic and one basic keratin is required for proper in vivo polymerization, isolated acidic and basic keratins in vitro can pair promiscuously. Epithelial cells, depending on their stage of differentiation, maturity, and cell type, express specific pairs of keratins, e.g., K14 (Type I) and K5 (Type II) predominate in the epidermal basal cells and K1 and K10 in the suprabasal cells. An abnormal expression of the keratin genes that encode K5-K14 impairs tonofilament formation and produces mechanical instability of the basal cells, manifested clinically as epidermolysis bullosa simplex, a disease characterized by excessive skin blistering following trivial trauma (23). Defective encoding, similarly, of the collagen VII genes disrupts anchoring fibrils formation and the resultant severe skin fragility is the characteristic feature of

one of a genetically inherited skin disease, dystrophic epidermolysis bullosa (24).

Collectively, the three structures, i.e., hemidesmosomes that form the cytoskeleton, anchoring filaments that connect the plasma membrane of a cell to its lamina lucida, and anchoring fibrils that underlie the lamina densa and anchor the entire cell to the underlying dermis (25,26), have been termed the "adhesion complex" (27). Defects in the adhesion complex producing skin fragility can be traced to abnormalities in the basal cell layer of the skin. The basal cell defects that produce an abnormal adhesion complex in epidermolysis bullosa may also be the basis for poor graft take of cultured keratinocytes in humans. Other factors besides anchoring fibrils are deficient in the engrafted cultured keratinocytes (28) and need study.

These studies indicate that the epidermal cells require a signal for basal lamina formation to anchor the epidermis to the dermis, and it appears that the signal is produced by the living substrate. If full-thickness explants of skin containing the substrate essential for basal lamina production are transplanted to the excised burn wound, they may regenerate relatively more mature skin in a shorter time than isolated cultured epidermal keratinocytes that depend on the host wound bed for their supply. If implanting full-thickness skin explants is shown to work in this manner, substantial shortening of the time required for satisfactory, durable closure of excised wounds may be possible.

To enhance understanding of cell-to-cell adhesion, we would like to develop a simple model of wound healing in which to study the fate of full-thickness skin explants containing all the elements essential to basal lamina production. In such a model, we will assess components of the adhesion complex in tissues treated with specific fluorescent antibodies, using electron microscopy.

MATERIALS AND METHODS

Experimental Design. Complete healing times for surgically excised wounds immediately seeded with species-specific preparations of full-thickness skin explants will be assessed. The explant-seeded excised wounds are covered with allogeneic skin stapled in place to protect the explants against mechanical trauma and to provide them with a natural environment of controlled pH, humidity, and gas content that may be essential for their optimal growth. After the allogeneic skin separates spontaneously, the progress of wound healing with explanted skin is visually monitored. Time to complete wound closure is recorded for each individual animal. To document healing, photographs of the wound are taken and wound biopsies are performed at appropriate intervals. Horizontal sections of biopsy specimens are examined with routine and electron microscopy. Animals are sacrificed upon complete resurfacing of the wound. Pelts are harvested for further

histopathologic studies with light and electron microscopy to determine the morphologic characteristics of the grafted wound.

Description of Procedures. Male Sprague-Dawley rats weighing 180-200 g are anesthetized with sodium pentobarbital (35 mg/kg IP) through a 25-ga needle. Animals are then assigned to study groups as indicated at Table 1.

TABLE 1. Grouping of Animals

Group	n=	Source of Dressing	Source of Explant
I	25	Group II	Group I (Autologous)
II	25	Group IV	Group IV (Allogeneic)
III	25	Group I	Groups I + III (Mixed)
IV	25	Group III	None

The dorsal surface of each animal is shaved. A 2 X 2-cm full-thickness skin strip is removed from the dorsal area. A 2 X 0.5-cm strip is removed from the edge of this skin strip. This smaller skin strip (2 X 0.5-cm^2) is diced into 1-mm cubes to be used as explants to seed the excised wounds, and the larger skin strip (2 X 1.5-cm^2) provides the wound dressing. Animals in Group IV receive no skin explants, while those in Group I receive explants of their own skin; animals in Group II receive skin explants from Group IV and those in Group III receive a 50:50 mix of their own skin and the skin of Group I animals. After undermining the wound edges to facilitate closure, the larger skin strips are stapled in place over the wounds. Wound dressings for Group I come from Group II, Group II from Group IV, Group III from Group I, and Group IV from Group III. Thus, all dorsal wounds are covered with allogenic skin grafts. The edges of the excised wounds are tattooed with india ink to assess healing by wound contracture. Animals are housed individually in cages where they have free access to food and water. Following separation of the allogeneic skin dressings, the wounds are photographed and biopsied and the procedures repeated at weekly intervals. Full-thickness wound biopsies include two contiguous skin explants to assess basement membrane formation and to study the morphologic characteristics of the cells migrating from the explants. Upon complete healing of the excised wounds, the animals are sacrificed by sodium pentobarbital overdose (60 mg/kg IP) through a 25-ga needle. The pelts are harvested for further histopathologic study.

Determination of Number of Animals Required. This is a pilot study. Wound healing will be evaluated in four groups of animals with a minimum for scientific validity of 25 animals per group for a total of 100 rats.

Data Analysis Plan. Significance of differences among groups will be determined using ANOVA and covariance analysis.

RESULTS

When staples securing the allogeneic skin cover were removed on the seventh postoperative day, the skin cover remained firmly adherent to the underlying excised edges of the wound for an additional three weeks. By then, however, the underlying excised wounds healed and progress of wound healing could not be monitored visually.

DISCUSSION

From these studies, we could not determine whether healing ensued from the outgrowth of epithelium from the wound edges, or from the skin explants, or both. Since allogeneic skin does not suit the needs of this study, a search is underway to find a nontoxic, tissue-nonreactive, transparent, durable, flexible, synthetic material that will allow the study of explanted skin. In the interim, however, studies will be initiated using sheets of transparent Silastic™ that keep the wound edges apart, prevent wound contraction, and allow visual monitoring. The amount of explanted skin will also be reduced in each successive study in order to determine the minimum amount of the explanted skin needed to satisfactorily close a full-thickness skin defect in a reasonable period of time.

PRESENTATIONS/PUBLICATIONS

None.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

ACCESSION: DA336049

SUMMARY DATE: 920311 **SUMMARY KIND:** A **PREV DATE:** **DISTRIBUTION:** CX

PROGRAM #: 61101A **PROJ #:** 30161101A91C **TASK AREA:** AZ **WORK UNIT:** 081

TITLE: D-Myo-Inositol-1,2,6-Triphosphate (PP56) and Burn Wound Edema in a Burned Rat Model

SUBJ1: 060500 - Medicine and Medical Research

START DATE: 9203 **END DATE:** 9309 **PERFORMANCE METHOD:** C

CONTRACT/GRANT NO:

CONT TOTAL: $
CUM TOTAL: $
TOTAL LAB FUNDS: $

RESOURCES ESTIMATE

FY	WORK YRS	$(Thousands)
91	0.0	$0
92	0.2	$19
93	0.2	$21

RESPONSIBLE DOD ORGANIZATION: 404885
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FT SAM HOUSTON, TX 78234
PRUITT, B A
210-221-2720

PERFORMING ORGANIZATION: 404885
MRDC INSTITUTE OF SURGICAL RESEARCH USAISR
FT SAM HOUSTON, TX 78234
MOZINGO, D W
210-221-3825

ASSOC1: BECKER, W K

ASSOC2: CIOFFI, W G

FOREIGN INTELLIGENCE CONSIDERED: Y **CIVILIAN APPLICABILITY:** M

KEYWORDS: ILIR; Lab Animals; Rats; Burns (Injuries); Edema; Healing; Therapy; Gravimetric Analysis; Histopathology; Electron Microscopy

OBJECTIVE: A DTIC literature search was conducted under DTIC request numbers W6K55A/W6K55E dated 4 March 1992. The objective of this work is to evaluate the effect of PP56 on burn wound edema in a burned rat model by administering PP56 at various doses and times after burn injury. Decreasing burn wound edema formation may simplify fluid resuscitation and reduce the morbidity and mortality of patients with thermal injury.

APPROACH: Animals will be anesthetized and a catheter will be placed into the right jugular vein. Four days after this procedure, the animals will again be anesthetized, the dorsal surface will be shaved, and a 20% full-thickness total body surface area scald burn will be inflicted. Animals will then be randomized to receive either PP56 or saline beginning at 0, 1, or 3 h postburn. Twelve animals from each subgroup will be sacrificed at 12, 24, or 48 h postburn. Burn wound edema will be evaluated by either Evans blue or by the gravimetric method. Before edema analysis, a weighed biopsy sample will be obtained from each burn wound for histopathological analysis of edema thickness and two samples from each group will be obtained for electron microscopy. Data will be analyzed by ANOVA as a 2 X 3 X 3 factorial design with appropriate consideration of all interactions. If appropriate, further analysis will be done using multiple regression techniques.

PROGRESS: 9203-9209. This project was approved by the USAISR Research Council and US Army Institute of Surgical Research Animal Use Committee during the second quarter of Fiscal Year 1992.

Equipment and supplies have been ordered and work will be initiated shortly. For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1992.*

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

ACCESSION: DA336049

SUMMARY DATE: 921001 **SUMMARY KIND:** D **PREV DATE:** 920311 **DISTRIBUTION:** CX

PROGRAM #: 61101A **PROJ #:** 30161101A91C **TASK AREA:** EE **WORK UNIT:** 081

TITLE: D-Myo-Inositol-1,2,6-Triphosphate (PP56) and Burn Wound Edema in a Burned Rat Model

SUBJ1: 060500 - Medicine and Medical Research

START DATE: 9203 **END DATE:** 9309 **PERFORMANCE METHOD:** C

CONTRACT/GRANT NO:

CONT TOTAL: $
CUM TOTAL: $
TOTAL LAB FUNDS: $

RESOURCES ESTIMATE

FY	WORK YRS	$(Thousands)
91	0.0	$0
92	0.3	$14
93	0.3	$16

RESPONSIBLE DOD ORGANIZATION: 404885
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PERFORMING ORGANIZATION: 404885
MRDC INSTITUTE OF SURGICAL RESEARCH USAISR
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MOZINGO, D W
210-221-3825

ASSOC1: BECKER, W K

ASSOC2: CIOFFI, W G

FOREIGN INTELLIGENCE CONSIDERED: Y **CIVILIAN APPLICABILITY:** M

KEYWORDS: ILIR; Lab Animals; Rats; Burns (Injuries); Edema; Healing; Therapy; Gravimetric Analysis; Histopathology; Electron Microscopy

OBJECTIVE: A DTIC literature search was conducted under DTIC request numbers W6K55A/W6K55E dated 4 March 1992. The objective of this work is to evaluate the effect of PP56 on burn wound edema in a burned rat model by administering PP56 at various doses and times after burn injury. Decreasing burn wound edema formation may simplify fluid resuscitation and reduce the morbidity and mortality of patients with thermal injury.

APPROACH: Animals will be anesthetized and a catheter will be placed into the right jugular vein. Four days after this procedure, the animals will again be anesthetized, the dorsal surface will be shaved, and a 20% full-thickness total body surface area scald burn will be inflicted. Animals will then be randomized to receive either PP56 or saline beginning at 0, 1, or 3 h postburn. Twelve animals from each subgroup will be sacrificed at 12, 24, or 48 h postburn. Burn wound edema will be evaluated by either Evans blue or by the gravimetric method. Before edema analysis, a weighed biopsy sample will be obtained from each burn wound for histopathological analysis of edema thickness and two samples from each group will be obtained for electron microscopy. Data will be analyzed by ANOVA as a 2 X 3 X 3 factorial design with appropriate consideration of all interactions. If appropriate, further analysis will be done using multiple regression techniques.

PROGRESS: 9203-9209. This project was approved by the USAISR Research Council and US Army Institute of Surgical Research Animal Use Committee during the second quarter of Fiscal Year 1992.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY (Continued)

Preliminary results confirm that PP56 is effective in reducing burn wound edema when administered 15 min postburn. For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1992.*

ABSTRACT

PROJECT NUMBER: 3M161101A91C-081, In-House Laboratory Independent Research

PROJECT TITLE: D-Myo-Inositol-1,2,6-Triphosphate (PP56) and Burn Wound Edema in a Burned Rat Model

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

PERIOD COVERED IN THIS REPORT: 11 March 1992 - 30 September 1992

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PP56, an enzymatically derived inositol phosphate derivative, has been shown to reduce burn wound edema and transcapillary albumin extravasation when administered immediately after burn injury. This study was performed to establish the effect of PP56 on burn wound edema in a burned rat model by evaluating the effect of the administration of PP56 at 15 min, 1 h, and 3 h postburn. The effect of the duration of drug infusion on edema formation was measured at 12, 24, and 48 h postburn. Sprague-Dawley rats were administered a 20% total body surface area full-thickness scald burn four days after implantation of a central venous infusion catheter. Animals were randomized to receive either PP56 or saline and then further randomized to receive the drug or saline beginning at 15 min, 1 h, or 3 h postburn. Animals from each group were sacrificed at 12, 24, and 48 h postburn. Burn wound edema was evaluated by the gravimetric method.

Preliminary results confirm that PP56 is effective in reducing burn edema when administered 15 min after thermal injury. However, no effect was observed when the drug was administered at 1 h or 3 h postburn. The beneficial effect was observed at 12 and 24 h postburn. Pending completion of data collection for all study groups, histologic effects and albumin extravasation by the Evans blue technique will be examined.

D-MYO-INOSITOL-1,2,6-TRIPHOSPHATE (PP56) AND BURN WOUND EDEMA IN A BURNED RAT MODEL

Thermal injury induces alterations of capillary permeability in the burn wound, which result in the extravasation of fluid into the extravascular space (1). Although some of the changes in capillary permeability in the burn wound can be explained by the direct effects of heat on the microcirculation, it is likely that other mechanisms, which are poorly understood, also alter capillary permeability in and adjacent to the burn wound. Loss of vascular integrity, with the subsequent development of burn wound edema, may have several deleterious consequences. These consequences include deficits in intravascular volume leading to burn shock, impaired tissue perfusion in the zone-of-stasis, and constricting edema developing in circumferential burn wounds (2). Because of these potential complications, it may be desirable to limit the development of edema in the burn wound.

PP56 is a product of hydrolysis of phytic acid by the phytase enzyme of yeast. This product has been shown to reduce several types of inflammation-induced edema (3). In preliminary studies, PP56 administered as a bolus of 30 mg/kg followed by an infusion of 0.6 mg/kg/min was associated with a decrease in burn wound edema following a full-thickness burn injury in rats (4,5). The mechanism of action of PP56 in eliciting this effect is poorly understood. In vitro studies indicate that PP56 has antioxidant properties, but only at levels that exceed those that can be safely achieved in plasma (3). Plasma levels in the rat model described above were in the range of 10-20 μM. In that study, PP56 was begun 15 min after the burn injury and was continued for only 2 h following injury, at which time tissue edema was measured by the extravasation of Evans blue. No other experimental results using this product have been reported in burned animals.

This study will attempt to establish the effect of D-myo-inositol 1,2,6-triphosphate (PP56) on burn wound edema in a burned rat model by evaluating the effect of the administration of PP56 at various doses and times following burn injury. Decreasing burn wound edema formation may simplify fluid resuscitation and reduce the morbidity and mortality of thermally injured soldiers.

MATERIALS AND METHODS

Experimental Design. Animals are anesthetized with sodium pentobarbital and a catheter is placed into the right jugular vein. Four days after this procedure, the animals are again anesthetized with sodium pentobarbital, the dorsal surface is shaved, and a 20% total body surface area burn is inflicted. Animals are then randomized to receive either PP56 or saline (see Table 1). Animals in each group are then further randomized to receive the drug or saline beginning at 0, 1, or 3 h postburn. Twelve animals from

each subgroup are sacrificed at 12, 24, and 48 h postburn. Burn wound edema is evaluated by either Evans blue or by the gravimetric method. Prior to edema analysis, a weighed biopsy sample is obtained from each burn wound for histopathological analysis of edema thickness and two samples from each group are obtained for electron microscopy.

TABLE 1. Number of Animals Per Group

Group	Time of Infusion (h Postburn)	Time of Sacrifice (h Postburn) 12	24	48
PP56	0	12	12	12
	1	12	12	12
	3	12	12	12
Saline	0	12	12	12
	1	12	12	12
	3	12	12	12

NOTE: Six animals from each subgroup will have burn edema determined by the Evans blue technique and 6 by the gravimetric method.

Description of Procedures. Two hundred and twenty-six adult male Sprague-Dawley rats weighing approximately 250 g will be used for this study. Animals are anesthetized with sodium pentobarbital (35 mg/kg IP) through a 25-ga needle. The first 10 animals were used to develop the catheterization procedure. Briefly, a longitudinal incision is made in the right neck and the right jugular vein identified by sharp dissection. Proximal distal control of the vein is obtained with 4-O silk suture. Through a venotomy, a 2F Silastic® grade catheter is advanced 1-1.5 cm into the superior vena cava. The silk suture is tied about the vein and catheter and the distal vein ligated with the silk suture. After good blood flow is documented, the catheter is tunnelled subcutaneously and exited through the skin at the base of the neck and connected to a heparin lock. The heparin lock and Silastic® tubing are securely sutured into place. The wound is closed with interrupted suture and the heparin lock flushed with dilute heparin solution (1 U heparin/ml saline). These 10 animals are then sacrificed with sodium pentobarbital (100 mg/kg IV). A thoracotomy is performed and the position of the Silastic® catheter in the superior vena cava confirmed. Adjustments to the length of catheter insertion in subsequent animals was made based on the findings at thoracotomy.

For animals assigned to study groups, the intravenous catheter is flushed with 1 cc of dilute heparin solution every 12 h until the infusion is started. No resuscitation is administered. Animals are housed for the remainder of the study in individual metabolic cages for quantitation of water intake and urine output. Four days after this procedure, the animals are again anesthetized with sodium pentobarbital (35 mg/kg IV) through a 25-ga needle. The dorsal surface is shaved and animals are placed in a plexiglass mold designed to expose 20% of the total body surface area. A scald burn is inflicted by immersion in 100°C water for 10 sec. The wound periphery is marked with permanent ink to ensure complete excision after sacrifice. Animals are then randomized to receive either a 30 mg/kg bolus of PP56 followed by an infusion of 0.6 mg/kg/min (0.36 ml/h of 10% PP56 in normal saline) or a saline bolus followed by an infusion of saline at the same rate. Animals in each group are then further randomized to receive the drug or placebo beginning at 0, 1, or 3 h postburn and continued until sacrifice. Twelve animals from each subgroup are sacrificed by sodium pentobarbital overdose (100 mg/kg IV) at 12, 24, and 48 h postburn.

Burn wound edema is evaluated by two techniques. The first involves the Evans blue technique. Evans blue (20 mg/kg IV) is administered 30 min prior to sacrifice. After sacrifice, the burn wound is excised from the animal. The extravasation of Evans blue into the wound will be quantitated by a spectrophotometric technique (4,5). The other technique for evaluating burn wound edema is gravimetric. Prior to edema analysis, a weighed biopsy specimen from each burn wound is obtained for histopathological analysis of edema thickness and two samples from each group are obtained for electron microscopy.

Determination of Number of Animals Required. Previous studies have shown that an estimate of 12 animals per group will be required for this study (4,5). Ten animals will be used to establish the cutdown and infusion procedure. A total of 226 animals are required.

Data Analysis Plan. These data will be analyzed by ANOVA as a 2 X 3 X 3 factorial with appropriate consideration of all interactors. If appropriate, further analysis will be done using multiple regression techniques.

RESULTS

The effect of the timing of dosage of PP56 on burn wound edema for animals sacrificed 12 h postburn appears in Figure 1. A significant reduction in the wet-to-dry ratio ($P = 0.003$) was observed when the drug was administered 15 min after thermal injury. Initial results did not support this finding when the drug was administered at 60 and 180 min postburn. Figure 2 demonstrates

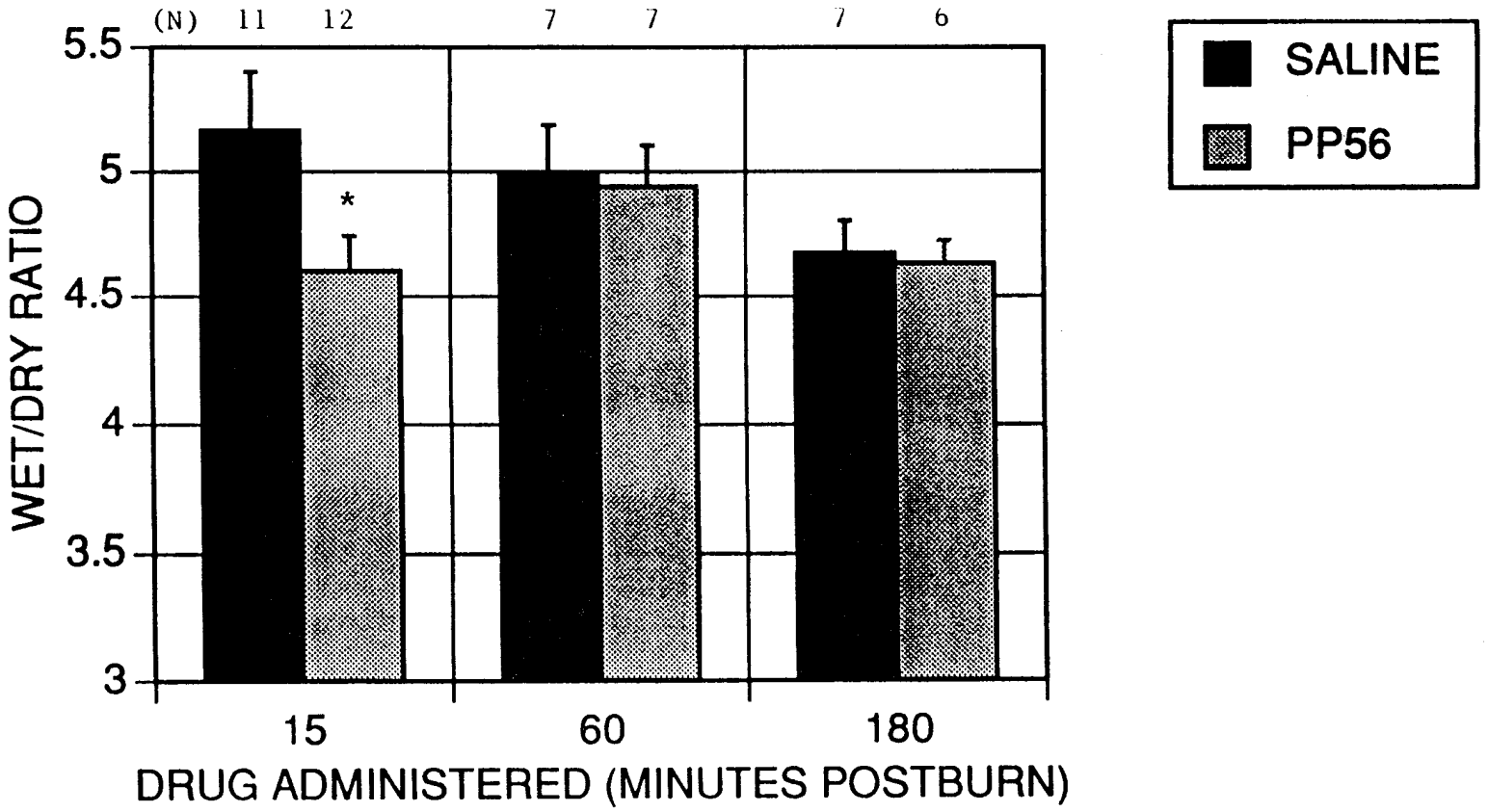


FIGURE 1. The effect of timing of dosage of PP56 on burn wound edema at 12 h postburn. The number of animals in each group appears at the top of the frame above each bar. *P = 0.003.

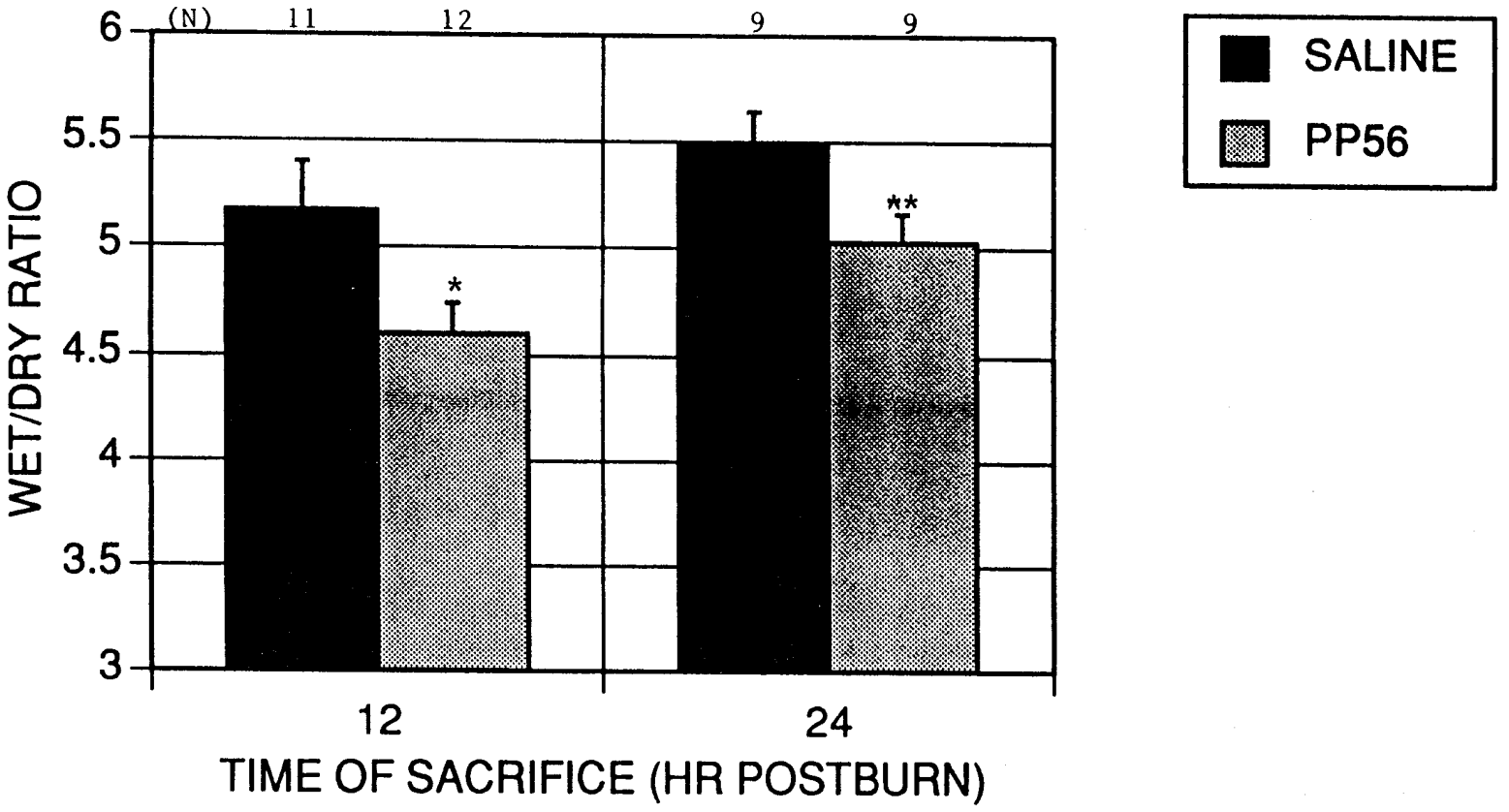


FIGURE 2. The duration of anti-edema effect of PP56 administered 15 min postburn. The number of animals in each group appears at the top of the frame above each bar. *P = 0.003, **P = 0.03.

the persistence of the antiedema effect of PP56 when administered 15 min postburn.

The histopathologic correlation with these findings is ongoing and data are not available for analysis at this time. Also, the technique of Evans Blue determination of postburn protein leakage is being refined and no data are available for analysis.

DISCUSSION

Initial results confirm that early administration of PP56 after thermal injury is capable of reducing burn wound edema. The mechanism by which this effect occurs is unclear. PP56 has been shown to have antioxidant properties, but only at levels that exceed those that can be safely achieved in the plasma (3). Also, PP56 has been shown to inhibit neuropeptide Y (6), which may be important in the regulation of cutaneous blood flow (7). The clinical usefulness of a drug that is only effective when administered 15 min postburn is uncertain. When the studies for all groups are completed, the data will be analyzed for the effect of PP56 when administered at various times and for various durations after burn injury.

PRESENTATIONS/PUBLICATIONS

None.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

ACCESSION: DA336063

SUMMARY DATE: 920408 **SUMMARY KIND:** A **PREV DATE:** **DISTRIBUTION:** CX

PROGRAM #: 61101A **PROJ #:** 30161101A91C **TASK AREA:** DA **WORK UNIT:** 082

TITLE: Kinetics of Nitric Oxide (NO) Production Following Thermal Injury in a Rat Model

SUBJ1: 060100 - Biochemistry
SUBJ2: 060500 - Medicine and Medical Research

START DATE: 9204 **END DATE:** 9309 **PERFORMANCE METHOD:** C

CONTRACT/GRANT NO:

CONT TOTAL: $
CUM TOTAL: $
TOTAL LAB FUNDS: $

RESOURCES ESTIMATE

FY	WORK YRS	$(Thousands)
91	0.0	$0
92	0.2	$13
93	0.2	$7

RESPONSIBLE DOD ORGANIZATION: 404885
MRDC INSTITUTE OF SURGICAL RESEARCH USAISR
FT SAM HOUSTON, TX 78234
PRUITT, B A
210-221-2720

PERFORMING ORGANIZATION: 404885
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BECKER, W K
210-221-3349

ASSOC1: CIOFFI, W G

ASSOC2: MCMANUS, A T

FOREIGN INTELLIGENCE CONSIDERED: Y **CIVILIAN APPLICABILITY:** M

KEYWORDS: ILIR; Lab Animals; Rats; Burns (Injuries); Kinetics; Metabolism; Gas Chromatography; Mass Spectrometry

OBJECTIVE: A DTIC literature search was conducted under DTIC request numbers W6K52A/W6K52E dated 4 March 1992. The objective of this work is to quantify the production of NO from arginine in vivo. Improved understanding of the pathophysiology of the response to burn injury may lead to improved treatment for patients with thermal injury.

APPROACH: Sprague-Dawley rats will be placed in individual metabolic cages and assigned to one of three groups. Group 1 will be fed a complete amino acid control diet and allowed free access to distilled water for 10 days. On days 11-15, each animal will be changed from distilled water to water containing NMMA; on days 16-20, the animals will receive daily injections of L-arginine. Group 2 will be fed a diet identical to that for Group 1 except that the arginine in the diet will be 99% ^{15}N-guanido-L-arginine. Animals in Group 3 will be equally divided into two subgroups, burn or sham-burn. The diet will be the same as for Group 2. The incorporation of ^{15}N label into nitrate will be calculated. Two-group comparisons will be performed by student's t test; multigroup or multiday comparisons will be performed by ANOVA with post-hoc tests of significance where indicated. Linear regression analysis of nitrate level by the Griess reagent technique and GC-MS or GC-NPD technique will be performed.

PROGRESS: 9204-9209. This study was approved by the USAISR Research Council and US Army Institute of Surgical Research Animal Care and Use Committee during the second and third quarters of Fiscal Year

1992. For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1992.*

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

ACCESSION: DA336063

SUMMARY DATE: 921001 **SUMMARY KIND:** D **PREV DATE:** 920408 **DISTRIBUTION:** CX

PROGRAM #: 61101A **PROJ #:** 30161101A91C **TASK AREA:** ED **WORK UNIT:** 082

TITLE: Kinetics of Nitric Oxide (NO) Production Following Thermal Injury in a Rat Model

SUBJ1: 060100 - Biochemistry
SUBJ2: 060500 - Medicine and Medical Research

START DATE: 9204 **END DATE:** 9309 **PERFORMANCE METHOD:** C

CONTRACT/GRANT NO:

CONT TOTAL: $
CUM TOTAL: $
TOTAL LAB FUNDS: $

RESOURCES ESTIMATE

FY	WORK YRS	$(Thousands)
91	0.0	$0
92	0.2	$13
93	0.2	$7

RESPONSIBLE DOD ORGANIZATION: 404885
MRDC INSTITUTE OF SURGICAL RESEARCH USAISR
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PERFORMING ORGANIZATION: 404885
MRDC INSTITUTE OF SURGICAL RESEARCH USAISR
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BECKER, W K
210-221-3349

ASSOC1: CIOFFI, W G

ASSOC2: MCMANUS, A T

FOREIGN INTELLIGENCE CONSIDERED: Y **CIVILIAN APPLICABILITY:** M

KEYWORDS: ILIR; Lab Animals; Rats; Burns (Injuries); Kinetics; Metabolism; Gas Chromatography; Mass Spectrometry

OBJECTIVE: A DTIC literature search was conducted under DTIC request numbers W6K52A/W6K52E dated 4 March 1992. The objective of this work is to quantify the production of NO from arginine in vivo. Improved understanding of the pathophysiology of the response to burn injury may lead to improved treatment for patients with thermal injury.

APPROACH: Sprague-Dawley rats will be placed in individual metabolic cages and assigned to one of three groups. Group 1 will be fed a complete amino acid control diet and allowed free access to distilled water for 10 days. On days 11-15, each animal will be changed from distilled water to water containing NMMA; on days 16-20, the animals will receive daily injections of L-arginine. Group 2 will be fed a diet identical to that for Group 1 except that the arginine in the diet will be 99% ^{15}N-guanido-L-arginine. Animals in Group 3 will be equally divided into two subgroups, burn or sham-burn. The diet will be the same as for Group 2. The incorporation of ^{15}N label into nitrate will be calculated. Two-group comparisons will be performed by student's t test; multigroup or multiday comparisons will be performed by ANOVA with post-hoc tests of significance where indicated. Linear regression analysis of nitrate level by the Griess reagent technique and GC-MS or GC-NPD technique will be performed.

PROGRESS: 9204-9209. This study was approved by the USAISR Research Council and US Army Institute of Surgical Research Animal Care and Use Committee during the second and third quarters of Fiscal Year

1992. Stable isotopic studies confirmed the origin of nitrate from L-arginine. Preliminary data analyses show an increase in urinary nitrate after burn injury. For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1992*.

ABSTRACT

PROJECT NUMBER: 3A161101A91C-082, In-House Laboratory Independent Research

PROJECT TITLE: Kinetics of Nitric Oxide (NO) Production Following Thermal Injury in a Rat Model

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

PERIOD COVERED IN THIS REPORT: 8 April 1992 - 30 September 1992

INVESTIGATORS: William K. Becker, MD, Lieutenant Colonel, MC
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Basil A. Pruitt, Jr., MD, Colonel, MC

NO is biosynthesized from the amino acid L-arginine by the enzyme nitric oxide synthase. NO is a vasodilator, a neurotransmitter, and may modulate immune function. This study was undertaken to determine whether the synthesis of NO is increased after experimental burn injury in a rat model. After a 30% total body surface area full-thickness burn in 300-g Lewis rats, the urinary output of nitrate, a stable metabolite of NO, was significantly increased for 8 days postburn compared with that of sham-burned control rats. The origin of the urinary output from L-arginine was demonstrated by administering the stable isotope $^{15}N_2$-guanido-arginine to burned and sham-burned rats and observing an immediate enrichment of 15N in nitrate. The amount of administered ^{15}N recovered as $^{15}NO_3$ was less than 1% of the administered arginine isotope in both burned and sham-burned rats. The recovery of the isotope increased tenfold over baseline recovery in burned rats. The arginine analog N-monomethyl-arginine, an inhibitor of the enzyme nitric oxide synthase, blocked the postburn rise in urinary NO_3 output in burned rats, but did not completely inhibit the output of NO_3 in burn wound-infected rats. Experimental burn injury in rats results in an increase in L-arginine-dependent NO production and urinary nitrate output.

KINETICS OF NITRIC OXIDE (NO) PRODUCTION FOLLOWING THERMAL INJURY IN A RAT MODEL

Burn injury is associated with a hypermetabolic response; the degree of hypermetabolism that follows burn injury is related to burn size (1). The hemodynamic component of the hypermetabolic response is characterized by an elevation of cardiac output and a depression of peripheral vascular resistance. Although the precise mechanisms that induce postburn hypermetabolism are not fully understood, catecholamines are considered to be one of the principal mediators of postburn hypermetabolism (2). When burn injury is complicated by the development of burn wound infection, there is often an associated increase in the magnitude of the hypermetabolic response (3).

NO, biosynthesized in mammalian species by the enzyme nitric oxide synthase (NOS, EC 1.14.14.39) from the amino acid L-arginine and molecular oxygen, is a vasodilator, a neurotransmitter, and may modulate immune function (4-7). NO is rapidly oxidized in vivo to the nitrogen oxides, nitrite (NO_2), and nitrate (NO_3), which are the stable metabolic end products of NO. The major route of elimination of these end products is by urinary excretion (8). Experimental studies in animals have demonstrated an increase in both arginine-dependent NO synthesis and the urinary excretion of NO_2/NO_3 following the administration of endotoxin (9). Also, increases in plasma levels of NO_2/NO_3 have been observed in humans with sepsis and in patients with advanced malignancies after the administration of IL2 (10,11). Blockade of NO synthesis, by stereospecific inhibitors of the enzyme NOS, has been studied in multiple species of experimental animals and results in an acute increase in blood pressure and peripheral vascular resistance, suggesting that the synthesis and release of NO may regulate, in part, both blood pressure and vascular tone (12,13).

NO is also produced by macrophages, and the production of NO by these cells is stimulated by mitogens (ConA), endotoxin, and cytokines (TNF, IL2, interferon-γ) (14). Production of NO by macrophages appears to play a role in the microbiostasis of intracellular pathogens, tumor cell cytostasis, and the macrophage response to alloantigens (15,16). The production of NO by macrophages in vitro can be blocked by stereospecific inhibitors of NOS; this blockage can be reversed by L-arginine.

It is possible that NO may be responsible, in part, for some of the hemodynamic and metabolic changes commonly observed after injury or infection. The studies reported here were performed to determine if arginine-dependent NO synthesis and the subsequent urinary excretion of NO_3, a stable metabolite end product of NO formation, were increased in a standard animal model of burn injury. Urinary NO_3 was measured in burned and sham-burned rats and the conversion of L-arginine to NO_3 was quantified, using

stable isotopic techniques, in burned and sham-burned rats. The conversion of arginine to an alternate metabolite, urea, was also measured in these rats by stable isotopic techniques and compared with the production of nitrogen oxides from L-arginine. Finally, the effect of n-monomethyl-arginine (NMMA), an inhibitor of the enzyme NOS, on the urinary excretion of NO_3 was studied in burned and burn wound-infected rats.

MATERIALS AND METHODS

Animal Model. Lewis rats (Harlan Sprague-Dawley, Inc., Indianapolis, IN) weighing approximately 300 g each were used for these studies. Animals were housed in individual plastic metabolic cages that allowed the measurement of food and water intake and quantitative collection of urine. The animals were allowed free access to a defined nitrate and nitrite-free amino acid diet that contained 12.1 g/kg L-arginine (Teklad Premier, TD 86529, Madison, WI). Nitrite-free and nitrate-free, distilled, deionized water was continuously available to each animal. A 30% total body surface area full-thickness dorsal scald burn was produced in anesthetized (sodium pentobarbital, 30 mg/kg IP) rats by immersion in 100°C water for 10 sec, using the procedure of Walker and Mason (17). Sham-burned (control) animals underwent identical anesthetic and handling procedures but were immersed in room-temperature water. No fluid resuscitation was given to these animals. Burn wound-infected animals underwent dorsal burn wound inoculation with 10^6 organisms of *Pseudomonas aeruginosa* (Strain 1244) immediately after burn injury (18). Urine was collected daily from each animal. Isopropyl alcohol (0.5 ml) was added daily to each urine collection container before the daily collection as a preservative. Urine samples were centrifuged at 300 g for 10 min and the supernatant frozen at -80°C before analysis. At the conclusion of the study, animals were sacrificed by sodium pentobarbital overdose (100 mg/kg IP).

Biochemical Procedures. Urinary NO_3 was determined by reaction with the Griess reagent (sulfanilamide and N-naphthyl ethylenediamine) following reduction of NO_3 to NO_2 by copper-coated cadmium, as previously described (19). This method measures both NO_2 and NO_3 present in the urine. Preliminary studies using urine from both burned and sham-burned rats demonstrated that urinary NO_2 (measured before reduction with cadmium) was uniformly less than 1% of the total nitrogen oxides (NO_2 + NO_3) present in urine and was frequently undetectable. Therefore, the urinary nitrogen oxide values reported here are equivalent to the urinary NO_3 levels. Urinary urea was determined on an automated analyzer by the urease method.

GC-MS Procedures. The atoms percent enrichment (APE) of urinary $^{15}N_2$-urea and $^{15}NO_3$ was measured by GC-MS using electron impact (urea) and chemical ionization (NO_3) techniques, as previously described (20,21). For $^{15}N_2$-urea, the trimethylsilyl

derivative was used and the mass/charge (m/z) ratios of 191 (M+2) and 189 (M) were measured. For urinary $^{15}NO_3$, the nitrobenzene derivative was used and m/z ratios of 124 (M+1) and 123 (M) were measured. All isotopic enrichments were corrected for the measured natural enrichment of each molecule. In the stable isotopic study, the percentage of administered isotope recovered in the end products urea or nitrate was calculated, as previously described by Wagner et al (20), on a daily basis.

Experimental Design

Phase I. Twenty-eight rats were divided into burn and sham-burn groups (n=14/group). Urine was collected from each animal for 24 h preburn to establish baseline NO_3 output and then daily for 8 days postburn. The urine was analyzed for NO_3 and the daily nitrate output for each animal (μmol NO_3/rat/day) was calculated from the urinary concentration of NO_3 and the daily urine output for each animal.

Phase II (Stable Isotopic Studies). The stable isotope, ^{15}N-guanido-arginine (99%, Cambridge Isotope Laboratories, Woburn, MA) was used for this phase of the study. The possible metabolic pathways for arginine and the flow of the guanido-^{15}N are shown in Figure 1. NO (and the stable metabolites NO_2 and NO_3) biosynthesized from this isotope will contain ^{15}N, since the guanido-nitrogen of arginine is the only known substrate for endogenous NO synthesis. Urea, derived from the arginine isotope by the action of the enzyme arginase, will contain both guanido-nitrogen atoms or two ^{15}N atoms per urea molecule. Measured $^{15}N_2$-urea (M+2) will be derived directly from the arginine isotope, since it is extremely unlikely, considering the large size of the exchangeable nitrogen pool, that any recycled ^{15}N will be incorporated simultaneously into both guanido positions of endogenously synthesized arginine.

In this study, 10 rats were divided into two equal groups, burn and sham burn. The diet for this phase of the study was altered; arginine was removed from the diet and replaced isonitrogenously with alanine (TD 91230, Teklad). This dietary manipulation was performed to enhance the plasma enrichment of arginine by the isotope by eliminating the dilution of isotope with dietary arginine. Previous studies by others (22) have demonstrated that elimination of dietary arginine had no effect on the NO response to infection in an animal model. Before burn or sham burn, the rats had free access to the arginine-free diet and to isotope-free distilled water for 2 days. The drinking water was then changed to a solution containing 5.7 mM $^{15}N_2$-guanido-arginine; animals were allowed free access to diet and to isotope-containing water for the remainder of the study. In contrast to the other amino acids, arginine is not involved in transamination reactions during intestinal uptake and transport; orally administered arginine will be absorbed into the bloodstream intact. Baseline

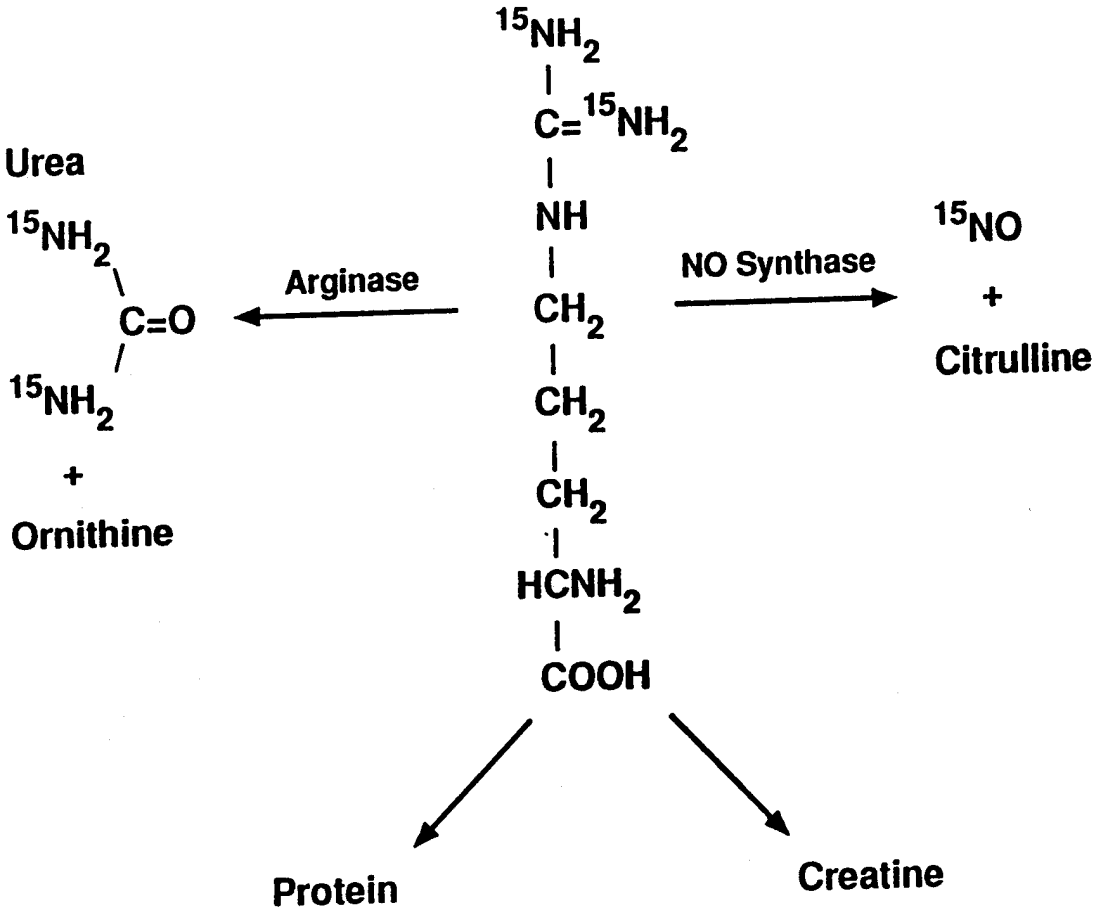


FIGURE 1. Possible metabolic pathways for L-arginine. The flow of ^{15}N-guanido-arginine is illustrated.

urine samples were collected while the animals were receiving isotope and an additional 3 days before burn or sham burn and urine samples were collected for 3 days postburn. Urine samples were analyzed for NO_3, urea, and the enrichment of $^{15}NO_3$ and $^{15}N_2$-urea. The daily intake of ^{15}N was calculated from the water intake and the concentration of isotope in the drinking water. The urinary content of ^{15}N isotope, in NO_3 (1 ^{15}N per molecule) or urea (2 ^{15}N per molecule) was calculated from the product of the quantity of each metabolite in the urine and the APE for $^{15}NO_3$ or $^{15}N_2$-urea.

Phase III. Eight rats were divided into two equal groups, burned and burn wound-infected. The NOS inhibitor NMMA was added to the animals' drinking water at a concentration of 50 mmol/l at the start of the study. Urine was collected daily for 4 days preinjury and for 5 days postinjury and analyzed for NO_3. The route of administration and concentration of NMMA were chosen because they had been previously demonstrated to block the NO response to infection in a murine model (22). The 30% total body

surface area burn injury that was used in the previous studies produces a nonlethal injury in the rat and is associated with a modest increase in metabolic rate. The burn wound infection model that was added for this phase of the study results in a progressive, lethal injury that is characterized by invasion of bacteria into viable tissue below and adjacent to the burn wound, bacteremia with the deposition of bacteria in distant organs, and a larger increase in metabolic rate than that observed in burned rats (18,23). The combination of burn injury and infection may be a more potent stimulus to NO production than burn injury alone. This possibility prompted the inclusion of this group in the study.

Statistical Analysis. Differences between two means were analyzed by the student's t test and a P value of less than 0.05 was used to denote statistical significance.

RESULTS

The baseline urinary output of NO_3 in normal rats was typically 1-3 μmole/rat/day. This is similar to the NO_3 production reported by Wagner et al (9) in similar sized normal rats (2-3 μmole/rat/day) but less than that reported by Leaf et al (9) (9.4 ± 3 μmole/rat/day in 280-300 g rats). The baseline output of NO_3 will depend, in part, on the amount of NO_2 or NO_3 present in the diet. The diets used in these studies were assayed for NO_2 and NO_3 by a commercial reference laboratory (Lancaster Laboratories, Lancaster, PA); neither NO_2 nor NO_3 were detectable in the diets. The urinary NO_3 values from Phase I are illustrated in Figure 2. After burn injury, there was an immediate increase in urinary NO_3 excretion that peaked at approximately 10 times the baseline value on postburn day 2. No increase in NO_3 was noted in sham-burned animals. Levels of urinary NO_3 output declined in burned animals after postburn day 2, but remained significantly elevated in burned animals compared with sham-burned control animals on each of the eight postburn study days.

The results of Phase II are listed in Tables 1 and 2. Baseline (preburn) output of NO_3 was similar in both the burn and sham-burn groups, and was also similar to the baseline values reported for Phase I. After burn injury, urinary NO_3 output rose to 20.18 μmol/rat/day on postburn day 3. No elevation of NO_3 output was noted in sham-burned rats. After the introduction of the arginine isotope into the drinking water on preburn day 3, there was an immediate enrichment of ^{15}N in NO_3. The oral route of intake for the isotope did result in some variability in isotope dose, since rats tended to drink more water after injury. The isotope intake ranged from 152 to 552 μmol of ^{15}N per day. The intake of arginine in this study was approximately one tenth to one third of that ingested by normal rats receiving the arginine-replete diet. Previous studies have demonstrated that isotope doses of $^{15}N_2$-guanido-arginine of up to 170 mg/kg in normal rats had no effect on urinary nitrate output (8). It is unlikely that the

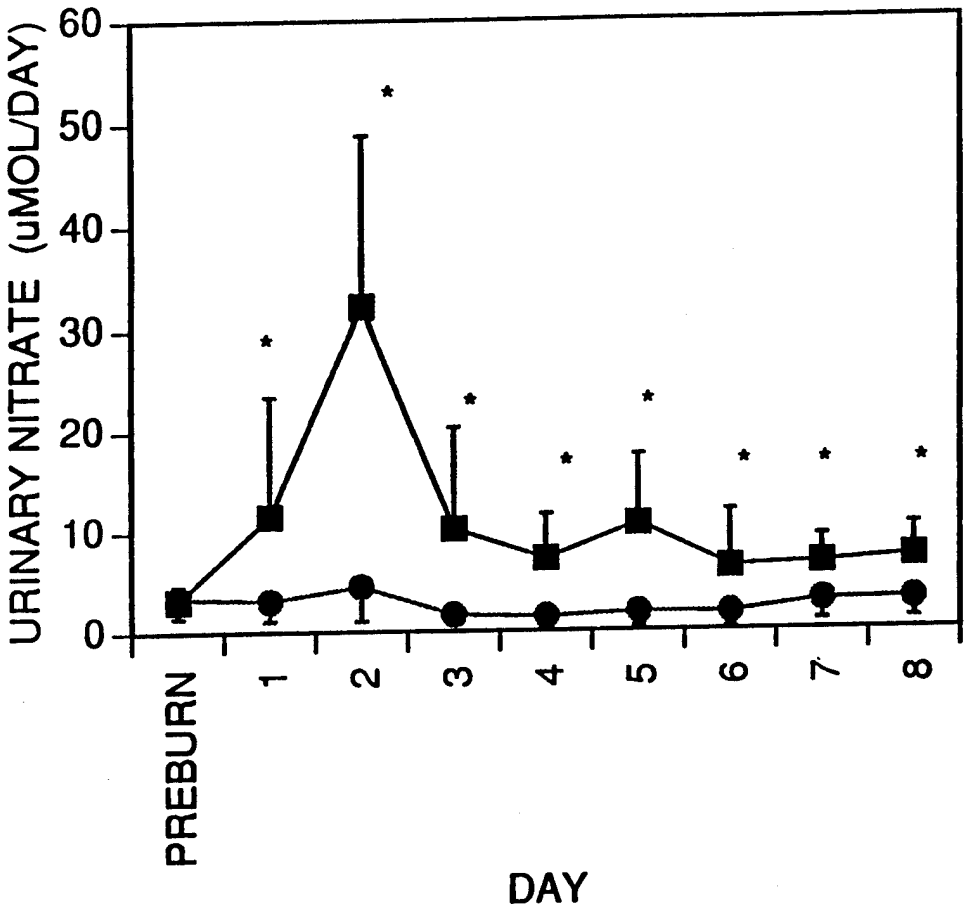


FIGURE 2. Urinary nitrate values (μmol/day) for burned (—■—) and sham-burned (—●—) rats in Phase I (n=14/group). *P <0.05.

variability in the quantity of isotopic arginine administered in these studies had any influence on the NO^3 output in these animals. The percentage of administered isotope recovered in NO_3 ranged from 0.019% to 0.051% in nonburned rats and increased to 0.175% and 0.167% in burned rats on postburn days 2 and 3. The limited availability of the $^{15}N_2$-guanido-arginine isotope at the time these studies were performed precluded studies beyond postburn day 3.

The APE of urinary $^{15}N_2$-urea was less than the sensitivity of the GC-MS (0.5 APE) in nonburned (both groups preburn) and sham-burned rats. The APE of $^{15}N_2$-urea and percentage of ^{15}N isotope recovered in $^{15}N_2$-urea in the burned animals are listed in Table 2. On postburn day 1, the recovery was 39% and it increased to 81% on postburn day 2, declining to 45% on postburn day 3. Urinary urea output (mmol/day) in the postburn period was postburn day 1, 9.50 ± 1.95; postburn day 2, 10.30 ± 2.27; and postburn day 3, 9.81 ± 1.54.

TABLE 1. Data from Stable Isotope Studies in Burned and Sham-Burned Rats (Mean ± SD)

Day		^{15}N-Nitrogen Intake (μmol ^{15}N/Day)	Urinary Nitrate (μmol/Day)	^{15}N-Nitrate (Atoms Percent Excess)	Recovery of ^{15}N in Nitrate (%)
			Burn Group		
Preburn	5	–	2.83 ± 1.45	–	–
	4	–	1.69 ± 0.59	–	–
	3	285 ± 69	1.95 ± 0.82	3.21 ± 1.5	0.021 ± 0.014
	2	217 ± 48	2.78 ± 1.67	4.29 ± 0.92	0.051 ± 0.026
	1	191 ± 51	1.07 ± 0.36	4.47 ± 2.34	0.024 ± 0.018
Postburn	0	182 ± 48	0.70 ± 0.09	3.48 ± 0.99	0.015 ± 0.011
	1	552 ± 178	6.20 ± 3.20*	2.82 ± 0.64	0.038 ± 0.025
	2	376 ± 95	17.17 ± 7.47*	3.61 ± 1.13	0.175 ± 0.085*
	3	458 ± 90	20.18 ± 11.68*	4.28 ± 1.22	0.167 ± 0.059*
			Sham-Burn Group		
Preburn	5	–	2.16 ± 1.82	–	–
	4	–	2.00 ± 1.15	–	–
	3	319 ± 95	3.05 ± 2.61	3.80 ± 1.91	0.041 ± 0.053
	2	182 ± 102	1.23 ± 0.54	3.90 ± 1.65	0.03 ± 0.02
	1	251 ± 86	2.10 ± 1.94	2.61 ± 1.33	0.02 ± 0.015
Postburn	0	205 ± 31	1.33 ± 1.43	3.06 ± 1.12	0.023 ± 0.032
	1	152 ± 65	1.19 ± 0.58	2.81 ± 0.72	0.023 ± 0.013
	2	266 ± 66	1.63 ± 0.63	3.67 ± 0.18	0.023 ± 0.009
	3	163 ± 69	1.02 ± 2.63	2.44 ± 0.31	0.019 ± 0.015

*P < 0.05 vs sham-burn group.

TABLE 2. Urea Kinetics in the Burned Rat (Mean ± SD)

Postburn Day	$^{15}N_2$–Urea (Atoms Percent Excess)	^{15}N (µmol/day)	Recovery of ^{15}N as Urea (%)
1	1.13 ± 0.70	115 ± 78	39 ± 21
2	1.46 ± 0.75	164 ± 104	81 ± 41
3	1.08 ± 0.46	103 ± 38	45 ± 17

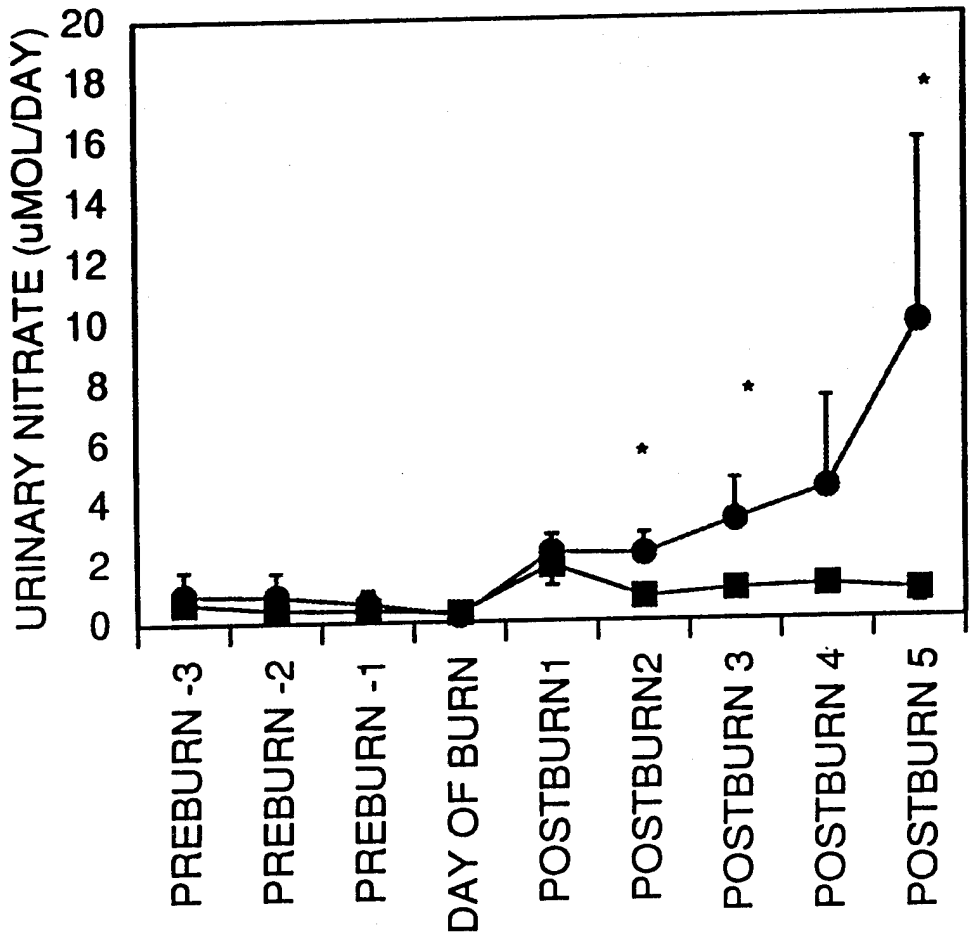


FIGURE 3. Urinary nitrate values for for burned (–■–) and burn-infected (–●–) rats in Phase III receiving the nitric oxide synthase inhibitor N-monomethyl-arginine. *P < 0.05.

The NO_3 output of the animals in Phase III is shown in Figure 3. Addition of NMMA to the animals' drinking water resulted in a decrease in the baseline (preburn) output of NO_3 to < 1 μmol/rat/day. The NMMA completely blocked the urinary NO_3 response to burn injury. In the burn wound-infected rats, there was a steady increase in the urinary output of NO_3 beginning on postburn day 2 and continuing until the end of the study on postburn day 5.

DISCUSSION

The origin, in mammalian species, of NO from the guanido-nitrogen of arginine and molecular oxygen with the production of citrulline as a byproduct catalyzed by the enzyme NOS is well established (6). The enzyme NOS has at least four isoforms (24,25). Several of the isoforms are constitutive, regulated by calcium/calmodulin, and are found in the brain, peripheral nerves, and vascular endothelial cells. Other isoforms of NOS are inducible, calmodulin-independent, require flavins as cofactors, and have been isolated primarily from macrophages, although these inducible isoforms also appear to be present in other tissues, including Kupffer's cells, hepatocytes, and chondrocytes (26-29). All isoforms require tetrahydrobiopterin and NADPH as cofactors (30).

In the central nervous system, NO functions as a neurotransmitter, the action of which is mediated by activation of soluble guanylate cyclase and the subsequent production of intracellular cGMP (31). In the peripheral nervous system, NO mediates nonadrenergic, noncholinergic neurotransmission in the gastrointestinal and genitourinary tracts (32-34). In both the gastrointestinal and genitourinary tracts, NO mediates smooth muscle relaxation; absence of NO-producing nerve fibers has been implicated in the development of infantile hypertrophic pyloric stenosis. NOS immunoreactivity has been demonstrated in the brain (cerebral cortex and cerebellum) and in the neurons of the myenteric plexus of the gastrointestinal tract (35).

Vascular endothelial cells contain a constitute form of the enzyme NOS and produce NO from L-arginine. NO, like other nitrates, is a vasodilator; endogenous production of NO by vascular endothelial cells appears to regulate, in part, blood pressure and vascular tone. The acute administration of stereospecific, competitive inhibitors of the enzyme NOS, such as NMMA or L-nitro-arginine, to experimental animals results in an increase in vascular tone and blood pressure (36-38). This increase in vascular tone and blood pressure can be reversed by the administration of L-arginine. Chronic blockade (2 months) of NOS in rats results in systemic hypertension, reduction in glomerular filtration, proteinuria, and histopathologic evidence of glomerulosclerosis (39). NOS inhibitors have been utilized to elevate blood pressure in animals with endotoxin and inhaled NO has been utilized to treat experimental pulmonary hypertension (40,41).

The current studies document a significant increase in the arginine-dependent urinary excretion of NO_3 after burn injury in rats. Sham-burned animals did not have an increase in urinary NO_3 excretion. The increase in NO_3 excretion in burned animals peaked in the early postburn period and persisted, at lower levels, for at least 8 days postinjury. The animals in these studies had no exogenous source of NO_2 or NO_3; therefore, the NO_3 recovered must have been produced endogenously. Synthesis of NO, by the enzyme NOS, with the subsequent oxidation of NO to NO_3, is the only known pathway for conversion of arginine guanido-nitrogen to NO_3. Seventy-five percent of NO_3 administered to experimental animals is recovered in the urine within 24 h (8). In the absence of renal dysfunction, renal excretion is the major pathway for the elimination of NO_3. Although NO was not directly measured in these studies, because of its short biologic half-life, the urinary NO_3 content that was measured is considered to be an index of NOS activity and NO production.

The administration of the stable isotope $^{15}N_2$-guanido-arginine to rats resulted in prompt enrichment of ^{15}N in urinary NO_3, providing evidence that the guanido-nitrogen of arginine was the source of the urinary NO_3. After burn injury, there was a tenfold increase in the recovery of the administered isotope as NO_3, providing additional evidence of the arginine-dependent origin of the urinary NO_3. The stable isotopic studies also demonstrated that NO synthesis represents only a small fraction (< 1%) of arginine metabolism. In contrast, after burn injury, a much larger quantity of the administered arginine isotope was recovered as an alternate arginine metabolite, $^{15}N_2$-urea. The metabolism of L-arginine in vivo is complex. Arginine can be synthesized in multiple tissues and there are several routes of catabolism (see fig 1). The stable isotopic studies performed in these studies allow a comparison of the relative activities of the various catabolic pathways of arginine. Because some of the $^{15}N_2$-urea synthesized from $^{15}N_2$-guanido-arginine will be hydrolyzed in the intestine and the ^{15}N recycled, the isotopic recovery calculation can only be used as an estimate of arginine catabolism. In addition, the arginine-free diet used in this study may have facilitated the incorporation of the isotopic arginine into urea; exogenous arginine is required for the efficient production of urea in rats (42).

The percent recovery of administered arginine guanido-nitrogen as $^{15}NO_3$ in these studies was similar to that reported by others. Wagner (9) reported a recovery of approximately 0.003% of administered ^{15}N-ammonia (a precursor of one of the two guanido-nitrogen of arginine) in normal rats; the recovery of this isotope increased 25-fold following the administration of endotoxin to the rats (9). Hibbs et al (10) measured the recovery of $^{15}N_2$-guanido-arginine as urinary $^{15}NO_3$ or ^{15}N-urea in two patients following the administration of IL2 to treat advanced cancer. In

these two patients, 0.7% of the isotope was recovered as NO_3 and 16% as urea (10).

The increase in NO production associated with a burn injury that was demonstrated in these studies could be blocked by the administration of the NOS inhibitor NMMA. The administration of NMMA to rats also resulted in a decrease in the baseline urinary output of NO_3. In burn wound-infected animals, the early NO response was blunted by NMMA, but an increase in NO production was noted on postburn day 2 and continued until the end of the study on postburn day 5, suggesting that the stimulus to NO production induced by burn wound infection was sufficient to overcome the effects of the NOS inhibitor. Burn wound infection in this rat model has previously been demonstrated to result in a greater increase in indices of hypermetabolism, including oxygen consumption and core temperature, compared with burn injury alone.

The current studies measured the dynamics of nitrogen oxide synthesis and excretion in a standard model of burn injury in rats. It was not possible, based on these studies, to determine the mechanism by which burn injury results in an increase in NO production. The physiologic significance (if any) of the increase in nitrogen oxide synthesis noted in these studies was not directly studied, nor was it possible to determine the specific tissue of origin of the excess nitrogen oxides generated postburn. Other studies suggest that macrophages are quantitatively the most important producers of NO, compared with other NO-producing tissues (43). Based on the physiologic properties of NO, as demonstrated by others in experimental studies, it is possible for us to speculate that the increase in NO synthesis observed after experimental burn injury may be responsible in part for some of the hemodynamic and metabolic changes classically observed after such injury. Further studies that directly investigate the physiologic and metabolic effects of NO production in the postburn period will be required to test this hypothesis.

PRESENTATIONS/PUBLICATIONS

Becker WK: Kinetics of nitrogen oxide production following experimental thermal injury in rats. Presented at the 52nd Annual Meeting of the American Association for the Surgery of Trauma, Louisville, Kentucky, 18 September 1992.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

ACCESSION: DA336100

SUMMARY DATE: 920610 **SUMMARY KIND:** A **PREV DATE:** **DISTRIBUTION:** CX

PROGRAM #: 61102A **PROJ #:** 30161102A91C **TASK AREA:** CA **WORK UNIT:** 083

TITLE: A New Ovine Model for Severe Smoke Inhalation Injury

SUBJ1: 060500 - Medicine and Medical Research

START DATE: 9206 **END DATE:** 9209 **PERFORMANCE METHOD:** C

CONTRACT/GRANT NO:

CONT TOTAL: $
CUM TOTAL: $
TOTAL LAB FUNDS: $

RESOURCES ESTIMATE

FY	WORK YRS	$(Thousands)
91	0.0	$0
92	0.5	$13
93	0.0	$0

RESPONSIBLE DOD ORGANIZATION: 404885
MRDC INSTITUTE OF SURGICAL RESEARCH USAISR
FT SAM HOUSTON, TX 78234
PRUITT, B A
210-221-2720

PERFORMING ORGANIZATION: 404885
MRDC INSTITUTE OF SURGICAL RESEARCH USAISR
FT SAM HOUSTON, TX 78234
OGURA, H
210-221-3349

ASSOC1: CIOFFI, W G

ASSOC2: JOHNSON, A A

FOREIGN INTELLIGENCE CONSIDERED: Y **CIVILIAN APPLICABILITY:** M

KEYWORDS: ILIR; Lab Animals; Sheep; Burns (Injuries); Inhalation; Pulmonary Edema; Pulmonary Insufficiency

OBJECTIVE: A DTIC literature search was conducted under DTIC request numbers W6N42N/W6N44J dated 27 May 1992. The objective of this work is to establish a new ovine model of severe smoke inhalation injury. A more reproducible animal model of severe smoke inhalation injury is necessary for the determination of the effects of medical interventions and new modes of mechanical ventilation for the treatment of patients with thermal injury.

APPROACH: Smoke inhalation injury will be produced in 24 sheep with a new smoke generator. After smoke exposure, the animals will be observed for 48 h. Cardiopulmonary variables will be measured and arterial and mixed-venous blood samples will be drawn presmoke and at 1, 3, 6, 12, 24, and 48 h after smoke exposure. At the end of 48 h, V_A/Q_C measurements will be obtained and bronchoalveolar lavage performed. Animals will then be sacrificed and extravascular lung water determined. ANOVA for a mixed factorial design and multivariate analysis (regression) will be used.

PROGRESS: 9206-9209. This project was approved by the USAISR Research Council and US Army Institute of Surgical Research Animal Care and Use Committee during the third quarter of Fiscal Year 1991. Equipment and supplies have been ordered and work will be initiated shortly. For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report* for fiscal years 1991 through 1992.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

ACCESSION: DA336100

SUMMARY DATE: 920930 **SUMMARY KIND:** K **PREV DATE:** 920610 **DISTRIBUTION:** CX

PROGRAM #: 61102A **PROJ #:** 30161102A91C **TASK AREA:** CA **WORK UNIT:** 083

TITLE: A New Ovine Model for Severe Smoke Inhalation Injury

SUBJ1: 060500 - Medicine and Medical Research

START DATE: 9206 **END DATE:** 9209 **PERFORMANCE METHOD:** C

CONTRACT/GRANT NO:

CONT TOTAL: $
CUM TOTAL: $
TOTAL LAB FUNDS: $

RESOURCES ESTIMATE

FY	WORK YRS	$(Thousands)
91	0.0	$0
92	0.1	$11
93	0.0	$0

RESPONSIBLE DOD ORGANIZATION: 404885
MRDC INSTITUTE OF SURGICAL RESEARCH USAISR
FT SAM HOUSTON, TX 78234
PRUITT, B A
210-221-2720

PERFORMING ORGANIZATION: 404885
MRDC INSTITUTE OF SURGICAL RESEARCH USAISR
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OGURA, H
210-221-3349

ASSOC1: CIOFFI, W G

ASSOC2: JOHNSON, A A

FOREIGN INTELLIGENCE CONSIDERED: Y **CIVILIAN APPLICABILITY:** M

KEYWORDS: ILIR; Lab Animals; Sheep; Burns (Injuries); Inhalation; Pulmonary Edema; Pulmonary Insufficiency

OBJECTIVE: A DTIC literature search was conducted under DTIC request numbers W6N42N/W6N44J dated 27 May 1992. The objective of this work was to establish a new ovine model of severe smoke inhalation injury. A more reproducible animal model of severe smoke inhalation injury was necessary for the determination of the effects of medical interventions and new modes of mechanical ventilation for the treatment of patients with thermal injury.

APPROACH: Smoke inhalation injury was produced in 24 sheep with a new smoke generator. After smoke exposure, the animals were observed for 48 h. Cardiopulmonary variables were measured and arterial and mixed-venous blood samples were drawn presmoke and at 1, 3, 6, 12, 24, and 48 h after smoke exposure. At the end of 48 h, V_A/Q_C measurements were obtained and bronchoalveolar lavage was performed. Animals were then sacrificed and extravascular lung water was determined. ANOVA for a mixed factorial design and multivariate analysis (regression) were used.

PROGRESS: 9206-9209. This project was approved by the USAISR Research Council and US Army Institute of Surgical Research Animal Care and Use Committee during the third quarter of Fiscal Year 1991. A reproducible ovine model of smoke inhalation injury that allows constant conditions of combustion and precise control of exposure volume and contact time was developed. Using this model, two different conditions of exposure, one producing moderate and the other severe injury, were examined. For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report* for fiscal years 1991 through 1992.

ABSTRACT

PROJECT NUMBER: 3A161101A91C-077, In-House Laboratory Independent Research

PROJECT TITLE: A New Ovine Model for Severe Smoke Inhalation Injury

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

PERIOD COVERED IN THIS REPORT: 1 October 1991 - 30 September 1992

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An ovine model of smoke inhalation injury with constant conditions of combustion and precise control of exposure volume and contact time was developed. Moderate injury (Group 1, n=7) was produced by alternating seven exposure units of wood smoke with seven units of air. Severe injury (Group 2, n=7) was produced with 10 exposure units of smoke mixed with oxygen. One exposure unit consisted of 5 breaths at a controlled tidal volume and breathhold. All animals were observed for 48 h while breathing spontaneously. After smoke exposure, animals in both groups developed progressive hypoxemia with increased pulmonary V_A/Q mismatching, pulmonary artery hypertension, increased extravascular lung water, increased pulmonary resistance, and decreased lung compliance. All of these physiologic deteriorations were more severe in Group 2 than in Group 1 ($P < 0.05$). Morphologic evidence of pulmonary injury was more pronounced in Group 2 ($P < 0.05$). This model is reproducible and can be used for studies of the pathophysiology or treatment of smoke inhalation injury.

A NEW OVINE MODEL FOR SEVERE SMOKE INHALATION INJURY

Smoke inhalation leads to deleterious bronchopulmonary changes, increased morbidity, and increased mortality in patients with thermal injury (1). Since the initial bronchopulmonary injury is triggered by noxious chemicals generated by incomplete combustion, the subsequent inflammatory process depends on the quality and the quantity of smoke to which an individual is exposed (2). Chemotactic signals from chemically stimulated airways may cause leukocyte activation and prostanoid production, leading to excessive inflammatory reaction in the lung (3,4). Progressive airway damage with subsequent pulmonary edema and deficient surface tension in alveoli not only worsen V_A/Q inequality and oxygenation, but increase the patient's susceptibility to pulmonary infection (5,6).

Due to technical difficulties, a reproducible model allowing careful control of thermolysis and exposure has not been thoroughly developed (7,8). In the present study, an ovine model of smoke inhalation injury utilizing constant conditions of combustion and precise control of exposure volume and contact time was developed.

MATERIALS AND METHODS

Experimental Design: Twenty-four 1- to 2-year-old neutered male sheep weighing 25-45 kg will be studied. Smoke inhalation injury will be produced with the new smoke generator. After smoke exposure, the animals will be extubated and observed for 48 h. Cardiopulmonary variables will be measured and arterial and mixed-venous blood samples will be drawn presmoke and at 1, 3, 6, 12, 24, and 48 h after smoke exposure. At the end of 48 h, V_A/Q_C measurements will be obtained and bronchoalveolar lavage will be performed. The animals will then be sacrificed and extravascular lung water will be determined.

Description of Procedures: Nineteen 1- to 2-year-old neutered male, commercially available, random source sheep weighing 27.2 ± 0.9 kg (range, 25-32) were studied. The animals were housed in covered outdoor runs, treated for parasites (1% ivermectin, 1 ml/75 lb), and fed commercial chow and water ad libitum. Baseline hematologic data (CBC, total proteins, and blood chemistries) were obtained 3 weeks before study. All animals were fasted for 24 h before instrumentation, smoke exposure, and use. The animals were anesthetized with sodium pentobarbital (25 mg/kg IV) administered through a 25-ga needle, orally intubated, mechanically ventilated, and placed in the supine position. A Silastic^R medical grade cannulae (30 cm) was inserted into a femoral artery and another into the femoral vein and one radiopaque sheath introducer (8.5F) was inserted into an external jugular vein using sterile technique. A Swan-Ganz catheter (7.5F, American Edwards Laboratories, Irvine, CA) will be inserted through the sheath into the jugular vein. The

arterial line was used for obtaining blood samples for blood gas analyses. The venous line was used for infusion of the solution containing the six inert gases to measure V_A/Q_C inequality using the multiple inert gas elimination technique.

Smoke inhalation injury was produced using a new smoke generator. A schematic representation of the smoke generator and delivery system is shown at Figure 1. Animals were randomized to one of three groups. Groups I and II were administered smoke inhalation injury and Group III served as the control group. For Group I, smoke was generated by thermolysis of pine woodchips (50 g) in a crucible furnace (Furnace Model 56622 and Control Console Model 58114, Lindberg, Watertown, WI) at a constant temperature of 400°C and an airflow of 3 l/min. Smoke was delivered into a reservoir and exposure was accomplished using a circuit equipped with a volume-controlled pump and a breathhold valve. Smoke injury was produced by alternating 7 exposure units of smoke with 7 units of air; one exposure unit consisted of 5 breaths at a constant tidal volume of 30 ml/kg with a constant breathhold of 4 sec, with a 5-sec pause between exposure units. Oxygen, carbon monoxide, and carbon dioxide contents of the smoke were measured with gas monitors (Model 5552, Hudson; Model CO101, Neotronics; and Datex, Puritan-Bennet; respectively). The temperature of the smoke used for exposure was measured using a Swan-Ganz catheter placed just proximal to the orotracheal tube. For Group II, smoke was generated by thermolysis of pine woodchips (60 g) in the crucible furnace at a constant temperature of 400°C and an air flow of 6 l/min. Smoke was delivered into the reservoir and mixed with a constant 2 l/min flow of 100% oxygen. Smoke injury was produced by 10 exposure units of this mixture, one exposure unit consisted of 5 breaths at a constant tidal volume of 30 ml/kg with a constant breathhold of 5 sec and a 5-sec rest between exposure units.

Immediately after smoke exposure, each animal was housed in an individual cage in climate-controlled facilities at 74-76°F (24-25°C) with a relative humidity of 40-50% and observed while spontaneously breathing in the awake state for 48 h after smoke insufflation. The animals received a maintenance infusion of lactated Ringer's (1.5 ml/kg/h).

Cardiopulmonary variables and blood gases were measured presmoke and at 1, 3, 6, 12, 24, 36, and 48 h after smoke exposure. Cardiopulmonary measurements included systemic blood pressure, heart rate, pulmonary artery pressure, pulmonary capillary wedge pressure, cardiac output, and pulmonary resistance. Pulmonary artery pressure was monitored with Statham P23Db transducers (Statham Instruments, Oxnard CA) and systemic arterial pressure with a Hewlett-Packard 1290-A quartz transducer (Hewlett-Packard Company, Waltham, MA). These pressures were recorded on a Hewlett-Packard four-channel recorder (Model 7754A). Cardiac output was measured in triplicate by the thermodilution technique

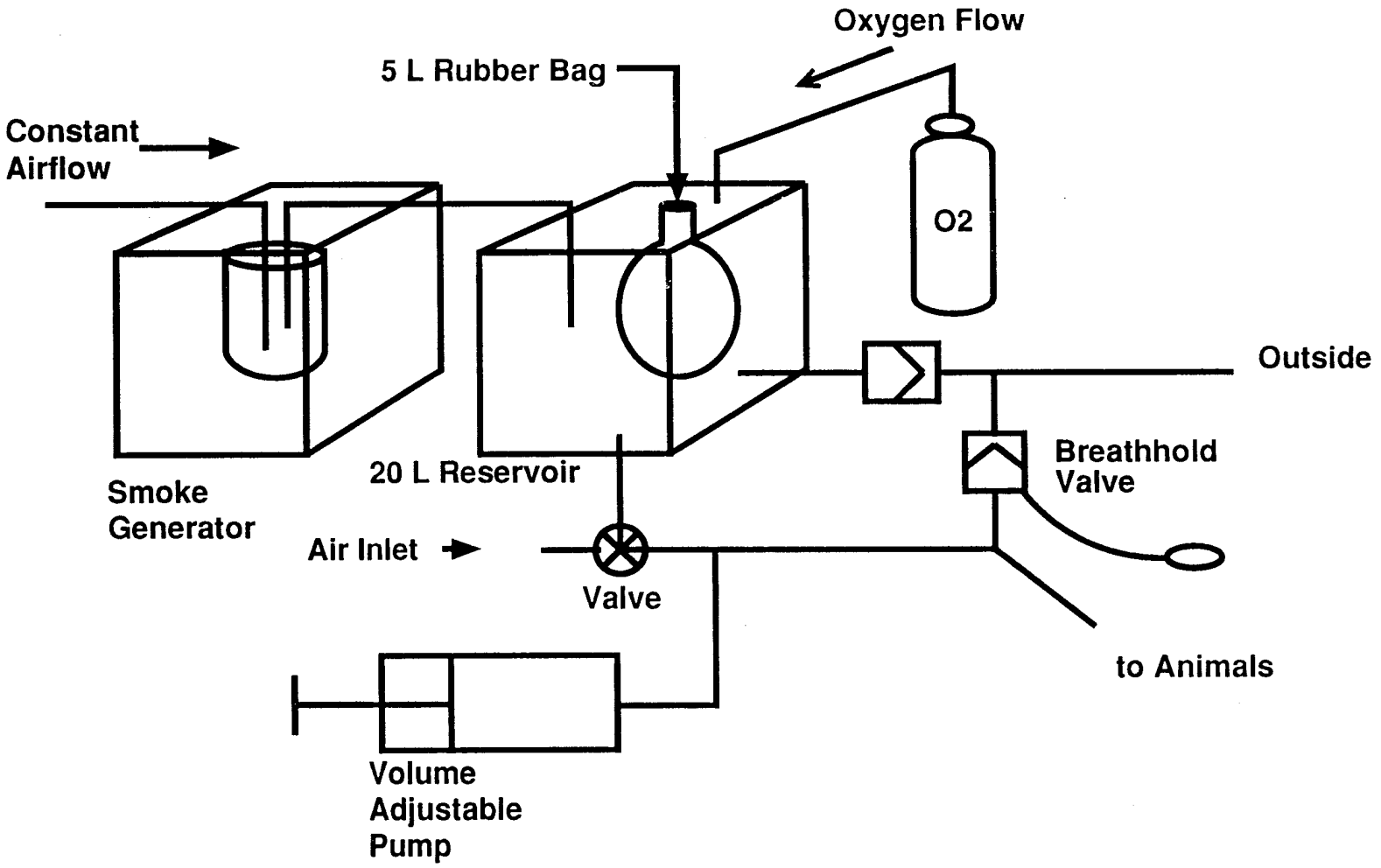


FIGURE 1. Schematic diagram of the smoke generator and delivery system. Air inlet was used for Group I animals. Oxygen flow was used for Group II animals.

(Model 9520A, Cardiac Output Computer, American Edwards Laboratories).

Static lung compliance and pulmonary resistance were measured presmoke and 48 h after smoke exposure while the animals were being mechanically ventilated. Using an esophageal balloon, transpulmonary pressure was measured with a differential transducer (MP-451, Validine Engineering Corporation). Inspiratory tidal volume was measured with a Wright spirometer. Air flow rate proximal to the orotracheal tube was measured with a pneumotachograph (Model 17212, Gould, Inc., The Netherlands). Static lung compliance was calculated by dividing the tidal volume by the transpulmonary pressure difference between the plateaued end-inspiratory pause phase and the end-expiratory-pause phase. Pulmonary resistance was calculated by dividing the inspiratory transpulmonary pressure change by the inspiratory air flow rate.

Respiratory index (RI), an oxygenation capacity index, was calculated by the following formula:

$$RI = \frac{PAO_2 - PaO_2}{PaO_2}$$

$$PAO_2 = 149 - 1.2 \times PaCO_2$$

where PAO_2 (torr) indicates alveolar oxygen pressure; PaO_2 (torr), arterial oxygen pressure; and $PaCO_2$ (mmHg), arterial CO2 pressure.

Arterial and mixed-venous blood samples were analyzed for blood gases. Blood gas analyses were performed using an IL 1303 pH/blood gas analyzer and an IL 282 CO-Oximeter (Instrumentation Laboratories, Inc., Lexington, MA). Blood samples for measurement of 6-keto-$PGF_{1\alpha}$, thromboxane B_2, and conjugated dienes were drawn at the same time and frozen at -70°C for later analyses. Thromboxane B_2 and 6-keto-$PGF_{1\alpha}$, the stable derivatives of thromboxane A_2 and PGI_2, will be measured by RIA (9). Conjugated dienes, products of lipid peroxidation, will be determined by the techniques of Ward et al (10). They will be read at an optical density of 233 nm in a spectrometer.

At the end of 48 h, the animals were anesthetized with sodium pentobarbital (25 mg/kg IV) administered through an existing intravenous line, paralyzed with pancuronium bromide (0.03-0.04 mg/kg, Pavulon^R, Organon Pharmaceuticals, West Orange NJ), orally intubated, and mechanically ventilated. During mechanical ventilation, the tidal volume was set at 15 ml/kg and the respiratory rate was 10 breaths per minute. PEEP was 5 cmH_2O and the FIO_2 was kept at 0.21 throughout the remainder of the study

period. After 30 min, measurement of V_A/Q_C using the multiple inert gas elimination technique was performed according to the method developed by Wagner et al (11). A lactated Ringer's solution containing six inert gases (sulfur hexafluoride, ethane, cyclopropane, halothane, diethyl ether, and acetone) was infused at a rate of 0.1 ml/kg/min. After 30 min when equilibrium of gas exchange occurred, arterial and mixed-venous blood samples were drawn anaerobically into preweighed, heparinized syringes (30 ml, matched, glass, Becton, Dickinson, and Company) simultaneously. Mixed-expired gas was collected from a temperature-controlled copper coil (OD = 3.5 cm, L = 550 cm) about 1 min after blood sampling, compensating for the delay of the mixing chamber. Duplicate blood and expired gas samples were immediately analyzed by GC (Model 5890, Series 2, Hewlett-Packard Company, Waltham, MS). Retention and excretion of the six gases were calculated and V_A/Q_C distribution was analyzed using a computer program designed specifically for MIGET.

After blood sample collections, bronchoalveolar lavage was performed with a bronchofiberscope (Olympus CLV-10) to obtain samples from the left lower lung lobe for measurement of 6-keto-$PGF_{1\alpha}$, thromboxane B_2, and conjugated dienes. Twenty milliliters of 0.9% saline were inserted to the left lower lobe and the fluid was immediately pulled back with suction. This process of lavage and suction was repeated three times (total fluid = 60 ml). The collected fluid was centrifuged and aliquots of the supernatant were stored at -70°C until measurement of total protein content. The cell pellet was resuspended with the same volume of saline as the supernatant, and total WBC count was determined using a hemocytometer. Differential cell counts were performed on Wright-Giemsa stained cytocentrifuge preparations. Total protein in the bronchoalveolar lavage fluid supernatant was measured using a spectrophotometric dye-binding protein assay (12).

The animal were then sacrificed with sodium pentobarbital (25 mg/kg) and a potassium chloride bolus (20 ml of a 200 g/l solution) administered through an existing intravenous line. The wet-to-dry lung weight ratio was determined by a modification of the gravimetric method of Drake et al (13). The right lung was removed after the bronchi and vessels were ligated. The entire right lung was homogenized with an identical weight of distilled water. Duplicate samples of the homogenate and arterial blood were weighed and dried at a constant temperature of 80°C. Dry weights were measured and the wet-to-dry ratios of the homogenate and blood were calculated. A sample of the homogenate was centrifuged at 14500 rpm for 1 h, and a blood sample was diluted with the same volume of distilled water. To determine the hemoglobin levels in the homogenate and blood, 20 μL of the homogenate supernatant or the diluted blood were added to 2.5 ml of Drabkin's solution. The absorbance of both solutions was measured spectrophotometrically at 540 nm. Then, the blood weight in the wet lung was calculated.

From these data, blood-free wet and dry weights of the right lung were calculated and the wet-to-dry lung weight ratio determined.

Histology. Histologic evaluation of injury in the tracheobronchial epithelium and lung parenchyma of each animal was performed by light microscopy using the criteria indicated in Tables 1 and 2.

TABLE 1. Tracheobronchoepithelial Damage Score

Score	Description
0	Normal
1	Normal height of epithelium with some loss of cilia
2	Superficial erosion of epithelium with complete loss of cilia
3	Severe erosion of epithelium
4	Complete ulceration of epithelium

TABLE 2. Lung Parenchymal Damage Score

Score	Description
0	Normal
1	A few inflammatory cells in alveolar septa
2	Multifocal areas with increased inflammatory cells in alveolar septa or a few inflammatory cells in alveoli
3	Disseminated inflammation and/or edema in alveolar septa and alveoli that affect less than half the section
4	Diffuse inflammation and/or edema in alveolar septa and alveoli that affect more than half the section

Statistical Analysis. Statistical analysis of the data was performed using the student's t test for comparisons between Groups I and II at equivalent time points and ANOVA (repeated measures) for comparisons of serial changes between the two groups. ANOVA was used to compare Groups I, II, and III with post hoc testing

using the Tukey method. Data are shown as mean ± SEM; significance was assigned at $P < 0.05$.

RESULTS

The mean contents of the smoke for Group I were 1.7% ± 0.1% carbon monoxide, 4.0% ± 0.5% carbon dioxide, and 15.5% ± 0.5% oxygen. For Group II, the contents were 0.9% ± 0.1% carbon monoxide, 2.3% ± 0.1% carbon dioxide, and 33.1% ± 0.6% oxygen. The temperature of the smoke used for exposure was higher than the ambient temperature (24°C) by 2.1°C ± 0.3°C in Group I and by 1.1°C ± 0.1°C in Group II.

The arterial COHb levels immediately after 3, 5, and 7 units of smoke in Group I were 24.2% ± 2.0%, 47.0% ± 3.3%, and 70.7% ± 2.2%, respectively, while the arterial COHb levels immediately after 4, 7, and 10 units of smoke in Group II were 25.5% ± 1.7%, 46.9% ± 2.5%, and 71.7% ± 2.3%, respectively. Arterial blood gas analyses just after smoke exposure revealed a PaO_2 of 118.0 ± 3.9 torr, a $PaCO_2$ of 31.7 ± 1.4 mmHg, and a pH of 7.51 ± 0.02 in Group I. In Group II, analyses revealed a PaO_2 of 174.1 ± 9.1 torr, a $PaCO_2$ of 36.0 ± 1.2 mmHg, and a pH of 7.42 ± 0.02. All animals survived the 48-h observation period.

Figure 2 depicts the serial determinations of PaO_2 after smoke exposure. Both groups developed a progressive hypoxemia, which was significantly worse in Group II compared to Group I during the second 24 h ($P < 0.05$, student's t test). The serial pattern of change was significantly different between the two groups ($P < 0.05$, ANOVA repeated measures).

Figure 3 depicts the serial pulmonary vascular resistance index for the two groups. Both groups showed a progressive elevation in the pulmonary vascular resistance index. In Group II, the pulmonary vascular resistance index was significantly higher than in Group I during the acute phase and at 48 h after smoke exposure. The serial change pattern differed significantly between the groups.

Table 3 shows other cardiopulmonary variables for the two groups. Both groups had an elevation of mean pulmonary artery pressure and pulmonary capillary wedge pressure after smoke exposure. In Group II, both values were significantly higher than in Group I during the second 24 h. The respiratory index (RI) was also increased in both groups after smoke exposure. In Group II, the respiratory index was significantly higher than in Group I during the second 24 h. $PaCO_2$, mean systemic arterial pressure, total peripheral resistance index, and cardiac index did not differ significantly between the two groups throughout the study.

The recovery of bronchoalveolar lavage fluid in this study was approximately 60%. Figure 4 shows the total WBC and PMNL counts in

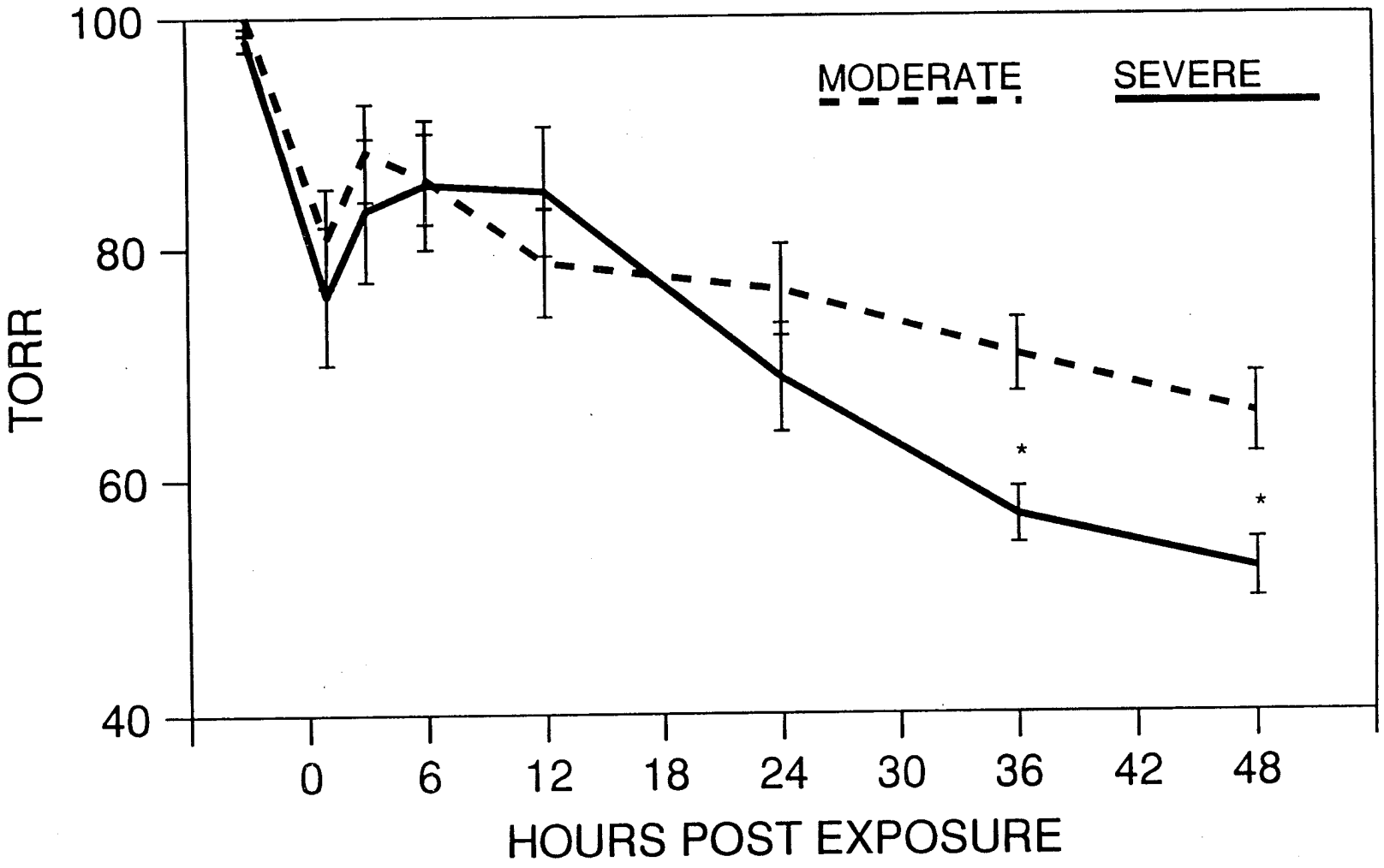


FIGURE 2. Serial PaO_2 (torr) for Groups I and II after smoke inhalation. Group I (MODERATE) is represented by the dotted line and Group 2 (SEVERE) by the solid line. Progressive hypoxemia in Group II was worse than in Group I during the second 24 h. *$P < 0.05$, student's t test at equivalent time.

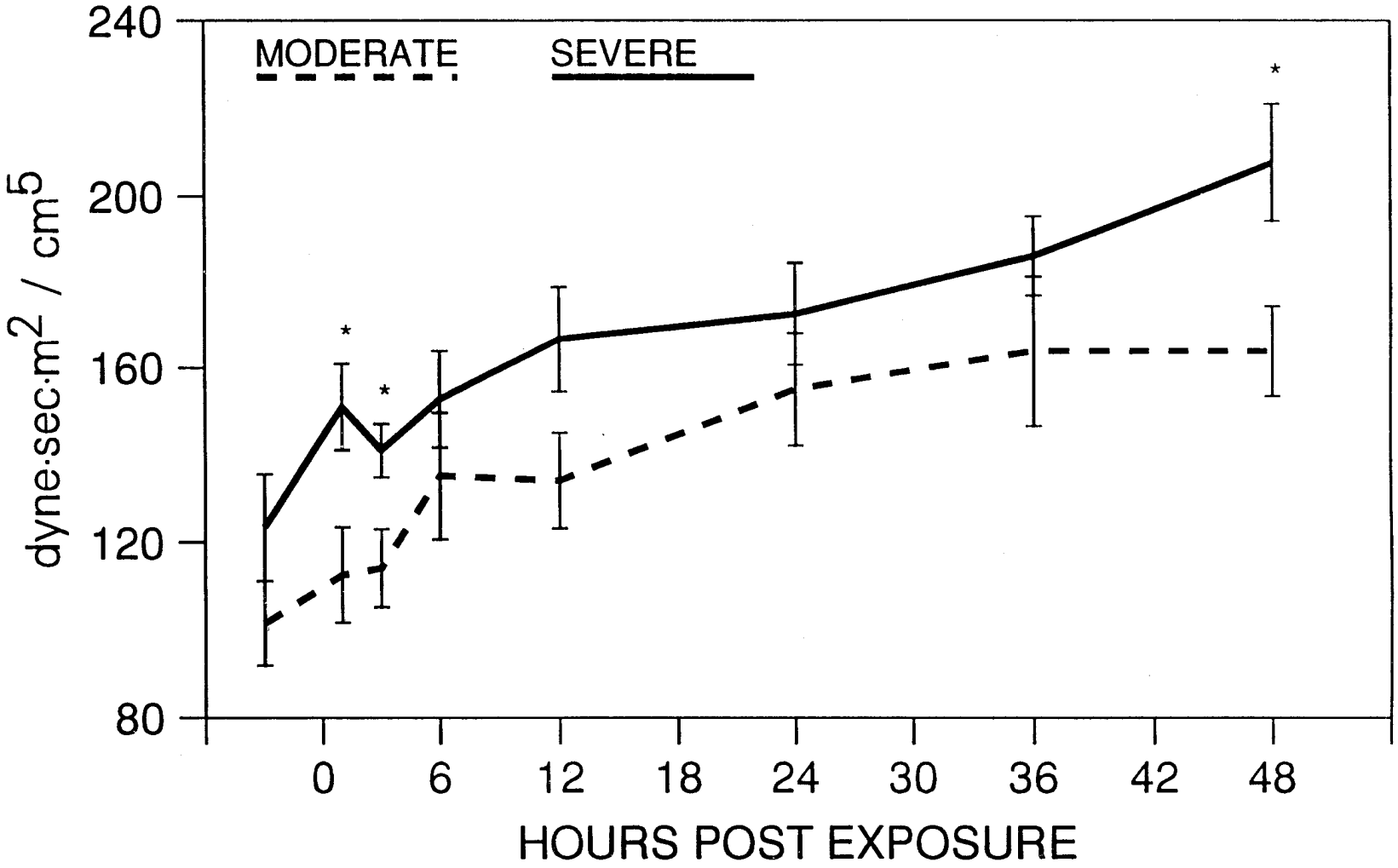


FIGURE 3. Serial pulmonary vascular resistance index (dyne·sec·m^2/cm^5) for Groups I and II after smoke inhalation. Group I (MODERATE) is represented by the dotted line and Group II (SEVERE) by the solid line. Pulmonary vascular resistance index in Group II was significantly higher than in Group I during the acute phase and at 48 h. *P < 0.05, student's t test at equivalent time.

TABLE 3. Cardiopulmonary Variables (Mean ± SEM)

	Presmoke	Postsmoke (h) 3	6	12	24	36	48
Group I							
MPAP (mmHg)	14.0 ± 0.7	16.4 ± 0.7	18.3 ± 1.4	18.6 ± 1.2	20.0 ± 1.2	20.7 ± 1.1	22.1 ± 1.0
PCWP (mmHg)	7.1 ± 0.3	7.7 ± 0.4	8.4 ± 0.6	8.9 ± 0.7	8.6 ± 0.6	9.1 ± 0.7	10.1 ± 0.5
RI	0.07 ± 0.02	0.23 ± 0.04	0.24 ± 0.04	0.34 ± 0.05	0.40 ± 0.05	0.50 ± 0.07	0.61 ± 0.08
PaCO2 (mmHg)	35.4 ± 1.5	34.3 ± 2.0	35.8 ± 1.8	37.2 ± 2.5	36.4 ± 2.2	36.6 ± 2.0	37.6 ± 2.1
MSAP (mmHg)	98.2 ± 3.4	102.7 ± 2.5	99.9 ± 3.8	102.7 ± 3.3	98.3 ± 2.5	97.2 ± 2.5	102.7 ± 3.1
TPRI (dyne·sec·m2/cm5)	1409 ± 82	1262 ± 109	1302 ± 102	1361 ± 97	1296 ± 159	1305 ± 89	1312 ± 64
CI (l/min/m2)	5.3 ± 0.3	6.2 ± 0.4	6.0 ± 0.5	5.9 ± 0.4	6.0 ± 0.5	5.9 ± 0.5	5.9 ± 0.1
Group II							
MPAP (mmHg)	15.7 ± 0.5	18.3 ± 0.3	19.0 ± 0.5	20.9 ± 0.8	22.1 ± 0.9	25.4 ± 0.9*	26.6 ± 1.0*
PCWP (mmHg)	8.0 ± 0.4	8.9 ± 0.5	9.1 ± 0.5	9.9 ± 0.3	10.7 ± 0.6*	11.6 ± 0.4*	12.1 ± 0.8
RI	0.09 ± 0.02	0.34 ± 0.09	0.27 ± 0.09	0.28 ± 0.09	0.56 ± 0.07	0.78 ± 0.06*	0.92 ± 0.08*
PaCO2 (mmHg)	33.0 ± 0.9	33.6 ± 0.9	33.5 ± 0.8	33.4 ± 1.6	36.7 ± 2.5	40.6 ± 2.5	41.4 ± 3.3
MSAP (mmHg)	97.5 ± 6.0	94.1 ± 4.4	94.7 ± 4.0	92.7 ± 3.1	91.4 ± 3.5	97.0 ± 3.8	94.9 ± 5.0
TPRI (dyne·sec·m2/cm5)	1557 ± 97	1357 ± 69	1420 ± 130	1352 ± 91	1414 ± 107	1309 ± 58	1331 ± 52
CI (l/min/m2)	5.1 ± 0.2	5.4 ± 0.2	5.3 ± 0.3	5.3 ± 0.2	5.3 ± 0.4	6.0 ± 0.3	5.6 ± 0.3

MPAP indicates mean pulmonary arterial pressure; PCWP, pulmonary capillary wedge pressure; RI, respiratory index; MSAP, mean systemic arterial pressure; TPRI, total peripheral resistance index; CI, cardiac index. *P <0.05, student's t test at equivalent time.

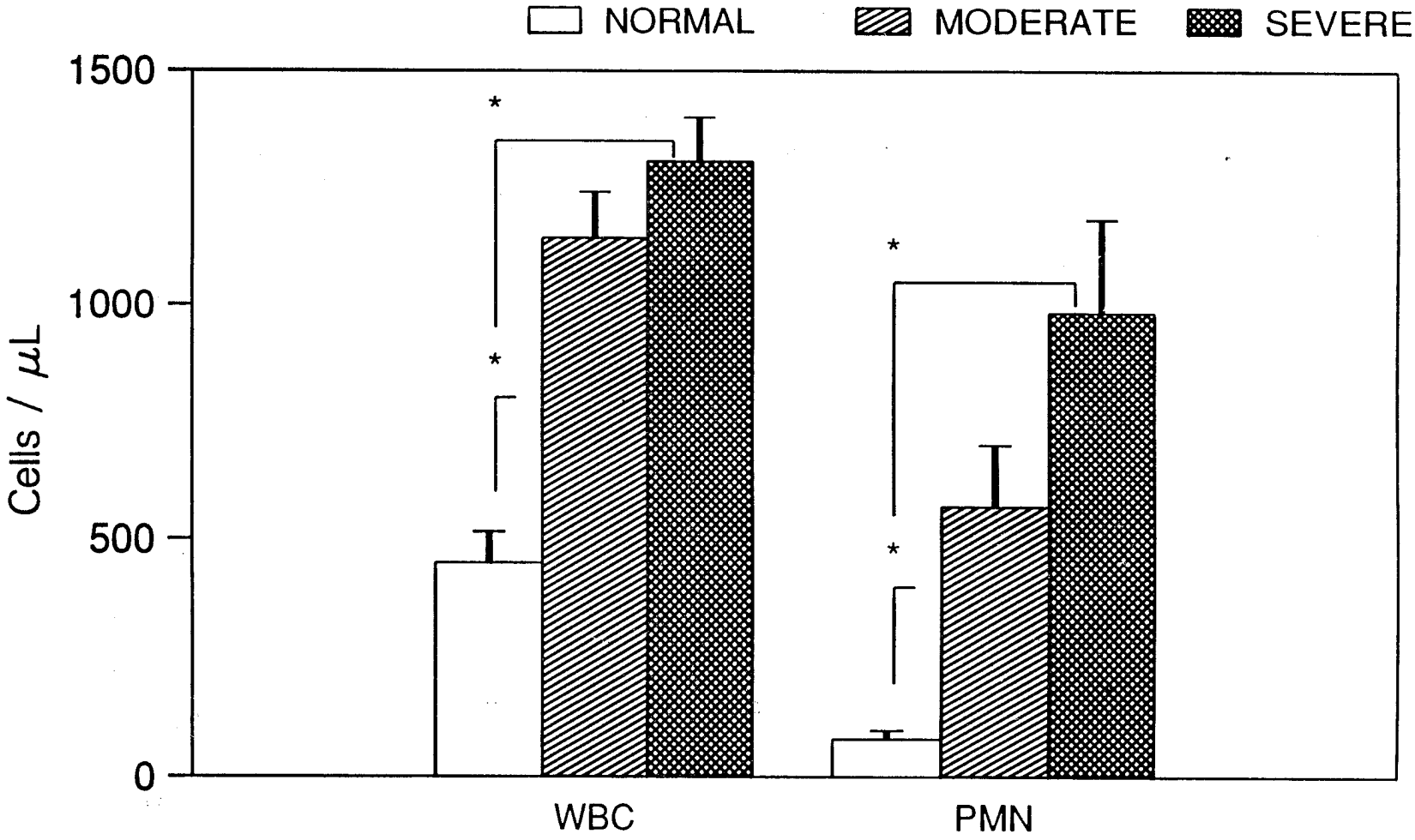


FIGURE 4. Total WBC and PMNL counts (cells/μl) in the bronchoalveolar lavage fluid from Groups I (MODERATE), II (SEVERE), and III (NORMAL). Both numbers for Group II were higher than Group I, but the differences between the two groups were not significant.

bronchoalveolar lavage fluid from Groups I and II, and from normal controls. Both numbers were significantly higher in Groups I and II compared to Group III ($P < 0.05$, ANOVA Tukey). In Group II, both values were higher than in Group I, but the differences between the two groups were not statistically significant.

Figure 5 depicts the total protein content in bronchoalveolar lavage fluid. The values for Groups I and II were significantly higher than that in Group III. In Group II, bronchoalveolar lavage total protein content was significantly higher than in Group I, while plasma albumin content at 48 h did not differ significantly between the two groups.

Figure 6 displays the wet-to-dry lung weight ratios for Groups I, II, and III. The ratio was significantly increased in Groups I and II compared to Group III. In Group II, the ratio was significantly higher than in Group I.

Figure 7 depicts the changes in static lung compliance and pulmonary resistance at 48 h compared to the presmoke values for Groups I and II. The changes in both variables were significantly greater in Group I than in Group I.

Table 4 represents the results of MIGET analysis for Groups I, II, and III. The mean V_A/Q of blood flow distribution was significantly decreased in Groups I and II as compared to Group III. In Group II, mean V_A/Q was significantly less than in Group I. The standard deviation of blood flow distribution on the log scale of V_A/Q axis was significantly increased in Groups I and II compared to Group III. In Group II, the standard deviation was significantly higher than in Group I. The total blood flow percentage to shunt and low V_A/Q area ($V_A/Q < 0.1$) was significantly increased in Groups I and II compared to Group III. In Group II, the percentage was significantly higher than in Group I. These results indicate that V_A/Q mismatching was increased after smoke inhalation and was significantly worse in Group II than in Group I.

Table 5 contains the histologic scores at the level of mid-trachea, left main bronchus, segmental bronchus, and left lower lobe parenchyma. The damage scores at each level were significantly higher in Group II than in Group I.

DISCUSSION

Smoke inhalation injury is initiated by exposure to noxious chemicals generated during incomplete combustion. Smoke exposure results in acute airway inflammation which histologically appears as a loss of cilia, erosion, and sloughing of bronchoepithelium, pseudomembrane formation with small airway occlusion and atelectasis, and morphologic changes in alveolar epithelial cells (14). After smoke exposure, activated leukocytes accumulate in the

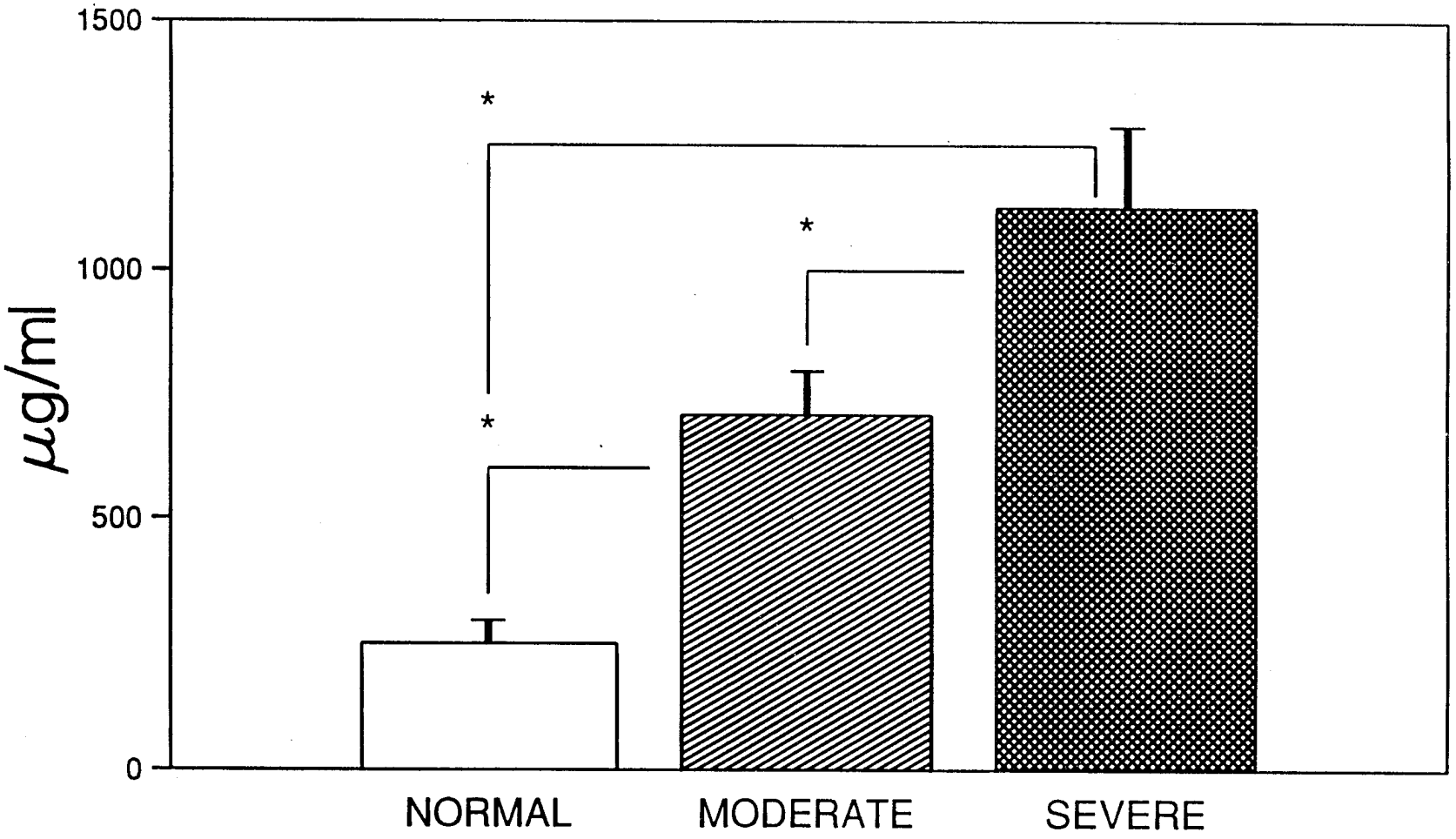


FIGURE 5. Total protein content (µg/ml) in the bronchoalveolar lavage fluid from Groups I (MODERATE), II (SEVERE), and III (NORMAL). The protein content for Group II was significantly greater compared to Group I. *P < 0.05, ANOVA (Tukey).

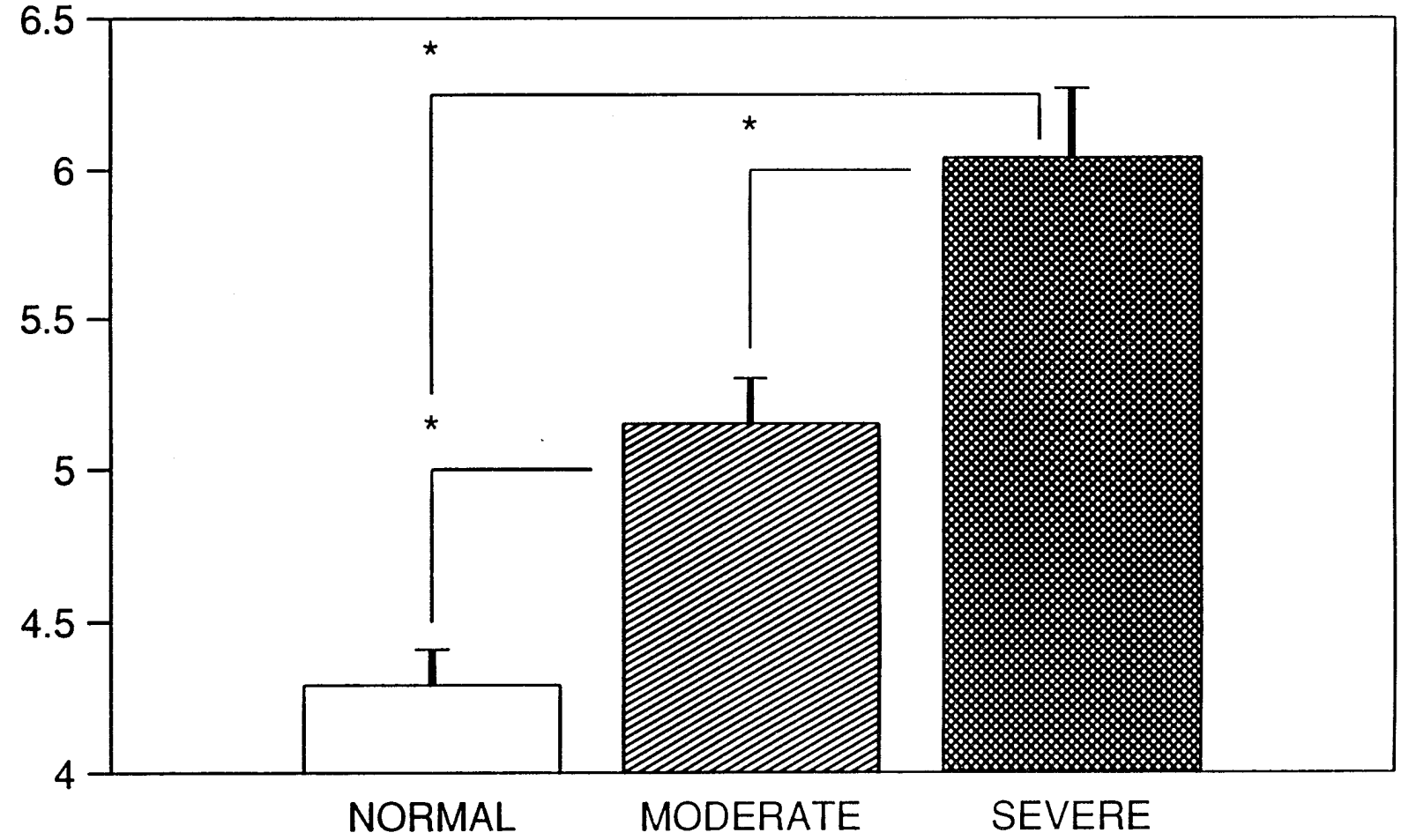


FIGURE 6. Wet-to-dry lung weight ratio for Groups I (MODERATE), II (SEVERE), and III (NORMAL). The ratio for Group II was significantly greater compared to Group I. *P < 0.05, ANOVA (Tukey).

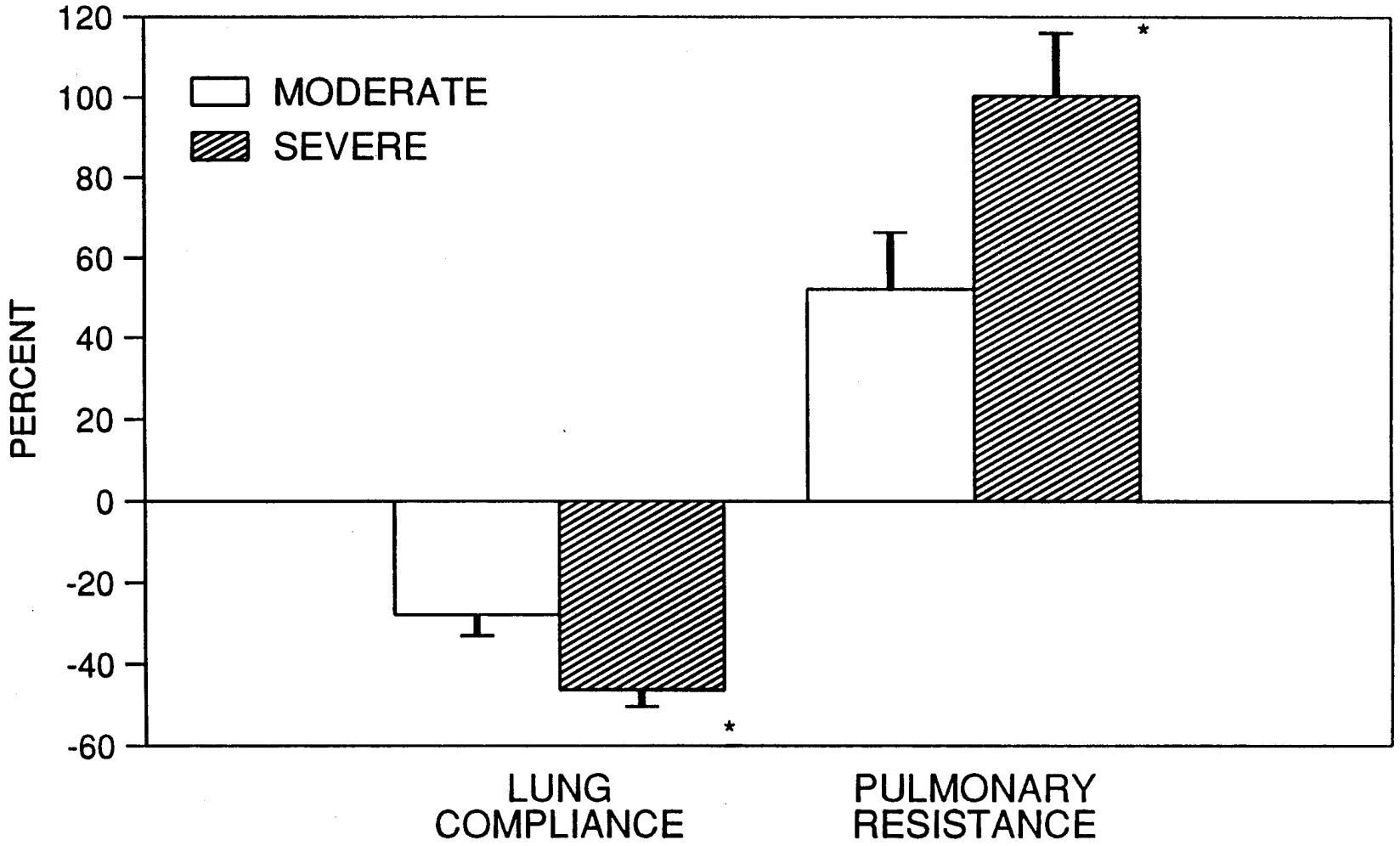


FIGURE 7. Changes (%) in static lung compliance and pulmonary resistance at 48 h compared to presmoke values. The changes in both variables were significantly greater in Group II (SEVERE) compared to Group I (MODERATE). *P < 0.05, student's t test).

TABLE 4. Ventilation Perfusion Ratio (V_A/Q) of Blood Flow Distribution as Determined by the Multiple Inert Gas Elimination Technique (Mean ± SEM)

	Group I	Group II	Group III
Mean VA/Q of Q	0.69 ± 0.04	0.41 ± 0.07*	0.95 ± 0.09*
Log SDQ	1.26 ± 0.10	2.32 ± 0.10*	0.44 ± 0.03*
VA/Q < 0.1 Area (%)	13.3 ± 1.9	29.6 ± 1.8*	0.1 ± 0.1*

Log SDQ indicates logarithmic standard deviation of blood flow distribution and VA/Q < 0.1 Area, total percentage of blood flow to shunt and very low VA/Q area. *P < 0.05 vs Group 1, ANOVA (Tukey).

TABLE 5. Histologic Damage Scores by Light Microscopy (Mean ± SEM)

Location	Group I	Group II	Group III
Mid-trachea	1.4 ± 0.2	3.0 ± 0.4*	0.0 ± 0.0*
Main bronchus	2.0 ± 0.2	3.1 ± 0.3*	0.2 ± 0.2*
Segmental bronchus	2.1 ± 0.3	3.4 ± 0.3*	0.2 ± 0.2*
Lower lobe parenchyma	1.6 ± 0.3	2.7 ± 0.4*	0.2 ± 0.2*

See Tables 1 and 2 for damage score criteria. *P < 0.05 vs Group I, ANOVA (Tukey).

bronchopulmonary region and are thought to produce progressive injury of the airway and lung parenchyma secondary to oxidant production and the products of degranulation (15,16). Inflammatory mediators such as thromboxanes, leukotrienes, neuropeptides, and platelet-activating factor have been reported to be involved in the pathophysiologic changes after smoke exposure (4,17-19). These mediators potentially modulate the increase in both pulmonary vascular permeability and pulmonary capillary hydrostatic pressure, as well as neutrophil activation (20). Bronchial blood flow and lung lymph flow of the smoke-exposed lung has been reported to be increased (21). Antiprotease activity is suppressed secondary to endogenous oxidant production and surface tension in the alveoli is decreased (22). The airway damage and subsequent pulmonary edema not only worsen ventilation perfusion distribution and oxygenation, but increase the susceptibility to pulmonary infection which increases morbidity and mortality in patients with combined smoke inhalation and thermal injury.

Smoke contains chemical gases, aerosols, particulates, and other organic molecules, including free radicals (23,24). These decomposition products and thus their toxicities depend principally on the material burned and prevailing thermal conditions. Using constant combustion conditions, the toxic effects of smoke generated from synthetic and natural polymers have been classified thoroughly in a rat model (25). Although thermal decomposition rates usually increase with increasing temperature, hydrocarbons have been noted to have a zone of negative temperature coefficient in which the rate of reaction decreases with increasing temperature (26). In addition, the nature of smoke can also depend upon factors such as oxygen supply, atmospheric pressure, space, humidity, decomposition mode, time duration from the initiation of thermolysis, and the rate of temperature increase (27).

Carbon monoxide and hydrogen cyanide, significant asphyxiants, are among the chemical gases generated following incomplete combustion (28,29). In spite of their marked suppressive effects on the central nervous system and cardiac function, carbon monoxide and hydrogen cyanide exert no significant pulmonary inflammatory effects when inhaled. In ovine models, the airway epithelial damage induced by smoke inhalation is not observed after carbon monoxide inhalation (30,31). Chemicals generated from common household materials which do induce airway inflammation include aldehydes, halogen acids, oxides of nitrogen, ammonia, sulfur dioxide, and phosgene (28). Wood smoke contains various chemicals including carbon monoxide and aldehydes; acrolein appears to be the most potent chemical initiating the cascade of airway inflammation (32,33). Inhaled aerosolized acrolein causes pulmonary edema in dog models and is thought to be a causative factor for the increase in pulmonary vascular permeability documented after wood smoke exposure (34,35).

The anatomic level of injury after smoke exposure is dependent upon the size and density of the particulates and aerosols which carry the noxious chemicals into the respiratory tract (36). The level of deposition is also influenced by the water and lipid solubility of the chemicals, as well as the airway moisture content and anatomical properties of the airway. The toxicity of smoke can be markedly reduced by removing particulate matter before exposure (37). Although free radicals have been detected by electron spin resonance spin-trapping methods in smoke after combustion of common household materials, the contributory effect of these radicals to smoke injury has not been clarified (37,38).

Animal models have been extensively used for studying the pathophysiology of smoke inhalation (7). Previous ovine models have lacked constant thermal conditions for combustion and precise control mechanisms for smoke exposure, limiting the reproducibility of the injury (19,39-43). Since the severity of injury depends principally on the nature of the smoke, exposure volume, and contact time, failure to control for these variables may limit the

usefulness of these models. Shimazu et al (42) developed a dose-responsive ovine model of smoke inhalation for the first time by controlling exposure, although thermal conditions for combustion were not thoroughly controlled. We modified this model by using a different smoke generating system. In the present study, the constant material and thermal conditions enabled us to generate smoke with relatively constant contents of carbon monoxide, carbon dioxide, and oxygen. Precise control of exposure volume and contact time resulted in a proportional relationship between smoke exposure units and arterial carboxyhemoglobin levels, as well as reproducible pathophysiologic changes. The addition of air breaths between smoke exposure, as well as mixing the generated smoke with oxygen, allowed maintenance of normal arterial blood gases throughout exposure. In spite of similar arterial carboxyhemoglobin levels after smoke exposure, progressive pulmonary deterioration occurred at different rates in Groups I and II. These differences were a result of the difference in the quantity and quality of the chemicals to which the pulmonary tree was exposed. Although the peak arterial COHb levels in both groups were higher than potentially lethal levels reported in human victims, all animals recovered from acute carbon monoxide toxicity. This is attributed to the short half life of COHb in sheep following acute carbon monoxide exposure (30).

Smoke exposure in the present study induced airway epithelial damage and pulmonary edema, leading to an increase in the shunt and low V_A/Q areas in the lung. The increased pulmonary resistance and decreased lung compliance resulted from the occlusion of small airways and collapse of alveoli. The increase in extravascular lung water may be a consequence of increased pulmonary vascular permeability and pulmonary capillary hypertension. The increased total protein content in the bronchoalveolar lavage fluid support the former mechanism, while the increased pulmonary arterial pressure and pulmonary capillary wedge pressure support the latter (20,44). All of these physiologic changes were more severe in Group II than in Group I, a finding consistent with the morphologic changes in the airway and lung parenchyma.

In summary, an ovine model of smoke inhalation injury has been developed using constant conditions of combustion and precise control of smoke exposure. This model is reproducible and can be used for studies of the pathophysiology or treatment of smoke inhalation injury.

PRESENTATIONS/PUBLICATIONS

None.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

ACCESSION: DA336118

SUMMARY DATE: 920812 **SUMMARY KIND:** A **PREV DATE:** **DISTRIBUTION:** CX

PROGRAM #: 61101A **PROJ #:** 30161101A91C **TASK AREA:** CA **WORK UNIT:** 084

TITLE: Effects of Inhaled Nitric Oxide (NO) on Smoke Inhalation Injury in an Ovine Model

SUBJ1: 060500 - Medicine and Medical Research

START DATE: 9208 **END DATE:** 9309 **PERFORMANCE METHOD:** C

CONTRACT/GRANT NO:

CONT TOTAL: $
CUM TOTAL: $
TOTAL LAB FUNDS: $

RESOURCES ESTIMATE

FY	WORK YRS	$(Thousands)
91	0.0	$0
92	0.1	$12
93	0.2	$28

RESPONSIBLE DOD ORGANIZATION: 404885
MRDC INSTITUTE OF SURGICAL RESEARCH USAISR
FT SAM HOUSTON, TX 78234
PRUITT, B A
210-221-2720

PERFORMING ORGANIZATION: 404885
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ASSOC1: CIOFFI, W G

ASSOC2: JORDAN, B S

FOREIGN INTELLIGENCE CONSIDERED: Y **CIVILIAN APPLICABILITY:** M

KEYWORDS: ILIR; Lab Animals; Sheep; Burns (Injuries); Lung; Inhalation; Therapy

OBJECTIVE: A DTIC literature search was conducted under DTIC request numbers W6Q05I/W6Q07I dated 13 August 1992. The objective of this work is to determine the pathophysiologic effects of inhaled NO on smoke inhalation injury in an ovine model. If inhaled NO can favorably affect the physiologic deterioration after smoke inhalation injury, the application of inhaled NO to patients with inhalation injury might be advantageous.

APPROACH: Twenty sheep will be divided into two groups. Group I (n=10) will be exposed to smoke and Group II (n=10) will be exposed to smoke and administered inhaled NO postinjury.

PROGRESS: 9208-9209. This study was approved by the USAISR Research Council and US Army Institute of Surgical Research Animal Care and Use Committee during the fourth quarter of Fiscal Year 1992. For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1992.*

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

ACCESSION: DA336118

SUMMARY DATE: 921001 **SUMMARY KIND:** D **PREV DATE:** 920812 **DISTRIBUTION:** CX

PROGRAM #: 61101A **PROJ #:** 30161101A91C **TASK AREA:** EC **WORK UNIT:** 084

TITLE: Effects of Inhaled Nitric Oxide (NO) on Smoke Inhalation Injury in an Ovine Model

SUBJ1: 060500 - Medicine and Medical Research

START DATE: 9208 **END DATE:** 9309 **PERFORMANCE METHOD:** C

CONTRACT/GRANT NO:

CONT TOTAL: $
CUM TOTAL: $
TOTAL LAB FUNDS: $

RESOURCES ESTIMATE

FY	WORK YRS	$(Thousands)
91	0.0	$0
92	0.1	$12
93	0.2	$28

RESPONSIBLE DOD ORGANIZATION: 404885
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PERFORMING ORGANIZATION: 404885
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210-221-3349

ASSOC1: CIOFFI, W G

ASSOC2: JORDAN, B S

FOREIGN INTELLIGENCE CONSIDERED: Y **CIVILIAN APPLICABILITY:** M

KEYWORDS: ILIR; Lab Animals; Sheep; Burns (Injuries); Lung; Inhalation; Therapy

OBJECTIVE: A DTIC literature search was conducted under DTIC request numbers W6Q05I/W6Q07I dated 13 August 1992. The objective of this work is to determine the pathophysiologic effects of inhaled NO on smoke inhalation injury in an ovine model. If inhaled NO can favorably affect the physiologic deterioration after smoke inhalation injury, the application of inhaled NO to patients with inhalation injury might be advantageous.

APPROACH: Twenty sheep will be divided into two groups. Group I (n=10) will be exposed to smoke and Group II (n=10) will be exposed to smoke and administered inhaled NO postinjury.

PROGRESS: 9208-9209. This study was approved by the USAISR Research Council and US Army Institute of Surgical Research Animal Care and Use Committee during the fourth quarter of Fiscal Year 1992. Equipment and supplies have been ordered and work will be initiated shortly. For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1992*.

ABSTRACT

PROJECT NUMBER: 3A161101A91C-084, In-House Laboratory Independent Research

PROJECT TITLE: Effects of Inhaled Nitric Oxide (NO) on Smoke Inhalation Injury in an Ovine Model

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

PERIOD COVERED IN THIS REPORT: 12 August 1992 - 30 September 1992

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No reports exist in the literature utilizing inhaled NO in the treatment of smoke inhalation injury. Recently, low levels of inhaled NO (10-80 ppm) has been reported as a selective pulmonary vasodilator without causing systemic vasodilation in hypoxic or thromboxane-induced pulmonary vasoconstriction lamb models. Similar effects of inhaled NO (20-40 ppm) were shown in patients with pulmonary hypertension or ARDS. However, effects of inhaled NO in acute lung injury have not been clarified. Smoke inhalation appears to cause mediator-induced pulmonary hypertension and airway inflammation, with overactivated neutrophils and platelets. Inhaled NO has the potential to attenuate pulmonary deterioration following smoke inhalation injury. Therefore, the objective of this study is determine the physiologic effects of inhaled NO on smoke inhalation injury in an ovine model.

This study was approved by the USAISR Research Council and US Army Institute of Surgical Research Animal Care and Use Committee during the fourth quarter of Fiscal Year 1992. Equipment and supplies have been ordered and work will be initiated shortly.

EFFECTS OF INHALED NITRIC OXIDE (NO) ON SMOKE INHALATION INJURY IN AN OVINE MODEL

No reports exist in the literature utilizing inhaled NO in the treatment of smoke inhalation injury. NO has been identified as the powerful endothelium-derived relaxing factor (1). One important biochemical property of NO is activation of soluble guanylate cyclase and elevation of tissue cGMP levels, which mediate vasorelaxation of vascular smooth muscle (2). NO also mediates nonadrenergic, noncholinergic relaxation in airway smooth muscle (3). Another important property of NO is inhibition of platelet and neutrophil aggregation and adhesion to endothelial cells (4-7). Exogenous NO improved survival time in a splanchnic artery occlusion shock model possibly secondary to cytoprotective effects of NO (8). NO also prevented changes in intestinal permeability after ischemia and reperfusion (9). Endogenous NO had a protective effect on pulmonary vascular permeability changes following vagal nerve stimulation (10).

Recently, low levels of inhaled NO (10-80 ppm) has been reported as a selective pulmonary vasodilator without causing systemic vasodilation in hypoxic or thromboxane induced pulmonary vasoconstriction lamb models (11,12). Similar effects of inhaled NO (20-40 ppm) were shown in patients with pulmonary hypertension or ARDS (13,14). In severe ARDS patients, inhaled NO decreased pulmonary arterial pressure and improved oxygenation (15). On the other hand, in normal lambs or healthy volunteers, inhaled NO (10-80 ppm) had no effects on cardiopulmonary function or pathological airway change (11,13). The United States Occupational Safety and Health Administration sets the time-weighted average NO value as 25 ppm. Inhaled NO combines with hemoglobin with great affinity, forming nitrosyl hemoglobin which is oxidized to methemoglobin. Methemoglobin is reduced to nitrate and hemoglobin and then excreted in urine (16). Methemoglobin levels after inhaled NO (80 ppm for 3 h), however, did not significantly increase in sheep (11).

Effects of inhaled NO in acute lung injury have not been clarified. Smoke inhalation appears to cause mediator-induced pulmonary hypertension and airway inflammation, with overactivated neutrophils and platelets. Inhaled NO has the potential to attenuate pulmonary deterioration following smoke inhalation injury. Therefore, the objective of this study is determine the physiologic effects of inhaled NO on smoke inhalation injury in an ovine model.

MATERIALS AND METHODS

Study Design. Twenty sheep will be divided into two groups. Group I (n=10) will be exposed to smoke and Group II (n=10) will be exposed to smoke and administered continuous inhaled NO postinjury.

Description of Procedures. Twenty 1- to 2-yr-old neutered male, commercially available, random source sheep weighing 25-45 kg will be studied. The animals will be housed in covered outdoor runs, treated for parasites (1% ivermectin, 1 ml/75 lb), and fed commercial chow and water ad libitum. Baseline hematologic data (CBC, total proteins, and blood chemistries) will be obtained 3 weeks prior to study. All animals will be fasted for 24 h before smoke exposure and use. The animals will be anesthetized with sodium pentobarbital (25 mg/kg IV) administered through a 25-ga needle via the external jugular vein, orally intubated, mechanically ventilated, and placed in the supine position. A 30-cm Silastic® medical grade cannula will be inserted into a femoral artery and another into a femoral vein. One radiopaque sheath introducer (8.5F, American Edwards Laboratories, Inc., Irvine, CA) will be inserted into an external jugular vein using sterile technique. The arterial line will be used for obtaining blood samples for blood gas analyses. The venous line will be used for infusion of the solution containing the six inert gases to measure V_A/Q_C inequality using the multiple inert gas elimination technique. A Swan-Ganz catheter (7F, American Edwards Laboratories) will be inserted through the sheath in the jugular vein. A tracheotomy will be performed using sterile technique and a 9-mm tracheal tube (Shiley Incorporated, Irvine, CA) will be placed.

Smoke insufflation resulting in a moderate inhalation injury will be produced by the method recently developed at this Institute. Immediately after smoke exposure, each animal will be housed in an individual cage in climate-controlled facilities at 74-76°F (24-25°C) with a relative humidity of 40-50% and observed while spontaneously breathing in the awake state for 24 h. During the study, the tracheal tube will be connected to a nonrebreathing circuit consisting of a 5-l reservoir bag and a one-way valve to separate inspired from expired gas. The inspired gas will be a precise mixture of oxygen and nitrogen for Group I (FIO_2 = 0.21) and a mixture of oxygen and nitrogen immediately diluted with NO to the correct inspired concentration for Group II (NO = 20 ppm, FIO_2 = 0.21). The residence half-time of NO in the reservoir bag will be ≤ 30 sec, with a fresh gas flow of 10 l/min. Expired gases will be scavenged and discarded.

Cardiopulmonary variables and blood gases will be measured presmoke and at 1, 3, 6, 12, 18, and 24 h after smoke exposure. Cardiopulmonary measurements will include systemic blood pressure, heart rate, pulmonary artery pressure, pulmonary capillary wedge pressure, cardiac output, and pulmonary vascular resistance.

Pulmonary artery pressure will be monitored with Statham P23Db transducers (Statham Instruments, Oxnard, CA) and systemic arterial pressure will be monitored with a Hewlett-Packard 1290-A quartz transducer (Hewlett-Packard Company, Waltham, MA). These pressures will be recorded on a Hewlett-Packard four-channel recorder (Model 7754A). Cardiac output will be measured in triplicate by the thermodilution technique (Cardiac Output Computer, Model 9520A, American Edwards Laboratories).

Arterial and mixed-venous blood samples will be analyzed for blood gases and methemoglobin levels. Blood gas analyses will be performed using an IL 1303 pH/blood gas analyzer and an IL 282 CO-oximeter (Instrumentation Laboratories, Inc., Lexington, MA). Blood samples for measurement of 6-keto-$PGF_{1\alpha}$, thromboxane B_2, conjugated dienes, and nitrate will be drawn at the same time. After centrifugation, the plasma of these samples will be stored at -70°C for later analyses. Due to the expense of such measurements, the assays will be performed only if expected pathophysiologic changes are seen. Thromboxane B_2 and 6-keto-$PGF_{1\alpha}$, the stable derivatives of thromboxane A_2 and PGI_2, will be measured by RIA (18). Conjugated dienes, products of lipid peroxidation, will be determined by techniques described by Ward et al (19). They will be read at an optical density of 233 nm in a spectrophotometer. Nitrate, the stable metabolite of nitric oxide, will be measured by GC using techniques modified from the method of Dunphy et al (20).

At the end of 24 h, the animals will again be anesthetized with sodium pentobarbital (25 mg/kg IV) through an existing intravenous line, paralyzed with pancuronium bromide (0.03-0.04 mg/kg, Pavulon®, Organon Pharmaceuticals, West Orange, NJ), and placed in the prone position. Measurement of V_A/Q_C using the multiple inert gas elimination technique will be performed (21). Lactated Ringer's solution containing six inert gases (sulfur hexafluoride, ethane, cyclopropane, halothane, diethyl ether, and acetone) will be infused at a rate of 0.1 ml/kg/min. After 30 min when equilibrium of gas exchange occurs, arterial and mixed-venous blood samples (10 cc each) will be drawn anaerobically into preweighed, heparinized syringes (30 ml, matched, glass, Becton Dickinson and Company, Franklin Lakes, NJ) simultaneously. Mixed-expired gas will be collected from a temperature-controlled copper coil (OD = 3.49 cm, L = 620 cm) about 1 min after blood sampling, compensating for the delay of the mixing chamber. Blood and expired gas samples will be immediately analyzed by GC.

After sample collections, bronchoalveolar lavage will be performed with a bronchoscope (Olympus CLV-10) to obtain samples from the left lower lung lobe for measurement of total protein, WBC and neutrophil counts, 6-keto-$PGF_{1\alpha}$, thromboxane B_2, and conjugated dines. Twenty milliliters of 0.9% sterile saline will be inserted to the left lower lung lobe and the fluid will be immediately pulled back through a suction port. This process of lavage and suction will be repeated three times (total fluid = 60 ml).

The animal will then be sacrificed with sodium pentobarbital (25 mg/kg IV) and a potassium sodium chloride bolus (20 ml of a 200 g/l solution) administered through an existing intravenous line. Extravascular lung water will be determined by a gravimetric method (17).

Determination of Number of Animals Required: This is a pilot study to determine the physiologic effects of inhaled NO on smoke inhalation injury using an ovine model. Based on previous experience using the ovine model for other studies, 20 animals should be satisfactory for adequate data collection.

Data Analysis Plan: ANOVA for a mixed factorial design and multivariate analysis (regression) will be utilized.

RESULTS

This study was approved by the USAISR Research Council and US Army Institute of Surgical Research Animal Care and Use Committee during the fourth quarter of Fiscal Year 1992. Equipment and supplies have been ordered and work will be initiated shortly.

DISCUSSION

Upon completion of data collection, the data will be analyzed as indicated and the results submitted for publication.

PRESENTATIONS/PUBLICATIONS

None.

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neutrophils in vitro. *Br J Haematol* 77:539-45, 1991.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

ACCESSION: DA336119

SUMMARY DATE: 920812 **SUMMARY KIND:** A **PREV DATE:** **DISTRIBUTION:** CX

PROGRAM #: 62787A **PROJ #:** 30162787A874 **TASK AREA:** CA **WORK UNIT:** 085

TITLE: A New Ovine Model of Smoke Inhalation Injury Combined with Thermal Burn

SUBJ1: 060500 - Medicine and Medical Research

START DATE: 9208 **END DATE:** 9309 **PERFORMANCE METHOD:** C

CONTRACT/GRANT NO:

CONT TOTAL: $
CUM TOTAL: $
TOTAL LAB FUNDS: $

RESOURCES ESTIMATE

FY	WORK YRS	$(Thousands)
91	0.0	$0
92	0.1	$17
93	0.3	$40

RESPONSIBLE DOD ORGANIZATION: 404885
MRDC INSTITUTE OF SURGICAL RESEARCH USAISR
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PRUITT, B A
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PERFORMING ORGANIZATION: 404885
MRDC INSTITUTE OF SURGICAL RESEARCH USAISR
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OGURA, H
210-221-3349

ASSOC1: CIOFFI, W G

ASSOC2: JORDAN, B S

FOREIGN INTELLIGENCE CONSIDERED: Y **CIVILIAN APPLICABILITY:** M

KEYWORDS: ILIR; Lab Animals; Sheep; Burns (Injuries); Inhalation

OBJECTIVE: A DTIC literature search was conducted under DTIC request numbers W6P54K/W6P49I dated 13 August 1992. The objective of this work is to establish a new ovine model of smoke inhalation injury combined with thermal burn. Since patients with smoke inhalation injury usually suffer from concomitant thermal injury, a reproducible ovine model of smoke inhalation injury combined with thermal injury would be useful for future studies of the effects of medical interventions and new modes of ventilatory support.

APPROACH: Six sheep will be used in a preliminary phase to determine adequate water temperature and contact time to produce a 30% total body surface area full-thickness scald burn. An additional 20 sheep will then be divided into two groups. Group I (n=10) will be administered a burn injury and Group II (n=10) will be administered a burn injury and exposed to smoke.

PROGRESS: 9208-9209. This study was approved by the USAISR Research Council and US Army Institute of Surgical Research Animal Care and Use Committee during the fourth quarter of Fiscal Year 1992. For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1992.*

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

ACCESSION: DA336119

SUMMARY DATE: 921001 **SUMMARY KIND:** D **PREV DATE:** 920812 **DISTRIBUTION:** CX

PROGRAM #: 62787A **PROJ #:** 30162787A874 **TASK AREA:** EC **WORK UNIT:** 085

TITLE: A New Ovine Model of Smoke Inhalation Injury Combined with Thermal Burn

SUBJ1: 060500 - Medicine and Medical Research

START DATE: 9208 **END DATE:** 9309 **PERFORMANCE METHOD:** C

CONTRACT/GRANT NO:

CONT TOTAL: $
CUM TOTAL: $
TOTAL LAB FUNDS: $

RESOURCES ESTIMATE

FY	WORK YRS	$(Thousands)
91	0.0	$0
92	0.1	$17
93	0.3	$40

RESPONSIBLE DOD ORGANIZATION: 404885
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PERFORMING ORGANIZATION: 404885
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210-221-3349

ASSOC1: CIOFFI, W G

ASSOC2: JORDAN, B S

FOREIGN INTELLIGENCE CONSIDERED: Y **CIVILIAN APPLICABILITY:** M

KEYWORDS: ILIR; Lab Animals; Sheep; Burns (Injuries); Inhalation

OBJECTIVE: A DTIC literature search was conducted under DTIC request numbers W6P54K/W6P49I dated 13 August 1992. The objective of this work is to establish a new ovine model of smoke inhalation injury combined with thermal burn. Since patients with smoke inhalation injury usually suffer from concomitant thermal injury, a reproducible ovine model of smoke inhalation injury combined with thermal injury would be useful for future studies of the effects of medical interventions and new modes of ventilatory support.

APPROACH: Six sheep will be used in a preliminary phase to determine adequate water temperature and contact time to produce a 30% total body surface area full-thickness scald burn. An additional 20 sheep will then be divided into two groups. Group I (n=10) will be administered a burn injury and Group II (n=10) will be administered a burn injury and exposed to smoke.

PROGRESS: 9208-9209. This study was approved by the USAISR Research Council and US Army Institute of Surgical Research Animal Care and Use Committee during the fourth quarter of Fiscal Year 1992. Equipment and supplies have been ordered and work will be initiated shortly. For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1992.*

ABSTRACT

PROJECT NUMBER: 3A161101A91C-162, In-House Laboratory Independent Research

PROJECT TITLE: A New Ovine Model of Smoke Inhalation Injury Combined with Thermal Burn

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

PERIOD COVERED IN THIS REPORT: 8 April 1992 - 30 September 1992

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Smoke inhalation injury causes airway inflammation in the early phase and pulmonary edema and infection in the later phase. On the other hand, thermal burn injury alone has been reported to induce pulmonary inflammation. The pulmonary change after combined injury, however, has not been thoroughly investigated due to the difficulty of controlling both injuries in large animals.

The objective of this work is to establish a new ovine model of smoke inhalation injury combined with thermal burn. Since patients with smoke inhalation injury usually suffer from concomitant thermal injury, a reproducible ovine model of smoke inhalation injury combined with thermal injury would be useful for future studies of the effects of medical interventions and new modes of ventilatory support.

A NEW OVINE MODEL OF SMOKE INHALATION INJURY COMBINED WITH THERMAL BURN

Smoke inhalation injury causes airway inflammation in the early phase and pulmonary edema and infection in the later phase. On the other hand, thermal burn injury alone has been reported to induce pulmonary inflammation (1). The pulmonary change after combined injury, however, has not been thoroughly investigated due to the difficulty of controlling both injuries in large animals.

A new ovine model of smoke inhalation injury has recently been developed at this Institute. In this model, smoke is generated by thermolysis of pine woodchips in a crucible furnace (Furnace Model 56622 and Control Console Model 58114, Lindberg) using a constant temperature and airflow rate, with which animals are exposed at a controlled tidal volume and breath hold time. The pathophysiologic changes after smoke inhalation are sufficiently reproducible within 48 h.

The degree of thermal burn primarily depends on contact temperature, time, and pressure. No reports exist in the literature in which these factors in an ovine model are well controlled. A reproducible thermal injury model could possibly be produced by a scald burn method with a constant contact time and temperature modified from the method of Walker and Mason (2).

A new ovine model of combined injury could also be developed by combining the above two methods. The objective of this study is to establish a reproducible ovine model of combined smoke inhalation injury and thermal injury by delineating the pathophysiologic changes.

MATERIALS AND METHODS

Study Design. Six sheep will be used in a preliminary phase to determine adequate water temperature and contact time to produce a 30% total body surface area full-thickness scald burn. An additional 20 sheep will then be divided into two groups. Group I (n=10) will be administered a burn injury and Group II (n=10) will be administered a burn injury and exposed to smoke.

Description of Procedures. Twenty-six 1- to 2-yr-old nonpregnant female, commercially available, random source sheep weighing 25-45 kg will be studied. The animals will be housed in covered outdoor runs, treated for parasites (1% ivermectin, 1 ml/75 lb), and fed commercial chow and water ad libitum. Baseline hematologic data (CBC, total proteins, and blood chemistries) will be obtained 3 weeks prior to study. All animals will be fasted for 24 h before use. The surfaces of both flanks will be shaved of all hair.

For the preliminary phase, 6 animals will be anesthetized with sodium pentobarbital (25 mg/kg IV) administered through a 25-ga needle via the external jugular vein, orally intubated, and mechanically ventilated. Animals will be placed in an asbestos-coated flexible mat containing two holes which expose a total of 30% of the total body surface area. The animals will be suspended on a 3-m metal bar and pressed tightly to the mold to protect unexposed skin from receiving scald injury due to water leakage. The mold will then be immersed into a tub containing heated water. Immediately after burn injury, each animal will be housed in an individual cage in climate-controlled facilities at 74-76°C (24-25°C) with a relative humidity of 40-50% and observed while spontaneously breathing in the awake state for 24 h. The animal will then be sacrificed with sodium pentobarbital (25 mg/kg IV) and a potassium chloride bolus (20 ml of a 200 g/l solution) administered via the external jugular vein. Histologic evaluation of burn depth will be conducted by LTC Okerberg to determine the extent of injury. Contact time and water temperature will be adjusted for each animal until a 30% total body surface area full-thickness scald burn is obtained.

Twenty additional animals will be anesthetized with sodium pentobarbital (25 mg/kg IV) administered through a 25-ga needle via the external jugular vein, orally intubated, mechanically ventilated, and placed in the supine position. A 30-cm Silastic® medical grade cannula will be inserted into a femoral artery and another into a femoral vein. One radiopaque sheath introducer (8.5F, American Edwards Laboratories, Inc., Irvine, CA) will be inserted into an external jugular vein using sterile technique. The arterial line will be used for obtaining blood samples for blood gas analyses. The venous line will be used for infusion of the solution containing the six inert gases to measure V_A/Q_C inequality using the multiple inert gas elimination technique. A Swan-Ganz catheter (7F, American Edwards Laboratories) will be inserted through the sheath in the jugular vein. A Foley catheter (10F, Catalog No. 4084, American Pharmaceutical Laboratories, Glendale, CA) will be placed in the bladder to monitor urine output and collect urine.

Just before burn injury, all animals will be administered additional sodium pentobarbital (10 mg/kg IV) to maintain deep anesthesia. Animals will be placed in an asbestos-coated flexible mat containing two holes which expose a total of 30% of the total body surface area. The animals will be suspended on a 3-m metal bar and pressed tightly to the mold to protect unexposed surface areas from receiving scald injury due to water leakage. The mold will then be immersed for the period of time as determined during the preliminary phase of this study into a tub of water at the temperature determined during the preliminary phase of this study. For animals randomized to Group 2, smoke insufflation resulting in a moderate inhalation injury will be produced by the method recently developed at this Institute.

Immediately after burn injury and/or smoke exposure, each animal will be housed in an individual cage in climate-controlled facilities at 74-76°F (24-25°C) with a relative humidity of 40-50% and observed while spontaneously breathing in the awake state for 48 h.

Cardiopulmonary variables and blood gases will be measured presmoke and at 1, 3, 6, 12, 24, 36, and 48 h after injury and/or smoke exposure. Cardiopulmonary measurements will include systemic blood pressure, heart rate, pulmonary artery pressure, pulmonary capillary wedge pressure, cardiac output, and pulmonary vascular resistance. Pulmonary artery pressure will be monitored with Statham P23Db transducers (Statham Instruments, Oxnard, CA) and systemic arterial pressure will be monitored with a Hewlett-Packard 1290-A quartz transducer (Hewlett-Packard Company, Waltham, MA). These pressures will be recorded on a Hewlett-Packard four-channel recorder (Model 7754A). Cardiac output will be measured in triplicate by the thermodilution technique (Cardiac Output Computer, Model 9520A, American Edwards Laboratories).

Arterial and mixed-venous blood samples will be analyzed for blood gases. Blood gas analyses will be performed using an IL 1303 pH/blood gas analyzer and an IL 282 CO-oximeter (Instrumentation Laboratories, Inc., Lexington, MA). Blood samples for measurement of 6-keto-$PGF_{1\alpha}$, thromboxane B_2, conjugated dienes, and nitrate will be drawn at the same time. After centrifugation, the plasma of these samples will be stored at -70°C for later analyses. Due to the expense of such measurements, the assays will be performed only if expected pathophysiologic changes are seen. Thromboxane B_2 and 6-keto-$PGF_{1\alpha}$, the stable derivatives of thromboxane A_2 and PGI_2, will be measured by RIA (2). Conjugated dienes, products of lipid peroxidation, will be determined by techniques described by Ward et al (3). They will be read at an optical density of 233 nm in a spectrophotometer. Nitrate, the stable metabolite of nitric oxide, will be measured by GC using techniques modified from the method of Dunphy et al (4).

At the end of 48 h, the animals will again be anesthetized with sodium pentobarbital (25 mg/kg IV) through an existing intravenous line, paralyzed with pancuronium bromide (0.03-0.04 mg/kg, Pavulon®, Organon Pharmaceuticals, West Orange, NJ), placed in the prone position, and mechanically ventilated. During mechanical ventilation, the tidal volume will be set at 15 ml/kg and the respiratory rate will be 10/min. PEEP will be 5 cmH_2O and the FIO_2 will be kept at 0.21 throughout the remainder of the study. Measurement of V_A/Q_C using the multiple inert gas elimination technique will be performed (5). Lactated Ringer's solution containing six inert gases (sulfur hexafluoride, ethane, cyclopropane, halothane, diethyl ether, and acetone) will be infused at a rate of 0.1 ml/kg/min. After 30 min when equilibrium of gas exchange occurs, arterial and mixed-venous blood samples (10 cc each) will be drawn anaerobically into preweighed, heparinized

syringes (30 ml, matched, glass, Becton Dickinson and Company, Franklin Lakes, NJ) simultaneously. Mixed-expired gas will be collected from a temperature-controlled copper coil (OD = 3.49 cm, L = 620 cm) about 1 min after blood sampling, compensating for the delay of the mixing chamber. Blood and expired gas samples will be immediately analyzed by GC.

After sample collections, bronchoalveolar lavage will be performed with a bronchoscope (Olympus CLV-10) to obtain samples from the left lower lung lobe for measurement of total protein, WBC and neutrophil counts, 6-keto-$PGF_{1\alpha}$, thromboxane B_2, and conjugated dines. Twenty milliliters of 0.9% sterile saline will be inserted to the left lower lung lobe and the fluid will be immediately pulled back through a suction port. This process of lavage and suction will be repeated three times (total fluid = 60 ml).

The animal will then be sacrificed with sodium pentobarbital (25 mg/kg IV) and a potassium chloride bolus (20 ml of a 200 g/l solution) administered through an existing intravenous line. Extravascular lung water will be determined by a gravimetric method (6). Water content of the burn wound and intact tissue will be measured as the difference between wet and dry weights (7).

Determination of Number of Animals Required. This is a pilot study to develop a new reproducible ovine model of combined burn injury. Six animals will be used for the preliminary phase only as needed. Based on previous experience using the ovine model for other studies, 26 animals should be satisfactory for adequate data collection.

Data Analysis Plan. ANOVA for a mixed factorial design and multivariate analysis (regression) will be utilized.

RESULTS

This study was approved by the USAISR Research Council and US Army Institute of Surgical Research Animal Care and Use Committee during the fourth quarter of Fiscal Year 1992. Equipment and supplies have been ordered and work will be initiated shortly.

DISCUSSION

Upon completion of data collection, the data will be analyzed as indicated and the results submitted for publication.

PRESENTATIONS/PUBLICATIONS

None.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

ACCESSION: DA346162

SUMMARY DATE: 920127 **SUMMARY KIND:** K **PREV DATE:** 911001 **DISTRIBUTION:** CX

PROGRAM #: 63002A **PROJ #:** 3M263002DB840 **TASK AREA:** CA **WORK UNIT:** 081

TITLE: The Effect of High-Frequency Ventilation on Smoke Inhalation Injury in Baboons

SUBJ1: 060500 - Medicine and Medical Research
SUBJ2: 061200 - Medical Facilities, Equipment, and Supplies

START DATE: 9005 **END DATE:** 9201 **PERFORMANCE METHOD:** C

CONTRACT/GRANT NO:

CONT TOTAL: $
CUM TOTAL: $
TOTAL LAB FUNDS: $

RESOURCES ESTIMATE

FY	WORK YRS	$(Thousands)
91	0.5	$88
92	0.0	$0
93	0.0	$0

RESPONSIBLE DOD ORGANIZATION: 404885
MRDC INSTITUTE OF SURGICAL RESEARCH USAISR
FT SAM HOUSTON, TX 78234
PRUITT, B A
210-221-2720

PERFORMING ORGANIZATION: 404885
MRDC INSTITUTE OF SURGICAL RESEARCH USAISR
FT SAM HOUSTON, TX 78234
CIOFFI, W G
210-221-8440

ASSOC1: RUE, L W

ASSOC2: JORDAN, B S

FOREIGN INTELLIGENCE CONSIDERED: Y **CIVILIAN APPLICABILITY:** M

KEYWORDS: RA II; Lab Animals; Baboons; Burns (Injuries); Morbidity; Inhalation; Pulmonary Edema; Pulmonary Function; Pulmonary Insufficiency; Respirations

OBJECTIVE: A DTIC literature search was conducted under DTIC request numbers W6L59B/W6M00C dated 18 April 1990. The objective of this work is to determine the effects of high-frequency oscillatory ventilation on pulmonary changes following smoke inhalation injury in a baboon model.

APPROACH: Twenty baboons were randomized to one of four study groups. Group I served as the control group. Groups II, III, and IV were exposed to smoke injury and then supported with one of three ventilators. Blood gas, blood pressure, airway pressures, and hemodynamic data were averaged over intervals and plotted at the midpoint of each interval. Physiologic and repetitive biochemical data were analyzed among groups using repeated measures ANOVA. Data were also compared at specific time points using ANOVA. Outcome data were analyzed using Chi square or the Fisher exact test. Nonparametric data were analyzed using the Kruskall-Wallis test.

PROGRESS: 9005-9201. High-frequency flow interruption was superior to conventional ventilation and high-frequency oscillatory ventilation in terms of amount of support required to maintain arterial blood gases in a normal range. For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report* for fiscal years 1990 through 1991.

ABSTRACT

PROJECT NUMBER: 3M263002D840-081, Advanced Development

PROJECT TITLE: The Effect of High-Frequency Oscillatory Ventilation on Smoke Inhalation Injury in Baboons

INSTITUTION: US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas 78234-5012,[1] and the Foundation for Biomedical Research, West Loop 410 at Military Drive, San Antonio, Texas 78284[2]

PERIOD COVERED IN THIS REPORT: 1 October 1991 - 27 January 1992

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Smoke inhalation leads to a complex sequence of pulmonary and pathophysiologic events which contribute to a high morbidity and mortality when combined with thermal injury. While the volume and composition of the inhaled material clearly plays a role in the severity of the pulmonary manifestations of inhalation injury, other data suggest that the mode of ventilatory support may also affect the severity of the disease process. Until recently, it had not been possible to use high-frequency oscillatory ventilation (HFOV) in large animals or adult humans because the efficiency of available oscillators was insufficient to deliver an adequate tidal volume. Development of a new form of HFOV has made it possible to study whether the dramatic results obtained in infant models of lung disease can be replicated in adult models. The purpose of the study was to compare the effects of two forms of high-frequency ventilation with conventional positive pressure ventilation in a primate model of moderate inhalation injury.

Eighteen baboons were randomized to one of four groups. Group I was not exposed to smoke and served as the control group. Groups II, III, and IV were subjected to a moderate smoke inhalation injury. Group II was placed on positive-pressure conventional ventilation, Group III on high-frequency flow interruption (HFFI), and Group IV on HFOV. The results from this study support our previous findings using HFFI in humans with smoke inhalation injury. The decreased incidence of pneumonia and mortality in patients treated with HFFI compared to a historical cohort of patients treated with positive-pressure ventilation may be secondary to a decrease in iatrogenic mechanical barotrauma which

is secondary to ventilatory mode. These data strongly support the continued use of HFFI in the support of patients with smoke inhalation injury, and offer an explanation for the observed decrease in morbidity and mortality.

THE EFFECT OF HIGH-FREQUENCY OSCILLATORY VENTILATION ON SMOKE INHALATION INJURY IN BABOONS

Smoke inhalation leads to a complex sequence of pulmonary and pathophysiologic events which contribute to a high morbidity and mortality when combined with thermal injury. While the volume and composition of the inhaled material clearly plays a role in the severity of the pulmonary manifestations of inhalation injury, other data suggest that the mode of ventilatory support may also affect the severity of the disease process (1).

High-frequency flow interruption (HFFI) is a form of high-frequency ventilation (HFV) in which exhalation is passive. Subdead-space tidal volumes are delivered into the airway at a predetermined frequency with a variable I:E ratio (usually 1:1). To prevent gas trapping, airway pressure is returned to baseline PEEP at scheduled intervals, usually every 2 sec. CO_2 clearance is controlled by varying peak pressures and the frequency at which airway pressure is returned to baseline. Oxygenation is controlled by frequency and peak pressures, which both effect mean airway pressure.

High-frequency oscillatory ventilation (HFOV) involves the active injection and withdrawal of gas from the lung. Active exhalation during HFOV is felt by some investigators to enhance gas egress, thus allowing the use of a higher frequency and lower tidal volume than conventional HFV with passive exhalation. The use of active exhalation allows peak and trough volumes and pressures to be held close to mean volumes and pressures, thus approaching a near constant lung volume. Since there is active withdrawal of gas, the oscillator itself results in no net input of gas. A source of fresh gas must be introduced distal to the mechanism which supplies the oscillatory excursions and adjustment of the gas flow and resistance will determine mean airway pressure. The major limiting factor of all types of HFV is inadvertent gas trapping. If the tidal volume injected is greater than that which can be eliminated during the expiratory interval, gas trapping and barotrauma will occur. The use of HFOV with active exhalation as well as prolonged lung I:E times may help to prevent this complication.

The use of HFOV and high mean airway pressures has been shown to markedly alter the progression of both ARDS and hyaline membrane disease in experimental animals. In rabbits, use of HFOV prevented edema, hyaline membrane formation, and the loss of membrane compliance seen in the surfactant-depleted animals treated with conventional ventilation following saline lung lavage (2). Even more dramatic findings have been documented in surfactant-deficient premature baboons treated with HFOV. The initiation of HFOV prior to the first breath prevented development of the pathologic, physiologic, and morphologic features of hyaline membrane disease

when compared to animals of comparable gestational age treated with conventional positive pressure ventilation and continual positive distending airway pressure (3,4). Of interest is the fact that the development of hyaline membrane disease was associated with increased levels of platelet-activating factor-like activity in the lung lavage while no increase in platelet-activating factor was seen in HFOV-treated animals. These data coupled with other experimental evidence have led to the hypothesis that in the face of surfactant deficiency, conventional tidal ventilation leads to epithelial injury, mediator release, and increased parenchymal injury.

Various forms of HFV have been shown to be efficacious in the management of infants and adults with bronchopleural fistula or respiratory failure unresponsive to conventional respiratory therapy. In some studies, HFOV has been efficacious in the management of infants with diffuse alveolar disease, although other studies have shown no dramatic improvement (5). HFFI has not been shown to be of benefit in the prevention of ARDS in adults (6). We have reported that the prophylactic use of HFFI was effective in reducing the incidence of pneumonia and mortality in patients with inhalation injury (1). Common to the success of HFV therapy in diffuse alveolar disease has been the use of a high mean airway pressure. Conversely, those studies in infants and adults where HFV has not been shown to be effective tended to use lower mean airway pressures than the conventional ventilatory support (7,8).

Until recently, it has not been possible to use HFOV in large animals or adult humans because the efficiency of available oscillators was insufficient to deliver an adequate tidal volume. Development of a new form of HFOV have made it possible to study whether the dramatic results obtained in infant models of lung disease can be replicated in adult models. The purpose of the study was to compare the effects of two forms of HFV with conventional positive pressure ventilation in a primate model of moderate inhalation injury.

MATERIALS AND METHODS

Study Design. Eighteen baboons were randomized to one of four groups. Group I (n=3) was not exposed to smoke and served as the control group. Groups II, III, and IV were subjected to a moderate smoke inhalation injury. Group II (n=5) was placed on positive-pressure conventional ventilation, Group III (n=5) on HFFI (Bird™, Percussionnaire Corporation); and Group IV (n=5) on HFOV (Foundation for Biomedical Research, San Antonio, TX).

Description of Procedures. At time 0, animals were anesthetized with ketamine hydrochloride (25-40 mg/kg IM), intubated, and paralyzed with pancuronium bromide (0.04-0.10 mg/kg IV). Thereafter, they were maintained under paralysis and sedated with diazepam (0.1 mg/kg IV or 7.5 mg/kg IM) as indicated by

clinical signs of anxiety, i.e., elevated pulse, blood pressure. If diazepam sedation was inadequate, additional sedation was accomplished with sodium pentobarbital (20–33 mg/kg IV).

A Swan-Ganz catheter (7F, American Edwards Company, Irvine, CA) was placed via a femoral vein using local anesthesia (1% lidocaine, 1–2 cc SC). Peripheral venous and arterial lines were placed for measurement of blood gases and administration of fluids. Fluids were administered at a rate of 80 ml/kg/day and consisted of 5% dextrose and 1/2N saline with 1 U heparin sodium per milliliter at 2 ml/h. Changes in fluid composition and infusion rate were based on hemodynamic data and electrolyte composition.

Baseline cardiac output was determined by thermodilution and arterial and venous blood gases were obtained. Blood was drawn for CBC and routine chemistries. Pulmonary function tests were performed. The right lower lung lobe was instrumented using a flexible bronchoscope and lavaged with two 50–cc aliquots of physiologic saline. The recovered lavage fluid was combined and an aliquot removed for cell count with the remainder centrifuged and the supernatant decanted. Differential count was performed on the cell plug. The supernatant was frozen at −70°C for later assay for total protein, total phosphatidylcholine content, and elastase content. After completion of baseline studies, the animals were allowed to recover for 1 h. At the end of 1 h, blood was again drawn for determination of arterial blood gases. The animals were then exposed to a moderate smoke inhalation injury using the techniques previously validated and described by this Institute.

All animals remained intubated postsmoke and allowed to breathe spontaneously. They were treated with 100% oxygen for 1 h to reduce the COHb level. Carbon monoxide levels were measured immediately before smoke exposure, immediately after smoke exposure, every 30 min for 2 h, and every 4 h for 24 h. Levels were obtained daily for the remainder of the study.

Arterial and venous blood gases were obtained hourly until the animal was stable and then every 6 h. Electrolytes, BUN, creatinine, CBC, and platelet counts were obtained every 12 h. Chest roentgenograms were obtained daily. Pulmonary function tests were performed at 0, 24, 72, and 130 h postsmoke. Pulmonary function tests consisted of measurement of functional residual capacity using helium dilution, passive exhalation resistance and compliance, pulmonary diffusing capacity, inspiratory capacity, and expiratory reserve. Lung lavage with saline was performed following the pulmonary function tests at each time point. Bronchoalveolar lavage was collected and frozen for later analysis of cell counts, elastase, total protein, and quantitative bacterial cultures.

Animals were turned from side–to–side every 4 h. Tracheal toilet was performed every 4 h and as clinically indicated. All

animals received gentamicin (2.5 mg/kg IV) every 8 h throughout the study.

Following the pulmonary function tests and determination of preintervention cardiac output and wedge pressure, the animals were placed on preassigned ventilators.

Animals in Group II were placed on positive-pressure ventilation with a tidal volume of 10 ml/kg. Ventilator adjustments were made in response to blood gas determination. PCO_2 was maintained between 35-45 torr by adjustment of tidal volume and frequency. PEEP was maintained at 3 cmH_2O unless the animal required an $FIO_2 > 0.6$ in order to maintain a PaO_2 in the protocol range. In that case, PEEP was increased to increase mean airway pressure.

Animals assigned to Group III were placed on HFFI. Frequency was set at 10 Hz with a 2-sec inspiratory time. Expiratory time was set to result in a return to baseline PEEP rate of 8/min. The I:E ratio of subtidal breaths was 1:1. PEEP, oscillatory in nature, was set at 5 cmH_2O. Peak inspiratory pressure was set at 24 cmH_2O. Adjustments in support were made according to blood gas determinations.

Group IV animals were placed on HFOV. Initial frequency was set at 10 Hz with oscillatory amplitude sufficient to produce detectable chest wall motion. Mean airway pressure was adjusted to maintain PaO_2 between 80 and 100 torr. The FIO_2, following 1 h of 100% O_2, was set at 0.21. Oxygenation was optimized by adjustment of mean airway pressure and FIO_2. Ventilation was optimized by adjustment of the oscillatory amplitude. If CO_2 clearance was inadequate on maximal HFOV settings, then conventional tidal breaths were superimposed upon the high-frequency oscillations. If there was a question about the adequacy of the mean airway pressure, then the animal was manually sighed and pre- and postsigh arterial blood gases were obtained. If the sigh resulted in an increase of 10 torr or greater in arterial PaO_2, then the mean airway pressure was adjusted upwards in increments of 2 cmH_2O.

All animals were supported for 154 h after injury. At the conclusion of the study or, in the opinion of the principal investigator, the animal was in irreversible cardiopulmonary failure, the animal was anesthetized with sodium pentobarbital (50 mg/kg IV) and exsanguinated.

Standard necropsy was performed on all animals. Sections of all organs were obtained and fixed for light and electron microscopy. The left lower lobe was inflated to 20 cmH_2O and fixed by the endotracheal instillation of Carnoy's solution. The trachea was examined for evidence of gross lesions. A ligature was placed at the site of the tip of the endotracheal tube before fixation. The trachea was fixed in its entirety, sectioned longitudinally,

and examined. A section of each of the remaining lobes was removed and frozen in liquid nitrogen and stored at -80°C. Carcasses were removed and destroyed by incineration.

Determination of Number of Animals Required. Previous studies using the adult baboon have substantiated the reproducibility of this model (9). Studies using HFOV in various animal ARDS or hyaline membrane disease models have shown marked intergroup differences with significance at this number (9). This, coupled with cost and ethical concerns, lead us to conclude that 5 animals per group were sufficient to allow us to conclude whether there were advantages/disadvantages to ventilator strategy.

Data Analysis Plan. Blood gas, blood pressure, airway pressures, and hemodynamic data were averaged over intervals and plotted at the midpoint of each interval. Physiologic and repetitive biochemical data were analyzed among groups using ANOVA for repeated measures. Data was also compared at specific time points using ANOVA.

Pathologic data was analyzed by three "blinded" graders using a semiquantitative technique, the panel of standards, which was used to determine the degree of parenchymal lung injury. This technique consisted of comparing the microscopic lung sections to one of seven panels that depicted a spectrum of pulmonary lesions (Grades 1-7) from mild to most severe. A Zeiss™ photomicroscope fitted with a 1X objective was used to photograph the entire cross section of the lobe. The 35-mm negatives were photographically enlarged to yield a 4" X 5.5" black and white photograph. Each photograph (not identified as to animal or treatment group) was graded independently by each of the three different observers using the panel of standards. The mean of the rater scores was calculated and the lobe score summed for each animal. Agreement among observers was determined by the Chronbach alpha test. The RIDIT test was used to test for any ventilator effect on the degree of lung parenchymal injury (4,10). All tests were considered significant for $P < 0.05$.

RESULTS

COHb concentrations for Groups II, III, and IV immediately after injury were similar. The mean COHb concentration for Group II was 42.4 ± 1.6; Group III, 44.8 ± 2.1; and Group IV, 45.8 ± 2.1. Two animals in Group IV did not survive the 6-day study period. All other animals completed the study.

Hemodynamics. Routine hemodynamic data are contained in Figures 1 through 5. Throughout the course of the study , mean systemic blood pressure did not change over time nor was it different between groups (fig 1). All animals became progressively tachycardic over the course of the study, a change which was statistically different. There was no difference between groups in

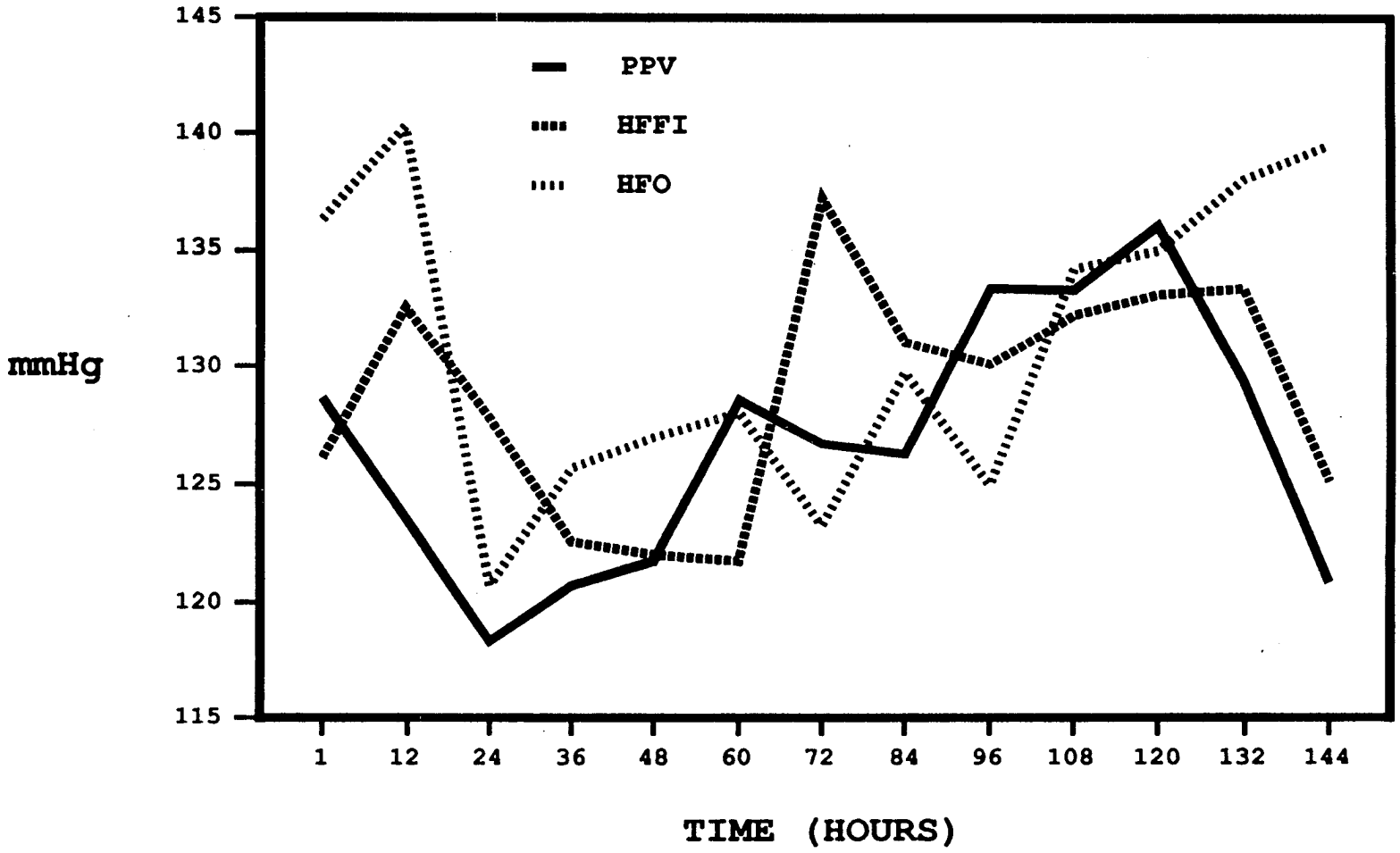


FIGURE 1. Mean blood pressure (mmHg). PPV indicates positive-pressure ventilation; HFFI, high-frequency flow interruption; and HFO, high-frequency oscillatory ventilation.

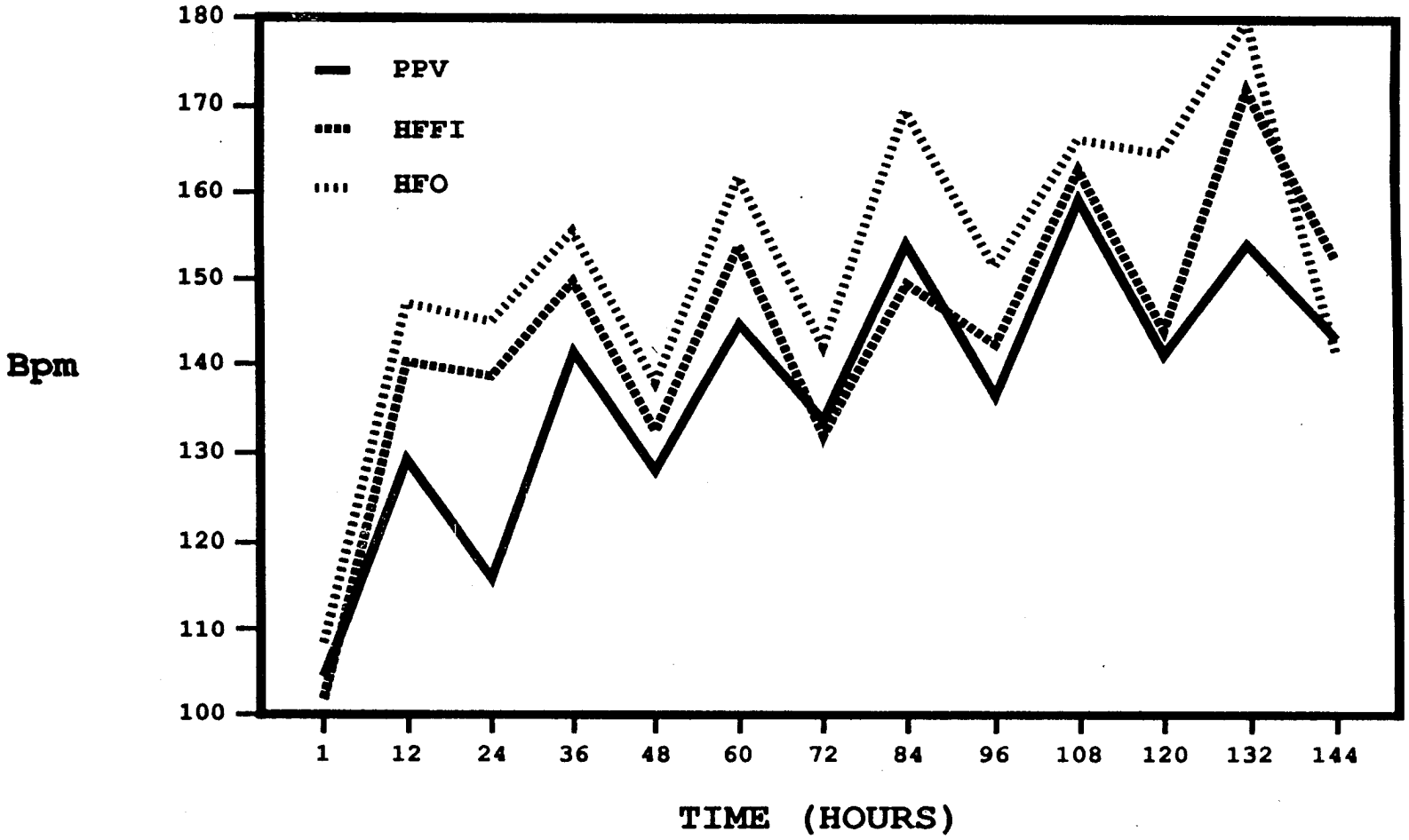


FIGURE 2. Heart rate (beats/min). PPV indicates positive-pressure ventilation; HFFI, high-frequency flow interruption; and HFO, high-frequency oscillatory ventilation.

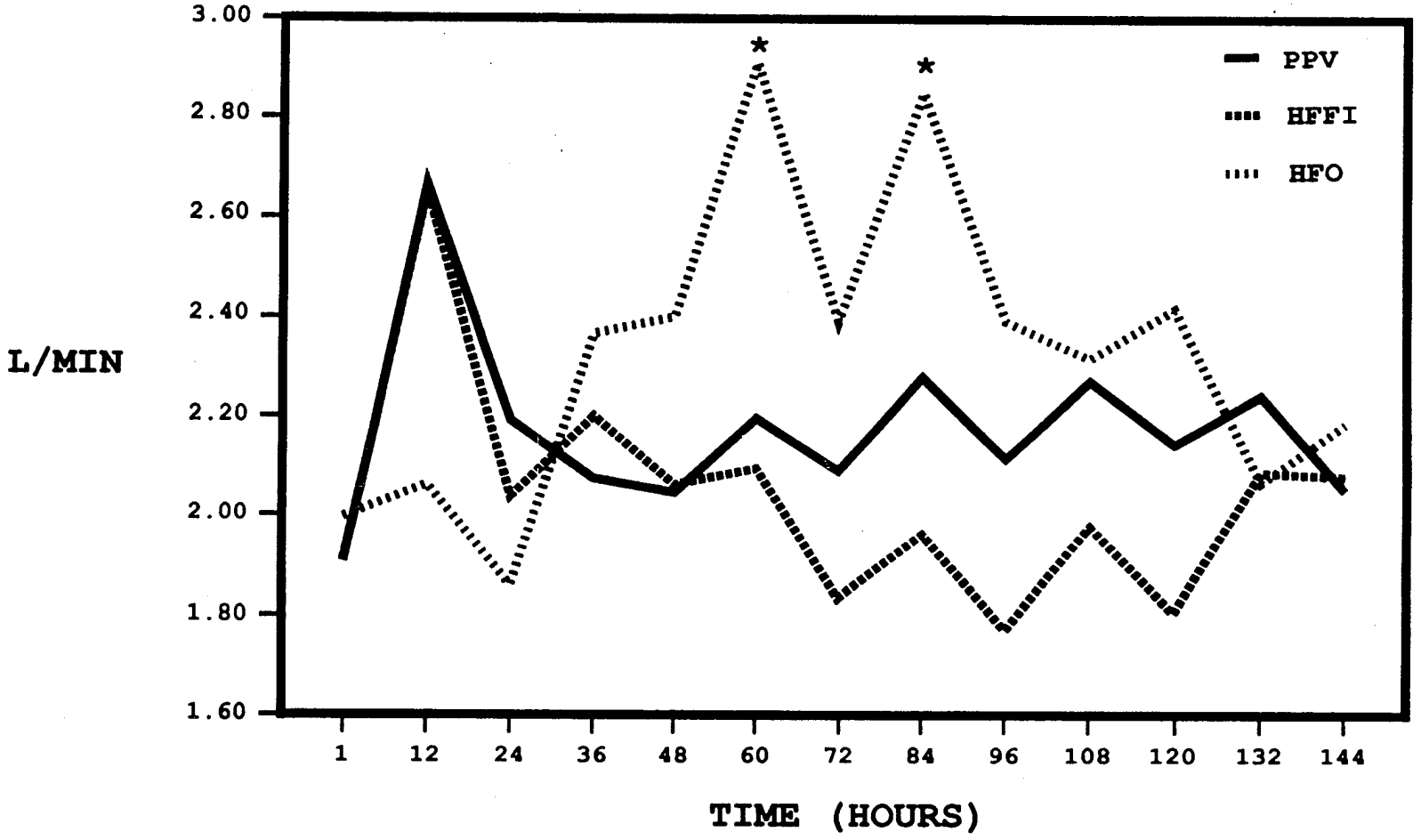


FIGURE 3. Cardiac output (l/min). PPV indicates positive-pressure ventilation; HFFI, high-frequency flow interruption; and HFO, high-frequency oscillatory ventilation. *P < 0.05 vs PPV and HFFI by ANOVA.

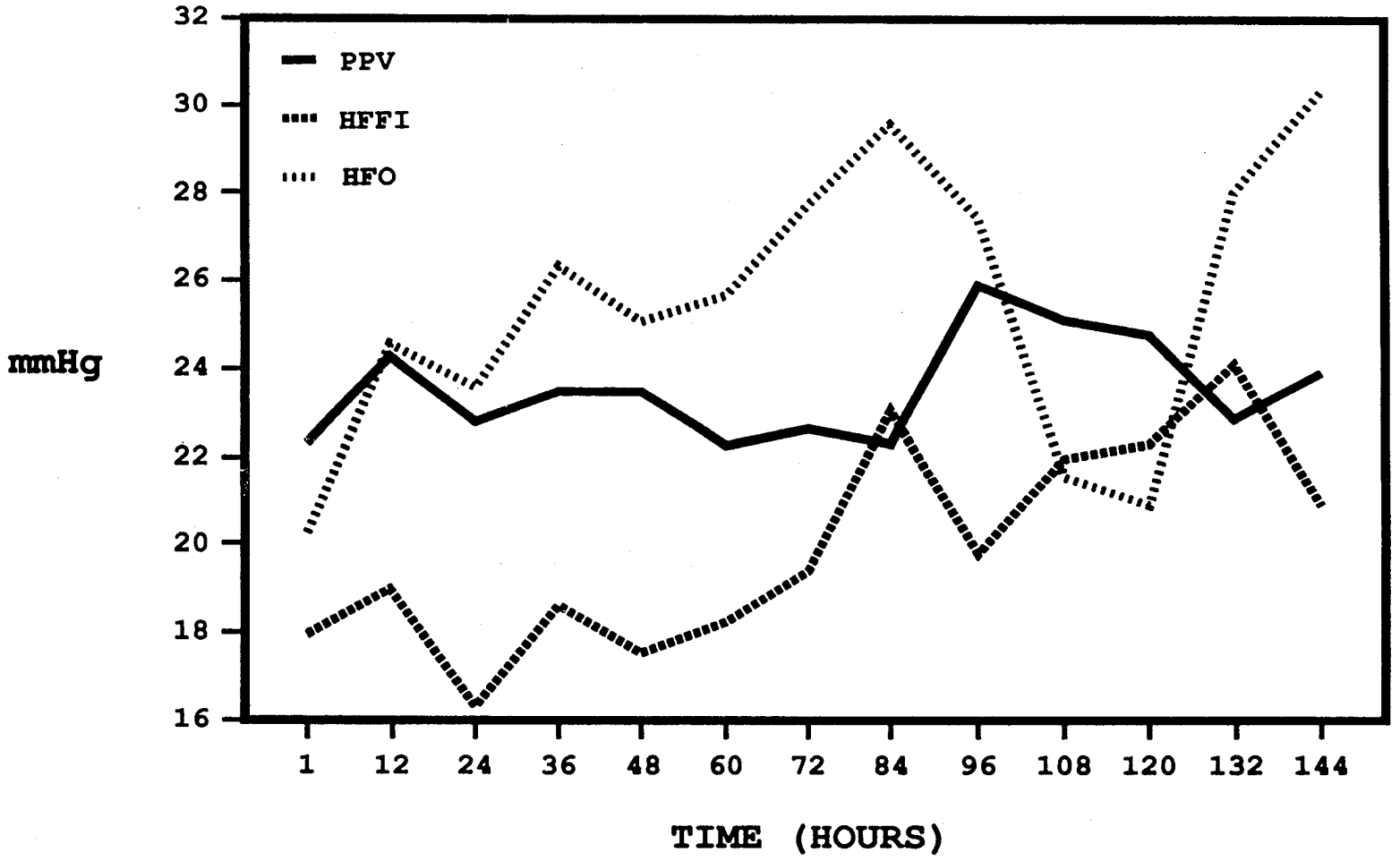


FIGURE 4. Mean pulmonary artery pressure (mmHg). PPV indicates positive-pressure ventilation; HFFI, high-frequency flow interruption; and HFO, high-frequency oscillatory ventilation.

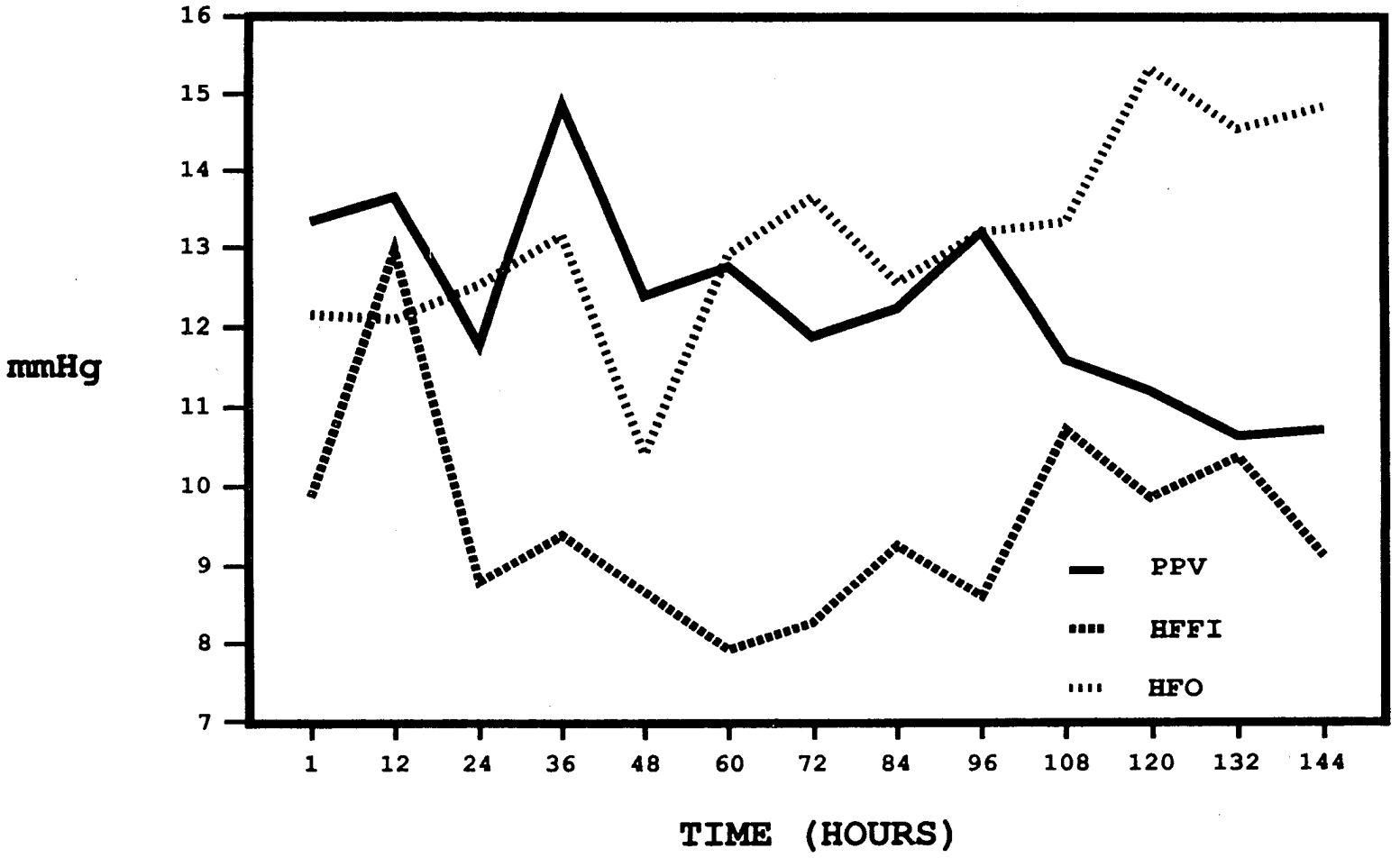


FIGURE 5. Mean pulmonary artery occlusion pressure (mmHg). PPV indicates positive-pressure ventilation; HFFI, high-frequency flow interruption; and HFO, high-frequency oscillatory ventilation.

the degree of tachycardia (fig 2). Although cardiac output tended to vary over time, it did not vary in a statistically significant consistent manner over time in any one group nor did it vary between groups at any time interval (fig 3). Mean pulmonary artery pressures significantly increased over time in all groups. There was no significant difference between groups in the rate of change of mean pulmonary artery pressure (fig 4). Pulmonary artery occlusion pressures did not vary significantly between groups or over time (fig 5).

Ventilatory Support. Data for ventilatory support are shown in Figures 6 through 10. The FIO_2 concentration necessary to maintain oxygenation within the prescribed range slightly but significantly increased over time in all groups. The rate of change was not different between groups, and the only significant difference between groups occurred at 84 h after injury when Group IV required significantly higher FIO_2 than Groups II and III (fig 6). The conventional respiratory rates for Groups II and III are compared in Figure 7. Group II required significantly greater rates than Group III at all time points. In addition, the rate required by Group II increased significantly over time, while that required by Group III did not (fig 7). Peak airway pressures were significantly greater for Group IV compared to Groups II and III. Additionally, peak airway pressures significantly increased over time for Group IV, but not for Groups II and III (fig 8). PEEP requirements were different between Groups II and III because of the preselected PEEP levels which were preset at the beginning of the study. PEEP requirements did not increase significantly over time in either of these groups (fig 9).

Arterial Blood Gas Data. PaO_2 did not vary between groups or over time in this study after the FIO_2 was decreased from 100% (fig 10). Arterial PCO_2 was maintained relatively constant throughout the study for all three groups once ventilatory support was initiated (fig 11). Only at 60 and 84 h was PCO_2 significantly higher in Group IV than the other two groups. Arterial pH data are depicted in Figure 12. Despite a relatively constant PCO_2 over the course of the study, the arterial pH tended to increase in all groups, indicating the presence of a metabolic alkalosis. Only at 60 and 84 h, the same time point in which arterial PCO_2 increased in Group IV, were there differences between groups. The alveolar arterial O_2 ratio did not vary significantly for the three groups over time. Only at 72 and 84 h was the Group IV mean alveolar–arterial O_2 ratio significantly less than the other two groups.

Intravenous Intake and Urine Output. Intravenous fluid administration did not vary between groups and did not vary significantly over time. Urine output significantly increased in all groups by the second 24 h, but did not vary between groups (fig 13).

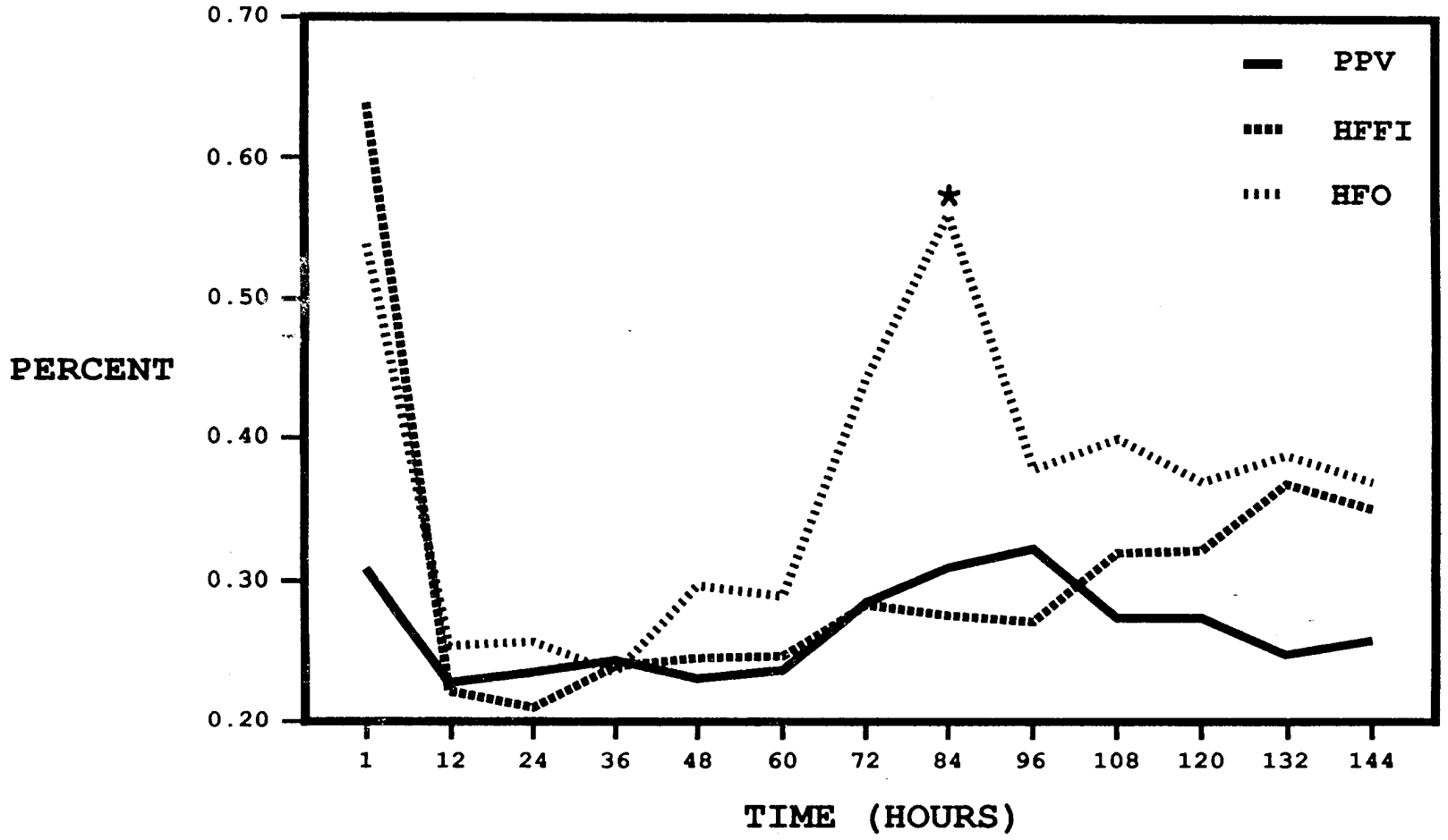


FIGURE 6. FIO_2 (%). PPV indicates positive-pressure ventilation; HFFI, high-frequency flow interruption; and HFO, high-frequency oscillatory ventilation. *P < 0.05 vs PPV and HFFI by ANOVA.

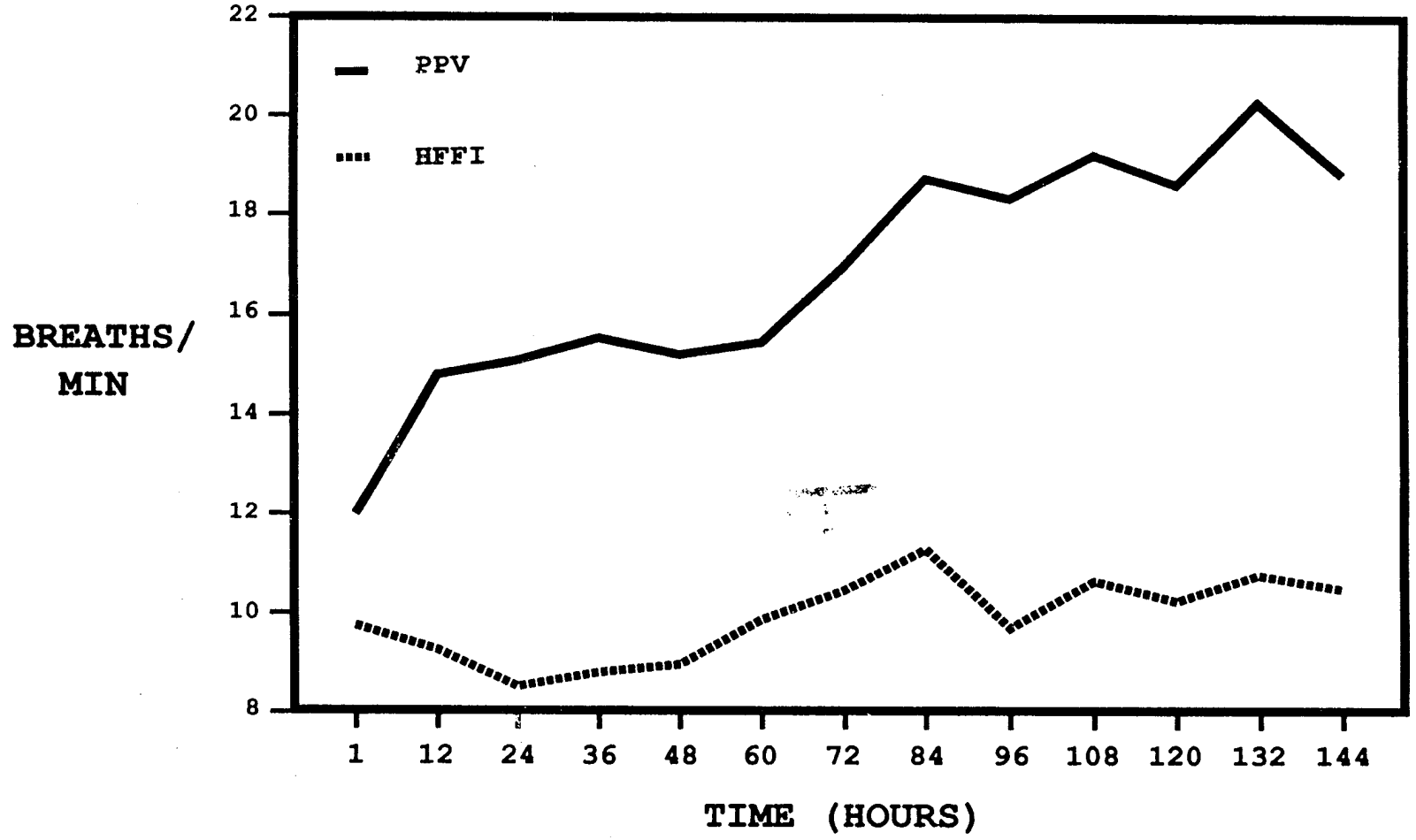


FIGURE 7. Respiratory rate (breaths/min). PPV indicates positive-pressure ventilation, and HFFI, high-frequency flow interruption.

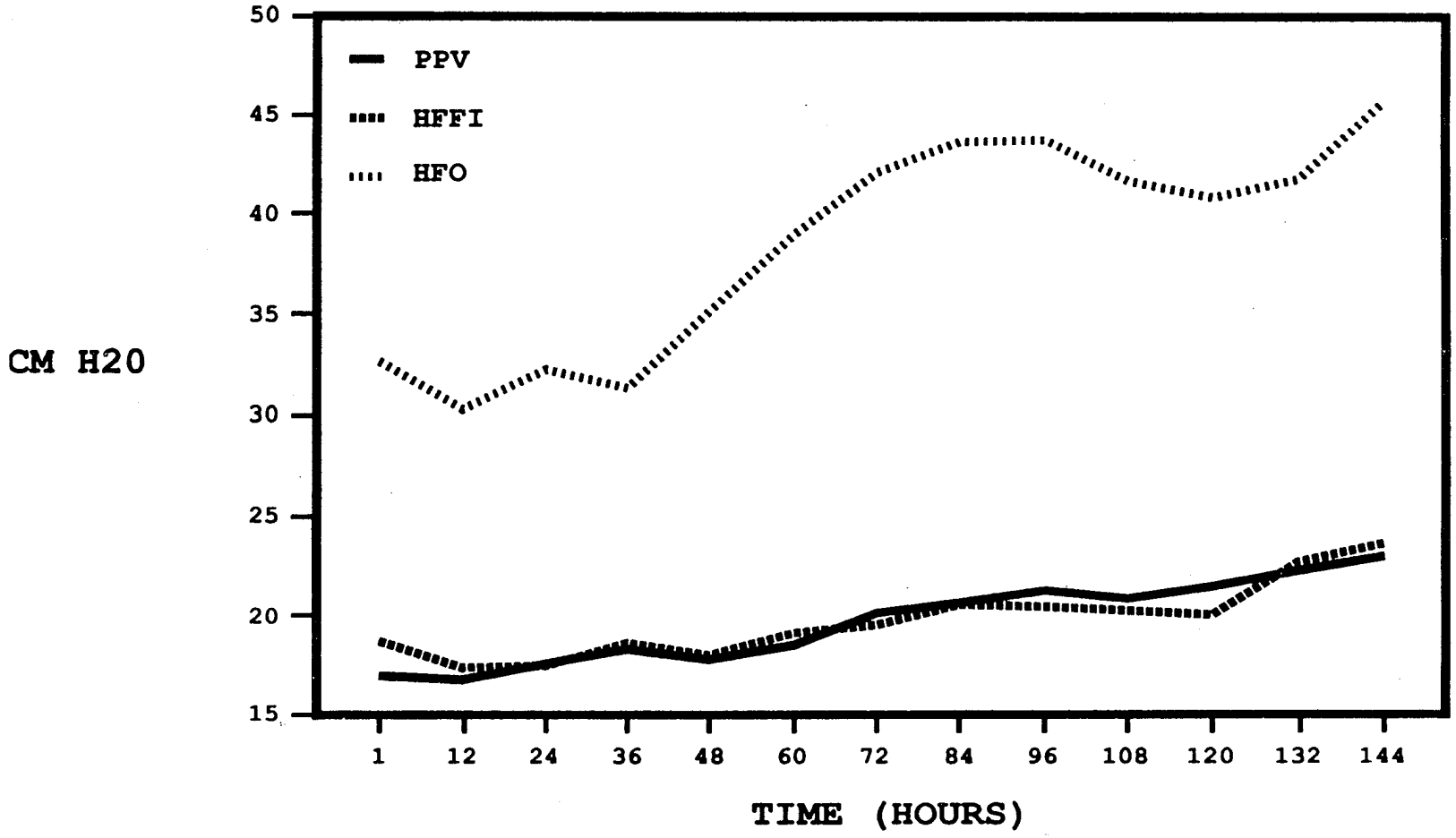


FIGURE 8. Peak airway pressures (cmH_2O). PPV indicates positive-pressure ventilation; HFFI, high-frequency flow interruption; and HFO, high-frequency oscillatory ventilation.

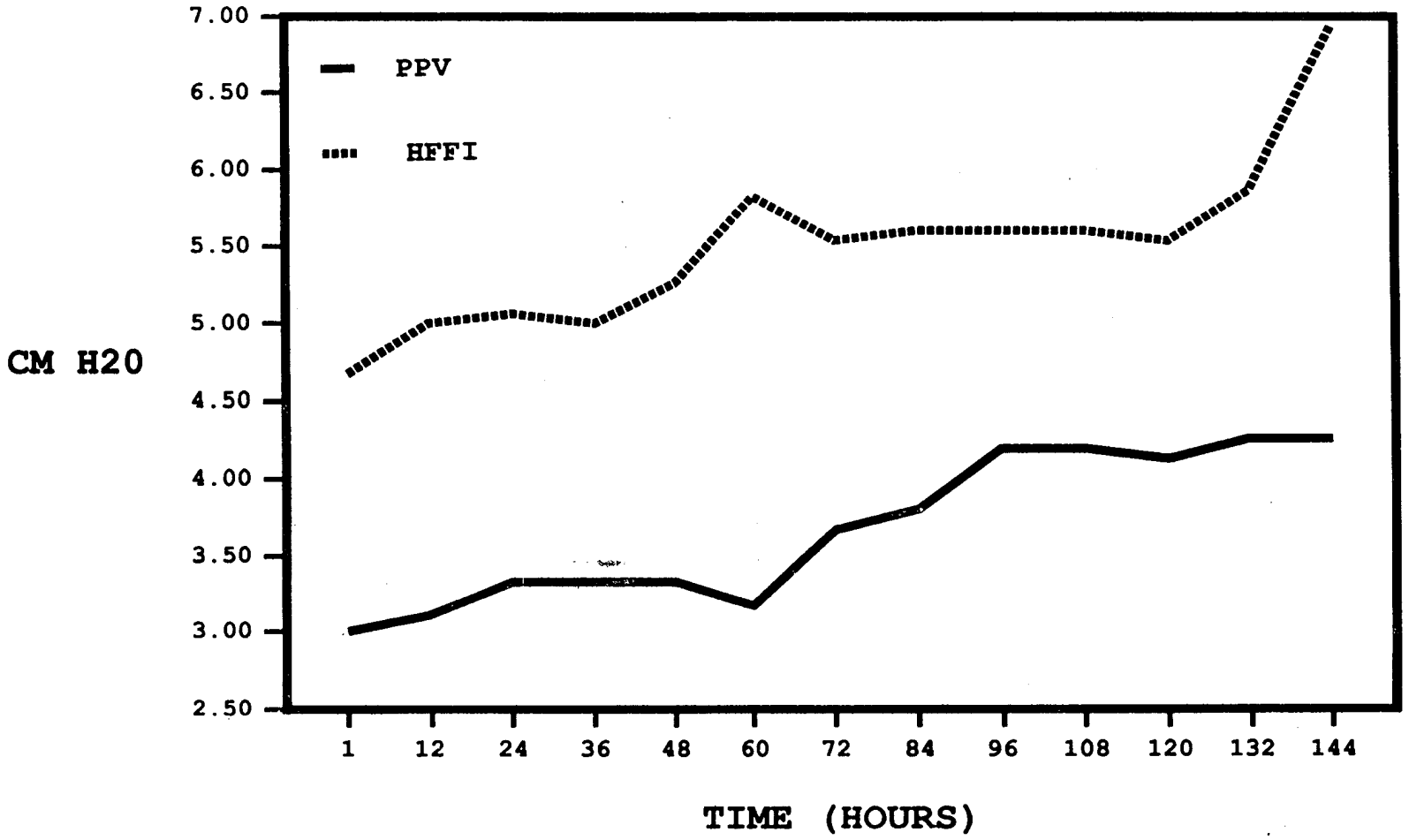


FIGURE 9. Positive-end expiratory pressure (cmH_2O). PPV indicates positive-pressure ventilation, and HFFI, high-frequency flow interruption.

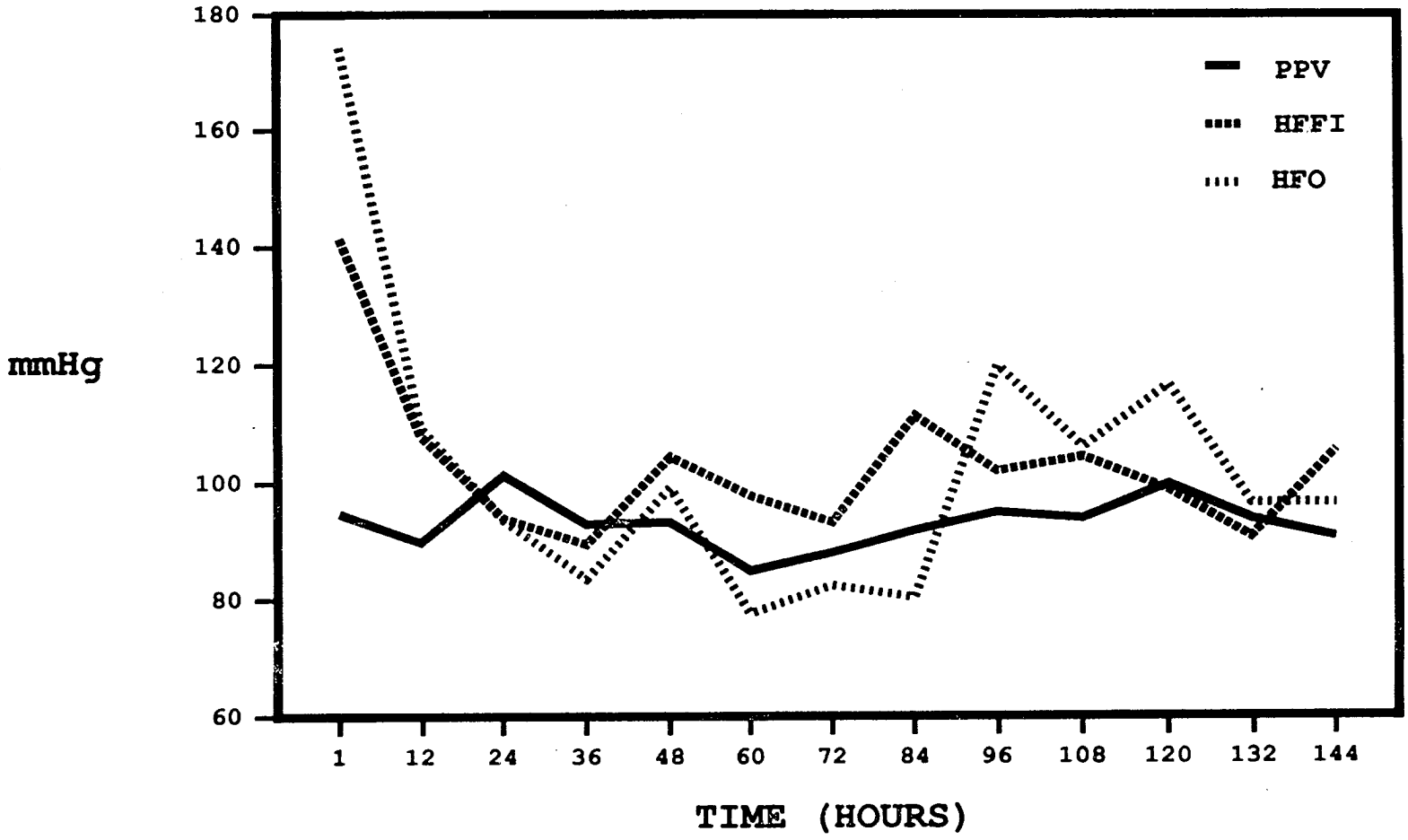


FIGURE 10. PaO_2 (mmHg). PPV indicates positive-pressure ventilation; HFFI, high-frequency flow interruption; and HFO, high-frequency oscillatory ventilation.

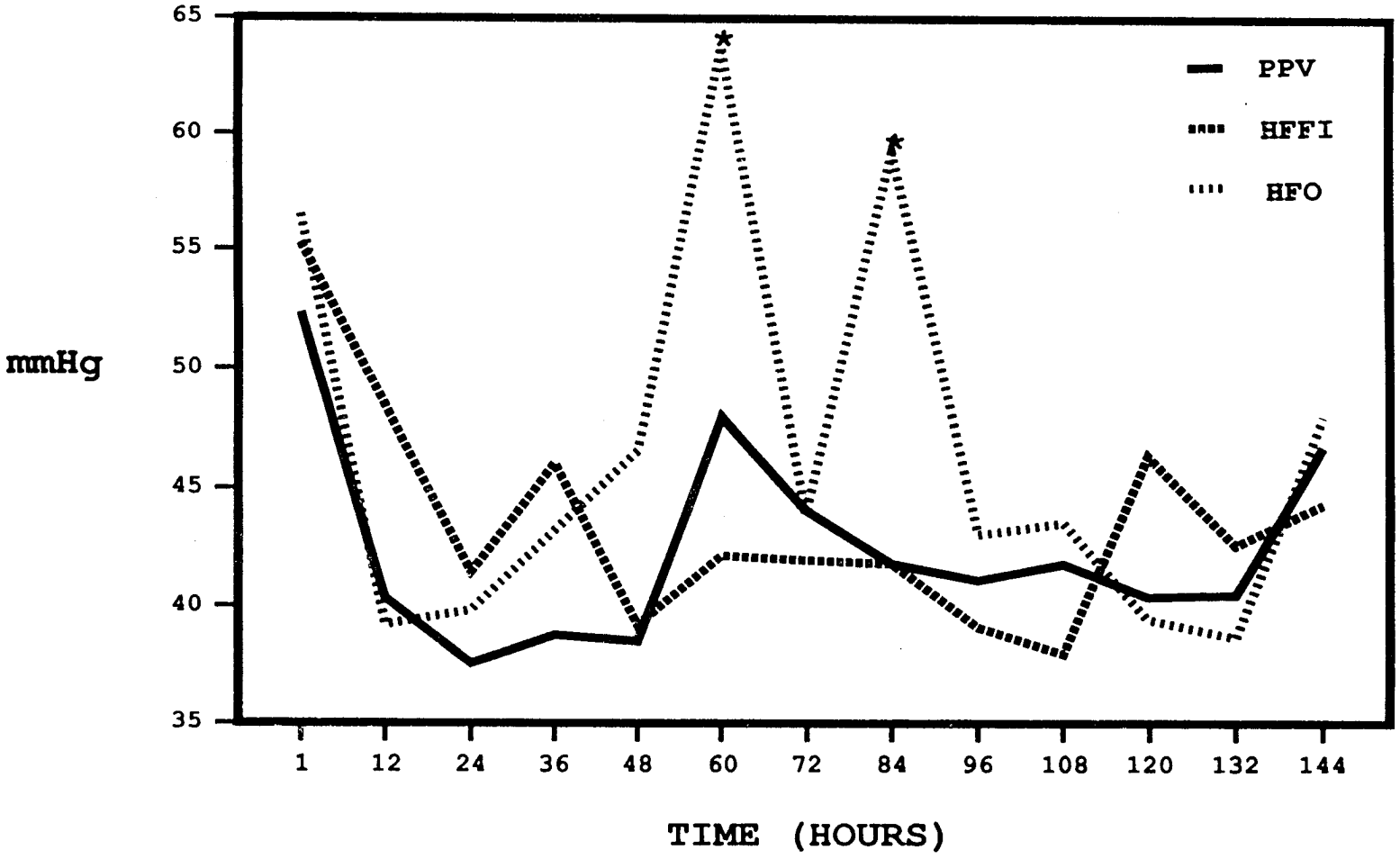


FIGURE 11. $PaCO_2$ (mmHg). PPV indicates positive-pressure ventilation; HFFI, high-frequency flow interruption; and HFO, high-frequency oscillatory ventilation. *P < 0.05 vs PPV and HFFI by ANOVA.

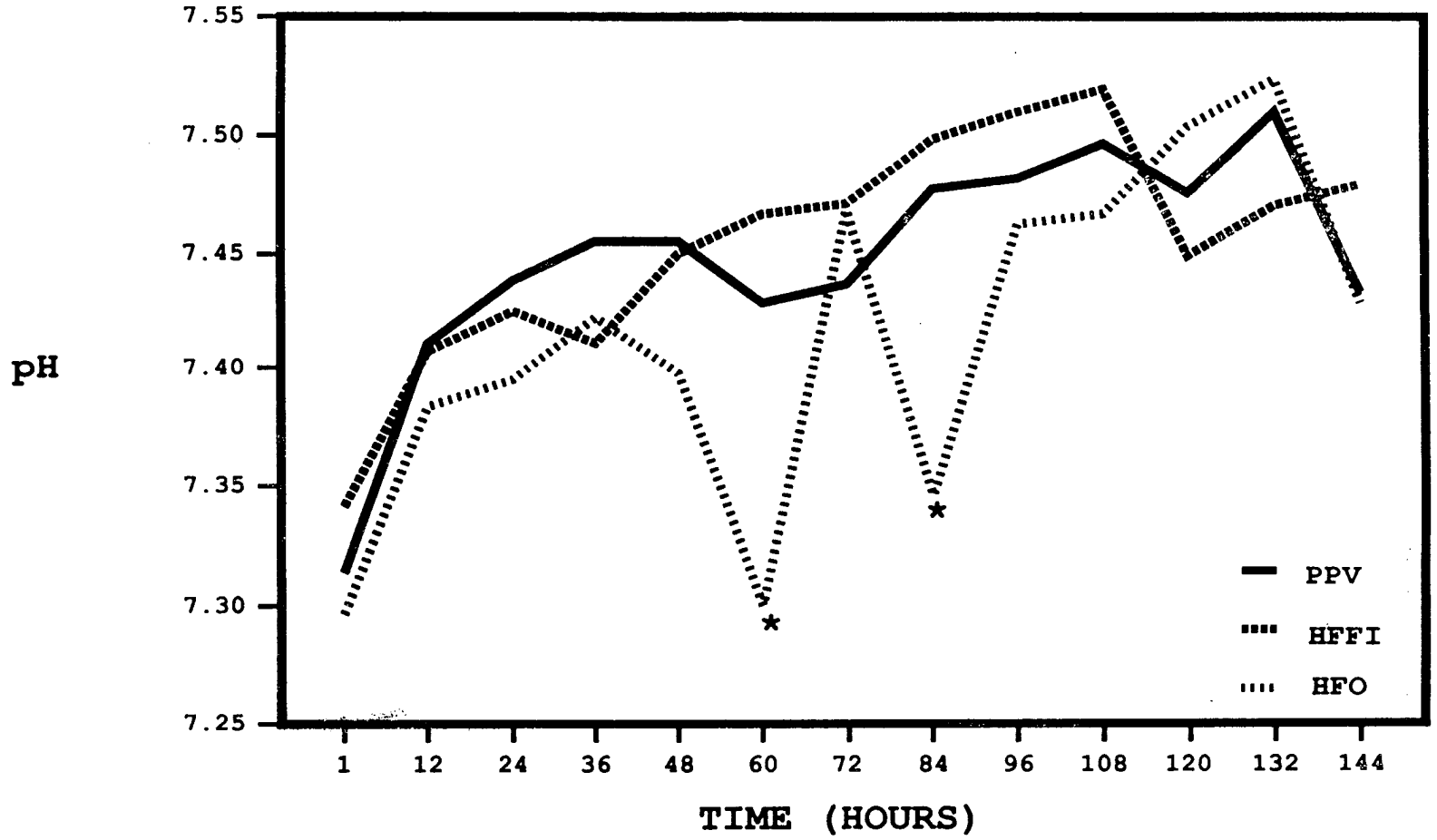


FIGURE 12. pH. PPV indicates positive-pressure ventilation; HFFI, high-frequency flow interruption; and HFO, high-frequency oscillatory ventilation.

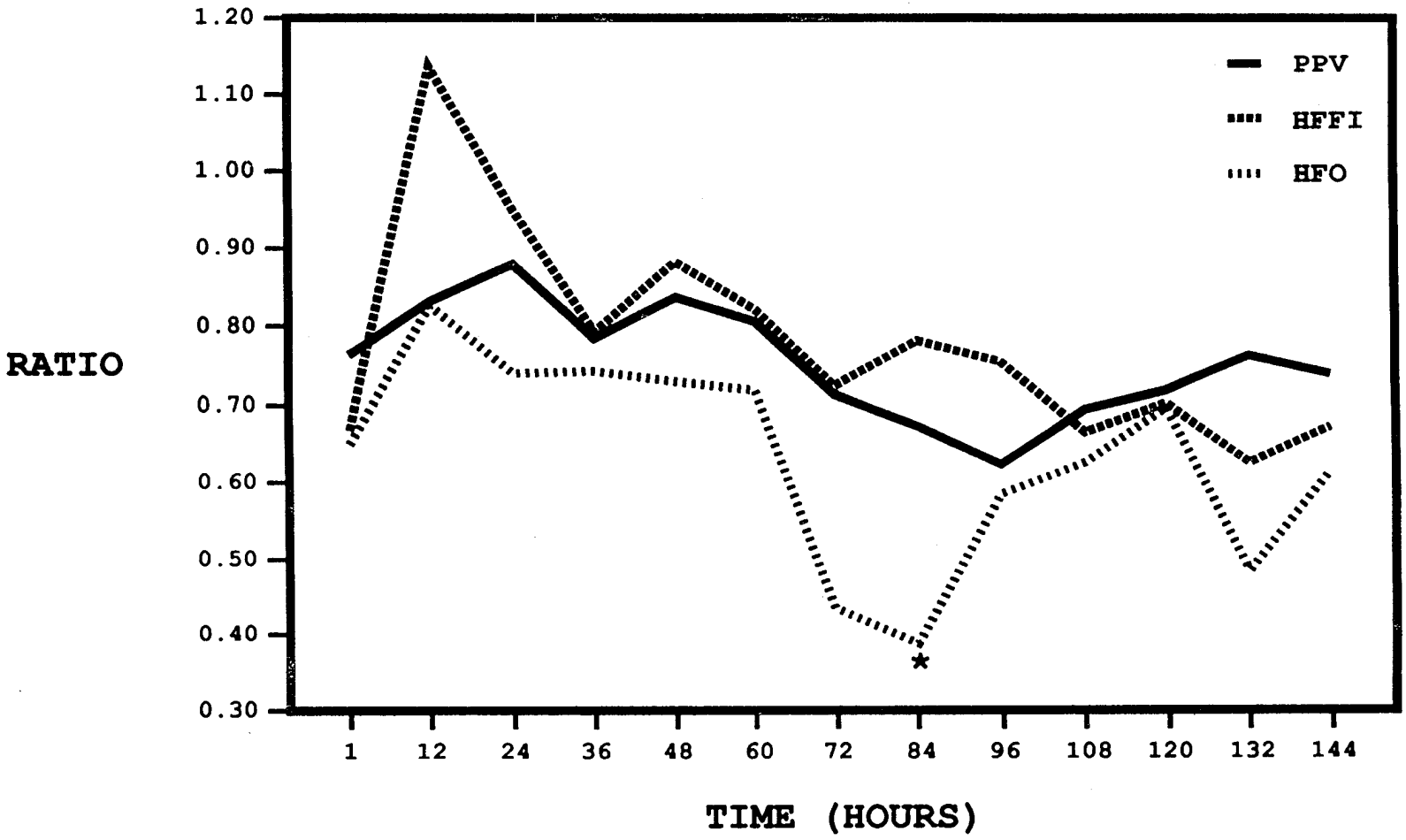


FIGURE 13. Alveolar-arterial O_2 ratio. PPV indicates positive-pressure ventilation; HFFI, high-frequency flow interruption; and HFO, high-frequency oscillatory ventilation. *P < 0.05 vs PPV and HFFI by ANOVA.

Chest Roentgenographs. There were no significant intergroup differences when daily chest roentgenographs were graded for atelectasis or pneumonia, although Group IV tended to have more atelectasis than either Group II or Group III (P = 0.1).

Pulmonary Function Tests. Pulmonary function data are depicted in Figures 14 through 18. Vital capacity, inspiratory capacity, functional residual capacity, and total lung capacity all decreased significantly over time, although there were no intergroup differences (figs 14-17). Effective residual volume also decreased significantly over time, but the rate of change was not different between treatment groups (fig 18). Diffusion capacity (fig 19) significantly decreased over time in all groups; however, no intergroup differences were noted.

Bronchoalveolar Lavage. Bronchoalveolar lavage was performed before injury and on postinjury days 1, 3, and 6. The lavage effluent was assayed for cell count, elastase activity, total protein, and total phosphatidylcholine content (figs 20-23). Group I animals were lavaged at the same time intervals. No differences between successive lavage and measured values were noted in Group I; therefore, each figure contains pooled control data. Bronchoalveolar lavage WBC counts increased significantly in Groups II, III, and IV over time. However, the rate of increase was not significantly different between groups. In addition, there were no intergroup differences at any of the postinjury time points. Similarly, bronchoalveolar lavage protein content increased significantly in all groups over time. The increase in bronchoalveolar lavage protein content was sustained in both high-frequency groups, but returned to baseline in Group II at 6 days after injury. ANOVA analysis confirmed significant differences between control values and Groups II and III at postinjury day 1. At postinjury day 6, Group III and Group IV levels were significantly elevated over control values. Bronchoalveolar lavage elastase increased significantly over baseline values in Groups II and IV compared to Group I. This increase in measurable elastase was sustained at 6 days postinjury only in Group IV. Group III had a small but insignificant increase in elastase compared to Group I. Bronchoalveolar lavage phosphatidylcholine content was measured on postinjury days 0, 1, and 6. There was no significant difference in bronchoalveolar lavage phosphatidylcholine content between any groups at any time point. The lack of difference between Group IV and other groups at postinjury day 6 is most likely due to a small number of measurements, as only three Group IV animals were alive on postinjury day 6.

Pathology. Parenchymal pathologic changes were scored using a panel of standards as previously described. Using RIDIT analysis, no significant intra-observer differences were noted. RIDIT analysis was then performed to determine if there were pathologic differences between groups. Group III had significantly less

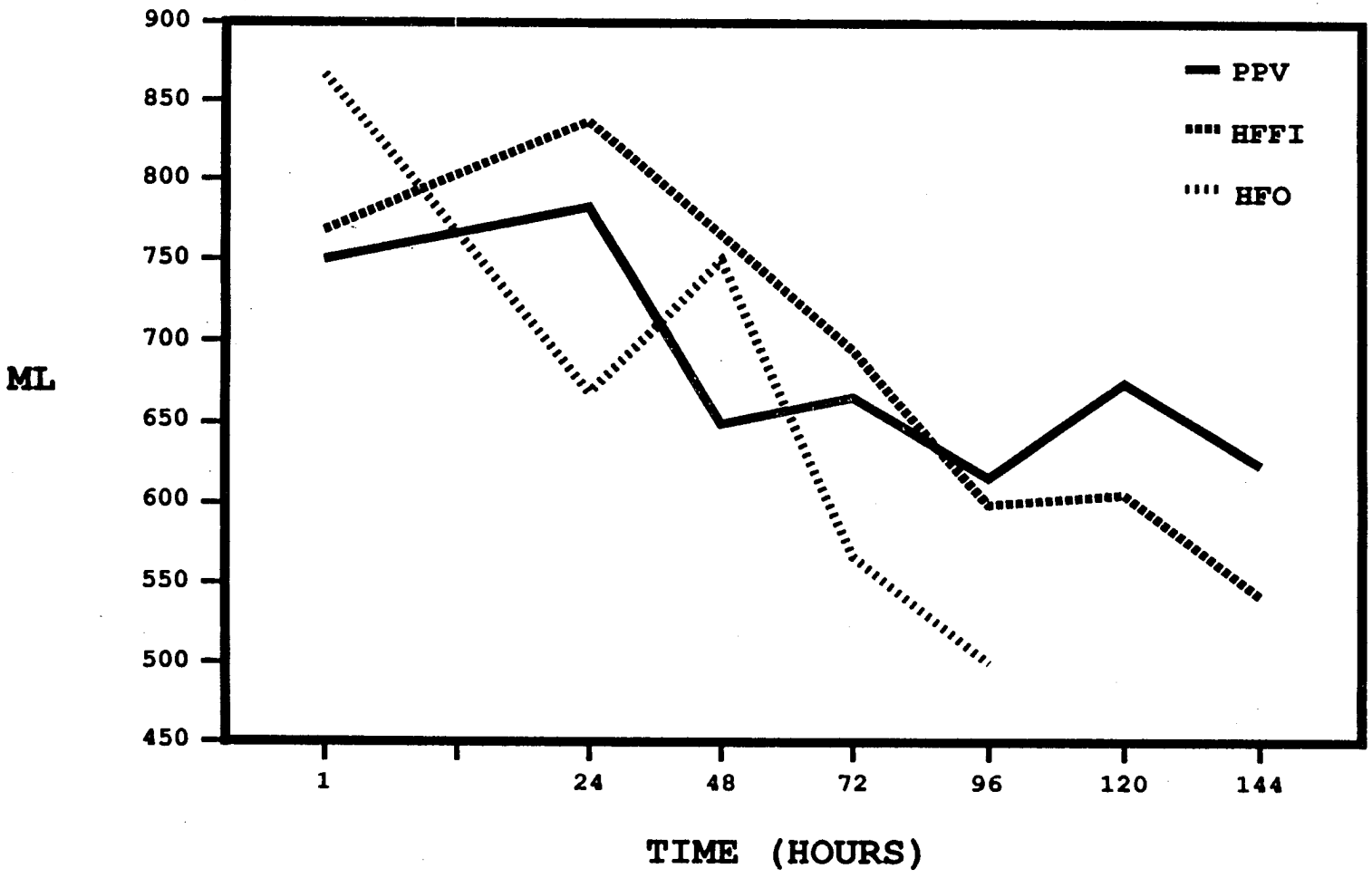


FIGURE 14. Vital capacity (ml). PPV indicates positive-pressure ventilation; HFFI, high-frequency flow interruption; and HFO, high-frequency oscillatory ventilation.

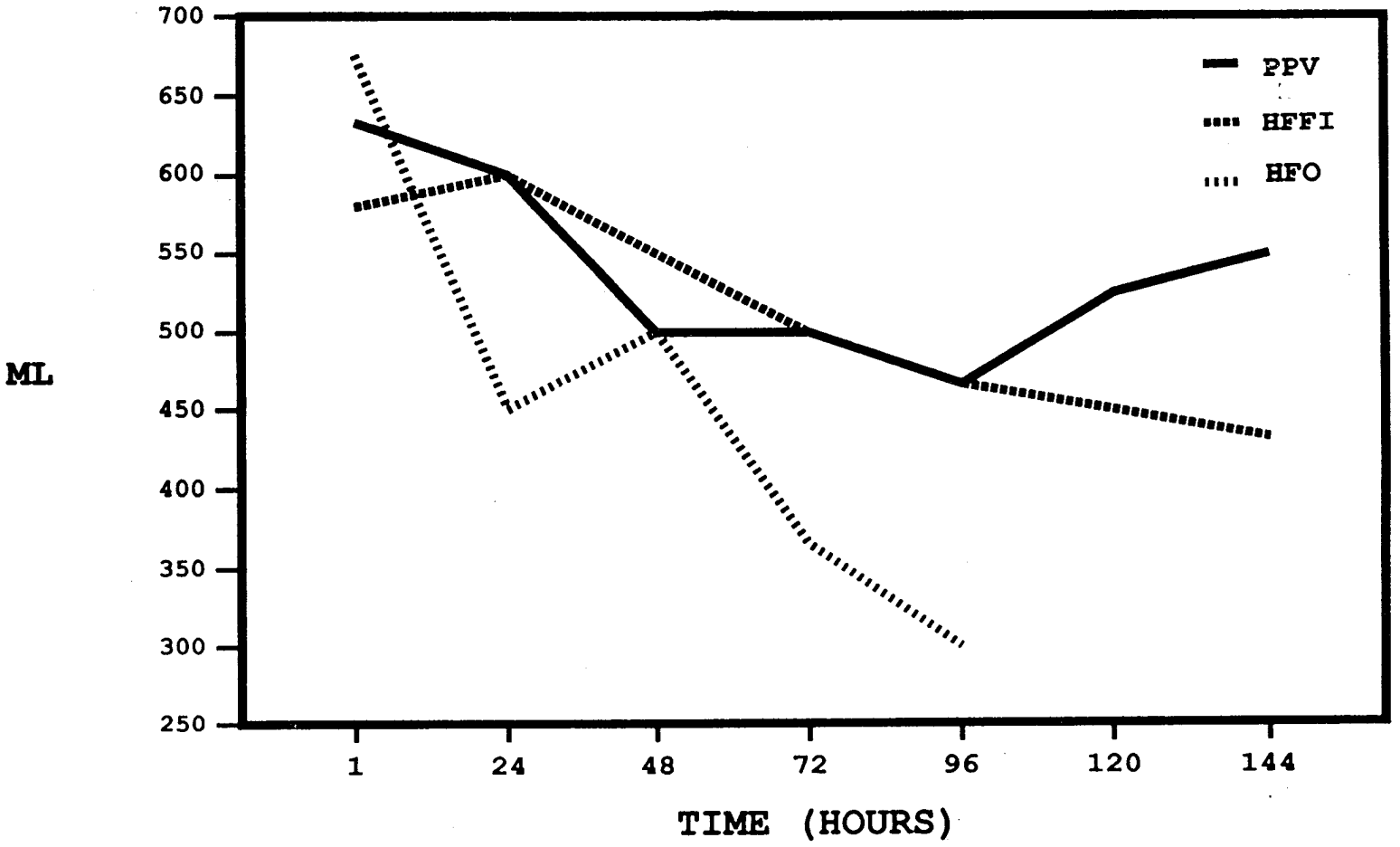


FIGURE 15. Inspiratory capacity (ml). PPV indicates positive-pressure ventilation; HFFI, high-frequency flow interruption; and HFO, high-frequency oscillatory ventilation.

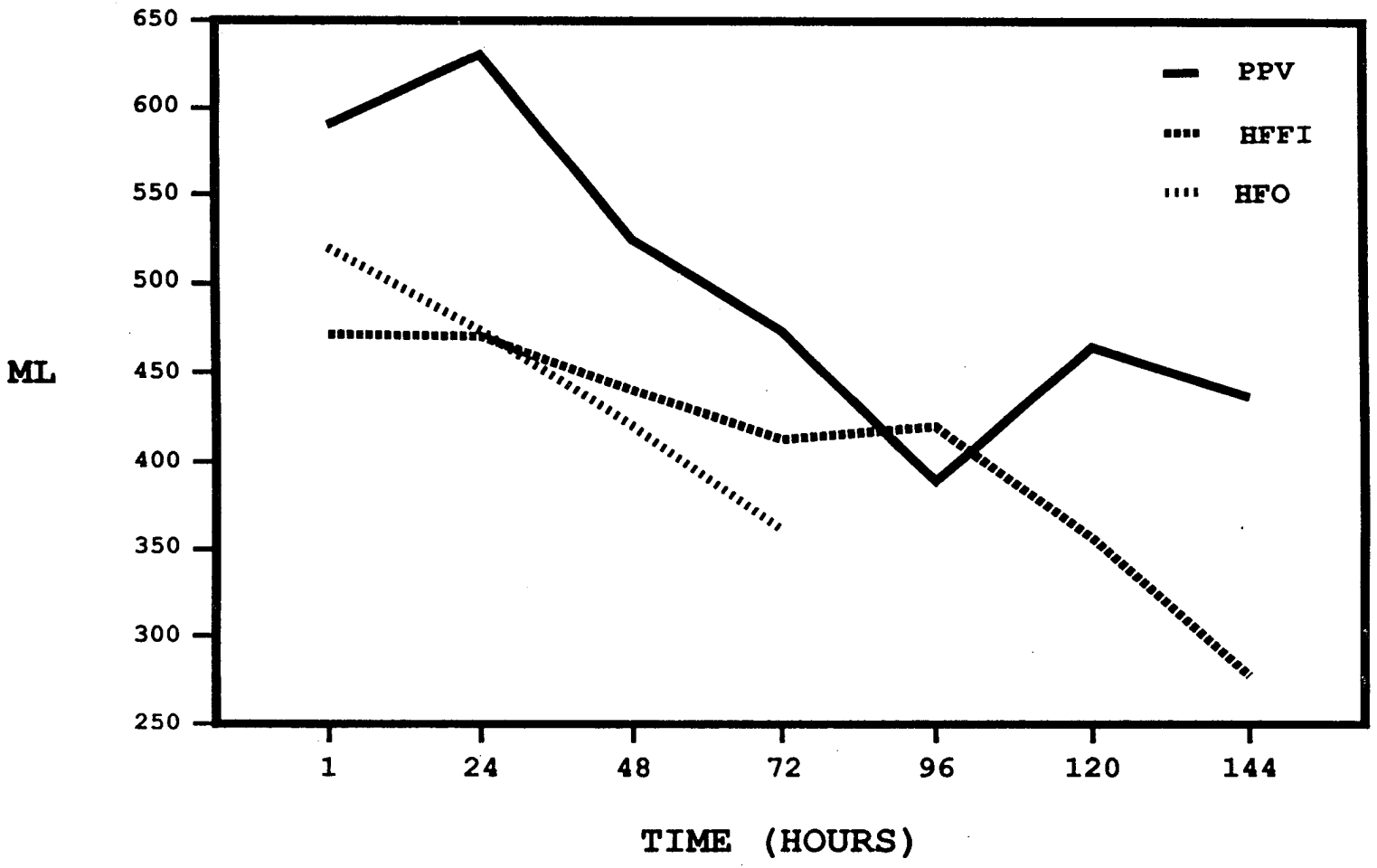


FIGURE 16. Functional residual capacity (ml). PPV indicates positive-pressure ventilation; HFFI, high-frequency flow interruption; and HFO, high-frequency oscillatory ventilation.

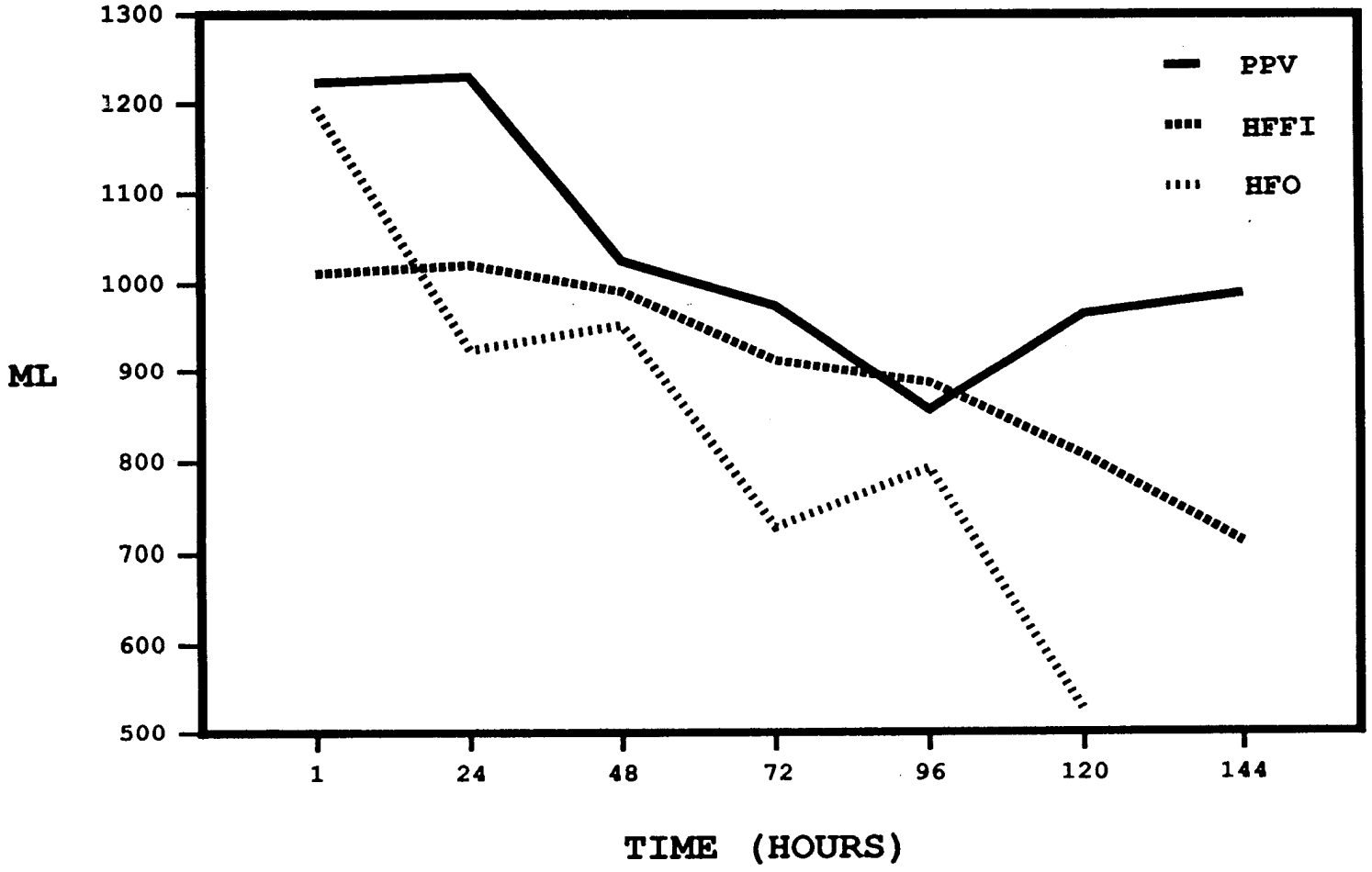


FIGURE 17. Total lung capacity (ml). PPV indicates positive-pressure ventilation; HFFI, high-frequency flow interruption; and HFO, high-frequency oscillatory ventilation.

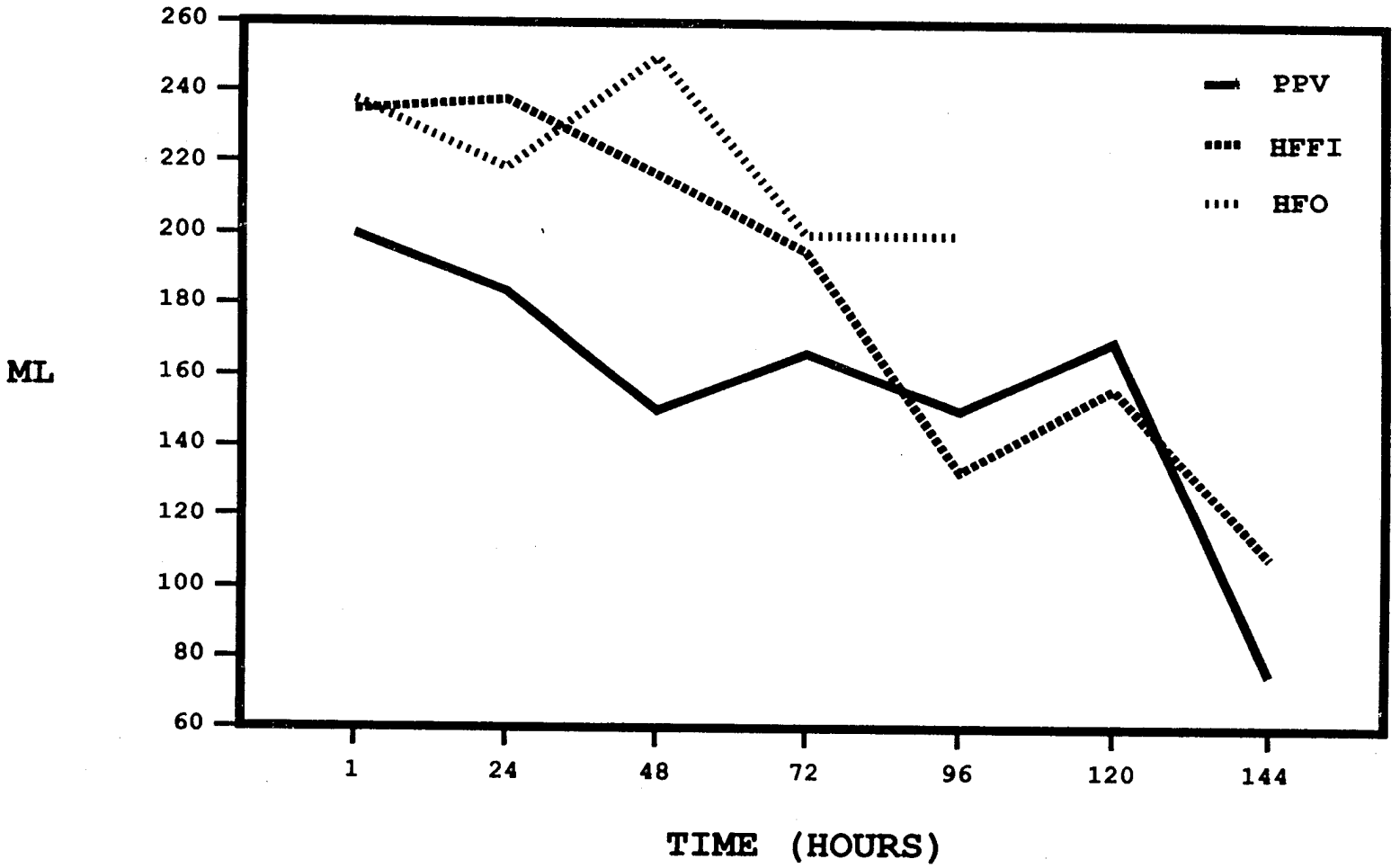


FIGURE 18. Effective residual volume (ml). PPV indicates positive-pressure ventilation; HFFI, high-frequency flow interruption; and HFO, high-frequency oscillatory ventilation.

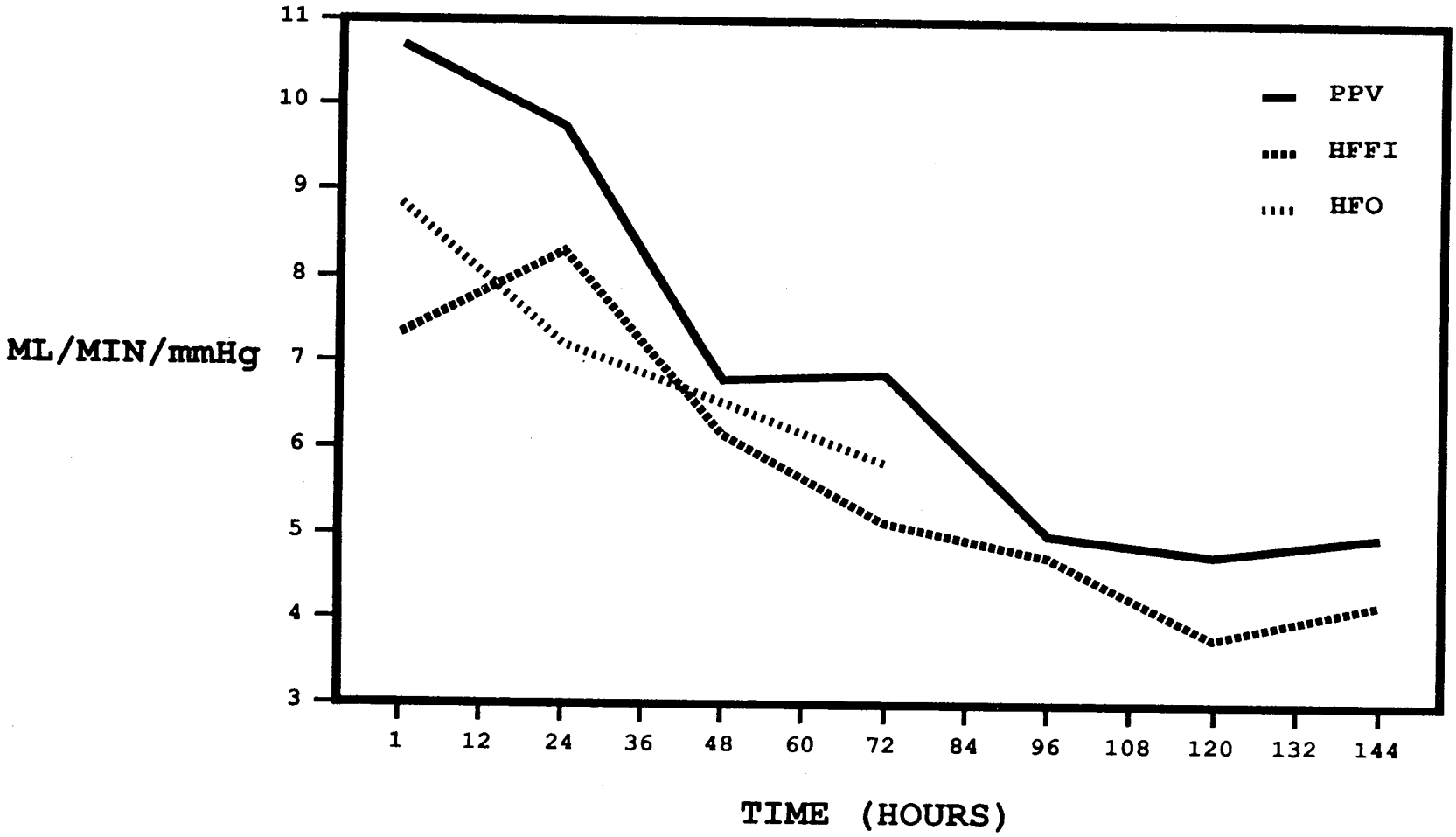


FIGURE 19. Diffusing capacity (ml/min/mmHg). PPV indicates positive-pressure ventilation; HFFI, high-frequency flow interruption; and HFO, high-frequency oscillatory ventilation.

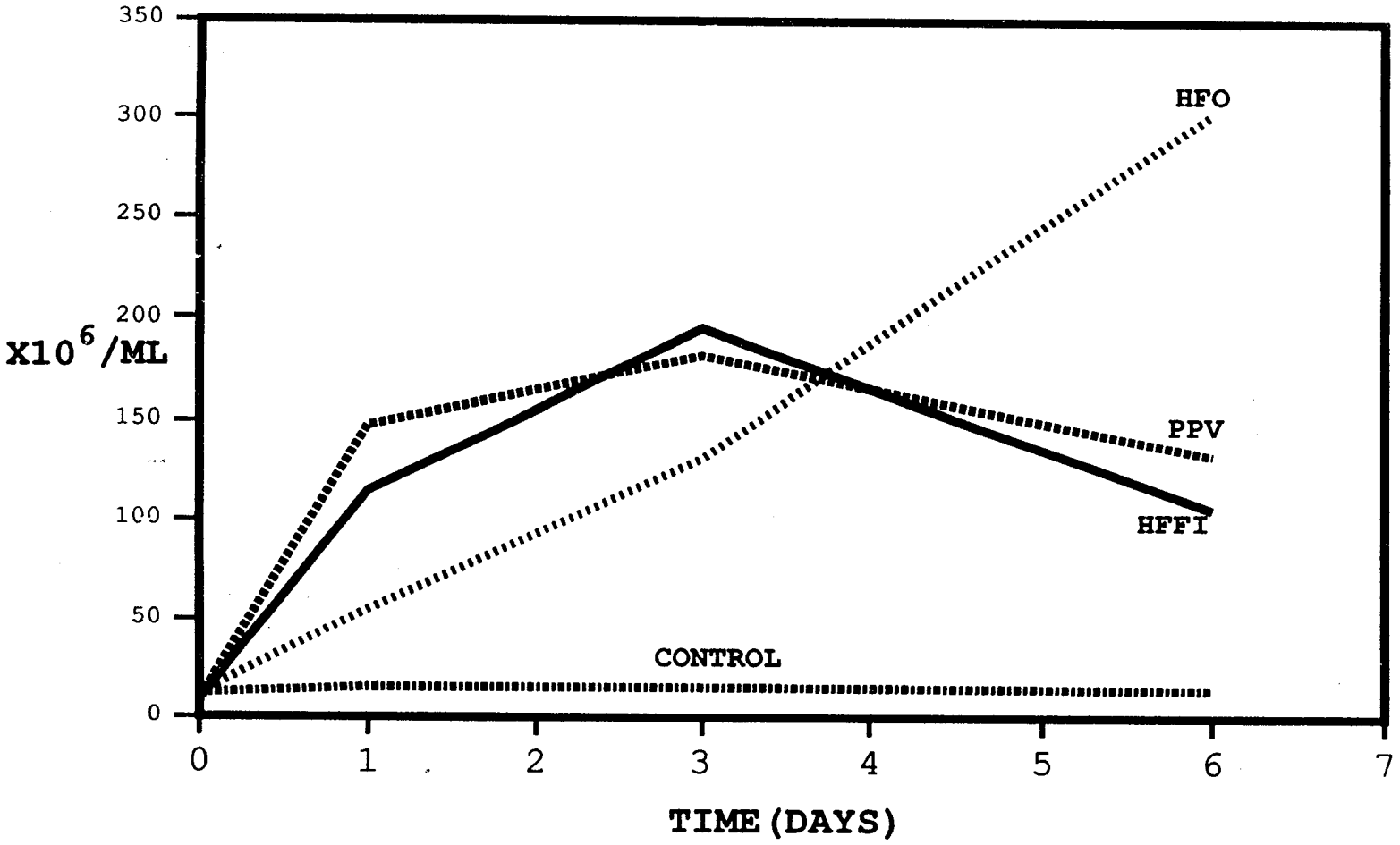


FIGURE 20. Bronchoalveolar lavage WBC count (10^6/ml). PPV indicates positive-pressure ventilation; HFFI, high-frequency flow interruption; and HFO, high-frequency oscillatory ventilation.

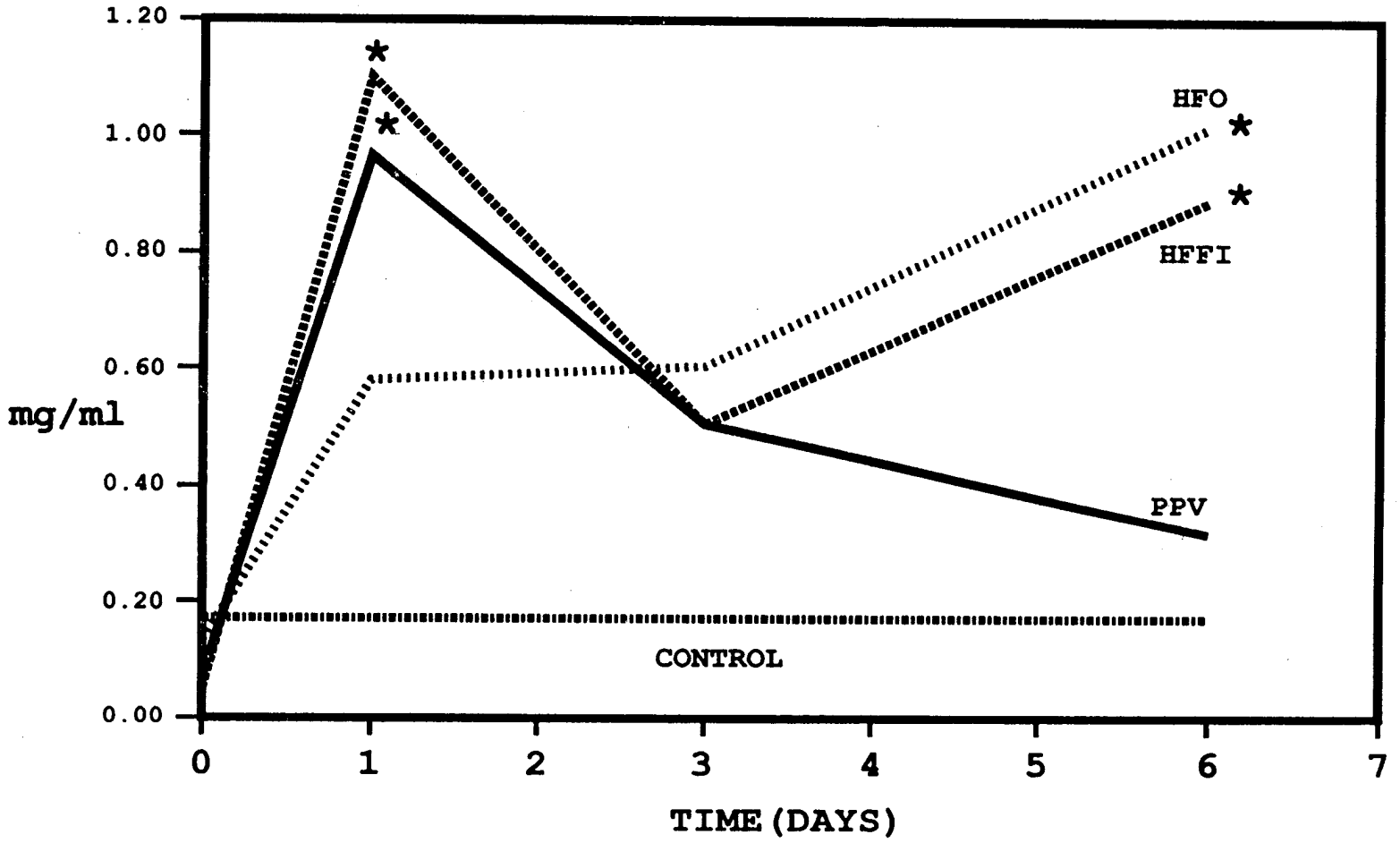


FIGURE 21. Bronchoalveolar lavage protein (mg/ml). PPV indicates positive-pressure ventilation; HFFI, high-frequency flow interruption; and HFO, high-frequency oscillatory ventilation.

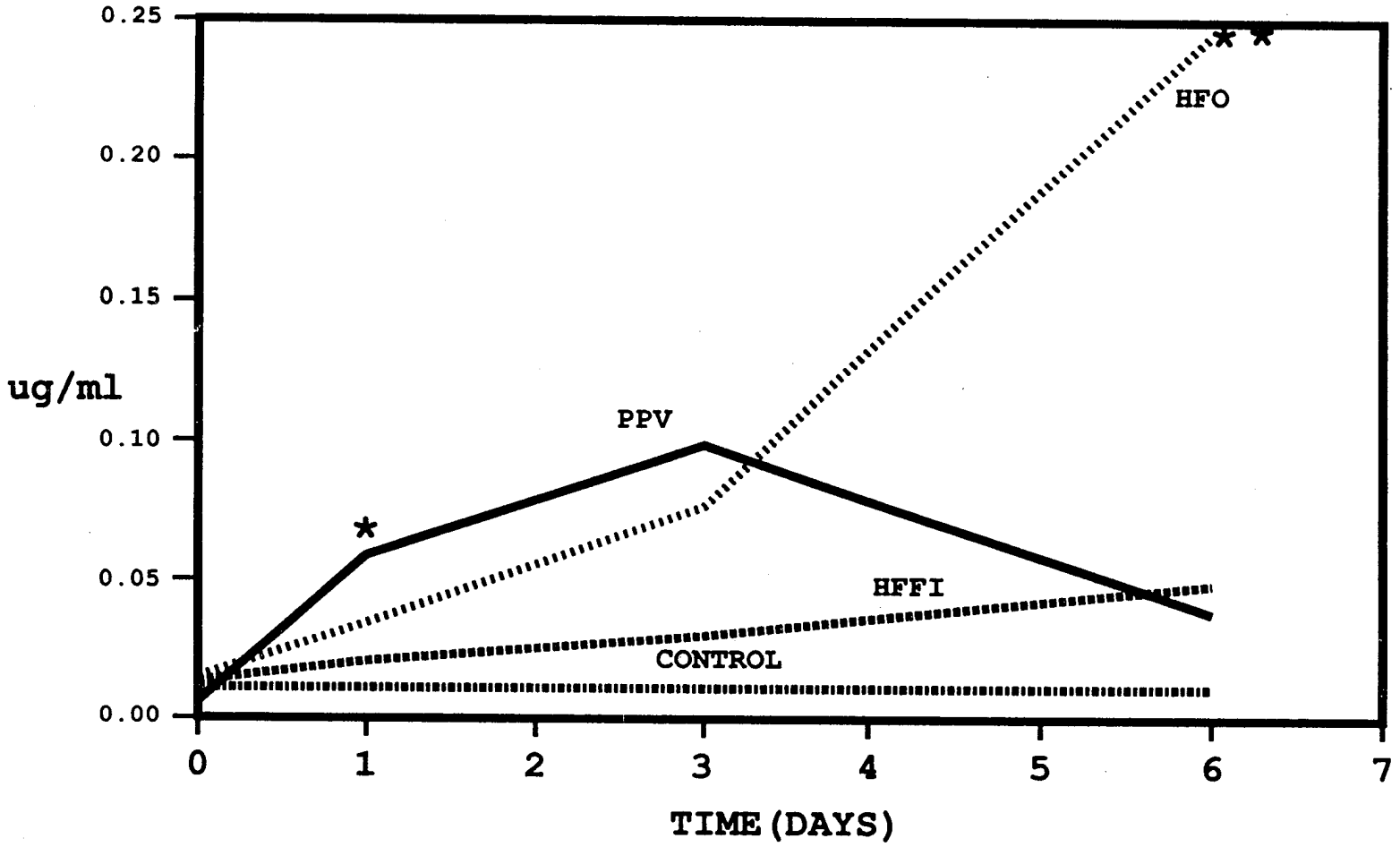


FIGURE 22. Bronchoalveolar lavage elastase (μg/ml). PPV indicates positive-pressure ventilation; HFFI, high-frequency flow interruption; and HFO, high-frequency oscillatory ventilation.

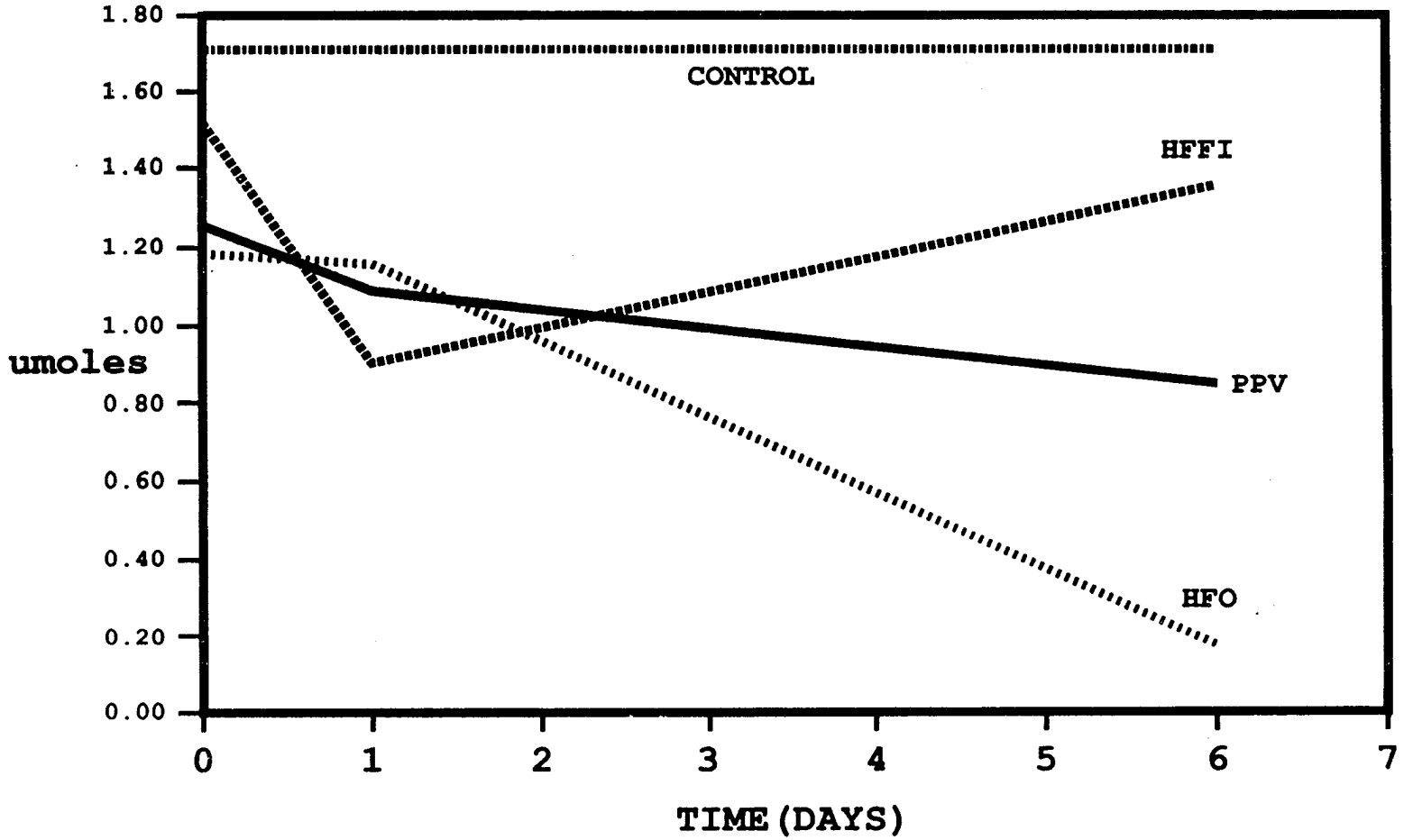


FIGURE 23. Bronchoalveolar lavage surfactant (μmoles). PPV indicates positive-pressure ventilation; HFFI, high-frequency flow interruption; and HFO, high-frequency oscillatory ventilation.

histopathologic parenchymal damage than Group II ($P = 0.03$) and Group IV ($P = 0.0008$). One animal in Group III was an extreme outlier and all sections showed severe diffuse damage to a much greater extent than any other animal in any group. Removal of this animal from analysis greatly increased the differences between Group III and Groups II and IV ($P < 0.0001$).

DISCUSSION

If any conclusions are to be drawn from this study, it is important that the original insult be consistent in all animals. Immediate postinjury carboxyhemoglobin levels after a moderate smoke injury were identical for the three groups. In addition, pulmonary function tests deteriorated at a similar rate in the three groups. These data indicate that the original insult was quite similar between animals and groups.

Maintenance of normal acid base balance and arterial blood gases was possible over the 6-day period utilizing either positive-pressure ventilation (Group II) or HFFI (Group III) but not HFOV (Group IV). The failure of HFOV in this model may be secondary to the heterogeneity of the disease process. In Group IV, small airway plugging appeared to result in a lack of consistent recruitment of collapsed lung segments, which may have resulted in overdistention of well ventilated lung segments. This lack of recruitment was apparent by the consistent radiographic appearance of atelectasis in Group IV. Failure of HFOV to maintain normal oxygenation and ventilation in this model is distinctly different than what has been reported in other models of ARDS (2-4). This may be because the typical ARDS insult is more homogeneous and predominantly an endothelial, not an epithelial cell injury.

Comparison of the level of support required by Groups II and III revealed one difference. A significantly higher ventilatory rate at the same peak inspiratory pressure was required for animals treated with positive-pressure ventilation (Group II) when compared to animals treated with HFFI (Group III). Construction of a barotrauma index (rate/pressure product) indicates that positive-pressure ventilated animals (Group II) were at significantly higher risk for parenchymal barotrauma than animals treated with HFFI (fig 24), despite maintenance of ventilation and oxygenation at the same predetermined limits.

Positive-pressure ventilation at relatively modest peak inspiratory pressures (30 cmH_2O) has been associated with significant alterations in lung pathology and physiology in an ovine model in which no concurrent lung insult was present (11). Although the etiology of the damage is unclear, other investigators have suggested that the repeated opening and closing of airway/alveolar units from a pressure below the opening pressure to one above may result in significant lung damage which can be

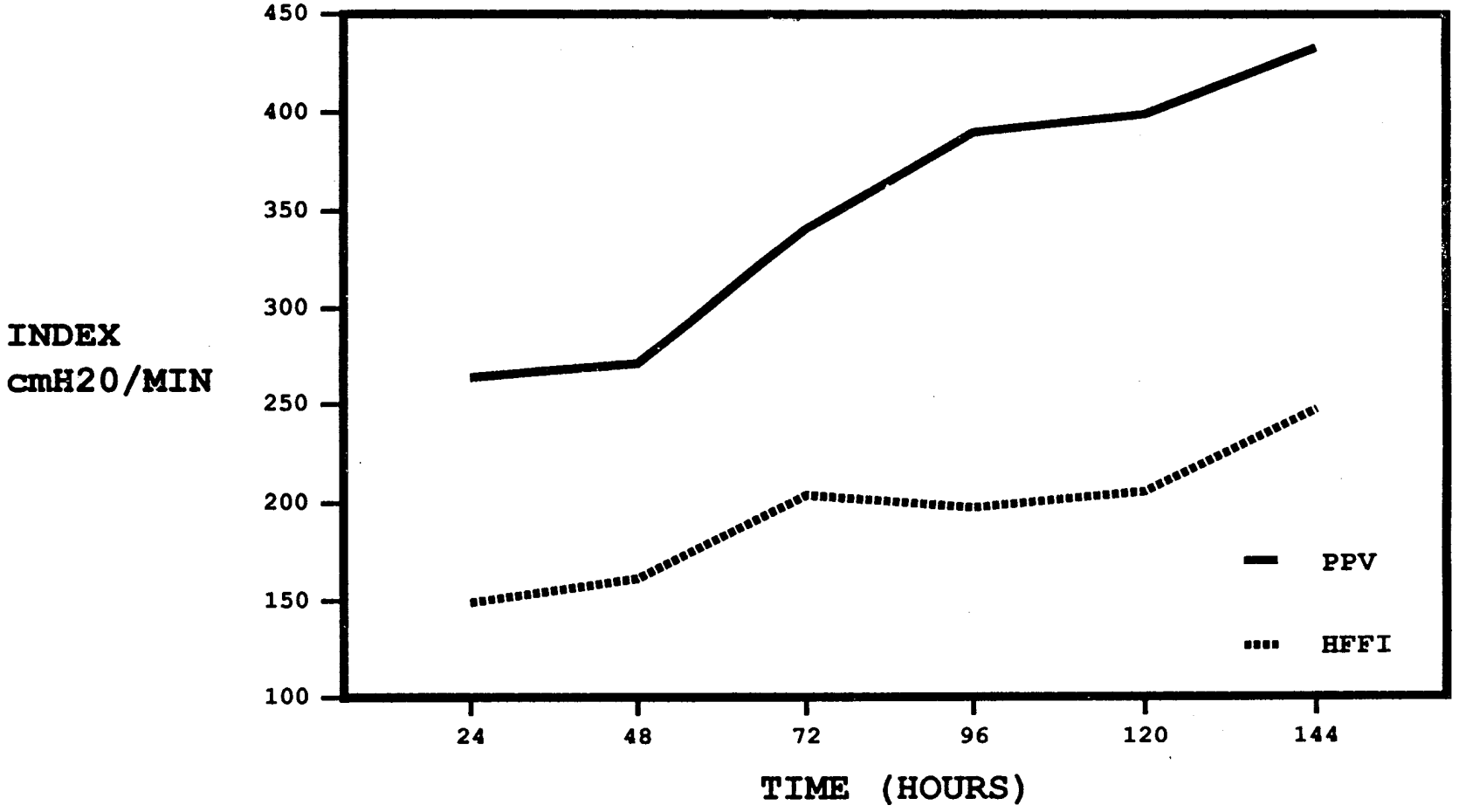


FIGURE 24. Barotrauma index (cmH_2O). PPV indicates positive-pressure ventilation, and HFFI, high-frequency flow interruption.

partially prevented by depletion of granulocytes prior to ventilatory support (12,13). HFFI, in our model, significantly decreased the number of times per minute that such cycles may occur. HFFI is also associated with auto-PEEP, which may prevent the cyclical opening and closing of diseased lung units.

Although no statistically significant differences in bronchoalveolar lavage findings were found comparing groups, there were several trends. All groups had a significant increase in bronchoalveolar lavage WBC counts; however, bronchoalveolar lavage elastase content tended to be less in Group III animals treated with HFFI. It has been suggested that bronchoalveolar lavage elastase is a marker of granulocyte activation (14). The findings of decreased levels of elastase in the animals with the mildest lung damage suggests a potential mechanism of ventilatory mode-induced, granulocyte-mediated lung damage.

These data support our previous findings using HFFI in humans with smoke inhalation injury. The decreased incidence of pneumonia and mortality in patients treated with HFFI compared to a historical cohort of patients treated with positive-pressure ventilation may be secondary to a decrease in iatrogenic mechanical barotrauma which is secondary to ventilatory mode. These data strongly support the continued use of HFFI in the support of patients with smoke inhalation injury, and offer an explanation for the observed decrease in morbidity and mortality.

PRESENTATIONS/PUBLICATIONS

None.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

ACCESSION: DA336099

SUMMARY DATE: 920528 **SUMMARY KIND:** A **PREV DATE:** **DISTRIBUTION:** CX

PROGRAM #: 63002A **PROJ #:** 3M263002DB840 **TASK AREA:** CA **WORK UNIT:** 082

TITLE: High-Frequency Ventilation in the Management of Acute Respiratory Failure Due to Diffuse Alveolar Damage in Adult Baboons

SUBJ1: 060500 - Medicine and Medical Research
SUBJ2: 061200 - Medical Facilities, Equipment, and Supplies

START DATE: 9205 **END DATE:** 9309 **PERFORMANCE METHOD:** C

CONTRACT/GRANT NO:

CONT TOTAL: $
CUM TOTAL: $
TOTAL LAB FUNDS: $

RESOURCES ESTIMATE

FY	WORK YRS	$(Thousands)
91	0.0	$0
92	0.5	$52
93	0.5	$54

RESPONSIBLE DOD ORGANIZATION: 404885
MRDC INSTITUTE OF SURGICAL RESEARCH USAISR
FT SAM HOUSTON, TX 78234
PRUITT, B A
210-221-2720

PERFORMING ORGANIZATION: 404885
MRDC INSTITUTE OF SURGICAL RESEARCH USAISR
FT SAM HOUSTON, TX 78234
CIOFFI, W G
210-221-8440

ASSOC1: DELEMOS, R A

ASSOC2: WINTER, D

FOREIGN INTELLIGENCE CONSIDERED: Y **CIVILIAN APPLICABILITY:** M

KEYWORDS: RA II; Lab Animals; Baboons; Burns (Injuries); Inhalation; Pulmonary Edema; Pulmonary Function; Pulmonary Insufficiency; Respirations; Morbidity

OBJECTIVE: A DTIC literature search was conducted under DTIC request numbers W6K45A/W6K47E dated 4 March 1992. The objective of this work is to determine the efficacy and safety of high-frequency ventilation in the management of acute respiratory failure/diffuse alveolar damage in the adult baboon. If these ventilatory strategies improve oxygenation and decrease barotrauma compared to conventional mechanical ventilation, treatment regimens can be revised to reduce mortality and morbidity in patients with inhalation injury.

APPROACH: Fifteen baboons will be randomized to one of three study groups, i.e., conventional mechanical ventilation with continual distending airway pressure, high-frequency oscillatory ventilation, or high-frequency flow interruption. Blood gas, blood pressure, airway pressure, and hemodynamic data will be averaged over intervals and plotted at midpoint of each interval. Physiologic and repetitive biochemical data will then be analyzed among groups using ANOVA for repeated measures and one-way ANOVA at each time point. Outcome data will be analyzed using Chi square or the Fisher exact test. Nonparametric data will be analyzed using Ridit or the Kruskall-Wallis statistics.

PROGRESS: 9205-9209. This study was approved by the USAISR Research Council, US Army Institute of Surgical Research Animal Care and Use Committee, and US Army Medical Research and Development Command Animal Use Review Office during the second and third

quarters of fiscal year 1992. For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1992.*

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

ACCESSION: DA336099

SUMMARY DATE: 921001 **SUMMARY KIND:** D **PREV DATE:** 920528 **DISTRIBUTION:** CX

PROGRAM #: 63002A **PROJ #:** 3M263002DB840 **TASK AREA:** EC **WORK UNIT:** 082

TITLE: High-Frequency Ventilation in the Management of Acute Respiratory Failure Due to Diffuse Alveolar Damage in Adult Baboons

SUBJ1: 060500 - Medicine and Medical Research
SUBJ2: 061200 - Medical Facilities, Equipment, and Supplies

START DATE: 9205 **END DATE:** 9309 **PERFORMANCE METHOD:** C

CONTRACT/GRANT NO:

CONT TOTAL: $
CUM TOTAL: $
TOTAL LAB FUNDS: $

RESOURCES ESTIMATE

FY	WORK YRS	$(Thousands)
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93	0.5	$323

RESPONSIBLE DOD ORGANIZATION: 404885
MRDC INSTITUTE OF SURGICAL RESEARCH USAISR
FT SAM HOUSTON, TX 78234
PRUITT, B A
210-221-2720

PERFORMING ORGANIZATION: 404885
MRDC INSTITUTE OF SURGICAL RESEARCH USAISR
FT SAM HOUSTON, TX 78234
CIOFFI, W G
210-221-8440

ASSOC1: DELEMOS, R A

ASSOC2: WINTER, D

FOREIGN INTELLIGENCE CONSIDERED: Y **CIVILIAN APPLICABILITY:** M

KEYWORDS: RA II; Lab Animals; Baboons; Burns (Injuries); Inhalation; Pulmonary Edema; Pulmonary Function; Pulmonary Insufficiency; Respirations; Morbidity

OBJECTIVE: A DTIC literature search was conducted under DTIC request numbers W6K45A/W6K47E dated 4 March 1992. The objective of this work is to determine the efficacy and safety of high-frequency ventilation in the management of acute respiratory failure/diffuse alveolar damage in the adult baboon. If these ventilatory strategies improve oxygenation and decrease barotrauma compared to conventional mechanical ventilation, treatment regimens can be revised to reduce mortality and morbidity in patients with inhalation injury.

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PROGRESS: 9205-9209. This study was approved by the USAISR Research Council, US Army Institute of Surgical Research Animal Care and Use Committee, and US Army Medical Research and Development Command Animal Use Review Office during the second and third

quarters of fiscal year 1992. Equipment and supplies have been ordered and work will be initiated shortly. For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1992*.

ABSTRACT

PROJECT NUMBER: 3M263002D840-081, Advanced Development

PROJECT TITLE: High-Frequency Ventilation (HFV) in the Management of Acute Respiratory Failure (ARF) Due to Diffuse Alveolar Damage (DAD) in Adult Baboons

INSTITUTION: US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas 78234-5012,[1] and the Foundation for Biomedical Research, West Loop 410 at Military Drive, San Antonio, Texas 78284[2]

PERIOD COVERED IN THIS REPORT: 1 October 1991 - 27 January 1992

INVESTIGATORS: William G. Cioffi, Jr., MD, Major, MC[1]
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ARF due to DAD occurs commonly following severe burns and/or smoke inhalation. In spite of advances in critical care medicine, mortality from ARF remains high. The use of high levels of positive-end expiratory pressure to prevent atelectasis and edema and to minimize the intrapulmonary shunt has been the most successful ventilatory strategy to date, but cardiovascular and air leak problems have limited its application.

HFV is a technique that accomplishes adequate ventilation/oxygenation at low tidal volumes and supraphysiologic respiratory frequencies. Because it produces minimal volume/pressure excursions around the mean pressure, HFV provides a mechanism whereby high mean airway pressures can be accomplished with minimal peak pressures. Thus, in theory, the benefits accomplished with high positive-end expiratory pressure could be accomplished without the associated risk of barotrauma.

The primary objective of this study is to determine the efficacy and safety of HFV in the management of acute respiratory failure/diffuse alveolar damage in the adult baboon. Fifteen baboons will be randomized to one of three study groups, i.e., conventional mechanical ventilation with continual distending airway pressure, high-frequency oscillatory ventilation, or high-frequency flow interruption. Blood gas, blood pressure, airway pressure, and hemodynamic data will be averaged over intervals and plotted at midpoint of each interval. Physiologic and repetitive biochemical data will then be analyzed among groups

using ANOVA for repeated measures and one-way ANOVA at each time point. Outcome data will be analyzed using Chi square or the Fisher exact test. Nonparametric data will be analyzed using Ridit or the Kruskall-Wallis statistics.

This study was approved by the USAISR Research Council, US Army Institute of Surgical Research Animal Care and Use Committee, and US Army Medical Research and Development Command Animal Use Review Office during the second and third quarters of fiscal year 1992. Equipment and supplies have been ordered and work will be initiated shortly. Upon completion of data collection, the data will be analyzed to determine the efficacy and safety of HFV in the management of acute respiratory failure/diffuse alveolar damage in the adult baboon.

HIGH-FREQUENCY VENTILATION (HFV) IN THE MANAGEMENT OF ACUTE RESPIRATORY FAILURE (ARF) DUE TO DIFFUSE ALVEOLAR DAMAGE (DAD) IN ADULT BABOONS

ARF due to DAD occurs commonly following severe burns and/or smoke inhalation. In spite of advances in critical care medicine, mortality from ARF remains high. The use of high levels of positive-end expiratory pressure (PEEP) to prevent atelectasis and edema and to minimize the intrapulmonary shunt has been the most successful ventilatory strategy to date, but cardiovascular and air leak problems have limited its application.

HFV is a technique that accomplishes adequate ventilation/oxygenation at low tidal volumes and supraphysiologic respiratory frequencies. Because it produces minimal volume/pressure excursions around the mean pressure, HFV provides a mechanism whereby high mean airway pressures can be accomplished with minimal peak pressures. Thus, in theory, the benefits accomplished with high PEEP could be accomplished without the associated risk of barotrauma.

Hamilton et al (1) demonstrated in an adult lavage model of ARF/DAD that HFOV at a high mean airway pressure resulted in improved oxygenation and prevented the pathologic evolution of DAD characteristic of that model. Froese (2) confirmed these observations and established that the same endpoint could be reached with any of the high-frequency techniques provided that the strategy was to maintain lung volume greater than closing volume. In another study, over 90% of term infants with ARDS/pneumonia and severe respiratory failure responded to HFOV and avoided the need for extracorporeal membrane oxygenation (ECMO) (3,4). There also have been anecdotal reports of older children with ARF/DAD who have been successfully rescued with HFOV. Adult experience with HFOV and ARF has been limited. Carlon et al (5) reported no benefit from high-frequency jet ventilation (HFJV) in a series of adults with ARDS. However, the strategy employed in that study was to use lower rather than higher mean pressures. This approach subsequently has been shown to be ineffective in animal models of ARF/DAD as well.

To date, there have been no trials of HFOV in ARF in adults primarily because the available oscillators have insufficient volume output to ventilate a sick adult. Based on the human newborn experience in patients with identical pathophysiology, there is every reason to expect that, should an appropriate oscillator be developed, the results in adult application should be equally dramatic.

The primary objective of this study is to determine the efficacy and safety of HFV in the management of acute respiratory failure/diffuse alveolar damage in the adult baboon, i.e., to use

an established reproducible model of ARF/DAD in the adult baboon; to compare the efficacy/safety of conventional ventilation with continual distending airway pressure (CV), high-frequency oscillatory ventilation (HFOV), and high-frequency flow interruption (HFFI) in the management of ARF/DAD in the adult baboon, and to assess the effect of each ventilator mode on morphologic, biochemical, and physiologic parameters reflecting lung injury.

Medical Application. The mode of ventilatory support may affect the severity and outcome of various pulmonary diseases, including inhalation injury (1,6,7). Lung injury following burns/smoke inhalation is a complex pathophysiologic process which may respond differently to different forms of mechanical ventilation. We have previously studied the effect of CV, HFOV, and HFFI on experimental smoke inhalation in the adult baboon and have shown that HFFI was preferable when airway injury was the predominant factor in overall pathophysiology. Since burns/inhalation injury are often associated with ARF/DAD, we now propose to study the effect of these ventilatory strategies on the progression and resolution of the ARF/DAD lesion.

Selection of Appropriate Animal Model. The cardiopulmonary physiology of baboons is similar to that of humans. In particular, autonomic nervous system control of cardiovascular function in the baboon (unlike that of smaller monkeys) is identical to that of man. The response of the baboon to various injuries, such as oxygen, radiation, etc., is more similar to the human response than that of sheep or smaller mammals. Because of the high degree of homology between baboon and man, most immunologic reagents developed for humans will also cross-react with the baboon. Extensive studies have been performed defining the course and characteristics of pulmonary oxygen toxicity in the baboon (8,9). Its morphologic and clinical features are similar to those of DAD in man (8,9). Three mechanisms have been widely used to produce animal models of ARF/DAD, i.e., lung lavage, injection of fatty acids, and pulmonary oxidant injury. Lung lavage removes/alters surfactant and produces a short-lived DAD lesion. Because surfactant synthesis is not altered and inhibitors are not induced, functional recovery begins almost immediately. Fatty acids induce an ARF/DAD lesion in a mechanism similar to that of fat emboli. Oleic acid has been used most commonly but other acids such as linoleic acid have also been used. These models can develop significant cardiovascular instability with perturbations of pulmonary blood flow. In addition, the dose/response is unpredictable. Because of the variability of response, the number of animals would have to be increased to generate meaningful results. Pulmonary oxidant injury produces a reproducible and stable lesion (9). The major drawback is the extended experimental time required to induce the lesion before one can test the intervention. Based on previous experience in the adult baboon, animals will be treated with an F_IO_2 of 1.0 for 5-6 days before

they are sufficiently ill to allow meaningful studies of interventions. This adds to the overall costs of the study because each animal will require 24-h supervision for 11-12 days. In spite of these limitations, the pulmonary oxygen toxicity model will allow the most easily interpretable results at the lowest cost in dollars and in number of animals.

MATERIALS AND METHODS

Study Design. Fifteen adult baboons will be selected for study and randomly assigned to one of three groups, i.e., conventional mechanical ventilation with continual distending airway pressure, HFOV, or HFFI.

Description of Procedures

Protocol for Injury Induction. Animals will be anesthetized with ketamine hydrochloride (25-40 mg/kg IM), intubated, and paralyzed with pancuronium bromide (0.04-0.10 mg/kg IV). Thereafter, they will be maintained under paralysis and sedated with diazepam (0.1 mg/kg IV or 7.5 mg/kg IM) as indicated by clinical signs of anxiety, i.e., elevated pulse, blood pressure. If diazepam sedation is inadequate, additional sedation will be accomplished with sodium pentobarbital (20-33 mg/kg IV).

Peripheral venous and arterial lines will be placed for measurement of blood gases and administration of fluids. Fluids will be administered at a rate of 80 ml/kg/day and will consist of 5% dextrose and normal saline with 1 U heparin sodium per milliliter at 2 ml/h. Changes in fluid composition and infusion rate will be based on hemodynamic data and electrolyte composition.

Arterial blood pressure, heart rate, and respiratory rate will be continuously monitored. Blood gases will be obtained every 4 h for the first day, every 6 h on days 2-4, and every 4 h thereafter. Electrolytes, BUN, creatinine, CBC, and platelet counts will be obtained daily. Chest roentgenograms will also be obtained daily. Pulmonary function tests will be performed at 0 h, 2 days, 4 days, at the time of study entry, 2 days after study entry, 4 days after study entry, and immediately prior to sacrifice. Pulmonary function tests will consist of measurement of functional residual capacity using helium dilution, passive exhalation resistance and compliance, pulmonary diffusing capacity, inspiratory capacity, and expiratory reserve. Lung lavage with saline will be performed following the pulmonary function tests at each time point. Bronchoalveolar lavage will be collected and frozen for later analysis of cell counts, elastase, total protein, and quantitative bacterial cultures.

Animals will be placed on CV using a Siemens-900™ ventilator. Proximal and distal airway pressures will be monitored via the double lumen endotracheal tube using a Validyne™ pressure

transducer. Initial tidal volume will be set at 10 m/kg with a frequency of 20 breaths per minute and a PEEP of 4 cmH_2O. Frequency and tidal volume will be adjusted based on blood gas determinations to keep the $PaCO_2$ between 35 and 45 torr. FIO_2 will be maintained at 1.0 and PEEP at 4 cmH_20 throughout the injury induction phase.

Animals will be turned from side to side every 4 h. Tracheal toilet will be performed every 4 h and as clinically indicated. Animals will receive gentamicin (2.5 mg/kg) every 8 h for the duration of the study or until therapy is changed based on clinical indications and/or cultures.

Induction of injury will be considered to be completed when an animal has radiographic findings of ARDS and a fall in PaO_2/FIO_2 to 40% of baseline. This will usually mean a PaO_2 between 150 and 175 torr. Alternatively, a fall in PaO_2/FIO_2 to 45% of baseline will be considered a sufficient endpoint if accompanied by a fall in respiratory system compliance to < 50% of baseline.

Protocol for Experimental Interventions. Once an animal has reached study entry criteria, pulmonary function tests will be performed as noted above. The animal will be anesthetized with ketamine hydrochloride (25-40 mg/kg IM) and a 7F Swan-Ganz catheter placed via a femoral vein using local anesthesia (1% lidocaine, 1-2 cc SC). Pulmonary artery pressure will be monitored continuously. Cardiac output will be measured every 6 h using thermodilution and pulmonary wedge pressure.

Following the pulmonary function tests and determination of preintervention cardiac output and wedge pressure, the animals will be placed on their preassigned ventilators.

CV will be accomplished using a volume ventilator. Oxygenation will be maximized by alterations of mean airway pressure. When ventilation is adequate, this will be accomplished by increases in PEEP. When ventilation is inadequate, tidal volume and PEEP will be increased. The goal will be to bring the FIO_2 below 0.4 and maintain a PaO_2 between 80-100 torr. PCO_2 will be maintained between 35-45 torr, if possible, by adjustment of rate and tidal volume.

HFOV will be accomplished using a diaphragm oscillator. Frequency will be set at 10 Hz with oscillatory amplitude sufficient to produce detectable chest wall motion. An I:E of 1:2 will be used. Initial mean airway pressure (P_{AW}) will be used on CV. P_{AW} will then be increased in increments of 2 cmH_2O until there is a rapid rise in PaO_2 or oxygen saturation. PaO_2 will then be maintained between 80-100 torr with an FIO_2 of 0.4 or less. If there is a question about further increases in PAW, the animal can be sighed and trends in oxygenation determined. If sigh results in an increased PaO_2, P_{AW} should be further increased incrementally.

If sigh results in no further increase in PaO_2, P_{aw} should not be increased. Chest roentgenograms will be used as a guide to degree of lung inflation. At any given frequency, $PaCO_2$ will be kept between 35-45 torr by adjusting the oscillatory amplitude.

Mixed-mode ventilation will be accomplished using the Bird™ percussionaire ventilator (HFFI). Ventilation rate, maximum and minimum airway pressure (P_{max} and P_{min}, respectively) will be set at a frequency and pressures similar to those on CV. HFFI will be superimposed at 10 Hz and blood gas obtained. Oxygenation will be optimized by adjustment of P_{aw} (PEEP and/or P_{max}). Ventilation will be optimized by adjustment of HFFI amplitude and frequency and peak pressure of tidal breaths. In general, the goal will be to reduce the frequency and peak pressure of the tidal breaths as much as possible while maintaining optimal oxygenation at a high mean airway pressure.

If at any time in the study, the diagnosis of pneumonia is entertained, a sputum gram stain and culture will be obtained to document the diagnosis and appropriate antibiotics instituted.

All animals will be supported for 120 h following the induction of injury. At the conclusion of the study or, in the opinion of the principal investigator, the animal is in irreversible cardiopulmonary failure, the animal will be anesthetized with sodium pentobarbital (50 mg/kg IV) and exsanguinated. Necropsy will be performed in the standard manner on all animals by Dr. Jackie J. Coalson, PhD, from the Department of Pathology, University of Texas Health Science Center, San Antonio, Texas. Sections of all organs will be obtained and fixed for light and electron microscopy. The left lower lobe will be inflated to 20 cmH_2O and fixed by the endotracheal instillation of Carnoy's solution. The trachea will be examined for evidence of gross lesions. The trachea will be fixed, sectioned longitudinally, and examined. A section of each of the remaining lobes will be removed and frozen in liquid nitrogen and stored at -80°C.

Determination of Number of Animals Required. Previous studies using the adult baboon have substantiated the reproducibility of this model (9). Studies using HFOV in various animal ARDS or HMD models have shown marked intergroup differences with significance at this number (9). This, coupled with cost and ethical concerns, leads us to conclude that 5 animals per group will be sufficient to allow us to conclude whether there are advantages/disadvantages to any ventilator strategy.

Data Analysis Plan. Blood gas, blood pressure, airway pressures and hemodynamic data will be averaged over intervals and plotted at the midpoint of each interval. Physiologic and repetitive biochemical data will then be analyzed among groups using ANOVA for repeated measures and one-way ANOVA at each time

point. Outcome data will be analyzed using Chi square or the Fisher exact test. Nonparametric data will be analyzed using Ridit or the Kruskall-Wallis statistics.

RESULTS

This study was approved by the USAISR Research Council, US Army Institute of Surgical Research Animal Care and Use Committee, and US Army Medical Research and Development Command Animal Use Review Office during the second and third quarters of fiscal year 1992. Equipment and supplies have been ordered and work will be initiated shortly.

DISCUSSION

Upon completion of data collection, the data will be analyzed to determine the efficacy and safety of HFV in the management of acute respiratory failure/diffuse alveolar damage in the adult baboon.

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None.

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Driscoll DM: Burn management. Presented to the National Teaching Institute, New Orleans, Louisiana, 23 May 1992.

Pruitt BA: Boston City Hospital and modern burn and trauma care. Presented as part of Boston City Hospital Clinical Day, Boston, Massachusetts, 23 May 1992.

Maroun CA: USAISR overview. Presented as part of Educator Tour, Brooke Army Medical Center, Fort Sam Houston, San Antonio, Texas, 28 May 1992.

Mozingo DW: Skin substitutes and cultured keratinocytes. Presented at the 21st Annual Educational and Scientific Symposium, Society of Critical Care Medicine, San Antonio, Texas, 25 May 1992.

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