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

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

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This publication was compiled by Christine C. Davis, Research Protocol Coordinator, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas 78234-5012.

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FOREWORD

This Institute's plan for system-wide burn care in support of Operation Desert Shield/Storm, formulated in August and September 1990, was activated in January 1991. When Desert Shield became Desert Storm, ISR burn teams were in place in Dhahran, Riyadh, and King Khalid Military City to provide theater-wide burn care. One of the Institute's burn surgeons had also established a burn-holding unit at the 2nd General Hospital, Landstuhl, Germany, and the ISR burn center promptly expanded to provide an 80-bed tertiary care capacity with the potential of maximum expansion to 200 beds. With the assistance of the American Burn Association, sufficient available tertiary burn care beds in civilian burn centers within a 50-mile radius of the anticipated casualty-receiving centers were also identified to accommodate the projected "overflow" of burn casualties.

The composition of the in-theater burn teams represented a variant of the Institute's "standard" flight team and the teams previously deployed in response to civilian and military mass casualty situations, with staffing modifications necessitated by expansion of the ISR burn center. The aeromedical transfer procedures for burn patients (developed in collaboration with the United States Air Force) and the staging of aeromedical transport, with final triage carried out at the burn-holding unit in Germany, were established to ensure continuity and quality of burn care. The transfer system was based on the Institute's experience in the virtually mortality-free aeromedical transfer of over 800 patients from a burn-holding unit in Japan during the Vietnam conflict and of scores of burn patients each year before and since then.

The preparations for the care of projected combat-incurred burns included activation of the Institute's IMA physicians and nurses experienced in burn care to meet anticipated clinical needs. Those personnel also participated in the Institute's ever-expanding professional education program in which, during this year of wartime activity, four medical students, seven PGY-1 physicians, 28 general surgery, plastic surgery, emergency medicine, and pediatric residents, and one surgical critical care fellow as well as 252 nursing personnel were accommodated. These graduate-level physicians and nurses joined a progressively larger cohort of military professionals familiar with the medical needs of burn patients.

Wartime preparations and establishment of the military's burn care system had, by virtue of the industry and expertise of the Institute's laboratory and clinical personnel, little impact on the research mission of the Institute. In fact, 11 new research projects were approved and initiated to bring the total number of research projects to 52. Research productivity and quality were maintained at the highest level, as indexed by numerous papers

competitively selected for presentation at professional meetings of national and international scope.

The reports in this volume reflect a successful integration of wartime mission and peacetime activities made possible only by the fact that the Institute, in its day-to-day activities, does in small scale what would have been necessary on a larger scale had there been numerous combat casualties. The institutional memory that resides in the Institute (the only tertiary burn treatment facility in the entire United States military) permitted the establishment of in-theater facilities for initial burn care and an aeromedical transfer system for burn patients, the effectiveness of which had been documented in prior conflicts and in numerous peacetime disasters. The dedication and unequalled professional capabilities of both the clinical and laboratory staff enabled them to maintain the research productivity documented by this volume while providing care that ensured the survival of all the casualties with burn injuries cared for at the Institute.

Basil A. Pruitt Jr.

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Commander and Director

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11. TITLE *(Precede with Security Classification Code)*
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b. CONTRACT/GRANT NUMBER	91	47.2	4393
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23. TECHNICAL OBJECTIVE 24. APPROACH 25. PROGRESS *(Precede text of each with Security Classification Code)*

23/24. (U) The objectives of this work are to provide specialized care for thermally injured patients, investigate diagnostic and therapeutic technics to improve the survival and function of thermally injured patients, and to promulgate scientific medical information to health professionals. Thermally injured patients from the 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.

25. (U) 9001 - 9012. Two hundred and sixteen seriously burned patients were admitted and treated at this Institute during calendar year 1990. Current clinical research activities include studies of host resistance, endocrine changes following injury, nutritional support regimens, skin substitutes, and postinjury infection control.

SUPPLEMENTAL WORK UNIT INFORMATION FOR THE PROJECT ENTITLED "CLINICAL OPERATION, CENTER FOR TREATMENT OF BURNED PATIENTS"

Subrecord/Linking Accession Number: Not applicable.

Search Control Data: 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.

Product Identification: For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report* for fiscal years 1950-91.

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

ABSTRACT

PROJECT NUMBER: 3M162787A874, 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

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Two hundred and sixteen patients were admitted to this Institute during calendar year 1990. 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 growth hormone 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, and a project to characterize certain biochemical indicators of infection in the thermally injured.

CLINICAL OPERATION
CENTER FOR TREATMENT OF BURNED PATIENTS

The Clinical Division of this Institute admitted 216 soldiers and other authorized patients with thermal, chemical, or electric injury during calendar year 1990. Aeromedical teams from the Institute conducted 56 missions to transfer 69 (31.9%) of the admitted patients. Fifty-six missions were within the continental United States and 1 was to an area overseas. Thirty-three missions (57.9%) were carried out by rotary-wing aircraft and 24 (42.1%) by fixed-wing aircraft. Ninety patients (41.7%) were admitted within 24 h of injury and 143 (66.2%) were admitted within 48 h of injury. One hundred and seventy-five patients (81%) were male and 41 (19%) were female.

The ages of these patients ranged from 6 months to 92 yr, with an average age of 28.8 yr. Burn sizes averaged 23.7% of the total body surface area, with an average full-thickness component of 10.2%. Fifty-three patients (24.5%) were in the pediatric age group (< 15 yr), with an average age of 4.3 yr and an average burn size of 14.4% of the total body surface area. There were 19 patients (8.8%) with high voltage electric injury and 4 patients (3.7%) with chemical injury. The average hospital stay of all patients, excluding convalescent leave for active duty military patients, was 39 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 59 patients (27.3%). Ninety-six patients (44.4%) had some associated injury (includes 59 patients with inhalation injury) which included fractures or dislocations in 6 patients, lacerations in 8 patients, and head injuries in 6 patients.

During calendar year 1990, 850 operative procedures were performed on 159 patients, an average of 5.3 operative procedures per patient.

Morbidity and Mortality. Seventeen patients (7.9%) died during calendar year 1990. Autopsies were performed in 12 (70.6%) of these hospital deaths. The average burn size of patients who died was 56.7% of the total body surface area and the full-thickness average was 35.2% of the total body surface area. Age ranged from 22 to 92 yr. Eight of these patients (47.1%) had inhalation injury as a primary or contributing cause of death. Thirteen patients (76.5%) had burn injuries ≥ 30% of the total body surface area, 10 (58.8%) exceeding 40%, and 9 (52.9%) exceeding 50% of the total body surface area. None of the 17 deaths occurred in pediatric patients.

Infection was once again the most common complication following thermal injury, with pneumonia occurring in 40 patients (18.5%).

TABLE 1. Sources of Admission (1990)

AREA	A	AD	AF	AFD	N/MC	ND	VAB	OTHER	TOTAL
First Army	1	–	–	1	1	–	1	1	5
Third Army	4	1	1	–	–	–	–	1	7
Fifth Army	22	17	8	8	3	–	8	110	176
Sixth Army	–	–	–	1	1	1	–	2	5
Belgium	1	–	–	–	–	–	–	–	1
Belize	–	–	–	–	–	–	–	5	5
Germany	1	–	–	1	–	–	–	–	2
Hawaii	–	–	–	–	–	1	–	–	1
Honduras	2	–	–	–	–	–	–	1	3
Japan	–	–	–	–	7	–	–	–	7
Mexico	–	–	–	–	–	–	–	1	1
Panama	1	–	–	–	–	–	–	–	1
Saudi Arabia	–	–	–	–	2	–	–	–	2
TOTAL	**32**	**18**	**9**	**11**	**14**	**2**	**9**	**121**	**216**

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 (1990)

Causes	Number of Patients	Percentage of Admissions	Deaths	Mortality (%)
Hot liquids	53	24.5	-	-
Gasoline, diesel, and kerosene	40	18.5	2	11.8
Open flames	21	9.7	2	11.8
Electrical	19	8.8	-	-
Structural fires	19	8.8	8	47.1
Bomb, shell, simulator grenade, and gunpowder explosions	12	5.6	-	-
Contact	12	5.6	1	5.9
Motor vehicle accidents	11	5.1	1	5.9
Butane, propane, or natural/sewer gas explosions	10	4.6	1	5.9
Chemical	8	3.7	-	-
Self-inflicted	7	3.2	2	11.8
Welding	2	0.9	-	-
Smoking, clothes ignition	1	0.5	-	-
Other	1	0.5	-	-
TOTAL	**216**	**100.0**	**17**	**7.9**

The most common organism isolated in patients with bacterial pneumonia was *Staphylococcus aureus* in 25 patients. Gram-negative organisms were responsible for the pneumonias in the remaining 15 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 Candida in 1. No patients had suppurative thrombophlebitis during this reporting period.

Table 3 lists the effect of age and extent of injury on survival and Table 4 lists mortality rates associated with increments of 10% of the total body surface area for the years 1986-90. Table 5 summarizes the survival of patients with extensive burns from 1963-90. 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 1990.

Educational Activities. During calendar year 1990, the professional staff of the Clinical Division continued to provide education to professional an paraprofessional groups at the local, national, and international levels. A total of 39 resident physicians were attached for periods of 1-2 months, including 10 from the University of Texas Health Science Center (San Antonio TX), 7 from Wilford Hall USAF Medical Center, 5 from Pensacola Naval Air Station, 4 from Brooke Army Medical Center, 3 each from Fitzsimons Army Medical Center and Providence Hospital (Southfield MI), 2 from Travis Air Force Base Medical Center, and 1 each from Brooks Aerospace Hospital (San Antonio TX), Letterman Army Medical Center, Staten Island Hospital, Columbus Hospital (Chicago IL), and Albert Einstein Hospital. Six interns from Brooke Army Medical Center rotated through the Institute. A total of 7 medical students rotated through the Institute, including 5 students from the Uniformed Services University of Health Sciences and 1 each from the University of Iowa and the University of Indiana College of Medicine. In addition, 3 critical care fellows from Brooke Army Medical Center rotated through the Institute. A total of 16 physicians visited from foreign countries for periods ranging from 1 day to 2 months, which included 5 from Korea, 2 each from Pakistan and Egypt, and 1 each from Northern Ireland, Singapore, Norway, the Philippines, Japan, Switzerland, and Germany. One foreign medical student from the United Kingdom visited the Institute for a period of 3 months. The Respiratory Therapy Branch had 46 trainees, the Physical Therapy Branch had 60 trainees, and the Occupational Therapy Branch had 109 trainees. Thirty-five scientific publications appeared in refereed medical journals and 183 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 (1990)

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	1	2	3	1	-	-	-	-	-	-	7	-	-
1	6	5	3	2	-	-	-	-	-	-	16	-	-
2	4	2	-	-	-	-	-	-	-	-	6	-	-
3	3	1	-	-	-	-	-	-	-	-	4	-	-
4	1	1	1	-	-	-	-	-	-	-	3	-	-
5 - 9	5	1	3	-	-	-	-	-	-	-	9	-	-
10 - 14	4	-	1	2	-	-	-	1	-	-	8	-	-
15 - 19	4	2	2	-	-	-	1	2	-	-	11	-	-
20 - 29	21	15	8	6	6	3	1	2	2	1	65	2	3.1
30 - 39	7	4	5	3	3	1	3	2	4	1	33	4	12.1
40 - 49	6	3	6	2	1	1	1	-	-	-	20	-	-
50 - 59	7	3	1	1	2	-	-	-	1	-	15	2	13.3
60 - 69	3	2	-	1	1	1	1	-	1	-	10	4	40.0
70 - 79	3	1	2	-	-	-	-	-	-	-	6	3	50.0
80 - 89	-	-	1	1	-	-	-	-	-	-	2	1	50.0
90 - 100	-	1	-	-	-	-	-	-	-	-	1	1	100.0
Total Cases	75	43	36	19	13	6	7	7	8	2	216		
Total 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

TABLE 4. Total Body Surface Area Burn Involvement (%) and Mortality (1986-90)

	0-9	10-19	20-29	30-39	40-49	50-59	60-69	70-79	80-89	91-100	Total
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
1986											
Number of Patients	61	40	32	21	19	7	11	7	4	5	207
Number of Deaths	1	2	2	2	5	2	4	2	4	5	29
Mortality (%)	1.6	5.0	6.3	9.5	26.3	28.6	36.4	28.6	100.0	100.00	14.0

TABLE 5. Survival and Nonsurvival by Year for Patients with Burns ≥ 30% of the Total Body Surface Area (1963-90)

	SURVIVORS			NONSURVIVORS		
	Number	Average Burn (%)		Number	Average Burn (%)	
Year	of Cases	Total	3°	of Cases	Total	3°
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
1964-89	3,620	131	3.6	932	164	17.6	734	220	30.0	515	234	45.4	940	760	80.9
1990	154	4	2.6	19	3	15.8	13	1	7.7	6	-	-	24	9	37.5

TABLE 7. Causes of Death (1990)

Patient	Age	Sex	BURN SIZE (%) Total	3°	Postburn Day	Cause of Death
1	24	M	96	90	1	*96% total body surface area burn with severe inhalation injury.
2	36	M	91	72	72	91% total body surface area burn with severe inhalation injury.
3	22	M	85	85	5	*85% total body surface area burn with severe inhalation injury.
4	60	M	91	91	36	91% total body surface area burn with severe inhalation injury.
5	36	M	82	82	0	82% total body surface area burn with severe inhalation injury.
6	35	F	81	65	15	*81% total body surface area burn with inhalation injury and bronchopneumonia.
7	66	M	60	30	3	60% total body surface area burn with acute myocardial infarction.
8	65	F	50	45	2	*50% total body surface area burn with inhalation injury.
9	83	M	41	29	33	41% total body surface area burn with acute myocardial infarction.
10	82	M	37	1	41	37% total body surface area burn with acute myocardial infarction.
11	53	M	32	14	13	32% total body surface area burn with hepatic cirrhosis/active hepatitis.

TABLE 7 (Continued)

Patient	Age	Sex	BURN SIZE (%) Total	3°	Postburn Day	Cause of Death
12	77	M	32	11	70	32% total body surface area burn with acute myocardial infarction.
13	63	F	30	25	28	30% total body surface area burn with massive pulmonary embolism.
14	80	F	23	22	0	*23% total body surface area burn with acute myocardial infarction.
15	70	M	24	20	5	24% total body surface area burn with acute myocardial infarction.
16	73	M	19	13	55	19% total body surface area burn with acute myocardial infarction.
17	92	F	19	14	17	19% total body surface area burn with acute necrotizing enterocolitis and peritonitis.

*Autopsy not performed.

PRESENTATIONS

Duncan DJ: Initial management of burn victims. Presented as part of the Critical Care Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 11 January 1990.

Pruitt BA Jr: Progress and problems in burn care. Presented to the San Antonio Surgical Society, San Antonio, Texas, 16 January 1990.

Pruitt BA Jr: The pathophysiology of burn injury. Presented to the TSI/DNA Combined Injured Core Group, Orlando, Florida, 17 January 1990.

Keenan J: Compassion knows no boundaries: USSR burn mission. Presented to the South Central Emergency Management Association, San Antonio, Texas, 19 January 1990.

Burleson DG: Longitudinal changes in T lymphocyte phenotype and function after thermal injury. Presented at the Conference on the Immunocompromised Surgical Patient: Mechanisms and Therapy in Trauma and Burns, Snowbird, Utah, 24 January 1990.

Driscoll DM: Compassion knows no boundaries: USSR burn mission. Presented at the Southwest Texas Methodist Hospital, San Antonio, Texas, 25 January 1990.

Molter NC: Needs of families of critically ill patients. Presented at the University of Texas Health Science Center, San Antonio, Texas, 30 January 1990.

Becker WK: Burn injury pathophysiology. Presented as part of the OT/PT Management of Burns Course, United States Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 5 February 1990.

Becker WK: Burn wound management. Presented as part of the OT/PT Management of Burns Course, United States Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 5 February 1990.

Cioffi WG Jr: Inhalation injury. Presented as part of the OT/PT Management of Burns, United States Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 5 February 1990.

Jordan BS: Review of current research at the United States Army Institute of Surgical Research. Presented as part of the OT/PT Management of Burns, United States Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 5 February 1990.

Summers TM: Psychosocial aspects of burn care. Presented as part of the OT/PT Management of Burns, United States Army Institute

of Surgical Research, Fort Sam Houston, San Antonio, Texas, 6 February 1990.

Molter NC: Principles of pain management. Presented as part of the OT/PT Management of Burns, United States Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 6 February 1990.

Carlson DE: Nutritional management. Presented as part of the OT/PT Management of Burns, United States Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 6 February 1990.

Hollan E: Infection control principles in burn care. Presented as part of the OT/PT Management of Burns, United States Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 7 February 1990.

Matta CB: Initial management of burn injuries in the theater of operation. Presented as part of the United States Air Force Battlefield Nursing Course, Brooks Air Force Base, San Antonio, Texas, 13 February 1990.

Pruitt BA Jr: Burns complicated by pregnancy and mechanical injury. Presented as part of the OT/PT Management of Burns Course, Fort Sam Houston, San Antonio, Texas, 15 February 1990.

McManus WF: Burn mass casualty care in Ufa, USSR. Presented to the International Society of Burns, Maui, Hawaii, 18 February 1990.

Becker WK: American military response to the Ufa train disaster. Presented at the Conference on Catastrophic Medicine, Ufa, USSR, 22 February 1990.

McManus AT: Microbiologic observations made by the laboratory component of the American burn team sent to aid victims of the Baskarian burn disaster. Presented at the Congress on Catastrophic Medicine, Ufa, Russia, 22 February 1990.

Cioffi WG Jr: The use of surfactant replacement in ARDS and smoke inhalation. Presented at the United States Army Institute of Surgical Research, 7 March 1990.

Duncan DJ: Initial management of burn victims in the theater of operation. Presented at the Tri-Service Emergency Medical Conference, San Antonio, Texas, 7 March 1990.

Chapman T: Burns/hazardous materials. Presented as part of the Emergency Medical Technician Course, Fort Sam Houston, San Antonio, Texas, 8 March 1990.

Keenan J: Compassion knows no boundaries: USSR burn mission. Presented at the FORESCOM Conference, Atlanta, Georgia, 9 March 1990.

Pruitt BA Jr: Shock and fluid resuscitation. Presented as part of the Advanced Burn Life Support Provider Course, Los Angeles, California, 14 March 1990.

Becker WK: Mass casualty experience in the Soviet Union. Presented to the Committee on Trauma at the 68th Annual Meeting of the American College of Surgeons, Washington, DC, 15 March 1990.

Burleson DG: IL2-receptor expression by stimulated lymphocytes from burned patients. Presented at the 14th International Meeting of the Society for Analytical Cytology, Ashville, North Carolina, 18 March 1990.

Shippee RL: Effect of zinc nutriture on the primary humoral response in a burn rat model. Presented at the 73rd Annual Meeting of the Federation of American Societies for Experimental Biology, New Orleans, Louisiana, 20 March 1989.

Burleson DG: Identification of neopterin in burned patient sera. Presented at the 73rd Annual Meeting of the Federation of American Societies for Experimental Biology, New Orleans, Louisiana, 22 March 1989.

Molter NC: United States Army Institute of Surgical Research. Presented as part of the Nurse Education Tour, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 26 March 1990.

Pruitt BA Jr: Shock and fluid resuscitation. Presented as part of the Advanced Burn Life Support Provider Course, Las Vegas, Nevada, 26 March 1990.

Pruitt BA Jr: Epidemiology, pathophysiology, and classification of chemical injury. Presented at the 22nd Annual Meeting of the American Burn Association, Las Vegas, Nevada, 27 March 1990.

Waymack JP: Effect of prostaglandin E (PGE) on resistance to sepsis. Presented at the 22nd Annual Meeting of the American Burn Association, Las Vegas, Nevada, 28 March 1990.

Waymack JP: Effect of prostaglandin E (PGE) on endotoxin/tumor necrosis factor (TNF) metabolism. Presented at the 22nd Annual Meeting of the American Burn Association, Las Vegas, Nevada, 28 March 1990.

Cioffi WG Jr: Advanced burn life support. Presented at the 22nd Annual Meeting of the American Burn Association, Las Vegas, Nevada, 29 March 1990.

Cioffi WG Jr: Granulocyte function following thermal injury. Presented at the 22nd Annual Meeting of the American Burn Association, Las Vegas, Nevada, 29 March 1990.

Driscoll DM: Compassion knows no boundaries: USSR burn mission. Presented at the San Antonio College, San Antonio, Texas, 30 March 1990.

Ikeuchi H: The effect of platelet-activating factor (PAF) and a PAF antagonist (CV-3988) on smoke inhalation injury using an ovine model - physiological change. Presented at the 22nd Annual Meeting of the American Burn Association, Las Vegas, Nevada, 30 March 1990.

McManus AT: A survey of blood culture data collected from 49 North American burn units with 8642 admissions. Presented at the 22nd Annual Meeting of the American Burn Association, Las Vegas, Nevada, 30 March 1990.

Shippee RL: Quality control of automated clinical chemistry data using general purpose commercial software. Presented to the Society of Armed Forces Military Laboratory Scientists, March 1989.

Driscoll DM: Compassion knows no boundaries: USSR burn mission. Presented at the Brooke Army Medical Center, Fort Sam Houston, San Antonio, Texas, 2 April 1990.

Keenan J: Compassion knows no boundaries: USSR burn mission. Presented at the Brooke Army Medical Center, Fort Sam Houston, San Antonio, Texas, 2 April 1990.

Pruitt BA Jr: Clinical and laboratory studies of inhalation injury. Presented at the 1990 Gary P. Wratten Surgical Symposium, Walter Reed Army Medical Center, Washington, DC, 4 April 1990.

Duncan DJ: Emergency management of the burn trauma victim. Presented at the Texas Nursing in 1990s Symposium, Texas State Emergency Nurses Association Convention, San Antonio, Texas, 5 April 1990.

McManus WT: Aeromedical transport of severely burned patients. Presented at the United States Air Force School of Aerospace Medicine, Brooks Air Force Base, San Antonio, Texas, 6 April 1990.

Matta CB: Initial management of burn victims in the theater of operation. Presented as part of the United States Air Force Battlefield Nursing Course, Brooks Air Force Base, San Antonio, Texas, 6 April 1990.

McManus AT: Silver compounds in serious burns. Presented at the 2nd International Conference on Gold and Silver in Medicine, Manchester, United Kingdom, 10 April 1990.

Becker WK: Hemorrhage and resuscitation. Presented to the Southern Society of Clinical Surgeons, San Antonio, Texas, 11 April 1990.

Cioffi WG Jr: High-frequency ventilation for inhalation injury: clinical and laboratory studies. Presented to the Southern Society of Clinical Surgeons, San Antonio, Texas, 11 April 1990.

Buescher TM: Antithrombin III in burn patients. Presented to the Southern Society of Clinical Surgeons, San Antonio, Texas, 11 April 1990.

McManus WF: Limitations of burn wound excision. Presented to the Southern Society of Clinical Surgeons, San Antonio, Texas, 11 April 1990.

Vaughan GM: Postburn endocrinologic changes. Presented to the Southern Society of Clinical Surgeons, San Antonio, Texas, 11 April 1990.

Waymack JP: Effects of arachidonic acid metabolites on the response to infection and sepsis. Presented to the Southern Society of Clinical Surgeons, San Antonio, Texas, 11 April 1990.

Duncan DJ: Initial management of burn victims in the theater of operation. Presented at the 217th Evacuation Hospital, Fort Sam Houston, San Antonio, Texas, 22 April 1990.

Summers TM: Management of stress and crisis. Presented as part of the Critical Care Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 23 April 1990.

Becker WK: Initial management of burn injuries. Presented at the Acute Burn Trauma: Meeting the Challenge – United States Army Institute of Surgical Research Burn Symposium, San Antonio, Texas, 24 April 1990.

Buescher TM: Burn wound care: topicals, excision, and skin substitutes. Presented at the Acute Burn Trauma: Meeting the Challenge – United States Army Institute of Surgical Research Burn Symposium, San Antonio, Texas, 24 April 1990.

Buescher TM: Management of chemical burns. Presented at the Acute Burn Trauma: Meeting the Challenge – United States Army Institute of Surgical Research Burn Symposium, San Antonio, Texas, 24 April 1990.

McManus WF: Burns in children: how are they different? Presented at the Acute Burn Trauma: Meeting the Challenge – United States Army Institute of Surgical Research Burn Symposium, San Antonio, Texas, 24 April 1990.

McManus WF: Aeromedical transport. Presented at the Acute Burn Trauma: Meeting the Challenge - United States Army Institute of Surgical Research Burn Symposium, San Antonio, Texas, 24 April 1990.

Pruitt BA Jr: Incidence and pathophysiology of burns. Presented at the Acute Burn Trauma: Meeting the Challenge - United States Army Institute of Surgical Research Burn Symposium, San Antonio, Texas, 24 April 1990.

Rue LW 3d: Management of electrical injury. Presented at the Acute Burn Trauma: Meeting the Challenge - United States Army Institute of Surgical Research Burn Symposium, San Antonio, Texas, 24 April 1990.

Waymack JP: Diagnosis and treatment of burn wounds infections. Presented at the Acute Burn Trauma: Meeting the Challenge - United States Army Institute of Surgical Research Burn Symposium, San Antonio, Texas, 24 April 1990.

DePew CL: Standards of nursing care for the burn patient in the resuscitative phase. Presented at the Acute Burn Trauma: Meeting the Challenge - United States Army Institute of Surgical Research Burn Symposium, San Antonio, Texas, 25 April 1990.

Harden N: Physical and occupational therapy roles in burn care. Presented at the Acute Burn Trauma: Meeting the Challenge - United States Army Institute of Surgical Research Burn Symposium, San Antonio, Texas, 25 April 1990.

Hollan E: Infection control in the burn unit. Presented at the Acute Burn Trauma: Meeting the Challenge - United States Army Institute of Surgical Research Burn Symposium, San Antonio, Texas, 25 April 1990.

Milner EA: Nutritional considerations following burn injuries. Presented at the Acute Burn Trauma: Meeting the Challenge - United States Army Institute of Surgical Research Burn Symposium, San Antonio, Texas, 25 April 1990.

Molter NC: Pain management. Presented at the Acute Burn Trauma: Meeting the Challenge - United States Army Institute of Surgical Research Burn Symposium, San Antonio, Texas, 25 April 1990.

Rue LW 3d: Management of inhalation injury. Presented at the Acute Burn Trauma: Meeting the Challenge - United States Army Institute of Surgical Research Burn Symposium, San Antonio, Texas, 25 April 1990.

Summers TM: Psychosocial aspects of burn care. Presented at the Acute Burn Trauma: Meeting the Challenge - United States Army

Institute of Surgical Research Burn Symposium, San Antonio, Texas, 25 April 1990.

Pruitt BA Jr: Infection problems of burn patients. Presented at the Second Scandinavian Burn Conference, Bergen, Norway, 26 April 1990.

Pruitt BA Jr: Burns in the high risk patient. Presented at the Second Scandinavian Burn Conference, Bergen, Norway, 27 April 1990.

Chapman T: Initial management of burn victims in the theater of operation. Presented at the Field Nursing Symposium, Darnell Army Community Hospital, Fort Hood, Killeen, Texas, 27 April 1990.

Chapman T: Initial management of the burn victim. Presented at the University of Wisconsin School of Nursing, Madison, Wisconsin, 30 April 1990.

Chapman T: Initial management of the burn victim. Presented at University Hospital, Madison, Wisconsin, 2 May 1990.

Chapman T: Initial management of the burn victim. Presented at Marshfield General Hospital, Marshfield, Wisconsin, 1 May 1990.

Becker WK: Disaster burn care: recent experiences. Presented to the United States Section of the International College of Surgeons, San Antonio, Texas, 4 May 1990.

Buescher TM: Wound coverage. Presented to the United States Section of the International College of Surgeons, San Antonio, Texas, 4 May 1990.

Cioffi WG: Inhalation injury. Presented to the United States Section of the International College of Surgeons, San Antonio, Texas, 4 May 1990.

McManus WF: Burn wound excision. Presented to the United States Section of the International College of Surgeons, San Antonio, Texas, 4 May 1990.

Rue LW 3d: Metabolic support of the burn patients. Presented to the United States Section of the International College of Surgeons, San Antonio, Texas, 4 May 1990.

Pruitt BA Jr: Current treatment of patients with extensive burns. Presented at the Surgical Grand Rounds, Mt. Sinai Medical Center, Miami Beach, Florida, 4 May 1990.

Waymack JP: Postburn immunologic changes. Presented to the United States Section of the International College of Surgeons, San Antonio, Texas, 4 May 1990.

Pruitt BA Jr: Care of the extensively burned patient. Presented to the United States Section of the International College of Surgeons, San Antonio, Texas, 5 May 1990.

Pruitt BA Jr: Initial care and fluid resuscitation. Presented to the United States Section of the International College of Surgeons, San Antonio, Texas, 5 May 1990.

Pruitt BA Jr: Antimicrobial therapy and wound monitoring. Presented to the United States Section of the International College of Surgeons, San Antonio, Texas, 6 May 1990.

Becker WK: Disaster burn care. Presented as part of the AMEDD Officers' Advanced Course, Fort Sam Houston, San Antonio, Texas, 11 May 1990.

Buescher TM: Disaster burn care. Presented as part of the AMEDD Officers' Advanced Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 11 May 1990.

Cioffi WG Jr: Inhalation injury in burn patients. Presented as part of the AMEDD Officers' Advanced Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 11 May 1990.

McManus WF: Emergency care and resuscitation of burn patients. Presented as part of the AMEDD Officers' Advanced Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 11 May 1990.

Rue LW 3d: Burn patient transfer. Presented as part of the AMEDD Officers' Advanced Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 11 May 1990.

McManus AT: *Klebsiella pneumoniae* in burned patients: relationship of colonization and infection to severity of injury. Presented at the 1990 Annual Meeting of the American Society of Microbiologists, Anaheim, California, 14 May 1990.

Chu C-S: Iontophoretic treatment of *Proteus-mirabilis* burn wound sepsis using silver nylon dressings. Presented at the 90th Annual Meeting of the American Society for Microbiology, Anaheim, California, 14 May 1990.

Keenan J: Compassion knows no boundaries: USSR burn mission. Presented as part of the AMEDD Officers' Advanced Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 16 May 1990.

McManus WF: Advances in burn care. Presented to the Department of Surgery, Staten Island University Hospital, Staten Island, New York, 17 May 1990.

McManus WF: Evolution of burn care. Presented to the Department of Surgery, New York Hospital-Cornell University Medical Center, New York, New York, 17 May 1990.

McManus AT: Microbiologic observations made by the laboratory component of the American burn team sent to aid victims of the Bashkarian burn disaster. Presented at the International Conference on "Disaster Medicine", Moscow, USSR, 22 May 1990.

Becker WK: Mass casualty burn experience. Presented at the International Conference on "Disaster Medicine", Moscow, USSR, 23 May 1990.

McManus AT: Microbiologic observations made by the laboratory component of the American burn team sent to aid victims of the Bashkarian burn disaster. Presented at the International Conference on "Disaster Medicine", Moscow, USSR, 23 May 1990.

Cioffi WG Jr: Burn care update - 1990. Presented to the 5th International Medical Congress, Hospital General DCD Victoria, Tamaplupas, Mexico, May 1990.

Becker WK: Military experience with mass casualty. Presented at the 19th Annual Meeting of the Society of Critical Care Medicine, San Francisco, California, 1 June 1990.

Pruitt BA Jr: Current treatment of patients with extensive burns. Presented at the Surgical Grand Rounds, Mt. Sinai Medical Center, Miami Beach, Florida, 4 June 1990.

Burleson DG: IL-1β measured by ELISA in plasma from patients with thermal injury. Presented at the joint meeting of the American Society for Biochemistry and Molecular Biology and the American Association of Immunologists, New Orleans, Louisiana, 5 June 1990.

McManus AT: Limiting *Pseudomonas aeruginosa* in burn units: a matter of timing? Presented at the 6th International Symposium on Infections in the Immunocompromised Host, Peebles, Scotland, 5 June 1990.

Becker WK: Fungal burn wound infection: a ten year experience. Presented at the 10th Annual Meeting of the Surgical Infection Society, Cincinnati, Ohio, 14 June 1990.

Cioffi WG Jr: The effect of GM-CSF on granulocyte function following thermal injury. Presented at the 10th Annual Meeting of the Surgical Infection Society, Cincinnati, Ohio, 15 June 1990.

Pruitt BA Jr: Methicillin-resistant Staphylococcus: its epidemiologic significance. Presented at the 10th Annual Meeting of the Surgical Infection Society, Cincinnati, Ohio, 15 June 1990.

Waymack JP: Effect of anesthesia and blood transfusions on host response to endotoxin. Presented at the 10th Annual Meeting of the Surgical Infection Society, Cincinnati, Ohio, 15 June 1990.

Pruitt BA Jr: Evaluation and management of patients with inhalation injury. Presented at the NIH Conference on Advances in the Understanding of Trauma and Burn Injury, Washington, DC, 22 June 1990.

Summers TM: Psychosocial aspects of thermal injury. Presented as part of the Clinical Pastoral Education Course, Brooke Army Medical Center, Fort Sam Houston, Texas, 26 June 1990.

Cioffi WG Jr: Inhalation injury. Presented to the Southwest Surgical Society, San Antonio, Texas, June 1990.

Stetz C: Assessment and initial management of the burn victim. Presented as part of the Advanced Burn Life Support Course, Naval School of Health Science, Portsmouth, Virginia, 28 July 1990.

Molter NC: Pain management. Presented to the University of Texas Health Science Center Nursing Students, United States Army Institute of Surgical Research, Fort Sam Houston, Texas, 30 July 1990.

Summers TM: Psychosocial aspects of thermal injury. Presented to the University of Texas Health Science Center Nursing Students, United States Army Institute of Surgical Research, Fort Sam Houston, Texas, 30 July 1990.

Matta CB: Initial management of burn victims in the theater of operation. Presented as part of the United States Air Force Battlefield Nursing Course, Brooks Air Force Base, San Antonio, Texas, 31 July 1990.

Rue LW 3d: Current strategies in burn care. Presented as part of the United States Army Subject Matter Expert Exchange Program, Montevideo, Uruguay, South America, 5 August 1990.

Milner EA: Research opportunities for AMSC officers at the United States Army Institute of Surgical Research. Presented at the Army Medical Specialist Corps Mary Lipscomb Hamrick Research Conference, Xerox Training Center, Leesburg, Virginia, 6 August 1990.

Rue LW 3d: Inhalation injury in current research directives. Presented as part of the United States Army Subject Matter Expert Exchange Program, Montevideo, Uruguay, South America, 6 August 1990.

Stetz C: Research activities at the United States Army Institute of Surgical Research. Presented to the University of

Texas Health Science Center Nursing Students, United States Army Institute of Surgical Research, Fort Sam Houston, Texas, 6 August 1990.

Stetz C: Multidisciplinary aspects of burn care. Presented to the University of Texas Health Science Center Nursing Students, United States Army Institute of Surgical Research, Fort Sam Houston, Texas, 6 August 1990.

Rue LW 3d: Planning a burn unit. Presented as part of the United States Army Subject Matter Expert Exchange Program, Montevideo, Uruguay, South America, 7 August 1990.

Rue LW 3d: Current strategies in burn care. Presented as part of the United States Army Subject Matter Expert Exchange Program, Asunction, Paraguay, South America, 8 August 1990.

Rue LW 3d: Inhalation injury in current research directives. Presented as part of the United States Army Subject Matter Expert Exchange Program, Asunction, Paraguay, South America, 8 August 1990.

Rue LW 3d: Planning a burn unit. Presented as part of the United States Army Subject Matter Expert Exchange Program, Asunction, Paraguay, South America, 9 August 1990.

Pruitt BA Jr: Physiology of sepsis. Presented as part of the Surgical Critical Care Course, Indiana University Medical Center, Indianapolis, Indiana, 11 August 1990.

Pruitt BA Jr: The treatment of septic shock. Presented as part of the Surgical Critical Care Course, Indiana University Medical Center, Indianapolis, Indiana, 11 August 1990.

Pruitt BA Jr: Pulmonary complications of burn patients. Presented at the Pulmonary Grand Rounds, Cedar-Sinai Medical Cener, Los Angeles, California, 15 August 1990.

Summers TM: Crisis and family. Presented as part of the Critical Care Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 24 August 1990.

Pruitt BA Jr: Shock and fluid resuscitation. Presented as part of the Advanced Burn Life Support Provider/Instructor Course, Lincoln, Nebraska, 27 August 1990.

Matta CB: Initial management of burn victims in the theater of operation. Presented as part of the United States Air Force Battlefield Nursing Course, Brooks Air Force Base, San Antonio, Texas, 28 August 1990.

Pruitt BA Jr: Shock and fluid resuscitation. Presented as part of the Advanced Burn Life Support Provider/Instructor Course, Lincoln, Nebraska, 28 August 1990.

Summers TM: Management of stress and crisis. Presented as part of the Critical Care Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 29 August 1990.

Vaughan GM: Syrian hamster pineal isoproterenol responsiveness extends into the early light phase. Presented at the 5th Triannual Colloquium of the European Pineal Study Group, Guildford, Surrey, England, 3 September 1990.

McManus AT: Environmental effects on the incidence and outcome of gram(-) bacteremia in burns. Presented at the 2nd International Conference of the Hospital Infection Society, Kensington, London, England, 4 September 1990.

Pruitt BA Jr: The electric bandaid. Presented to the International Surgical Group, Montreal, Canada, 14 September 1990.

Pruitt BA Jr: Stress-related lesions of the lower GI tract in severely injury patients. Presented to the Halsted Society, Cincinnati, Ohio, 15 September 1990.

Pruitt BA Jr: Fluid resuscitation following injury. Presented to the Department of Surgery, Truman Medical Center, Kansas City, Missouri, 27 September 1990.

Pruitt BA Jr: The diagnosis and treatment of opportunistic infections in injured man. Presented at the Medicine Grand Rounds, Department of Surgery, Truman Medical Center, Kansas City, Missouri, 28 September 1990.

Pruitt BA Jr: Current treatment of the extensively burned patient. Presented at St. Joseph's Hospital, Kansas City, Missouri, 29 September 1990.

Pruitt BA Jr: Current treatment of the extensively burned patient. Presented at St. Luke's Hospital, Kansas City, Missouri, 29 September 1990.

Cioffi WG: Disaster management. Presented at the 1st International Meeting on Burns and Fire Disasters, Palermo, Sicily, September 1990.

Driscoll DM: Compassion knows no boundaries: USSR burn mission. Presented at the Emergency Nurses Association Teaching Institute, Chicago, Illinois, 4 October 1990.

Pruitt BA Jr: The changing epidemiology of infection in burn patients. Presented at the 3rd Surgical Infections Seminar,

Uniformed Services University of the Health Sciences, Bethesda, Maryland, 4 October 1990.

Cioffi WG Jr: High-frequency ventilation Presented as part of the Staff In-Service, United States Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 12 October 1990.

Kim SH: Viral infection in severely burned patients: a review of seven year's experience (1981-7). Presented at the 84th Annual Scientific Assembly of the Southern Medical Association, Nashville, Tennessee, 12 October 1990.

Burleson DG: Relationship of lymphocyte subpopulations to severity of injury in burned patients. Presented at the 27th National Meeting of the Society for Leukocyte Biology, 12th Annual International Research Congress and 12th Leukocyte Culture Conference, Heraklion, Crete, Greece, 16 October 1990.

Driscoll DM: Management of burn victims in the theater of operation. Presented as part of the United States Air Force Battlefield Nursing Course, Brooks Air Force Base, San Antonio, Texas, 23 October 1990.

Duncan DJ: Aeromedical transport of the burn patient. Presented to the Recruiting Command, Montgomery, Alabama, 23 October 1990.

Duncan DJ: Initial management of the burn trauma patient. Presented to the Recruiting Command, Montgomery, Alabama, 23 October 1990.

Stetz C: Nursing care of burn wounds. Presented to the Recruiting Command, Montgomery, Alabama, 23 October 1990.

Summers TM: Psychosocial aspects of thermal injuries. Presented to the Recruiting Command, Montgomery, Alabama, 23 October 1990.

Pruitt BA Jr: History of burn care. Presented to the Fresno Surgical Society, Fresno, California, 2 November 1990.

Pruitt BA Jr: Pulmonary complications in burn patients. Presented at the Valley Medical Center, Fresno, California, 2 November 1990.

McManus WF: Management of mass casualties. Presented at the University of Texas Health Sciences Center at Dallas, Richardson, Texas, 2 November 1990.

Cioffi WG Jr: Alternatives to conventional ventilatory support. Presented as part of Staff In-Service, United States Army

Institute of Surgical Research, Fort Sam Houston, San Antonio. Texas, 5 November 1990.

Becker WK: Fungal colonization of the burn wound. Presented at the 8th International Congress on Burn Injuries, New Delhi, India, 14 November 1990.

Burleson DG: The effect of GM-CSF on lymphocyte subpopulations in burned patients. Presented at the 8th International Congress on Burn Injuries, New Delhi, India, 14 November 1990.

Cioffi WG Jr: Alternatives to conventional ventilatory support. Presented at Loyola University Symposium on Infection and Critical Care, Chicago, Illinois, 14 November 1990.

Pruitt BA Jr: The feedback loop of burn research. Presented at the 8th International Congress on Burn Injuries, New Delhi, India, 14 November 1990.

Vaughan GM: Altered TSH-thyroid axis control in burned rats. Presented at the 8th International Congress on Burn Injuries, New Delhi, India, 14 November 1990.

Chu C-S: Iontophoretic treatment of burn wound sepsis using silver-nylon dressings. Presented at the 8th International Congress on Burn Injuries, New Delhi, India, 15 November 1990.

Hander EW: Design of a comprehensive clinical and research data system for a burn center. Presented at the 8th International Congress on Burn Injuries, New Delhi, India, 15 November 1990.

Pruitt BA Jr: Recent developments in burn care. Presented at Sion Hopsital and Medical School, Bombay, India, 15 November 1990.

Kim SH: Pitfalls in the burn wound biopsy interpretation. Presented at the 8th International Congress on Burn Injuries, New Delhi, India, 16 November 1990.

McManus AT: *Klebsiella pneumoniae* in burned patients: relationship of colonization and infection to severity of injury. Presented at the 8th International Congress on Burn Injuries, New Delhi, India, 16 November 1990.

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ABSTRACT

PROJECT NUMBER: 3M162787A874, 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 1990 - 31 December 1990

INVESTIGATORS: Roger L. Wesley, MD, Captain, MC
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, 159 patients were anesthetized a total of 436 times, an average of 2.7 times per patient. The most commonly used anesthetic agent was narcotics (88.8%) followed by isoflurane (18.3%).

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 and 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 (1987-90)

Agent	1987		1988		1989		1990*	
	Number	%	Number	%	Number	%	Number	%
Enflurane	129	27.8	23	4.9	3	0.6	-	-
Halothane	29	6.3	7	1.5	12	2.4	12	2.8
Isoflurane	196	42.3	278	59.5	167	33.9	80	18.3
Ketamine hydrochloride	57	12.3	35	7.5	36	7.3	21	4.8
Local	9	1.9	9	1.9	6	1.2	9	2.1
Narcotics	39	8.6	114	24.5	269	54.7	387	88.8
Nitrous oxide	4	0.9	1	0.2	1	0.2	94	21.6
TOTAL	**463**	**100.0**	**467**	**100.0**	**493**	**100.0**	*	*

*Two separate methods were used to enter data into the data base. MAJ Wesley 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 1990.

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 50-60% 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 used in 1 patient during this reporting period. This was for a patient with a full stomach (rapid sequence induction) with a recent (8 h postburn) small electrical burn. Thus, succinylcholine was not contraindicated, i.e., no risk of hyperkalemia. This 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 93% 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 is a serious problem. Several methods were employed to maintain body temperature during anesthesia. Ambient temperatures were maintained between 85°F and 90°F. Anesthetic gases were heated and humidified and radiant heat lamps were used when necessary. Disposable K-thermia™ heating blankets were also helpful and were most effective when used on children. 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* (1988-90)

Monitor/Parameter	1988		1989		1990	
	Number	%	Number	%	Number	%
Pulse oximeter (hemoglobin saturation)	465	99.8	488	99.0	435	99.8
Inspired oxygen concentration	461	98.9	493	100.0	430	98.6
Temperature	460	98.7	485	98.4	429	98.4
End-tidal carbon dioxide	459	98.5	473	95.9	415	95.2
Pulmonary function	423	90.8	461	93.5	403	92.4
Arterial line	138	29.6	124	25.1	147	33.7
Swan-Ganz catheter	29	6.2	42	8.5	51	11.7
Central venous pressure	14	3.0	21	4.3	54	12.4

*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 (1971-90)

Year	Number of Patients	Number of Patients Anesthetized	% of All Patients	Number of Times Anesthetized	Number of Times Anesthetized Per Patient
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 (1986-90)

Procedure	1986		1987		1988		1989		1990	
	Number	%	Number	%	Number	%	Number	%	Number	%
Autograft	372	47.0	389	43.8	395	42.7	424	43.6	385	45.3
Chondrectomy	1	0.1	5	0.6	2	0.2	6	0.6	5	0.5
Excision	303	38.3	397	44.7	421	45.6	453	46.6	398	46.8
Eye and lid	19	2.4	9	1.0	11	1.2	1	0.1	1	0.1
Intra-abdominal	4	0.5	7	0.8	6	0.6	17	1.7	5	0.5
Orthopedic	29	3.7	27	3.0	36	3.9	25	2.6	23	2.7
Plastic	5	0.6	5	0.6	12	1.3	11	1.1	21	2.5
Other	58	7.3	50	5.6	41	4.4	36	3.7	12	1.4
TOTAL	**791**	**100.0**	**889**	**100.0**	**924**	**100.0**	**973**	**100.0**	**850**	**100.0**

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
					15 Oct 90	DD-DR&E(AR) 636
3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5 SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISP'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
NONE	A. New	U	U		CX	

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62787A	3M162787A874	CK	161
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code) (U) Effect of Nutritional Support on Immune Function in Patients with Thermal Injury - A Component Study

12. SUBJECT AREAS
0605 Medicine and Medical Research 0608 Food, Food Service, and Nutrition

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
9010	9209	DA	C. In-House

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

18. RESOURCES ESTIMATE

FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
90	0.0	0
91	0.1	50

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME: US Army Institute of Surgical Research	a. NAME: US Army Institute of Surgical Research
b. ADDRESS (Include zip code): Fort Sam Houston, San Antonio, Texas 78234-5012	b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-5012
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR: BECKER, W K
d. TELEPHONE NUMBER (Include area code): 512-221-2720	d. TELEPHONE NUMBER (Include area code): 512-221-3349
	f. NAME OF ASSOCIATE INVESTIGATOR (If available): CIOFFI, W G
	g. NAME OF ASSOCIATE INVESTIGATOR (If available): MILNER, E A

21. GENERAL USE: FINA
MILITARY/CIVILIAN APPLICATION: M

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Burns (Injuries); (U) Nutrients; (U) Nutrition; (U) Hypermetabolism; (U) Immunosuppression; (U) Therapy

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

23/24. (U) The objective of this work is to compare the efficacy of various nutritional components specifically designed to promote immune function in hypermetabolic patients. 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), 7, 14, 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, REE, 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 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 will occur; however, these variables will be monitored in case such a difference should occur.

25. (U) 8810 - 8909. Not applicable.

(U) 8910 - 9009. Not applicable.

(U) 9010 - 9109. 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 1991.

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

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
				DA335682	01 Oct 91	DD-DR&E(AR) 636
3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISP'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
15 Oct 90	D. Chg	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62787A	3M162787A874	CK	161
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c. CONTRIBUTING				

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9010	9209	DA	C. In-House

17. CONTRACT/GRANT MILITARY RELEVANCY CERTIFIED APPROVED BY Basil A Pruitt

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

18. RESOURCES ESTIMATE

a. FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
91	0.5	27
92	0.5	28

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME: US Army Institute of Surgical Research	a. NAME: US Army Institute of Surgical Research
b. ADDRESS (Include zip code): Fort Sam Houston, San Antonio, Texas 78234-5012	b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-5012
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR: BECKER, W K
d. TELEPHONE NUMBER (Include area code): 512-221-2720	d. TELEPHONE NUMBER (Include area code): 512-221-3349
21. GENERAL USE: FIC MILITARY/CIVILIAN APPLICATION: M	e. NAME OF ASSOCIATE INVESTIGATOR (If available): CIOFFI, W G f. NAME OF ASSOCIATE INVESTIGATOR (If available): MILNER, E A

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Burns (Injuries); (U) Nutrients; (U) Nutrition, (U) Hypermetabolism; (U) Immunosuppression; (U) Therapy

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

23/24. (U) The objective of this work is to compare the efficacy of various nutritional components specifically designed to promote immune function in hypermetabolic patients. 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), 7, 14, 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, REE, 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 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 will occur; however, these variables will be monitored in case such a difference should occur.

25. (U) 9010 - 9109. 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 1991. Equipment and supplies have been ordered and work will be initiated shortly.

DD FORM 1498 83 MAR — EDITION OF MAR 66 IS OBSOLETE — * U.S.G.P.O. 1988-491-003/50329

SUPPLEMENTAL WORK UNIT INFORMATION FOR THE PROJECT ENTITLED "EFFECT OF NUTRITIONAL SUPPORT ON IMMUNE FUNCTION IN PATIENTS WITH THERMAL INJURY - A COMPONENT STUDY"

Subrecord/Linking Accession Number: Not applicable.

Search Control Data: W6R09K/W6R10L, 9 October 1990.

Product Identification: For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1991*.

Unclassified Special Categories: Volunteers: Adults; RA II.

ABSTRACT

PROJECT NUMBER: 3M162787A874, 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

PERIOD COVERED IN THIS REPORT: 15 October 1990 - 30 September 1991

INVESTIGATORS: William K. Becker, MD, Lieutenant Colonel, MC
William G. Cioffi, Jr., MD, Major, MC
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Ronald L. Shippee, PhD, Major, MS
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Basil A. Pruitt, Jr., MD, Colonel, MC

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 (malto dextran)	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 of age. Female patients must have been surgically sterilized, be postmenopausal (> 45 yr of age 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 of age.

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

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 1991. Equipment and supplies have been ordered and work will be initiated 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				1. AGENCY ACCESSION DAOG6971	2. DATE OF SUMMARY 01 Oct 91	REPORT CONTROL SYMBOL DD-DRA&E(AR) 636
3. DATE PREV SUMRY 01 Oct 90	4. KIND OF SUMMARY D. Chg	5. SUMMARY SCTY U	6. WORK SECURITY U	7. REGRADING	8. DISP'N INSTR'N CX	9. LEVEL OF SUM A. WORK UNIT

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

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

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

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

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

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

18. RESOURCES ESTIMATE

a. FISCAL YEARS	a. PROFESSIONAL WORK YEARS	b. FUNDS (In thousands)
91	0.5	185
92	0.5	193

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME: US Army Institute of Surgical Research	a. NAME: US Army Institute of Surgical Research
b. ADDRESS (Include zip code): Fort Sam Houston, San Antonio, Texas 78234-5012	b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-5012
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR: MC MANUS, W F
d. TELEPHONE NUMBER (Include area code): 512-221-2720	d. TELEPHONE NUMBER (Include area code): 512-221-8301
21. GENERAL USE: FIC MILITARY/CIVILIAN APPLICATION: M	e. NAME OF ASSOCIATE INVESTIGATOR (If available) g. NAME OF ASSOCIATE INVESTIGATOR (If available)

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

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

23/24. (U) The objective of this work is to evaluate the efficacy of 5% aqueous mafenide acetate-soaked dressings, employed either for final debridement of burn wounds or following application of meshed cutaneous autograft to prevent infection and desiccation of the tissue exposed in the interstices of such grafts. Patients admitted to this Institute for care following thermal, chemical, or electric injury will be treated with 5% aqueous mafenide acetate soaks daily.

25. (U) 9010 - 9109. One hundred and twenty-eight patients were treated with 5% aqueous mafenide acetate soaks. Four of these patients exhibited mild cutaneous atopy. This low incidence of mild side effects of 5% aqueous mafenide acetate and its continued clinical effectiveness speak for the continued use of this valuable therapeutic agent.

DD FORM 1498 83 MAR — EDITION OF MAR 68 IS OBSOLETE — * USGPO: 1988-491-003/10329

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

Subrecord/Linking Accession Number: Not applicable.

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

Product Identification: For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report* for fiscal years 1977-91.

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

ABSTRACT

PROJECT NUMBER: 3M162787A874-00, Applied Research and Exploratory Development

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

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

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

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

During this reporting period, 5% aqueous mafenide acetate dressings have continued to be efficacious in the care of the burn wound. One hundred and twenty-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 3.1% incidence of skin rash (a opy) 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 128 of 216 patients (59.2%). The 5% aqueous mafenide acetate-soaked dressings are used as wet-to-dry dressings to debride nonviable tissue elements in preparation for split-thickness autograft procedures or as continuous wet dressings to protect freshly excised wounds that are not autografted. In addition, when meshed cutaneous autografts are applied, dressings are soaked with 5% aqueous mafenide acetate solution to decrease the rate of bacterial growth and to prevent desiccation of tissue exposed in the interstices of such grafts.

Four patients (3.1%) 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				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
				DAOG6970	01 Oct 91	DD-DR&E(AR) 636
3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
01 Oct 90	D. Chg	U	U		CX	

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

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

12. SUBJECT AREAS
0605 Medicine and Medical Research 0615 Pharmacology

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

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

a. DATE EFFECTIVE		18. RESOURCES ESTIMATE		
b. CONTRACT/GRANT NUMBER		a. FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS *(In thousands)*
c. TYPE	d. AMOUNT	91	0.5	194
e. KIND OF AWARD	f. CUM/TOTAL	92	0.5	36

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

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

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

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

25. (U) 9010 - 9109. In order to investigate the pineal model of sympathetic responsiveness, it will be necessary to assess circulating levels of melatonin both during the day and night and in vitro from incubated pineals. A new assay system with iodinated tracer and ammonium sulfate precipitation improved detectability and was able to define the response of incubated rat pineals to isoproterenol. Use of tritiated melatonin allowed the determination that melatonin recovery from buffer and plasma in the extraction procedure did not differ. The large within-assay coefficient of variation (often 20%) was improved (to 10%) by use of a second antibody and one-half the concentration of ammonium sulfate for precipitation, which reduced the nonspecific binding from 8-9% to 1.7%. The least detectable was lowered to 2 pg/ml for assay of 0.25 ml of sample. The problem of altered assay recovery upon dilution of hamster serum appears to have been improved or resolved.

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

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

Subrecord/Linking Accession Number: Not applicable.

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

Product Identification: For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report* for fiscal years 1980-91.

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

ABSTRACT

PROJECT NUMBER: 3M162787A874, Applied Research and Exploratory Development

PROJECT TITLE: STUDIES OF THE NEUROENDOCRINE ABNORMALITIES IN BURN INJURY: In vitro Response of Burned Rat Pineals to Isoproterenol (ISO) - Use of a New Melatonin (MEL) Assay and Its Further Development

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

INVESTIGATORS: George M. Vaughan, MD, Colonel, MC
Basil A. Pruitt, Jr., MD, Colonel, MC

The Stockgrand MEL antibody was used in a RIA procedure involving sample extraction, antibody reaction with eluates and radioiodinated MEL tracer, and ammonium sulfate separation. Single rat pineals taken during daytime and incubated in vitro produced approximately 12 ng MEL (measured in the medium) by 4 h and 20 ng by 8 h of incubation. With 10^{-6} M ISO in the medium, production was 60 ng by 4 h and 150 ng by 8 h. The production at 4 h with and without ISO was the same for pineals taken from rats with a 22% total body surface area burn as for pineals from rats with a sham burn. Assessment of a modified procedure for MEL assay in serum (involving a second-antibody system and a lesser concentration of ammonium sulfate as separant) showed a marked reduction in nonspecific binding and in assay variation. The least detectable concentration in buffer (2.8 pg/ml) allowed estimation of nocturnal serum values higher than that. Such samples (pooled) for Syrian hamsters were estimated at 13.8 pg/ml with 100% assay recovery. Serial dilutions of this serum source approached the least detectable and roughly approximated parallelism with the projected standard curve.

IN VITRO RESPONSE OF BURNED RAT PINEALS TO ISOPROTERENOL (ISO): USE OF A NEW MELATONIN (MEL) ASSAY AND ITS FURTHER DEVELOPMENT

Abnormalities of sympathetic function occur in burn injury, with increased sympathetic tone underlying further increases occurring episodically during care of patients and terminal sympathetic unresponsiveness in those who do not survive (1). MEL is produced in the pineal gland and is the only known hormone secreted systemically and whose primary stimulus for production is norepinephrine. Sympathetic stimulation of the pineal has been reviewed previously (2,3). MEL may provide a useful index of sympathetic function to employ in studies of the control of sympathetic activity and responsiveness. An earlier study (4) disclosed what appeared to be a blunting of the nocturnal MEL surge in burn patients. That study employed an assay which, though perhaps the best available one at the time, was not optimal for serum samples. This was partly because nonspecific serum elements appear to provide sufficient "noise" that values (primarily those from daytime with low MEL) cannot adequately be assessed in that RIA. For the Syrian hamster, a model of pineal function similar to that in humans, this problem is magnified in that the nocturnal serum MEL peak is apparently lower in this species than in rats and humans and probably cannot be assessed adequately with that assay. In fact, an adequate assessment of the magnitude of serum MEL concentration in the Syrian hamster has not been available.

We previously developed another RIA for MEL (5) with use of a more specific antibody (6) which allowed greater sensitivity and a more reliable assessment of lower levels. As a further general test of this assay system, we have employed ISO stimulation of rat pineals in vitro. We obtained initial results of in vitro ISO stimulation of pineals in the burn rat model. Finally, we modified the assay to address some of its remaining shortcomings and report the concentration found in several sources of serum.

MATERIALS AND METHODS

The MEL RIA (5) employed the Stockgrand (6) ovine antiserum 704/8483 (Stockgrand Ltd, United Kingdom) diluted 1:3000 in 0.1 M tricine buffer in saline, 0.1% gelatin, and 100 mg/L thimerosal, pH 7.0 (the assay buffer). In this buffer was separately also dissolved the tracer, ^{125}I-iodomelatonin (IM.215, 2000 Ci/mmol (Amersham Corporation, Arlington Heights, IL)). The standard MEL concentrations were diluted either in this buffer or in phosphate-buffered saline gelatin. Samples (0.25 ml standards or unknowns) were extracted in 12 X 75-mm glass tubes with 2 ml chloroform (certified ACS, assay 99.9%, ethanol preservative 0.75%, ORM-A UN1888 (Fisher Scientific Company, Fair Lawn, NJ)). The chloroform phase was washed (vortexed) successively with 0.25 ml 0.1 N NaOH, then deionized H_2O. Another aliquot of H_2O (without vortexing) was used to attempt to remove the last traces of NaOH.

The chloroform was evaporated (approximately 1 h) in a vacuum centrifuge, followed by elution in 0.5 ml assay buffer at 4°C overnight, followed by a wash with 2 ml petroleum ether (certified ACS, E139-1 (Fisher Scientific Company)) and transfer of 0.4 ml washed eluate to another set of glass tubes in an ice bath. To these tubes were added 0.05 ml 1% physiologic sheep gamma globulin carrier in assay buffer; 0.05 ml 1:3000 antiserum; and 0.05 ml tracer, a constant 4000-8000 cpm. The mixture was incubated overnight at 4°C followed by addition of 2 ml cold ammonium sulfate 35 g/dl in H_2O, another incubation (30 min) at 4°C, and centrifugation at 4°C and 2000 g for 30 min. The precipitates (bound cpm) were counted in a gamma scintillation counter, and the cpm data (corrected only for background) were reduced by four-parameter logistic regression with the preextraction standard concentrations followed by use of the parameters and cpm of unknowns to estimate MEL in pg/ml for samples as they existed prior to extraction.

Male rats weighing a mean 396 g were maintained on a light/dark cycle of 14:10 h and sacrificed at approximately 3-4 h into the light phase. Pineals were taken after decapitation and placed separately in 1 ml minimal essential medium (No. 11-100-22 (Flow Laboratories, Inc., McLean, VA)), Auto Pow, without glutamine, with phenol red and with our addition of $NaHCO_3$ 2 g/L, gentamicin 50 mg/L, and then fetal calf serum to 10% of the total volume, final pH 7.4) for pre-gassing, then incubation at 37°C with or without ISO, under an atmosphere of 95% O_2 and 5% CO_2, as previously described (7,8). In another experiment, rats (mean weight 430 g) were maintained as above and divided into those receiving a standard full-thickness scald burn (22% of the total body surface area) under sodium pentobarbital (35 mg/kg) anesthesia with 15 ml physiologic saline resuscitation given between the times of back and front components of the burn, and those receiving only anesthesia, hair clipping, and resuscitation (sham burn). Burned areas were traced onto paper. These were cut out and weighed (along with area standards for the same paper) in order to determine the burned area. Total body surface area was determined as 10 times the body weight in grams raised to the two-thirds power. On postburn day 8, rats were decapitated as above and pineals were placed in vials for a 4-h incubation as above with or without ISO. The medium was sampled at various times in all incubation studies and frozen at -60°C. Suitable dilutions were made for MEL assay so that most samples gave 20-80% inhibition of tracer binding by antibody.

Following this, because of rather high nonspecific binding and within-assay coefficients of variation (CV), the assay was modified by adding anti-sheep-gamma globulin (second antibody) to the reaction mixture either at the time when the first (ovine) antibody and carrier were added or at other times. Additionally, various concentrations of separant (ammonium sulfate) and buffer substitution were assessed.

Data analyses were performed with the BMDP statistical package (9) on a VAX 4000 system.

RESULTS

For the assay as used in the first part of these studies (without second antibody), the nonspecific binding (NSB_0) cpm in tubes without antibody or MEL was approximately 6% of total cpm. Another estimate (NSB_B) of nonspecific binding, the logistic projection of cpm for infinite MEL concentration, was approximately 9%. Neither estimate was used in calculating MEL concentration, because this was done by logistic regression of the uncorrected cpm on preextraction concentration. The B_0 (proportion of NSB_B-corrected cpm bound by antibody out of the total cpm in the absence of MEL) was approximately 33%. The analytic least detectable (MEL concentration at a cpm equal to the mean minus 2SD for cpm of 9 tubes with no MEL) was usually about 5 pg/ml of buffer (before extraction) but occasionally extended higher, up 13 pg/ml, depending on the variability of response to the zero-MEL standards. The ED_{50} (MEL concentration producing cpm halfway between those for zero-MEL and NSB_B) was usually about 30 pg/ml of buffer before extraction. Based on buffer samples containing between 12.5 and 250 pg/ml, the mean within-assay CV was usually 20% but often lower in the 25-100 pg/ml range.

Pooled hamster night serum (collected near the time of the expected peak nocturnal serum MEL) gave values ranging 14 to 22 pg/ml in different runs. Dilution of serum aliquots 1:2 and 1:4 in buffer resulted in progressively lower MEL values with highly variable dilution-corrected recovery. This variability reflected the assay "noise" in the output from samples with small amounts of MEL and less than 10-20% inhibition of tracer binding, at a position on the curve where relatively small changes in binding produce large changes in calculated concentration. Subsequent use of this procedure was confined to samples with sufficient MEL to produce usually more than 20% inhibition.

Figure 1 shows the effect of ISO in the medium of incubating pineals from normal rats. The largest rise in MEL production occurred at 10^{-6} M ISO. Previous results indicated that rat pineals such as these weigh approximately 1.5 mg. As can be seen, they can produce about 20 ng/pineal by 8 h in the unstimulated state and 150 ng/pineal by 8 h when stimulated by ISO. Table 1 indicates that 10^{-6} M ISO stimulates in vitro production of MEL by rat pineals with no discernable effect of a 22% burn at postburn day 8.

It is to be noted that, if in the assay as used above without second antibody, one uses > 35 g/dl ammonium sulfate as the concentration for the separant (2 ml), the NSB_0 rises with no appreciable augmentation of B_0. On the other hand, when the separant concentration is < 35 g/dl, both NSB_0 and B_0 fall, such

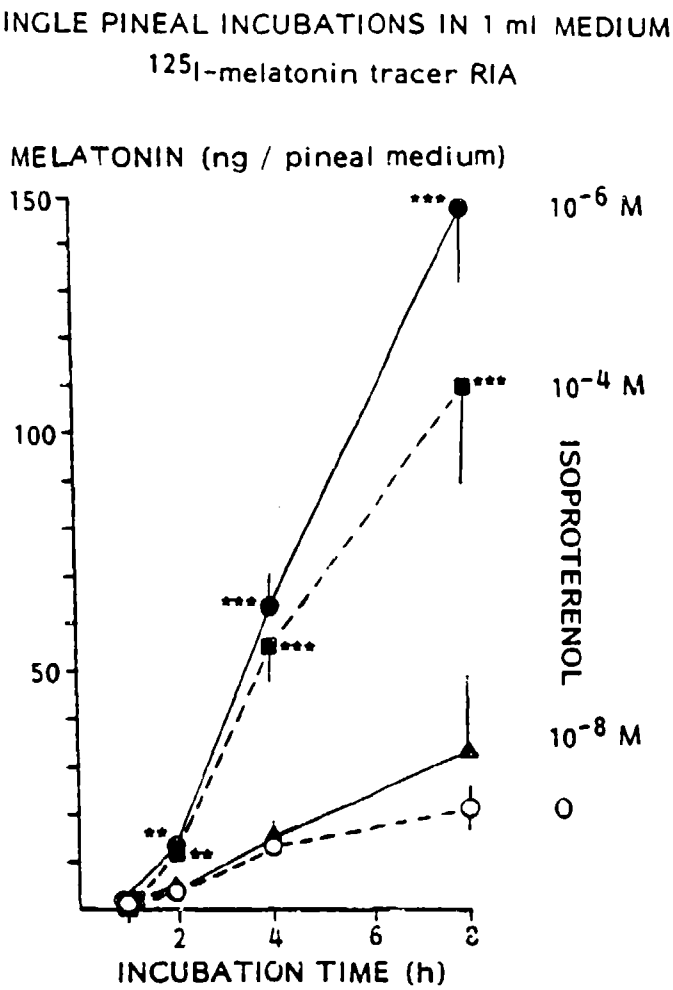

FIGURE 1. Mean ± SE MEL accumulated in the medium with a pineal and several concentrations of (or no) ISO, sampled at various times (n=4/group). **P < 0.01, ***P < 0.001 vs zero ISO at the same time point (separate variance t test).

that almost none of the specifically bound tracer is precipitated when 1 ml of buffer is substituted for the ammonium sulfate separant.

In the following procedures, 0.04 ml unextracted buffer was used in place of the eluate for addition of assay components in the absence of MEL. Initial trials of further modification of the assay by adding 0.05 ml of various dilutions of a second antibody (Ab_2) were made using 1 ml cold buffer in place of the ammonium sulfate separant with immediate centrifugation and decanting. Anti-sheep-gamma globulin from two sources (Stockgrand Ltd, United Kingdom, and Sigma Chemical Company, St. Louis, MO) produced no

TABLE 1. Melatonin ± SE in Single Pineal Incubations from Burned and Sham-Burned Rats - Absence (CON) and Presence (ISO) of 10^{-6} M ISO

	Incubation Time (h) =	2	2	4	4
	Medium =	CON	ISO	CON	ISO
	n =	4	4	4	4
Cumulative production (ng/pineal medium)	Burn	3.0 ± 0.5	13.1 ± 2.9*	8.4 ± 0.9	63.0 ± 10.5*
	Sham-burn	2.9 ± 0.4	12.1 ± 1.0**	7.4 ± 0.5	62.8 ± 7.0**
	Incubation Period (h) =	0-2	0-2	2-4	2-4
Production rate (ng/pineal/h)	Burn	1.5 ± 0.2	6.5 ± 1.5*	2.7 ± 0.3	25.0 ± 3.8**
	Sham-burn	1.4 ± 0.2	6.1 ± 0.5**	2.3 ± 0.2	25.3 ± 3.4**

*$P < 0.05$, **$P < 0.01$ vs CON (separate variance t test). There was no influence of burn (full-thickness, 22% total body surface area) detected by two-way ANOVA after accounting for the effect of ISO, whether the variances were or were not (Brown-Forsythe) assumed to be equal.

usable precipitation of specifically bound tracer, as indicated by B_0 of < 9%. However, donkey anti-sheep-gamma globulin from Fitzgerald (40-DS40, Fitzgerald Industries International, Inc., Chelmsford, MA) produced a B_0 of 22% when added undiluted (0.05 ml) at the beginning of the first antibody incubation extended for 24 or 48 h, or when added at 24 h into a 48-h incubation. When this Ab_2 was added after 24 h of first incubation and left for only 60 min before addition of buffer for decantation, B_0 was 15%. Thus, this Ab_2 could most conveniently be added at the beginning of the incubation, in sequence after carrier (sheep gamma globulin, 15131, 0.2 mg/ml, 1% physiologic in buffer (Sigma Chemical Company), first antibody, and tracer. The Ab_2 from Fitzgerald was used in subsequent studies.

With this AB_2 system, 2 ml of several concentrations of cold ammonium sulfate (separant) with immediate centrifugation and decanting was used instead of the buffer before centrifugation and decanting. As seen in Table 2, 35 g/dl ammonium sulfate detected B_0 at 30.3%. But, this was apparent only after subtracting an unacceptable 20% (of total counts) as NSB_0. The optimal concentration appeared to be 17.5 g/dl, at which the B_0 was still 29% (also comparable to that in the previous system with no second antibody), but the NSB_0 fell to 1.1%. An effect of ammonium sulfate in the presence of second antibody was suggested by the fall in B_0 with smaller separant concentrations.

With use of 2 ml 17.5 g/dl ammonium sulfate, different dilutions of the Ab_2 were tested (fig 2). For comparison, 1 ml of buffer was used instead of ammonium sulfate. In both cases, centrifugation and decanting followed immediately. Ammonium sulfate was more effective as a separant than buffer, and undiluted Ab_2 (0.05 ml) seemed optimal.

As seen in Figure 3, with use of 1 ml buffer, precipitation of the bound tracer decays with more time elapsed before centrifugation and decanting. However, with use of 2 ml 17.5 mg/dl ammonium sulfate, the amount of first-antibody bound tracer that was precipitated was higher and stable.

For further study, undiluted Ab_2 (0.05 ml) was added for the entire first antibody incubation and 2 ml cold 17.5 g/dl ammonium sulfate (with 30 min further incubation at 4°C) was used as separant. This modified system was then employed with the usual eluate from extracted samples and MEL standards. In essence, this procedure modification involves use of Ab_2 and 17.5 g/dl ammonium sulfate instead of the previous 35 g/dl ammonium sulfate alone to precipitate bound tracer. Calculations were all based on preextraction concentrations. With this modified (Ab_2) system, five runs were made and all standards and samples were extracted in triplicate in a run.

TABLE 2. Influence of Ammonium Sulfate on Melatonin Tracer Precipitation in the Presence of Undiluted Ab_2

Ammonium Sulfate (g/dl) =	35	17.5	8.75	4.38
B_o (%)	30.3	29.1	26.2	25.4
NSB_o (%)	20.0	1.1	1.5	1.0

Ab_2 indicates second antibody (donkey anti-sheep); B_o, proportion of total tracer counts precipitated in the absence of added nonradioactive melatonin, corrected by subtraction of NSB_o; and NSB_o, proportion of counts precipitated in the absence of both melatonin and first antibody.

Table 3 gives some performance data for this system. Though the B_o and ED_{50} were about the same as those for the previous assay system, other parameters showed considerable improvement. NSB_o and NSB_B showed near-agreement and both were below 2%. Variability was reduced so that the within-assay CV was 5.6% for buffer and 7.5% for serum samples. The between-assay CV was approximately 5%. The least detectable in buffer was 2.8 pg/ml of unextracted standard equivalent.

Pooled hamster night serum MEL was estimated at 13.8 pg/ml, and recovery of MEL added to aliquots of this appeared to be 100%. However, when aliquots of the hamster samples were diluted with buffer, dilution-corrected recovery was above 100% as the expected MEL value approached the least detectable. For burned human serum and normal rat night serum with greater starting concentrations of MEL, dilution-corrected recovery was approximately 100% of predicted. Figures 4 and 5 give the binding profiles for two runs which included serial dilutions of serum samples. The dashed line extends from the point for the undiluted sample in a line ideally parallel to the standard curve. The observed dilution points apparently fell off this line for hamster serum more than did those for the other serum sources. Nevertheless, even with the hamster serum dilutions above 80% B/B_o (bound cpm as a fraction of bound cpm in the absence of MEL), the response was qualitatively in the expected direction approaching parallelism.

DISCUSSION

The presently explored MEL assay as used without an Ab_2 system and with relatively concentrated ammonium sulfate as the separant was adequate to assess the high concentrations of MEL found in medium surrounding an incubated pineal. The resulting estimates agreed closely with those found previously with another assay for incubated normal rat pineals with and without a β-adrenergic stimulus such as norepinephrine or ISO (7,8). These studies showed

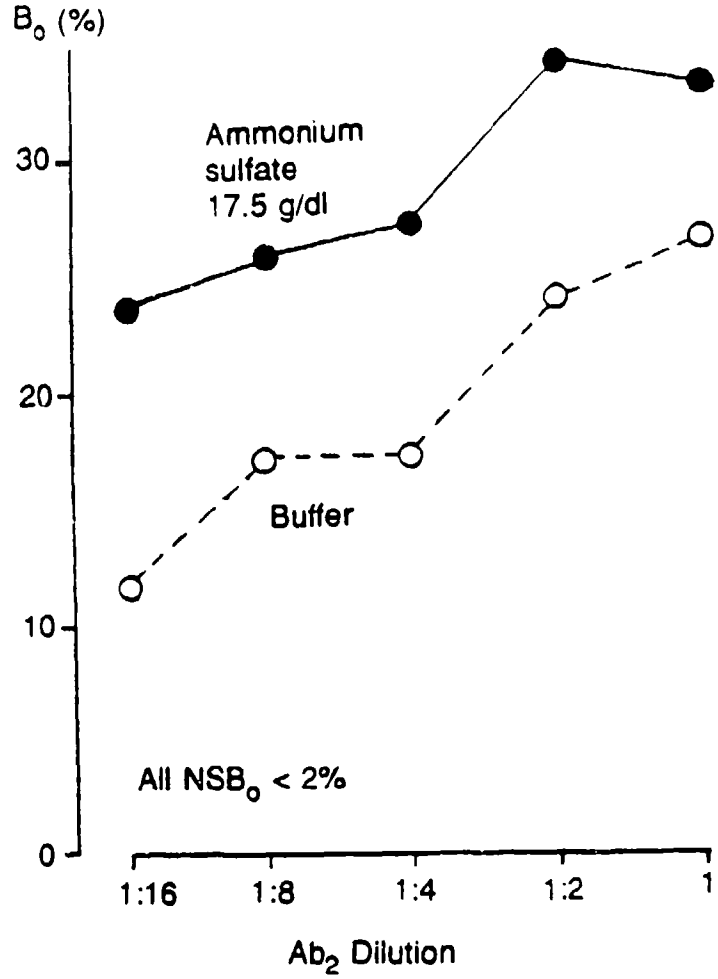

FIGURE 2. Effect of Ab_2 and separant on MEL tracer precipitation. Means of triplicates with 2 ml ammonium sulfate or 1 ml buffer (each at 4–6°C) added as separant at the end of the 20-h incubation with first antibody, carrier, tracer, and second antibody at 4°C. Binding was assessed as counts precipitated by centrifugation and measured after decanting.

that, as expected for most tissues that receive noradrenergic postganglionic innervation, the protective nerve-ending uptake mechanism may interfere with the use of norepinephrine for stimulating the pineal gland (7). Consequently, ISO, which is not taken up in the nerve endings, provides an advantage and has been used (8). The absence of an effect of burn injury on the pineal response to ISO may be due to relatively small size of the burn (22%). This system should, though, provide a model for studying larger burns and the influence of infection and of the other (noncatechol) hormones that change in burn injury and sepsis (corticosteroids, iodothyronines, and glucagon). The blunting of

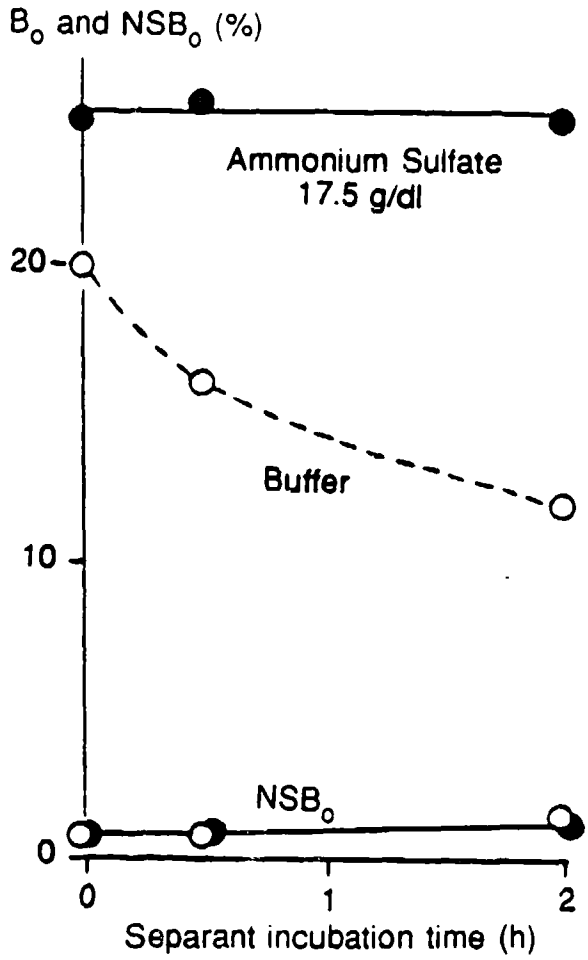

FIGURE 3. Stability of binding in the Ab_2 system with separant. Means of triplicates with 2 ml ammonium sulfate or 1 ml buffer added as separant after the 20-h antibody incubation which included undiluted Ab_2. Antibody and separant incubations were at 4°C.

the nocturnal surge of circulating MEL in burn patients previously observed (4) may have been due to the presence of large burns and sepsis in some of the patients, though this explanation remains to be established.

The antibody used in this assay was chosen on the basis of its specificity, which is more important when measuring MEL in serum. Serum has more antibody-interfering substances than medium from incubated pineals and the MEL concentrations are lower in serum. The presently used antibody had specificity sufficient to be used in unextracted serum (6) wherein it could detect changes in the range found in humans during the nocturnal MEL surge (40-80 pg/ml). However, as originally reported, even with the original use of large (0.5 ml) samples, the least detectable was 10 pg/ml, not

TABLE 3. Trials of the Melatonin Assay with the Ab_2 System

	n	mean	± SE
NSB_o (%)	5	1.10	0.04
NSB_B (%)	5	1.82	0.04
B_o (%)	5	28.0	2.4
Least detectable (pg/ml)	5	2.8	0.4
ED_{50} (pg/ml)	5	28.2	1.4
Within-assay coefficient of variation (%), buffer[a]	5	5.6	0.8
Within-assay coefficient of variation (%), serum[a]	5	7.5	1.0

Pooled Serum	Mean	Between-Assay Coefficient of Variation (%)	n	Mean Recovery (%) ± 50 pg/ml[b]		± 100 pg/ml[b]	Dilution-Corrected Mean Recovery (%) 1:2		1:4
Hamster, night (pg/ml)[c]	13.8	4.2	4	102	(n=2)	99	109	(n=3)	139
Burn patient (pg/ml)[d]	53.6	5.2	4	109		104	96		98
Human, day (pg/ml)[e]	3.4	-	1	96		95	-		-
Rat, night (pg/ml)[c]	128.0	-	2	-		-	104		101
Rat, day (pg/ml)[e]	14.2	-	2	86		93	-		-

[a]Samples containing 12.5-250 pg/ml.
[b]Amount of added MEL (pg/ml).
[c]Samples from normal animals collected near the time of the nocturnal MEL peak.
[d]Samples collected from patients 3-4 h past the nocturnal MEL peak, expected to be almost as high as at the peak.
[e]Samples from normal humans or rats collected during the day when MEL is expected to be low.

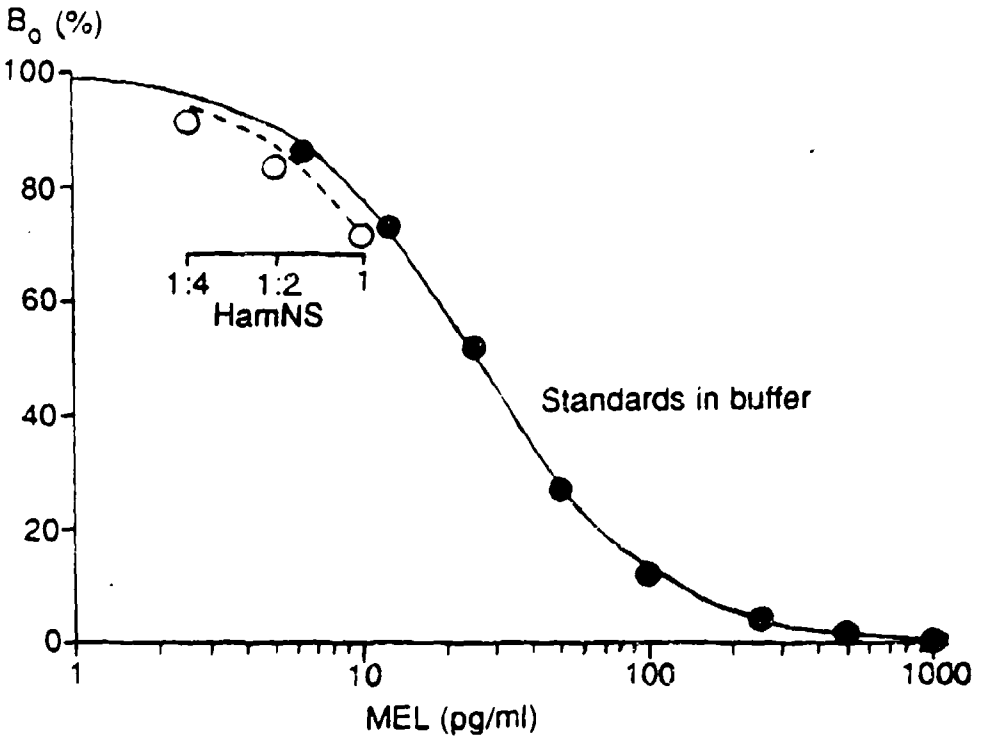

FIGURE 4. Melatonin tracer binding profile with the Ab_2 system. Means of triplicates for B/B_0, NSB_B-corrected proportion of MEL-tracer bound divided by that in the absence of added nonradioactive MEL. Standards are related to the preextraction MEL concentration in buffer. B/B_0 values for HamNS (pooled Syrian hamster night serum collected near the nocturnal MEL peak) dilutions in buffer before extraction are placed arbitrarily on the abscissa with appropriate spacing between dilutions.

practical for the Syrian hamster in which nocturnal MEL appears to be about 14 pg/ml. This least detectable likely resulted from the original use of charcoal-extracted serum as the matrix for standards as well as a large amount of antibody and MEL tracer. The latter was tritiated and of low specific activity compared to the iodomelatonin used currently. We also use one-fourth the amount of antibody. In our procedure including Ab_2, even with one-half the sample volume (herein 0.25 ml), the least detectable is reduced to about 3 pg/ml. Use of an Ab_2 system in the assay provides acceptable CV (5% between assay) for values in the range of nocturnal samples in several species. Though there appears to be some margin for fidelity with lower values, it is still not likely that changes in serum MEL in the range of daytime values can be estimated with fidelity.

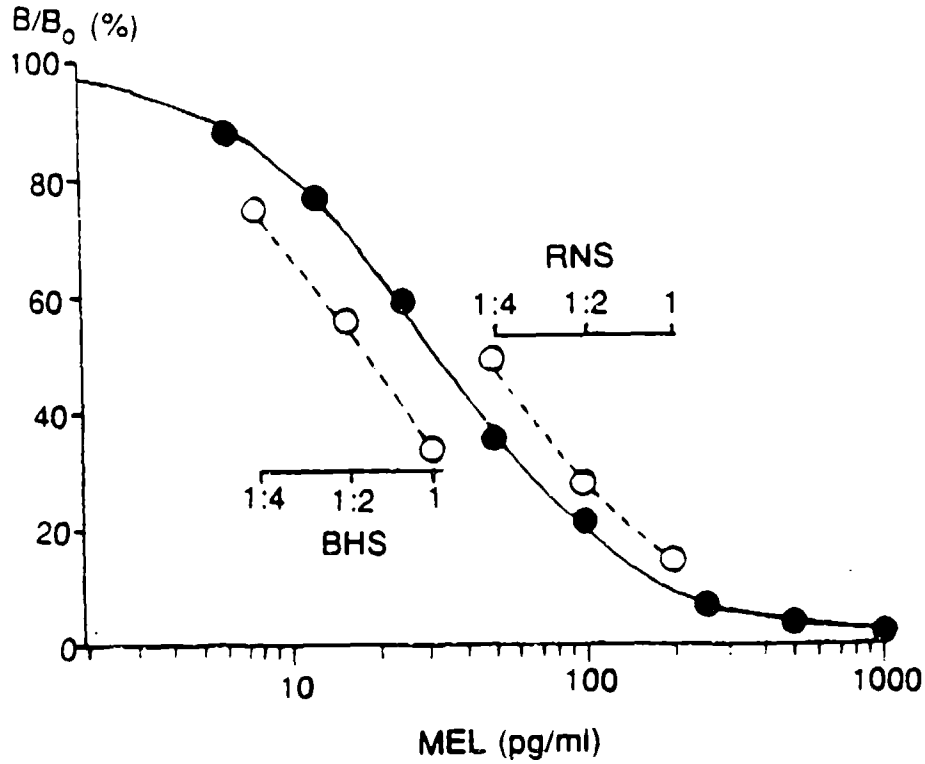

FIGURE 5. Melatonin tracer binding profile with the AB_2 system. Means of triplicates for B/B_0. Standard B/B_0 values in buffer (●) are related to the preextraction nontracer MEL concentration. BHS, pooled burned human serum, collected during the late part of the nocturnal MEL surge. RNS, pooled rat night serum, collected near the peak of the nocturnal surge. B/B_0 values for BHS and RNS dilutions in buffer before extraction are placed at an arbitrary position along the abscissa though the spacing between dilutions is set by the relative MEL concentrations expected from dilution.

PRESENTATIONS

Vaughan GM: Syrian hamster pineal sympathetic responsiveness in the early light phase. Presented at the Annual Meeting of the American Society of Zoologists, San Antonio, Texas, 28 December 1990.

Vaughan GM: Circadian thythms in Harderian gland porphyrins in Sprague-Dawley and Fischer-344 rats exposed to chronic long or short photoperiodic conditions. Presented at the Annual Meeting of the American Society of Zoologists, San Antonio, Texas, 28 December 1990.

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.

PUBLICATIONS

Vaughan GM: Neuroendocrine and sympathoadrenal response to thermal trauma. In Doleček R, Brizio-Molteni A, Molteni A, and Traber D (eds): *Endocrine Response to Thermal Trauma - Pathophysiologic Mechanisms and Clinical Interpretation.* Philadelphia: Lea & Febiger, 1990, Chap 13, pp 267-306.

Vaughan GM: Syrian-hamster pineal sympathetic responsiveness in the early light phase (abstr). *Am Zool* 30(4):PA25, 1990.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION DA314707	2. DATE OF SUMMARY 21 Mar 91	REPORT CONTROL SYMBOL DD-DR&E(AR) 636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISP INSTRN	9. LEVEL OF SUM A. WORK UNIT
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10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62787A	3M162787A874	EA	165
b. CONTRIBUTING				
c. CONTRIBUTING				

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

12. SUBJECT AREAS
0605 Medicine and Medical Research 0615 Pharmacology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
8805	9103	DA	C. In-House

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

a. DATE EFFECTIVE		18. RESOURCES ESTIMATE		
b. CONTRACT/GRANT NUMBER		a. FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
c. TYPE	d. AMOUNT	91	0.1	51
e. KIND OF AWARD	f. CUM/TOTAL	92	0.0	0

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

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

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

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

25. (U) 8805 - 9103. A total of 12 patients were enrolled in this study. This multicenter trial demonstrated no benefit in the rate of donor site healing in patients who received growth hormone versus those who received placebo. In addition, a consistent IgF1 effect could not be demonstrated in patients receiving growth hormone.

DD FORM 83 MAR 1498 EDITION OF MAR 68 IS OBSOLETE. * GPO: 1988 -A51-003/50329

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

Subrecord/Linking Accession Number: Not applicable.

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

Product Identification: For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report* for fiscal years 1988-1991.

Unclassified Special Categories: Volunteers: Adults; RA II.

ABSTRACT

PROJECT NUMBER: 3M162787A874, Applied Research and Exploratory Development

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

INSTITUTION: US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas 78234-5012;[1] Genentech, Inc., 460 Point San Bruno Boulevard, South San Francisco, California 94080;[2] and Harvard Medical School and Brigham and Women's Hospital, 75 Francis Street, Boston, Massachusetts 02115[3]

PERIOD COVERED IN THIS REPORT: 1 October 1990 - 31 March 1991

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Douglas W. Wilmore, MD[3]

Twelve patients from this Institute have been enrolled in this study. In patients receiving growth hormone, the administration of either 5 or 10 mg/day of recombinant human growth hormone resulted in a dose range of 0.05-0.15 mg/kg of ideal body weight. Analysis of the IGF-1 response in these patients indicated the lack of a consistent response as well as IGF levels not significantly elevated over control patients until postburn day 14. Since IGF-1 response is an indicator of growth hormone action, it is hypothesized that this lack of response may be secondary to inadequate drug dosing. There was a wide range of donor site healing times among all patients, ranging from 4-19 days for the treated group and 8-14 days for the control group. This lack of response may be due to inadequate drug dosing or the fact that therapy was initiated at variable times following resuscitation. No untoward effects secondary to administration of recombinant human growth hormone were noted in this group of patients.

The total number of patients required by the sponsor for completion of this multicenter study have been enrolled and data is currently being analyzing by the sponsor. The Institute's portion of the study was completed on 31 March 1991.

THE EFFECT OF RECOMBINANT HUMAN GROWTH HORMONE TREATMENT ON THE RATE OF HEALING ON BURN PATIENTS WHO REQUIRE SKIN GRAFTING

The development of genetic engineering techniques has made available large amounts of naturally occurring peptides. It is now possible to test the potential of such peptides, some of which may be clinically useful in situations which require tissue regeneration. This study was designed to evaluate the efficacy and safety of recombinant human growth hormone on the rate of healing in burn patients.

The overall effect of growth hormone on protein metabolism is illustrated by the well documented increase in linear growth that results from the administration of growth hormone to growth hormone-deficient children. Growth hormone administration improves nitrogen balance, increases somatomedin-C levels, and increases body cell mass in growth hormone-deficient children (1). Growth hormone also exerts a positive effect on nitrogen balance and somatomedin-C in healthy adults (2-4).

Recent studies using methionyl human growth hormone (Protropin®) in calorie-deprived human volunteers illustrated its anabolic effect. Hypocaloric parenteral nutrition resulted in negative balance of nitrogen and potassium. These trends were markedly reversed when growth hormone was given. Changes in body weight followed a similar pattern (5).

Stressed patients, whether septic, postoperative, or victims of burns or trauma, undergo well described metabolic changes that result in negative nitrogen balance and loss of body protein. There is evidence that as the catabolic state continues, it results in decreased resistance to infection, poor wound healing, and in general, prolonged recovery. When patients cannot eat, nutrients may be provided by intravenous feedings, but such an approach is not always successful and parenteral nutrition is associated with significant side effects.

Investigators have attempted to modify the metabolic response to stress with hormonal interventions, notably insulin (6), anabolic steroids (7-8), and growth hormone. Administration of insulin and anabolic steroids has met with little success and has not been generally used in clinical practice. Until recently, growth hormone was not available in sufficient quantity for use as an anabolic agent. The current availability of biosynthetically produced human growth hormone has made it possible to explore the clinical efficacy of this hormone.

A limited number of studies were carried out with pituitary growth hormone, primarily in burned patients and experimental animals. Gump et al (9) demonstrated that burned rats receiving

adequate nutrition and growth hormone did not suffer a catabolic response, but when the burned rats were starved, they lost weight at a greater rate than control animals. In 1960, Soroff et al (10) demonstrated similar positive effects of human growth hormone administration in patients during the anabolic phase of burn recovery.

Liljedahl et al (11) and Wilmore et al (12) both showed that growth hormone caused a significant improvement in nitrogen and potassium balance in the postburn period, the latter specifically with high calorie and protein intake.

The role of growth hormone in postoperative nutrition and nitrogen loss has been examined in a few studies. Rowe and Kinney (13) demonstrated an alteration in substrate utilization in postoperative orthopedic patients given growth hormone, with a fall in respiratory quotient and a shift to lipid substrate. Johnston and Hadden (14) showed no improvement in nitrogen balance after herniorrhaphy in patients treated with growth hormone compared to matched controls. However, nitrogen intakes were low, caloric provision was not measured, and only the immediate postoperative period was studied.

In recent work, Wilmore et al used methionyl human growth hormone (Protropin®) to evaluate whether growth hormone can promote anabolism in surgical patients. Patients (n=9) received a constant parenteral infusion of a hypocaloric diet which provided 1100 kcal/24 h and 1.3 g/kg/24 h protein for at least 2 weeks. During one week, Protropin® (10 mg SC) was given daily and the other week served as the control. Daily balance studies demonstrated that growth hormone resulted in significant retention of nitrogen (+3.4 g/24 h) and phosphorus (+218 mg/24 h) despite provision of only 60% of caloric requirements. Six patients received Protropin® daily (10 mg SC) for up to 25 consecutive days. Significant nitrogen and phosphorus retention occurred over the entire period of growth hormone administration and no significant side effects were observed.

It was the clinical impression of these investigators that the nitrogen retention associated with growth hormone administration was accompanied by accelerated wound healing and an apparent decrease in morbidity and duration of hospital stay.

It was the purpose of this double-blind, randomized, placebo-controlled study to determine whether administration of recombinant human growth hormone accelerates wound healing in burned patients. Because the heterogeneity of burns makes it difficult to evaluate healing among patients, this controlled study focuses on the rate of healing of the patients' skin graft donor sites. However, healing of the primary burn, duration of hospitalization, and mortality were also evaluated.

MATERIALS AND METHODS

Study Design. For this multicenter, randomized, double-blind study, patients were randomized to receive daily subcutaneous or intramuscular injections of 0.2 mg/kg recombinant human growth hormone or placebo until the end of hospitalization. Patients were randomized to treatment or control groups by the Biostatistics and Data Management Division of Genentech, Inc. (South San Francisco CA). Groups were balanced for age, cause, and extent of burn.

The "study wound" was a donor site from which skin was taken for grafting. The donor site was part of a planned and necessary surgical procedure and the care of the donor skin was not greatly altered from standard techniques. The methods for taking the skin and caring for the donor site wound were standardized for all patients. All wounds were inspected and evaluated by one observer.

Number of Patients. Up to 100 patients were authorized for enrollment in this study based on eligibility criteria and informed consent. For purposes of computing statistical power, an average healing time of 12 days for "young" patients and 16 days for "older" patients was used. A 25% reduction in healing time, 3-4 days, was considered clinically significant. Assuming a standard deviation of about 3 days after adjusting for age, cause, and extent of burn, a total of 100 patients would provide at least 95% power for one-tail t tests at the 0.05 α level comparing the treatment and control groups (including adjustments for multiple comparisons).

Patient Criteria. 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 considered for enrollment in the study:

1. Male or female patients $\geq$ 18 and $\leq$ 80 yr old. Female patients were either surgically sterile or postmenopausal (> 45 yr old and the lack of menstrual periods for > 1 yr) or had a negative pregnancy test prior to initiation into the study.

2. Patients with flame or scald burns requiring a skin graft from the anterior upper thigh, buttock, or lateral upper arm. This site was the "test wound" and was evaluated for time of healing.

3. Patients with burns < LD_{75}, the size of burn at which 75% of the patients die at any particular age, using the US Army Institute of Surgical Research probit analysis (15).

4. Patients with successful resuscitation without major complication.

5. Patients who were able to take a minimum of 80% of maintenance energy, protein, and other nutrient requirements by the enteral or parenteral route.

6. Patients with inhalation injuries were eligible. Patients maintained on mechanical ventilation were required to have a satisfactory PO_2 and PCO_2 on < 60% oxygen.

7. Patients with a prehospitalization weight between 80 and 140% normal body weight as determined from standard tables for age and sex (Desirable Weight Tables, Metropolitan Life Insurance Company, 1959).

8. Patients with a single uncomplicated fracture of a long-bone were eligible.

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

1. Patients < 18 or > 80 yr old.

2. Patients who were pregnant or nursing.

3. Patients without flame or scald burns requiring a skin graft from the anterior upper thigh, buttock, or lateral upper arm.

4. Patients with burns > LD_{75}, the size of burn at which 75% of the patients die at any particular age, using the US Army Institute of Surgical Research probit analysis (15).

5. Patients who had complications during the resuscitation period.

6. Patients who were not able to take a minimum of 80% of maintenance energy, protein, and other nutrient requirements by the enteral or parenteral route.

7. Patients with inhalation injuries on mechanical ventilation with unsatisfactory PO_2 and PCO_2 or on > 60% oxygen.

8. Patients with a prehospitalization weight < 80 and > 140% normal body weight as determined from standard tables for age and sex (Desirable Weight Tables, Metropolitan Life Insurance Company, 1959).

9. Patients with associated head injuries requiring specific therapy.

10. Patients with associated injuries to the chest or abdomen requiring surgery or tube drainage.

11. Patients with multiple fractures.

12. Patients with a history of cancer within 5 yr or active neoplasia.

13. Patients with insulin-dependent diabetes mellitus.

14. Patients with renal failure (creatinine > 1.5 mg/dl).

15. Patients with hepatic disease (bilirubin > 3.0 mg/dl).

16. Patients with a past history of chronic infection such as AIDS or tuberculosis.

17. Patients with uncompensated congestive heart failure.

18. Patients with other chronic illnesses such as arthritis, cirrhosis, hyperlipidemia, or autoimmune disease requiring drug therapy.

19. Patients who required chronic glucocorticoid or nonsteroidal anti-inflammatory drugs.

20. Patients with an established clinically significant nonburn wound-related infection.

21. Patients who received any other experimental drug therapy within 2 months of the study.

Medication, Dose, and Administration. Recombinant human growth hormone was supplied for this study as sterile, lyophilized powders in vials containing 5 mg growth hormone. The placebo consisted of excipient which was identical in appearance to the test drug. Each morning, patients received 0.2 mg/kg recombinant human growth hormone or placebo by subcutaneous or intramuscular injection. Treatment was begun as early as possible after resuscitation or stabilization (regardless of timing of surgery) and continued for the duration of hospitalization. Other medications were administered as needed, including histamine antagonist, insulin, antihypertensive, cardiac, pain, and sleeping medications.

Wound Care. Graft donor sites evaluated as part of this study were on the anterior upper thigh, buttock, or upper arm. These were the usual and preferred sites for taking skin for grafting. Skin was taken from the site with a dermatome set at 10/1000s of an inch in thickness and full width. Two designated dermatomes were used exclusively for patients enrolled in this study. In addition, skin from the donor site was harvested by only two investigators. Only the first harvest of a donor site was used for this study.

Fine-mesh gauze was applied to all donor sites. Bed cradles were used to assure that bed sheets did not displace the fine-mesh gauze and the patient was positioned so that the donor site was exposed to the air.

Laboratory Studies. Laboratory studies (see Table 1 for the study plan flow chart) were performed weekly and included CBC, serum chemistries, i.e., glucose, electrolytes, and liver and renal function tests, acute-phase proteins, i.e., transferrin and retinol-binding protein, urinalysis, free thyroxine, growth hormone antibodies (baseline and last day of study only), insulin levels (for patients not on exogenous insulin), and pharmacokinetics. Hematology, serum chemistries, thyroid function tests, and urinalyses were determined for all patients by Smith Kline Bioscience Laboratories (Philadelphia PA) and transferrin and retinol-binding protein by the Laboratory for Surgery Metabolism and Nutrition.

Following the tenth injection, a full 24-h pattern of endogenous and exogenous recombinant growth hormone levels were measured. A 3-ml sample was drawn into a standard tube without anticoagulants (red top) every 4 h and centrifuged, with samplings scheduled to include a sample drawn approximately 2 h after the onset of sleep when the usual normal surge of growth hormone occurs. Samples frozen on dry ice were then shipped to Genentech, Inc., via overnight delivery as soon as possible after the sample was drawn.

Somatomedin-C, one measure of the index of activity of the recombinant growth hormone, was measured at each blood draw. A 2-ml blood sample was drawn into a standard tube containing EDTA (purple top) and centrifuged. The plasma was then removed and immediately frozen. Samples frozen on dry ice were shipped to Genentech, Inc., via overnight delivery as soon as possible after the sample was drawn.

Physical Examinations. Physical examinations were performed daily and included weight, concomitant medications, vital signs, and any adverse events.

Nutrition. Near constant nutritional intake was provided beginning on the first postoperative day. This provided at least 80% of energy and protein requirements (calculated by standard formulae). The nutrients were provided in the same relative proportion throughout the study, with protein accounting for 15-25% total energy, carbohydrate providing 50-80%, and fat providing 3-40%. Additional calories and protein could be provided, but they were not to be more than 25% of the estimated total requirements. Carbohydrate intake did not exceed 6 mg/kg/min (about 300 g/day for the usual patient). Nutrients were provided by the enteral or enteral-parenteral routes throughout the study and the route was altered according to the clinical course of the patient. Intake

TABLE 1. Study Plan Flow Chart

	Baseline	Daily	Weekly
Physical examination*	X	X	
Burn evaluation	X		X
Vital signs	X	X	
Weight	X	X	
Concomitant medications	X	X	
Nutritional intake	X	X	
Complete blood count	X		X
Serum chemistries	X		X
Transferrin	X		X
Retinol-binding protein	X		X
Urinalysis	X		X
Free thyroxine	X		X
Somatomedin-C	X		X
Human growth hormone antibody test	X		X
Study medication		X	
Adverse events		X	

*Includes graft site evaluation with photographs beginning the third postoperative day.

was monitored by the hospital dietitian and nutrition was supervised by a single nutritionist.

End Points.

Donor sites. On the third postoperative day, the wound was examined by a trained evaluator. The donor site wound was measured and photographed using a standard camera and distance. Using sterile technique, each of the four corners of the fine-mesh gauze was gently lifted to determine if the dressing was adherent to the

underlying skin. This was done using sterile forceps and minimal tension. At the end of the examination, the unattached fine-mesh gauze was trimmed away with scissors and the dressing rephotographed. This examination procedure was performed each day until the fine-mesh gauze was completely removed. Complete removal indicated complete wound healing. Using the measurements and photographs, the fraction of the wound covered with fine-mesh gauze (unhealed) was plotted as a function of time. The time to 90% wound closure was compared between treatment and control groups.

Primary Burn. The extent of the burn was charted on graphs developed by the National Burn Information Exchange (16). Second and 3° burn areas were noted separately. The total area involved was calculated at the time of the first graft and weekly thereafter. The fraction of unhealed 2° and 3° sites was plotted as a function of time for the three treatment groups.

Length of Hospitalization. Length of hospitalization was defined as the time from admission until hospital discharge.

Nutrition. Nutritional intake was determined by daily calorie counts in patients who were eating spontaneously and/or from volume of parenteral nutrients.

Infection Rates/Mortality. Infection rates and mortality were compared among the three treatment groups.

Statistical Analyses. Mortality (survival time) and healing time of the skin graft donor site were the primary end points. Differences between treatment and control groups were assessed with respect to mortality using Cox model regression survival analysis, with age, cause of burn, and extent of burn as covariates. Differences between treatment and control groups with respect to healing time of the skin graft donor site for patients who did not die were also evaluated using analysis of covariance, with age, cause of burn, and extent of burn as covariates.

Healing time of the primary burn site and length of hospitalization were secondary end points, the analysis of which was similar to that for healing time of the skin graft donor site. Nutritional intake and infection rates were also secondary end points for which appropriate comparisons were made between treatment and control groups.

Adverse events were tabulated and appropriate comparisons were made between treatment and control groups. Laboratory safety data were tabulated and values outside normal limits identified.

RESULTS

Twelve patients from this Institute were enrolled in this study. In patients receiving growth hormone, the administration of

either 5 or 10 mg/day resulted in a dose range of 0.05-0.15 mg/kg of ideal body weight. Analysis of IGF-1 response in these patients indicated the lack of a consistent response as well as IGF levels not significantly elevated over control patients until postburn day 14. Since IGF-1 response is an indicator of growth hormone action, it is hypothesized that this lack of response may be due to inadequate drug dosing. There was a wide range of donor site healing times among all patients, ranging from 4-19 days for the treatment group and 8-14 days for the control group. This lack of response may be secondary to inadequate drug dosing or the fact that therapy was initiated at variable times following resuscitation. No untoward effects secondary to administration of recombinant human growth hormone were noted in this group of patients.

DISCUSSION

The total number of patients required by the sponsor for completion of this multicenter study have been enrolled and data is currently being analyzing by the sponsor. The Institute's portion of the study was completed on 31 March 1991.

PRESENTATIONS/PUBLICATIONS

None.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION	2. DATE OF SUMMARY: 19 Nov 90	REPORT CONTROL SYMBOL: DD-DRA&E(AR) 636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISP/INSTR'N	9. LEVEL OF SUM/A. WORK UNIT
NONE	A. New	U	U		CX	

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

11. TITLE (Precede with Security Classification Code) (U) Effect of Clotrimazole on the Prevention of Fungal Colonization and Infection in Thermally Injured Patients

12. SUBJECT AREAS
0605 Medicine and Medical Research

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
9011	9309	BA	C. In-House

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

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

18. RESOURCES ESTIMATE

a. FISCAL YEARS	b. PROFESSIONAL WORKYEARS	c. FUNDS (In thousands)
91	0.1	58
92	0.1	61

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

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Burns (Injuries); (U) Fungi; (U) Therapy; (U) Immunosuppression; (U) Chi Square Test

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

23/24. (U) The objective of this work is to assess the effectiveness of clotrimazole in preventing fungal colonization and infection in thermally injured patients. Two hundred and twenty patients will be 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 will be performed and, if clinically indicated by the appearance of the wound, biopsies will be obtained for histologic evaluation.

25. (U) 8810 - 8909. Not applicable.

(U) 8910 - 9009. Not applicable.

(U) 9010 - 9109. 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.

DD FORM 83 MAR 1498 — EDITION OF MAR 88 IS OBSOLETE

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
				DA335693	01 Oct 91	DD-DR&E(AR) 636
3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISP'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
19 Nov 90	D. Chg	U	U		CX	

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

11. TITLE *(Precede with Security Classification Code)* (U) Effect of Clotrimazole on the Prevention of Fungal Colonization and Infection in Thermally Injured Patients

12. SUBJECT AREAS
0605 Medicine and Medical Research

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
9011	9309	DA	C. In-House

MILITARY RELEVANCY CERTIFIED
APPROVED BY Paul A. Pruitt

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

18. RESOURCES ESTIMATE

a. FISCAL YEARS	b. PROFESSIONAL WORKYEARS	c. FUNDS (In thousands)
91	0.4	19
92	0.5	24

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME: US Army Institute of Surgical Research	a. NAME: US Army Institute of Surgical Research
b. ADDRESS (Include zip code): Fort Sam Houston, San Antonio, Texas 78234-5012	b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-5012
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR: RUE, L W
d. TELEPHONE NUMBER (Include area code): 512-221-2720	d. TELEPHONE NUMBER (Include area code): 512-221-8440
21. GENERAL USE: FIC	e. NAME OF ASSOCIATE INVESTIGATOR (If available): CIOFFI, W G
MILITARY/CIVILIAN APPLICATION: M	g. NAME OF ASSOCIATE INVESTIGATOR (If available): MC MANUS, A T

22. KEYWORDS *(Precede EACH with Security Classification Code)* (U) Burns (Injuries); (U) Fungi; (U) Therapy; (U) Immunosuppression; (U) Chi Square Test

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

23/24. (U) The objective of this work is to assess the effectiveness of clotrimazole in preventing fungal colonization and infection in thermally injured patients. Two hundred and twenty patients will be 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 will be performed and, if clinically indicated by the appearance of the wound, biopsies will be obtained for histologic evaluation.

25. (U) 9011 - 9109. 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. Patients meeting entry criteria are being randomly enrolled in the study. It is anticipated that another year or two of clinical experience will be required to obtain the requisite number of patients for meaningful statistical analysis.

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

SUPPLEMENTAL WORK UNIT INFORMATION FOR THE PROJECT ENTITLED "EFFECT OF CLOTRIMAZOLE ON THE PREVENTION OF FUNGAL COLONIZATION AND INFECTION IN THERMALLY INJURED PATIENTS"

Subrecord/Linking Accession Number: Not applicable.

Search Control Data: W6045B/W6046D, 6 November 1990.

Product Identification: For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1991*.

Unclassified Special Categories: Volunteers: Adults; RA II.

ABSTRACT

PROJECT NUMBER: 3M162787A874, 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 1990 - 30 September 1991

INVESTIGATORS: Loring W. Rue, III, MD, Major, MC
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Nineteen patients have been enrolled in this study to date. When the projected total of 220 patients have completed the study, the data will be analyzed as to the efficacy of clotrimazole in the prevention of fungal colonization and infection in thermally injured patients.

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 is to verify that clotrimazole is effective in preventing of fungal colonization and infection in thermally injured patients.

MATERIALS AND METHODS

Study Design. Two hundred and twenty patients will be 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 will be performed and, if clinically indicated by the appearance of the wound, biopsies will be obtained for histologic evaluation.

Description of Procedures. Two hundred and twenty patients will be 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 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 are examined by a physician on a daily basis for any evidence of burn wound infection. Routine microbiologic cultures are obtained every Monday, Wednesday, and Friday, and as clinically indicated. Isolates from each source are typed and compared and the timing of colonization for each source is also recorded. Wounds having a clinical appearance suggestive of invasive fungal infection will undergo a full-thickness lenticular biopsy for histologic evaluation. Evidence of invasive fungal burn wound infection will be considered an indication for surgical excision of the wound. Serial liver function tests, WBC counts, BUNs, and creatinines are monitored to rule out any toxic effect of the medication.

Patient Criteria. Two hundred and twenty 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 beginning the study.

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

1. Male or female patients $\geq$ 18 years of age. 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 are excluded from participation in this study:

1. Patients < 18 yr of age.

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 are pregnant or nursing.

Determination of Number of Subjects Required. The incidence of fungal colonization, as indicated by a review of microbiologic survey data from 1986-9 and reported by investigators, is 42%, and if 1% clotrimazole cream decreases the colonization rate by 50%, 110 patients in each group will be necessary for a type I error < 0.05 and a type II error < 0.1.

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

Data Analysis Plan. Differences between the treatment groups and the incidence of fungal colonization and/or fungal burn wound invasion will be evaluated for significance using a Chi-square technique.

RESULTS

Nineteen patients have been enrolled in this study in a pairwise fashion to date.

DISCUSSION

When the projected total of 220 patients have completed the study, the data will be analyzed as to the efficacy of clotrimazole in the prevention of fungal colonization and infection in thermally injured patients.

PRESENTATIONS/PUBLICATIONS

None.

REFERENCES

1. Pruitt BA Jr: Phycomycotic infections. *Probl Gen Surg* 1:664-78, 1984.

2. Brooke HM, Nash G, Foley FD, Pruitt BA Jr: Opportunistic fungal infection of the burn wound with phycomycetes and Aspergillus. *Arch Surg* 102:476-82, 1971.

3. Pruitt BA Jr: The burn patient. II. Later care and complications of thermal injury. *Curr Probl Surg* 16:1-95, 1979.

4. Nash G, Foley FD, Goodwin MN Jr, et al: Fungal burn wound infection. *JAMA* 215:1664-6, 1971.

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7. Gilman AG, Rall TW, Nies AS, et al (eds): *The Pharmacologic Basis of Therapeutics*. New York: Pergamon Press, 8th ed, 1990.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
					16 Jan 91	DD-DRAM(AR) 636
3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISP'N INSTR'N	9. LEVEL OF SUM / WORK UNIT
NONE	A. New	U	U		CX	

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62787A	3M162787A874	DA	168
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code) (U) Determination of Vecuronium Bromide Requirements in the Thermally Injured Patient

12. SUBJECT AREAS
0605 Medicine and Medical Research 0615 Pharmacology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
9101	9209	DA	C. In-House

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

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

18. RESOURCES ESTIMATE

a. FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
91	0.3	55
92	0.2	58

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME: US Army Institute of Surgical Research	a. NAME: US Army Institute of Surgical Research
b. ADDRESS (Include zip code): Fort Sam Houston, San Antonio, Texas 78234-5012	b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-5012
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR: WESLEY, R L
d. TELEPHONE NUMBER (Include area code): 512-221-2720	d. TELEPHONE NUMBER (Include area code): 512-221-3118
21. GENERAL USE: FINA MILITARY/CIVILIAN APPLICATION: M	e. NAME OF ASSOCIATE INVESTIGATOR (If available): MONGAN, P D f. NAME OF ASSOCIATE INVESTIGATOR (If available): THOMAS, J F

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Burns (Injuries); (U) Anesthesia; (U) Muscle Relaxants; (U) Dosage; (U) Pharmacokinetics

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

23/24. (U) The objectives of this work are to determine the ED_{95} of vecuronium bromide for train-of-four twitch height depression in thermally injured patients and to compare these data with historical data previously determined for nonthermally injured patients. 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.

25. (U) 8810 - 8909. Not applicable.

(U) 8910 - 9009. Not applicable.

DD FORM 1498 93 MAR — EDITION OF MAR 88 IS OBSOLETE

CONTINUATION OF DD FORM 1498 FOR THE PROJECT ENTITLED "DETERMINATION OF VECURONIUM BROMIDE REQUIREMENTS IN THE THERMALLY INJURED PATIENT"

(U) 9101 - 9109. This project was approved by the USAISR Research Council, the US Army Institute of Surgical Research Human Use Committee, and The Surgeon General's Human Subjects Research Review Board during the first and second quarters of Fiscal Year 1991.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1 AGENCY ACCESSIO: DA335914	2 DATE OF SUMMARY 31 Oct 91	REPORT CONTROL SYMBOL DD-DR&E(AR) 636

3 DATE PREV SUMRY	4 KIND OF SUMMARY	5 SUMMARY SCTY	6 WORK SECURITY	7 REGRADING	8 DISPOSITION	9 LEVEL OF SUM A WORK UNIT
16 Jan 91	D. Chg	U	U		CR	

10 C/CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62787A	3M162787A874	DA	168
b. CONTRIBUTING				
c. CONTRIBUTING				

11 TITLE (Precede with Security Classification Code) (U) Determination of Vecuronium Bromide Requirements in the Thermally Injured Patient

12 SUBJECT AREAS
0605 Medicine and Medical Research 0615 Pharmacology

13 START DATE	14 ESTIMATED COMPLETION DATE	15 FUNDING ORGANIZATION	16 PERFORMANCE METHOD
9101	9209	DA	C. In-House

17 CONTRACT/GRANT MILITARY RELEVANCY CERTIFIED APPROVED BY: Paul O Pruitt

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

18 RESOURCES ESTIMATE

a. FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
91	0.5	23
92	0.5	24

19 RESPONSIBLE DOD ORGANIZATION	20 PERFORMING ORGANIZATION
a. NAME: US Army Institute of Surgical Research	a. NAME: US Army Institute of Surgical Research
b. ADDRESS (Include zip code): Fort Sam Houston, San Antonio, Texas 78234-5012	b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-5012
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR: WESLEY, R L
d. TELEPHONE NUMBER (Include area code): 512-221-2720	d. TELEPHONE NUMBER (Include area code): 512-221-8118
21 GENERAL USE: FIC	e. NAME OF ASSOCIATE INVESTIGATOR (If available): MONGAN, P D
MILITARY/CIVILIAN APPLICATION: M	f. NAME OF ASSOCIATE INVESTIGATOR (If available): THOMAS, J F

22 KEYWORDS (Precede EACH with Security Classification Code) (U) Burns (Injuries); (U) Anesthesia; (U) Muscle Relaxants; (U) Dosage; (U) Pharmacokinetics

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

23/24. (U) The objectives of this work are to determine the ED_{95} of vecuronium bromide for train-of-four twitch height depression in thermally injured patients and to compare these data with historical data previously determined for nonthermally injured patients. 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.

25. (U) 9101 - 9109. This project was approved by the USAISR Research Council, the US Army Institute of Surgical Research Human Use Committee, and The Surgeon General's Human Subjects Research Review Board during the first and second quarters of Fiscal Year 1991. All necessary equipment has been

DD FORM 1498 83 MAR — EDITION OF MAR 88 IS OBSOLETE — GPO 1988 0-491-003/80329

CONTINUATION OF DD FORM 1498 FOR THE PROJECT ENTITLED "DETERMINATION OF VECURONIUM BROMIDE REQUIREMENTS IN THE THERMALLY INJURED PATIENT"

procured, set up, calibrated, and tested. Two patients have been enrolled in the study to date. A computer model for generation of subsequent vecuronium bromide dosage based on twitch-height suppression from the initial dose has been developed and is being used in the operating room.

SUPPLEMENTAL WORK UNIT INFORMATION FOR THE PROJECT ENTITLED "DETERMINATION OF VECURONIUM BROMIDE REQUIREMENTS IN THE THERMALLY INJURED PATIENT"

Subrecord/Linking Accession Number: Not applicable.

Search Control Data: W6025N/W6029N, 29 January 1990.

Product Identification: For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1991.*

Unclassified Special Categories: Volunteers: Adults; RA II.

ABSTRACT

PROJECT NUMBER: 3M162787A874, 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 1992 - 30 September 1991

INVESTIGATORS: Roger L. Wesley, MD, Major, MC[1]
Paul D. Mongan, MD, Captain, MC[2]
John G. Thomas, Major, MD[1]
Anthony Pellegrino, MD, Captain, MC[3]
William F. McManus, MD, Colonel, MC[1]
Basil A. Pruitt, Jr., MD, Colonel, MC[1]

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 old 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 old.

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

One patient has been enrolled in this study to date. There were no side effects or adverse reactions with this patient.

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.

REFERENCES

1. Martyn J, Goldhill DR, Goudsouzian NG: Clinical pharmacology of muscle relaxants in patients with burns. *J Clin Pharmacol* 26:680-5, 1986.

2. Martyn JA, Matteo RS, Szyfelbein SK, Kaplan RF: Unprecedented resistance to neuromuscular blocking effects of metocurine with persistence after complete recovery in a burned patient. *Anesth Analg* 61:614-7, 1982.

3. Martyn JA, Goudsouzian NG, Matteo RS, et al: Metocurine requirements and plasma concentrations in burned paediatric patients. *Br J Anaesth* 55:263-8, 1983.

4. Martyn JA, Liu LM, Szyfelbein SK, et al: The neuromuscular effects of pancuronium in burned children. *Anesthesiology* 59:561-4, 1983.

5. Martyn JA, Szyfelbein SK, Ali HH, et al: Increased d-tubocurarine requirement following major thermal injury. *Anesthesiology* 52:352-5, 1980.

6. Martyn JA, Matteo RSA, Greenblatt DJ, et al: Pharmacokinetics of d-tubocurarine in patients with thermal injury. *Anesth Analg* 61:241-6, 1982.

7. Dwersteg JF, Pavlin EG, Heimbach DM: Patients with burns are resistant to atracurium. *Anesthesiology* 65:517-20, 1986.

8. Meretoja OA, Wirtavuori K: Two-dose technique to create an individual dose-response curve for atracurium. *Anesthesiology* 70:732-6, 1989.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION	2. DATE OF SUMMARY: 28 Feb 91	REPORT CONTROL SYMBOL: DD-DR&E(AR) 636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISP'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
NONE	A. New	U	U		CX	

10. NO. CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62787A	3M162787A874	CK	169
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code) (U) Short-Term Anabolic Effects of Recombinant Human Insulin-Like Growth Factor I in Thermally Injured Patients

12. SUBJECT AREAS
0605 Medicine and Medical Research 0615 Pharmacology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
9102	9209	DA	C. In-House

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

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

18. RESOURCES ESTIMATE

a. FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
91	0.1	60
92	0.1	63

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

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Burns (Injuries); (U) Hormones; (U) Protein Metabolism; (U) Therapy

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

23/24. (U) The objective of this work is to assess the biochemical and physiologic efficacy of recombinant human insulin-like growth factor I in thermally injured patients. Fifteen patients will be studied between postburn days 7 and 15. The drug 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.

25. (U) 8810 - 8909. Not applicable.

(U) 8910 - 9009. Not applicable.

(U) 9102 - 9109. This project was approved by the USAISR Research Council, the US Army Institute of Surgical Research Human Use Committee, and The Surgeon General's Human Subjects Research Review Board during the first and second quarters of Fiscal Year 1991.

DD FORM 83 MAR 1498 EDITION OF MAR 80 IS OBSOLETE. * U.S.G.P.O. 1988-491-083/50329

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION DA335813	2. DATE OF SUMMARY 01 Oct 91	REPORT CONTROL SYMBOL DD-DRA&M(AR) 636
3. DATE PREV SUM'RY 28 Feb 91	4. KIND OF SUMMARY D. Chg	5. SUMMARY SCTY U	6. WORK SECURITY U	7. REGRADING	8. DISP'N INSTR'N CX	9. LEVEL OF SUM A. WORK UNIT

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62787A	3M162787A874	CK	169
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code) (U) Short-Term Anabolic Effects of Recombinant Human Insulin-Like Growth Factor I in Thermally Injured Patients

12. SUBJECT AREAS
0605 Medicine and Medical Research

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
9102	9209	DA	C. In-House

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

a. DATE EFFECTIVE	FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
b. CONTRACT/GRANT NUMBER			
c. TYPE / d. AMOUNT	91	0.3	16
e. KIND OF AWARD / f. CUM/TOTAL	92	0.5	24

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME US Army Institute of Surgical Research	a. NAME US Army Institute of Surgical Research
b. ADDRESS (Include zip code) Fort Sam Houston San Antonio, Texas 78234-5012	b. ADDRESS Fort Sam Houston San Antonio, Texas 78234-5012
c. NAME OF RESPONSIBLE INDIVIDUAL PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR CIOFFI, W G
d. TELEPHONE NUMBER (Include area code) 512-221-2720	d. TELEPHONE NUMBER (Include area code) 512-221-8440
21. GENERAL USE FIC MILITARY/CIVILIAN APPLICATION: M	f. NAME OF ASSOCIATE INVESTIGATOR (If available) GRAVES, T A g. NAME OF ASSOCIATE INVESTIGATOR (If available) RUE, L W

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Burns (Injuries); (U) Hormones (U) Protein Metabolism; (U) Therapy

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

23/24. (U) The objective of this work is to assess the biochemical and physiologic efficacy of recombinant human insulin-like growth factor I in thermally injured patients. Fifteen patients will be studied between postburn days 7 and 15. The drug 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.

25. (U) 9102 – 9109. This project was approved by the USAISR Research Council, the US Army Institute of Surgical Research Human Use Committee, and The Surgeon General's Human Subjects Research Review Board during the first and second quarters of Fiscal Year 1991. Four patients have been enrolled in the study to date.

DD FORM 93 MAR 1498 EDITION OF MAR 88 IS OBSOLETE. * U.S.G.P.O. 1991-491-003/30319

SUPPLEMENTAL WORK UNIT INFORMATION FOR THE PROJECT ENTITLED "SHORT-TERM ANABOLIC EFFECTS OF RECOMBINANT HUMAN INSULIN-LIKE GROWTH FACTOR I IN THERMALLY INJURED PATIENTS"

Subrecord/Linking Accession Number: Not applicable.

Search Control Data: W6P15L/W6P18N, 3 January 1991.

Product Identification: For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1991*.

Unclassified Special Categories: Volunteers: Adults; RA II.

ABSTRACT

PROJECT NUMBER: 3M162787A874, 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: 28 February 1991 - 30 September 1991

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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 (prior to 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.

Three patients have been enrolled in this study to date. 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 with these 3 patients. Stable isotope data have been collected and are currently being analyzed. REE 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 10 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

To date, 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 no increase in endogenous serum IGF-1 levels was noted. 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 contributes to the stress-induced insulin resistance in this type of patients. IGF-1 is an insulin agonist and most likely acts through its own receptor, the Type 1 IGF receptor. Studies in a burned rodent model suggest that recombinant human insulin-like growth factor I (rhIGF-1) was able to reduce the hypermetabolism which contributes to the loss of both body protein and body fat (4).

Insulin resistance is a common feature in catabolic patients. Since growth hormone is an insulin antagonist, it may contribute to the deterioration of glucose tolerance. IGF-1 is expected to have the opposite effect of growth hormone in this respect and, at least from a theoretical standpoint, may be favorable in regulating glucose metabolism.

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 a specific carrier protein, and < 20% of circulating IGF-1 is in the free state. The development of a specific RIA for this growth factor, and the biosynthesis of recombinant IGF-1, has provided the opportunity to study its biologic effects and therapeutic potential in humans.

In 1971, Daughaday et al (5), proposed that stimulation of growth hormone leads to the synthesis of IGF-1 in the liver and eventual secretion into the circulation. IGF-1 is considered to be

one of the potential mediators of growth hormone. The 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 rhIGF-1 in healthy patients. However, reports in animals demonstrate that recombinant human insulin-like growth factor 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 rhIGF-1. In summary, the in vivo pharmacologic effects of this compound in 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. In addition, rhIGF-1 was able to lower serum levels of triglycerides and total cholesterol. Additionally, glomerular filtration rate 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.

This trial was designed to determine the anabolic effects of rhIGF-1 in thermally injured patients. rhIGF-1 is administered for 3 days by continuous intravenous infusion. Patients are evaluated at baseline (prior to 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.

MATERIALS AND METHODS

Study Design. This is a pilot study which will 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 old. Female patients must have been surgically sterilized, be postmenopausal (≥ 45 yr of age and lack of menstrual periods for > 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 > 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 old.

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 REE), 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 REE 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 > 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 infusion of 5% glucose intravenously is determined by the patient's fluid needs, but is changed 2 h before each IVGTT 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.

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

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

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

Determination of Number of Subjects Required. This is a pilot study to judge the efficacy of rhIGF-1 in thermally injured patients. A series of 10 patients will allow a reasonable estimate of this compound's ability to reduce the catabolic response following thermal injury. However, 5 additional patients may be enrolled in the study since some patients may incur an unforeseen clinical need for surgery which would prevent completion of the study. This is unlikely for the time period postburn chosen (between 7 and 15 days postburn).

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, REE 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. Differences among pretreatment, during treatment, and posttreatment indices will be evaluated using ANOVA.

RESULTS

Three patients have been enrolled in this study to date. Patient demographics are outlined in Table 1. 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 with these 3 patients. Stable isotope data has been collected and are currently being analyzed. REE did not appear to change with rhIGF-1 infusion.

TABLE 1. Patient Demographics

Patient Number	Age (Yr)	Burn Size (% TBSA)	Study Initiation (Postburn Day)
1	68	49.75	8
2	35	48.50	19
3	53	48.50	13

TBSA indicates total body surface area.

Data from the IVGTT 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.

DISCUSSION

In the first 3 patients studied, no untoward effects of rhIGF-1 infusion administered over a 3-day period were noted. rhIGF-1 infusion appeared to have significant insulin-like effects, as shown by the suppression of serum insulin and C peptide levels during the infusion. In addition, the handling of a glucose load was not changed, although insulin secretion during the IVGTT was blunted.

When 10 patients have completed the study, all data will be analyzed so that the effect of rhIGF-1 infusion on protein kinetics can be ascertained.

PRESENTATIONS/PUBLICATIONS

Cioffi WG Jr: Preliminary results of the short-term anabolic effects of recombinant human insulin-like growth factor in thermally injured patients. Presented at the Ciba-Geigy Investigators Meeting, Summit, New Jersey, 30 September 1991.

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

12. SUBJECT AREAS
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a. NAME: US Army Institute of Surgical Research	a. NAME: US Army Institute of Surgical Research
b. ADDRESS (Include zip code): Fort Sam Houston, San Antonio, Texas 78234-5012	b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-5012
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR: RUE, L W
d. TELEPHONE NUMBER (Include area code): 512-221-2720	d. TELEPHONE NUMBER (Include area code): 512-221-8440
21. GENERAL USE: FIC MILITARY/CIVILIAN APPLICATION: M	f. NAME OF ASSOCIATE INVESTIGATOR (If available): CIOFFI, W G g. NAME OF ASSOCIATE INVESTIGATOR (If available): DRISCOLL, D M

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

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

25. (U) 9010 - 9109. Twenty-three applications have been performed on 17 patients, with an overall engraftment of 47.5%. It has been noted that patients undergoing excisions to fascial wound beds had graft takes of approximately 32.5% versus a 57.7% take on dermal wound beds. Patients with larger burn sizes (> 70% of the total body surface area) have had less than optimal graft take, averaging 30.4%, as compared to burns < 70% of the total body surface area in which graft takes have been approximately 74%. As a consequence of the variation in graft take, an evaluation of the maturation and differentiation of these applied epithelial autografts will be undertaken using histologic and immunologic techniques as additional patients are enrolled in the study.

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

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ABSTRACT

PROJECT NUMBER: 3M162787A874, 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 2 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.

Twenty-three applications have been performed on 17 patients. One patient underwent a single application of cultured keratinocytes to the open interstitial areas of previously applied meshed split-thickness skin autograft and was excluded from subsequent data analysis. Thirteen patients underwent a single application of cultured keratinocytes to freshly prepared wound beds. Two patients underwent 2 applications, and a single patient underwent 4 procedures. The mean age of the treated patients was 29.7 yr (range 10-56) with a mean burn size of 68.2% of the total body surface area (range 42-85%). Eleven patients had documented inhalation injury requiring mechanical ventilatory support. The mean length of hospitalization was 132 days (range 50-275). Two patients died, yielding a mortality of 12.5%.

EVALUATION OF in vitro CULTIVATED KERATINOCYTES AS EPITHELIAL AUTOGRAFTS FOR THE CLOSURE OF BURN WOUNDS

There are more than 2 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. 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).

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 ≥ 18 yr old and ≤ 65 yr old. Female patients must be previously surgically sterilized, be postmenopausal (> 45 yr old and lacked menstrual periods for > 1 yr), or have a negative pregnancy test.

3. Patients with burn wounds between 40% and 75% 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 yr old or > 65 yr old.

2. Patients who are pregnant or nursing.

3. Patients with burns of < 40% or > 75% 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 3 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 3 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 7-10 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 tc 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 0-10, 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

Twenty-three applications have been performed on 17 patients. One patient underwent a single application of cultured keratinocytes to the open interstitial areas of previously applied meshed split-thickness skin autograft and was excluded from subsequent data analysis. Thirteen patients underwent a single application of cultured keratinocytes to freshly prepared wound beds. Two patients underwent 2 applications, and a single patient underwent 4 procedures. The mean age of the treated patients was 29.7 yr (range 10–56) with a mean burn size of 68.2% of the total body surface area (range 42–85%). Eleven patients had documented inhalation injury requiring mechanical ventilatory support. The mean length of hospitalization was 132 days (range 50–275). Two patients died, yielding a mortality of 12.5%.

Keratinocyte grafts were applied to a mean of 15.9% of the body surface area (range 4–59%). The mean percentage of keratinocyte engraftment was 47.5%. Because the ultimate goal of this technology is definitive body surface area wound coverage, and because small surface areas of keratinocyte application with excellent engraftment may tend to skew the data in an artificially favorable direction if only mean percentage of engraftment is considered, it was felt that an assessment of actual body surface area coverage was a more objective means of assessing the impact of cultured keratinocytes. The tendency for large surface area applications to yield poor percentages of engraftment was reflected in the fact that the average body surface area of definitive wound coverage was 4.7% of the total body surface area as opposed to the expected 7.5% when mean surface area of application and mean percentage engraftment are considered.

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 1 summarizes our experience with respect to the excisional wound bed. Mean patient age and burn size were similar between both groups. Despite application of cultured epithelial autografts to a larger proportion of the 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. Table 2 summarizes the data with respect to the extent of burn injury. Again, the ages of the two patient groups were similar; however, patients with burn sizes in excess of 70% of the total body surface area had a similar extent of definitive wound coverage (4.6% vs 4.8%) when compared to patients with smaller burns despite over three times greater area of application of keratinocytes (21.5% vs 6.6%).

TABLE 1. Wound Bed Data

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.

TABLE 2. Burn Size Data

Burn Size (% TBSA)	n=	Mean Age (Yr)	Keratinocyte Application (% TBSA)	Definitive Coverage (% TBSA)
< 70	6	31 (18-48)	6.6 (4.0-8.8)	4.8 (2.8-7.8)
> 70	10	29 (10-56)	21.5 (7.4-59.2)	4.6 (0-18.6)

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

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). Reports of successful engraftment have ranged from 47% up to 75% of applied cells. On first inspection, our results with cultured keratinocytes were similar to published reports. However, rather than reporting percentage of successful engraftment, a more objective means of assessing the impact of this technology was to determine the actual body surface area closed by

the grafts. In this cohort of burn patients, only 4.7% (range 0-18.6%) of the total body surface area was definitively covered with cultured epithelial autografts despite application to an average of 15.9% of the body surface area. Further evaluation of the data reveals small areas of definitive wound coverage in patients with burns > 70% of the total body surface area, the target population for this technology. Although the overall patient experience with this technology has been less than encouraging, the data do reveal a wide range of keratinocyte engraftment, both in the population of patients with large burns and on fascial wound bed excisions. Future investigation into this wound care technology will focus upon means by which engraftment success can be optimized. Consequently, this protocol was amended to permit evaluation of maturation and differentiation of the applied epidermal cells with respect to the formation of basement membranes and anchoring fibrils as assessed by intermittent histologic examination of biopsies of the sites of engraftment. Also, investigation into the causes of delayed graft loss will be pursued by evaluation of the impact of microbial density and the possible role of autoimmune phenomenon.

PRESENTATIONS/PUBLICATIONS

None.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
				DA346158	01 Oct 91	DD-DR&E(AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISP'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
01 Oct 90	D. Chg	U	U		CX	

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

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

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

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

17. CONTRACT/GRANT — MILITARY RELEVANCY CERTIFIED — 18. RESOURCES ESTIMATE

a. DATE EFFECTIVE	APPROVED BY	FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS *(In thousands)*
b. CONTRACT/GRANT NUMBER		91	0.5	23
c. TYPE	d. AMOUNT	92	0.5	24
e. KIND OF AWARD	f. CUM/TOTAL			

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME: US Army Institute of Surgical Research	a. NAME: US Army Institute of Surgical Research
b. ADDRESS *(Include zip code)*: Fort Sam Houston, San Antonio, Texas 78234-5012	b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-5012
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR: WAGUESPACK, R L
d. TELEPHONE NUMBER *(Include area code)*: 512-221-2720	d. TELEPHONE NUMBER *(Include area code)*: 512-221-8440
21. GENERAL USE: FIC MILITARY/CIVILIAN APPLICATION: M	e. NAME OF ASSOCIATE INVESTIGATOR *(If available)*: RUE, L W g. NAME OF ASSOCIATE INVESTIGATOR *(If available)*: CIOFFI, W G

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

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

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

25. (U) 9010 - 9109. Thirteen patients have been enrolled in the study, 7 during this reporting period. Upon completion of enrollment, the data will be analyzed as indicated.

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

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

Subrecord/Linking Accession Number: Not applicable.

Search Control Data: W6O10D/W6OO7E, 6 March 1990.

Product Identification: For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1991.*

Unclassified Special Categories: Volunteers: Adults; RA II.

ABSTRACT

PROJECT NUMBER: 3M162787A874, 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: 15 October 1990 - 30 September 1991

INVESTIGATORS: Robert L. Waguespack, MD, Captain, MC
William G. Cioffi, Jr., MD, Major, MC
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 is to evaluate the efficacy of Eurothane® in the treatment of skin graft donor sites in comparison with fine-mesh gauze.

Thirteen patients have been enrolled in this study, 7 during this reporting period. Upon completion of enrollment, the data will be analyzed as indicated.

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 following by 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 is 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 < 70% of the total body surface area undergoing an initial split-thickness skin graft harvest from an anterior thigh will be enrolled in the study. One surgeon harvests the skin grafts utilizing the same dermatome at the same thickness (0.010 in). One-half of the donor site is treated with Eurothane® after obtaining hemostasis with warm saline laparotomy pads. The dressing extends at least 1 cm beyond the wound margin for optimal adherence. The other half of the donor site is covered with fine-mesh gauze and hemostasis is achieved with warm saline laparotomy pads. The fine-mesh gauze is applied to the wound edges without overlap. The Eurothane®-treated donor site areas are inspected after removal of the dressing on the seventh postoperative day or at the time of spontaneous separation of the dressing. If the wound is completely reepithelialized, it is exposed to air. If spontaneous separation occurs prior to the seventh postoperative day, and provided no contraindications exist, additional Eurothane® is applied.

Criteria for Admission to the Study. Patients admitted to the US Army Institute of Surgical Research are offered the opportunity to participate 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 enrolled in the study upon giving written informed consent:

1. Male or female patients $\geq$ 18 yr of age. Female patients must have been surgically sterilized, be postmenopausal (> 45 yr of age 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 < 70% of the total body surface area.

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

1. Patients < 18 yr of age.

2. Patients who are 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.

Determination of Number of Subjects Required. A total of 42 patients will be required to demonstrate a 25% treatment difference with a Type I error of 0.05 and a power of 90%.

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

Data Analysis Plan. Data analysis will be performed using the paired t test. Each patient will serve as his/her own control.

RESULTS

Thirteen patients have been enrolled in this study, 7 during this reporting period.

DISCUSSION

When the projected total of 42 patients have completed the study, the data will be analyzed as to the efficacy of Eurothane® in the treatment of skin graft donor sites in comparison with fine-mesh gauze.

PRESENTATIONS/PUBLICATIONS

None.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION DA318017	2. DATE OF SUMMARY 01 Oct 91	REPORT CONTROL SYMBOL DD-DRAB(AR) 636
3. DATE PREV SUM'RY 01 Oct 90	4. KIND OF SUMMARY D. Chg	5. SUMMARY SCTY U	6. WORK SECURITY U	7. REGRADING	8. DISP'N INSTR'N CX	9. LEVEL OF SUM A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62787A	3M162787A874	FA	172
b. CONTRIBUTING				
c. CONTRIBUTING				

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

12. SUBJECT AREAS
0605 Medicine and Medical Research

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
8902	9209	DA	C. In-House

17. CONTRACT/GRANT MILITARY RELEVANCY CERTIFIED APPROVED BY Paul Pruitt

a. DATE EFFECTIVE		b. RESOURCES ESTIMATE		
b. CONTRACT/GRANT NUMBER		FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
c. TYPE	d. AMOUNT	91	0.5	25
e. KIND OF AWARD	f. CUM/TOTAL	92	0.5	26

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

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

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

23/24. (U) The objective of this work is to determine the significance of 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. A 5-ml sample of whole blood will be drawn on a twice weekly basis from 100 consecutive burn patients with burns > 20% of the total body surface area. Patients will be monitored on a prospective basis for the development of infections and the clinical course will be correlated with the results of TNF assays.

25. (U) 9010 - 9109. Twenty-three patients were enrolled in the study to date and serial plasma samples were drawn for analysis by ELISA. This study was terminated at the request of the primary investigator.

DD FORM 83 MAR 1498 EDITION OF MAR 80 IS OBSOLETE * U.S.G.P.O. 1988-491-003/60329

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

Subrecord/Linking Accession Number: Not applicable.

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

Product Identification: For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report* for fiscal years 1989-91.

Unclassified Special Categories: Volunteers: Adults; RA II.

ABSTRACT

PROJECT NUMBER: 3M162787A874, 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 1990 - 30 September 1991

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

Burn patients have a significant infection diathesis due in part to the loss of the normal epithelial skin barrier and to the 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 have been enrolled in the study to date and serial plasma samples have been drawn for analysis by ELISA.

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

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

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

Despite these findings in animal models, very little investigation has been performed in human burn patients. Therefore, this study will attempt to delineate better the significance of TNF levels in burn patients, with special reference to the correlation of elevations in TNF levels and impending sepsis or recovery from infections.

MATERIALS AND METHODS

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

Criteria for Admission. Patients admitted to the US Army Institute of Surgical Research are offered the opportunity to participate in this study. 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 considered eligible for enrollment in the study after giving written informed consent:

1. Male or female patients ≥ 18 yr old.

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

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

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

1. Patients < 18 yr old.

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

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

4. Patients with toxic epidermal necrolysis.

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

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

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

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

FIGURE 1. Data collection scheme.

Postburn Day	TNF Level	WBC Count	Existing Infection	Maximum Temperature

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

RESULTS

Twenty-three patients have been enrolled in the study to date and serial plasma samples have been drawn for analysis by ELISA.

DISCUSSION

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

PRESENTATIONS/PUBLICATIONS

None.

REFERENCES

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2. Männel DN, Northoff H, Bauss F, Falk W: Tumor necrosis factor: a cytokine involved in toxic effects of endotoxin. *Rev Infect Dis* 9:S602-6, 1987.

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4. Dinarello CA, Cannon JG, Wolff SM, et al: Tumor necrosis factor (cachectin) is an endogenous pyrogen and induces production of interleukin 1. *J Exp Med* 163:1433-50, 1986.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
				DA346159	01 Oct 91	DD-DR&E(AR) 636
3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISP'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
01 Oct 90	D. Chg	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62787A	3M162787A874	DA	173
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE *(Precede with Security Classification Code)* (U) A Comprehensive Analysis of the Perceived Needs of Families of Critically Injured Burned Patients

12. SUBJECT AREAS
0605 Medicine and Medical Research

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
9003	9209	DA	C. In-House

MILITARY RELEVANCY CERTIFIED
APPROVED

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

RESOURCES ESTIMATE		
FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS *(In thousands)*
91	0.5	23
92	0.3	12

18. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME: US Army Institute of Surgical Research	a. NAME: US Army Institute of Surgical Research
b. ADDRESS *(Include zip code)*: Fort Sam Houston, San Antonio, Texas 78234-5012	b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-5012
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR: MOLTER, N C
d. TELEPHONE NUMBER *(Include area code)*: 512-221-2720	d. TELEPHONE NUMBER *(Include area code)*: 512-221-8024
21. GENERAL USE: FIC MILITARY/CIVILIAN APPLICATION: M	e. NAME OF ASSOCIATE INVESTIGATOR *(If available)*: SUMMERS, T M g. NAME OF ASSOCIATE INVESTIGATOR *(If available)*: LESKE, J

22. KEYWORDS *(Precede EACH with Security Classification Code)* (U) Burns (Injuries); (U) Hospitalization; (U) Medical Services; (U) Health Care Facilities

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

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

25. (U) 9010 - 9109. Twelve burn units, including this Institute, are participating in this study. Data collection for this Institute was completed in June 1991. Thirty-eight patients at this Institute were enrolled in the study, with 56 family members completing 94 questionnaires and 32 staff members completing 69 questionnaires. Analysis of data has begun. Data collection by other units will be completed by February 1992.

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

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

Subrecord/Linking Accession Number: Not applicable.

Search Control Data: W6R49L/W6R44M, 9 January 1990.

Product Identification: For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report* for fiscal years 1990-1.

Unclassified Special Categories: Volunteers: Adults; RA II.

ABSTRACT

PROJECT NUMBER: 3M162787A874, 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 1990 - 30 September 1991

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

Several studies have described the needs of families of critically ill patients as perceived by the family members. No studies have described such needs of families of critically injured burned patients. The objectives of this study are 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 6 months 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 are presently enrolling patients in the study.

Data collection at the Institute was completed during June 1990. Data at other burn units will be collected for 6 months or until February 1992. Data from all participating units will then be analyzed during the summer of 1992 to determine the needs of family members of critically injured burned patients as they perceive them across a span of time compared to the perceptions of the nurses who interact with them in the burn unit and to describe the psychometric properties of the CCFNI when used with the burn unit population.

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.

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

Leske (17) replicated part of Molter's study (14) using a heterogeneous sample from different institutions. Using a self-report questionnaire format versus the interview format used by Molter, families were surveyed within the first 72 h of the patient's admission to the ICU. The top nine needs identified in Leske's study were among the top 10 needs reported by Molter.

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

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

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

In a study using the CCFNI that compared needs of the family members of brain-injured patients versus those of family members of patients without brain injury, Mathis (21) found differences and similarities in family needs among varying patient populations. Although the small sample size raises questions about statistical

conclusion validity, determining if differences exist among varying patient populations is of interest to all specialties, including burn nursing.

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

Norris and Grove (23) used a variation of the CCFNI to compare needs reported by family members to those reported by critical care nurses. Findings indicated that there was a statistical difference between the perceptions of needs of the two groups on four items. This study served to sensitize nurses to the importance of assessing the family's perception of needs. In a similar study (24), the level of need satisfaction as perceived by 52 family members was compared to how accurately 92 nurses identified those areas of relatively high and low family satisfaction. The nurses were accurate (Spearman's at 0.50) at identifying the level at which family members perceived needs as being met. A limitation of the study was that there was no attempt to match the family's perceptions with those of a primary nurse for the patient/family unit. In addition, the importance of the needs to the family member was not evaluated.

A review of the literature over the past 15 years related to family needs of burn patients indicates that most studies/case reports are concerned with informational needs, usually at the time of discharge (25-28). A retrospective survey of 68 family members (29) did evaluate family needs related to receiving information prior to the initial visit. The most important topics identified were the patient's condition, chance of recovery, and a description of the injury. This study also identified aspects of the first visit that were disturbing to the family, i.e., the appearance of the patient and the environment in the unit. Families also identified that the physician (91.1%) and the nurses (42.6%) were the primary personnel who should meet these needs. No studies were reported that used the CCFNI to identify a more comprehensive data base of family needs in the burn unit population.

There is a comprehensive data base related to family needs in the critical care unit environment; however, very little research has been conducted describing the needs of 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.

MATERIALS AND METHODS

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

Study Design. This is a descriptive/comparative study to establish a comprehensive data base related to the perceived needs of family of critically burned patients. A letter was sent to all burn units within the United States currently listed in the directory of the American Burn Association inviting participation in this study. The primary benefit to participating units is that analyses of their unit-specific data can be compared with the total study sample analyses. The unit-specific analyses may also serve as baseline data for potential studies of specific interventions in that unit. In addition, all units participating will be listed in any published results. Once the units obtain permission from their institutional review board to conduct this study, data are collected for a 6-month period or until February 1992. The unit data collection coordinator codes all data forms and maintains the key to the data at the facility. Only coded forms are forwarded to the Institute for analyses. The principal investigators at the Institute have the specific unit code key for participating units and each unit receives their unit code number, study instruments, and specific data collection instructions upon enrollment in the study.

Description of Procedures. After the family member visits the patient at least once within the first 72 h postadmission to the critical care burn unit, and after verbal/written informed consent is obtained, the unit data collection coordinator has the family member complete the Family Member Data Form and the CCFNI. Any number of family members for a specific patient can participat in completing the first version of the CCFNI. One member of the family is then designated by the family as the "primary visitor". The family member designated as the primary visitor is 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 1).

TABLE 1. 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 (does not include death)

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

The data collection coordinator collects the data about the unit once using the Unit Demographic Data Form and data about each study patient using the Patient Data Form. Using both the Patient Data Form and Family Member Data Form, a code key sheet is completed for each patient. Only coded forms of data tools are sent to the Institute. The primary investigators at the Institute maintain a code key sheet for the unit code numbers only.

Data Analysis Plan. In order to pilot proper data collection techniques, data from the Institute was collected for 6 months prior to soliciting participation from other burn units within the United States. Descriptive and inferential statistical procedures were used for analyses. Selected psychometric properties of the

CCFNI were determined based on data obtained within the first 72 h postadmission from all burn units participating in the study. It is anticipated that 400-600 family members will be involved in the study as well as 250-500 health care personnel.

RESULTS

An initial 6 months 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. There were no reports from family or staff members of negative experiences associated with the study.

Based on the data collected during the pilot portion of the study, the decision was made not to request information concerning who met the family needs. In addition, clarification as to the time frame of when to administer the CCFNI to the staff was delineated in the protocol. All data collection tools were reviewed by an expert for ease of entry into a computer data base.

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 are presently enrolling patients in the study.

Data collection at the Institute was completed during June 1991. Preliminary demographic data indicate that family members completing the questionnaire consisted of 8 spouses (21.1%), 25 parents (65.8%), 2 siblings (5.3%), 2 children (5.3%), and 1 grandparent (2.6%). The mean age for these individuals was 41.6 yr (range 16-64). Thirty-four of these family members were female (89.5%) and 4 (10.5%) were male. These individuals ranged from manual laborers to administrative personnel, small business managers, and minor professionals. Educational levels ranged from high school graduate through partial college training. Seventy-two percent indicated that religion was important in their lives and 55% had prior experience with family members in an ICU; no one had prior experience with a family member in a burn unit. Thirty-two of these family members designated themselves as primary visitors, although 38 were considered primary visitors to complete subsequent questionnaires.

Data for the burn patients indicated a mean age of 27.2 yr (range < 1-93), a mean total body surface area burn size of 39.5% (range 7-85%), and a mean 3° burn size of 23.2% (range 0-85%). Inhalation injury was present in 47.4% of these patients, other injuries in 10.5%, and prior medical conditions in 21.1%. The assessment of severity of injury for the data collection coordinator and the family member are as indicated in Table 2.

TABLE 2. Assessment of Severity of Injury (%)

Assessment	Data Collection Coordinator	Family Member
Critical, may die	26.3	31.6
Ill, but will live	60.5	39.5
Stable	13.2	23.7

DISCUSSION

Data at other burn units will be collected for 6 months or until February 1992. Upon completion of enrollment at all burn units participating in the study, the data will be analyzed to determine the needs of family members of critically injured burned patients as they perceive them across a span of time compared to the perceptions of the nurses who interact with them in the burn unit and to describe the psychometric properties of the CCFNI when used with the burn unit population.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION: DA346179	2. DATE OF SUMMARY: 01 Oct 91	REPORT CONTROL SYMBOL: DD-DR&E(AR) 636
3. DATE PREV SUM'RY: 01 Oct 90	4. KIND OF SUMMARY: D. Chg	5. SUMMARY SCTY: U	6. WORK SECURITY: U	7. REGRADING:	8. DISP'N INSTR'N: CX	9. LEVEL OF SUM / A. WORK UNIT:

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62787A	3M162787A874	CG	174
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code) (U) Effect of Sucralfate on Prevention of Stress Ulcers and Nosocomial Pneumonia in Thermally Injured Patients

12. SUBJECT AREAS: 0605 Medicine and Medical Research

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
9001	9509	DA	C. In-House

17. CONTRACT/GRANT — MILITARY RELEVANCY CERTIFIED — APPROVED [signature]

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

18. RESOURCES ESTIMATE

FISCAL YEARS	a. PROFESSIONAL WORK YEARS	b. FUNDS (In thousands)
91	0.5	25
92	0.5	26

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME: US Army Institute of Surgical Research	a. NAME: US Army Institute of Surgical Research
b. ADDRESS (Include zip code): Fort Sam Houston, San Antonio, Texas 78234-5012	b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-5012
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR: CIOFFI, W G
d. TELEPHONE NUMBER (Include area code): 512-221-2720	d. TELEPHONE NUMBER (Include area code): 512-221-8440
21. GENERAL USE: FIC MILITARY/CIVILIAN APPLICATION: M	f. NAME OF ASSOCIATE INVESTIGATOR (If available): MC MANUS, A T g. NAME OF ASSOCIATE INVESTIGATOR (If available): MC MANUS, W F

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Burns (Injuries); (U) Ulcers; (U) Pneumonia; (U) Therapy

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

23/24. (U) The objective of this work is to determine whether sucralfate is effective in the prevention of stress ulcer-induced gastrointestinal bleeding and reduces the incidence of nosocomial pneumonia. Three hundred burn patients will be randomized to receive either standard prophylaxis (cimetidine and antacids) or sucralfate. Differences between treatment groups in the rate of occurrence of pneumonia and clinically evident gastrointestinal bleeding will be evaluated for significance using the Chi square technique.

25. (U) 9010 - 9109. Sixty-three patients have been enrolled in this study, 43 during this reporting period. Upon completion of enrollment, the data will be analyzed as indicated.

DD FORM 83 MAR 1498 — EDITION OF MAR 88 IS OBSOLETE — * U.S.G.P.O. 1988-491-003/50329

SUPPLEMENTAL WORK UNIT INFORMATION FOR THE PROJECT ENTITLED "EFFECT OF SUCRALFATE ON PREVENTION OF STRESS ULCERS AND NOSOCOMIAL PNEUMONIA IN THERMALLY INJURED PATIENTS"

Subrecord/Linking Accession Number: Not applicable.

Search Control Data: W6N47E/W6N40C, 30 May 1990.

Product Identification: For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report* for fiscal years 1990-1991.

Unclassified Special Categories: Volunteers: Adults; RA II.

ABSTRACT

PROJECT NUMBER: 3M162787A874, 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 1990 - 30 September 1991

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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).

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 old. Female patients must have been surgically sterilized, be postmenopausal (> 45 yr of age 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 old.

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 will be randomized to receive either standard prophylaxis or sucralfate. Standard anti-ulcer prophylaxis will consist of the administration of cimetidine and antacids. Cimetidine (300 mg IV) will be administered every 6 h. The dose of cimetidine will be adjusted depending upon the patient's renal function and gastric pH. Antacids will be administered as a 30-cc bolus via the nasogastric tube every 2 h. Any evidence of clinically significant upper gastrointestinal bleeding will result in withdrawal of the patient from the study. Withdrawal from the study for any reason will result in the classification of the patient as a treatment failure. Gastric pH will be checked every hour and if the pH is < 4.5, the dose of antacids will be doubled and administered on an hourly basis until the pH is ≥ 4.5. Sucralfate (1 g suspended in 20 cc water) will be administered via the nasogastric tube every 6 h. The tube will be clamped for 1 h following administration. The gastric pH of these patients will be checked and recorded every hour. The incidence of clinically evident gastrointestinal bleeding will be recorded. Any patient who demonstrates clinically evident bleeding will undergo upper gastrointestinal endoscopy to verify the source of the bleeding. The diagnosis of pneumonia will be 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 will be 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 will be recorded for each patient. Sputum Gram stain and cultures and gastric aspirate cultures will be obtained every Monday, Wednesday, and Friday and as clinically indicated. Isolates from each source will be typed and compared. The timing of colonization for each source will be 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 25-30%. 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 will be checked and recorded every hour. The incidence of clinically evident gastrointestinal bleeding, pneumonia, and tracheobronchitis will be recorded for each patient and the timing of colonization for each source will be recorded.

Data Analysis Plan. 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.

RESULTS

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.

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

None.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
				DA346178	01 Oct 91	DD-DR&E(AR) 636
3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
01 Oct 90	D. Chg	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62787A	3M162787A874	CA	175
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE *(Precede with Security Classification Code)* (U) Study of Effect of Intermittent vs. Continuous Administration of EXOSURF in Patients with ARDS Induced by Thermal Injury

12. SUBJECT AREAS
0605 Medicine and Medical Research

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
9001	9309	DA	C. In-House

MILITARY RELEVANCY CERTIFIED
APPROVED BY Basil M. Pruitt

17. CONTRACT/GRANT	18. RESOURCES ESTIMATE		
a. DATE EFFECTIVE	FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS *(In thousands)*
b. CONTRACT/GRANT NUMBER	91	0.5	25
c. TYPE / d. AMOUNT	92	0.5	27
e. KIND OF AWARD / f. CUM/TOTAL			

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME: US Army Institute of Surgical Research	a. NAME: US Army Institute of Surgical Research
b. ADDRESS *(Include zip code)*: Fort Sam Houston, San Antonio, Texas 78234-5012	b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-5012
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR: CIOFFI, W G
d. TELEPHONE NUMBER *(Include area code)*: 512-221-2720	d. TELEPHONE NUMBER *(Include area code)*: 512-221-8440
21. GENERAL USE: FIC MILITARY/CIVILIAN APPLICATION: M	f. NAME OF ASSOCIATE INVESTIGATOR *(If available)*: RUE, L W g. NAME OF ASSOCIATE INVESTIGATOR *(If available)*

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

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

23/24. (U) The objectives of this work are to determine whether a 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. Twenty-four patients with early onset ARDS secondary to thermal and smoke inhalation injury will be randomized to one of four treatment groups. Patients in each group will receive continuous aerosol administration of the appropriate agent for 5 days. A variety of ventilatory, hemodynamic, and blood gas data will be collected.

25. (U) 9010 - 9109. This study has been terminated due to problems encountered with the aerosol generator. No patients were enrolled in the study.

DD FORM 1498 83 MAR — EDITION OF MAR 88 IS OBSOLETE. — * U.S.G.P.O.: 1988 -491-003/50329

SUPPLEMENTAL WORK UNIT INFORMATION FOR THE PROJECT ENTITLED "STUDY OF EFFECT OF INTERMITTENT VS. CONTINUOUS ADMINISTRATION OF EXOSURF® IN PATIENTS WITH ARDS INDUCED BY THERMAL INJURY"

Subrecord/Linking Accession Number: Not applicable.

Search Control Data: W6N55B/W6N57E, 30 May 1990.

Product Identification: For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report* for fiscal years 1990-1.

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

ABSTRACT

PROJECT NUMBER: 3M162787A874, 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 1990 - 30 September 1991

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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 project was approved by the USAISR Research Council, the US Army Institute of Surgical Research Human Use Committee, and The Surgeon General's Human Subjects Research Review Board during the second quarter of fiscal year 1990. No patients have been enrolled in the study to date.

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 old. Female patients must have been surgically sterilized, be postmenopausal (> 45 yr old 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 old.

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 with evidence of hepatic failure as defined by bilirubin > 5 or SGOT or SGPT > 5 times the upper limit of the normal range.

12. Patients known to have AIDS.

13. Patients with a clinical diagnosis of septic syndrome at time of screening.

14. Patients with head injuries with a Glasgow coma score < 6.

15. Patients receiving chronic medications for COPD, asthma, or emphysema.

16. 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 have been enrolled in this study to date. Laboratory experience with the VISAN® nebulizer, which is to be used for the delivery of the aerosolized surfactant, has uncovered multiple inherent defects.

DISCUSSION

Until the nebulizer can be safely used in the animal model, patients will not be enrolled in this study.

PRESENTATIONS/PUBLICATIONS

None.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION: DA146781	2. DATE OF SUMMARY: 91 Oct 31	REPORT CONTROL SYMBOL: DD-DRSRDA(AR) 636
3. DATE PREV SUM'RY: 01 Oct 90	4. KIND OF SUMMARY: D. Chg	5. SUMMARY SCTY: U	6. WORK SECURITY: U	7. REGRADING	8. DISS'N INSTR'N: CX	9. LEVEL OF SUM: A. WORK UNIT

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62787A	3M162787A874	EA	176
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) A Clinical Study of the Efficacy of Topical Silicone Gel in the Prevention of Hypertrophic Burn Scar Formation

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

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
9006	9309	DA	C. In-House

17. CONTRACT/GRANT: MILITARY RELEVANCY STATEMENT Approved by Carol Thurston
a. DATE EFFECTIVE:
b. CONTRACT/GRANT NUMBER:
c. TYPE:
d. AMOUNT:
e. KIND OF AWARD:
f. CUM/TOTAL:

18. RESPONSIBLE DOD ORGANIZATION

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

19. RESOURCES ESTIMATE

FISCAL YEARS	a. PROFESSIONAL MAN-YEARS	b. FUNDS (In Thousands)
91	0.5	23
92	0.5	24

20. PERFORMING ORGANIZATION

a. NAME: US Army Institute of Surgical Research
b. ADDRESS: Fort Sam Houston
San Antonio, Texas 78234-5012
c. NAME OF PRINCIPAL INVESTIGATOR: HARVEY, J D
d. TELEPHONE NUMBER (Include area code): 512-221-8957
e. NAME OF ASSOCIATE INVESTIGATOR (If available): CIOFFI, W G
f. NAME OF ASSOCIATE INVESTIGATOR (If available): RUE, L W

21. GENERAL USE: FIC
MILITARY/CIVILIAN APPLICATION: M

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

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

23/24. (U) The objective of this work is to evaluate the efficacy of silicone gel in the prevention of hypertrophic burn scar formation. Improved management of hypertrophic scar formation will benefit military survivors of burn injury. Forty-two patients who have undergone meshed, split-thickness skin grafting of at least one upper extremity will be enrolled in this study. When the graft has healed and the wound is closed, Silastic™ gel sheeting will be applied to the grafted area. Data from treated and untreated sites of each patient will be subjected to a one-way, within subjects ANOVA. Data groups will be formed for treated and untreated sites and will undergo a one-way, between patients ANOVA.

25. (U) 9010 - 9109. Eleven patients were enrolled in the study during this reporting period. 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.

DD FORM 1498 82 MAR — EDITION OF MAR 80 IS OBSOLETE

SUPPLEMENTAL WORK UNIT INFORMATION FOR THE PROJECT ENTITLED "A CLINICAL STUDY OF THE EFFICACY OF TOPICAL SILICONE GEL IN THE PREVENTION OF HYPERTROPHIC BURN SCAR FORMATION"

Subrecord/Linking Accession Number: Not applicable.

Search Control Data: W6007D/W6008D, 30 May 1990.

Product Identification: For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1991.*

Unclassified Special Categories: Volunteers: Adults; RA II.

ABSTRACT

PROJECT NUMBER: 3M162787A874, 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 1990 - 30 September 1991

INVESTIGATORS: Karoline D. Harvey, OTR, Captain, SP
William G. Cioffi, Jr., MD, Major, MC
Loring W. Rue, III, MD, Major, MC
William F. McManus, MD, Colonel, MC
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 mechanisms of action of silicone gel on hypertrophic scar is unknown. After studying silicone gel sheets, Quinn et al (5) concluded that its mechanisms of action in altering scar tissue is not secondary to pressure, temperature, oxygen tension, or skin hydration and occlusion and may therefore involve a chemical factor. 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 old. Female patients must have been surgically sterilized, be postmenopausal (> 45 yr of old and lack of menstrual periods for > 1 yr), or have a negative pregnancy test prior to 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 old.

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 includes 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.

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.

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

Eleven patients have been enrolled in this study to date, all during this reporting period. Immediate visual smoothening and softening of treatment sites have 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.

REFERENCES

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3. Helal B, Chapman R, Ellis M, Gifford D: The use of silicone oil for mobilization of the hand. *J Bone Joint Surg* 64:67-9, 1982.

4. Ohmori S: Effectiveness of silastic sheet coverage in the treatment of scar keloid (hypertrophic scar). *Aesthetic Plast Surg* 12:95-9, 1988.

5. Quinn KJ, Evans JM, Courtney JM, et al: Non-pressure treatment of hypertrophic scars. *Burns* 12:102-8, 1985.

6. Quinn KJ: Silicone gel in scar treatment. *Burns* 13:S33-40, 1987.

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8. Esposito G, Ziccardi P, Scioli M, et al: The use of a modified tonometer in burn scar therapy. *J Burn Care Rehabil* 11:86-90, 1990.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION: DA335466	2. DATE OF SUMMARY: 01 Oct 91	REPORT CONTROL SYMBOL: DD-DR&E(AR) 436

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISP'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
01 Oct 90	D. Chg	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62787A	3M162787A874	AZ	177
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code) (U) A Clinical Study of the Efficacy of Low-Dose Dopamine Therapy in Hospitalized Burn Patients

12. SUBJECT AREAS
0605 Medicine and Medical Research

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
9007	9309	DA	C. In-House

17. CONTRACT/GRANT — MILITARY RELEVANCY CERTIFIED — APPROVED BY Barry A. Burtchi

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

18. RESOURCES ESTIMATE

a. FISCAL YEARS	b. PROFESSIONAL WORKYEARS	c. FUNDS (In thousands)
91	0.5	39
92	0.5	43

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME: US Army Institute of Surgical Research	a. NAME: US Army Institute of Surgical Research
b. ADDRESS (Include zip code): Fort Sam Houston, San Antonio, Texas 78234-5012	b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-5012
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR: CIOFFI, W G
d. TELEPHONE NUMBER (Include area code): 512-221-2720	d. TELEPHONE NUMBER (Include area code): 512-221-8440
21. GENERAL USE: FIC	e. NAME OF ASSOCIATE INVESTIGATOR (If available): HEIRONIMUS, J D
MILITARY-CIVILIAN APPLICATION: M	f. NAME OF ASSOCIATE INVESTIGATOR (If available): VAUGHAN, G M

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Burns (Injuries); (U) Dopamine; (U) Therapy; (U) Kidney Function Tests

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

23/24. (U) The objective of this work is to assess the short- and long-term usefulness of DermaGraft™ dermal replacement in burn patients. Thirty consecutive patients will be enrolled in the study as part of a multicenter study. Each patient's wounds will be excised in the operating room and one treatment wound site and one control site anatomically comparable as to location and of approximate equal dimensions will be identified. A biopsy for quantitative microbial culture and histology will be taken from each excised wound bed prior to application of grafts. The DermaGraft™ will then be placed onto the chosen site. Split-thickness autograft will then be placed over the treatment and control sites and secured and dressed using standard surgical techniques. Treatment and control wounds will be examined and photographed on postoperative days 5, 7, and 14 and at the time of discharge. Four-millimeter full-thickness punch biopsies will be taken from the center of the treatment and control sites on postoperative day 14. The wounds will be assessed for percent take, pigmentation, vascularity, and pliability and patients will be queried about pain and itching.

25. (U) 9106 - 9109. 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. Equipment and supplies have been ordered and work will be initiated shortly.

DD FORM 83 MAR 1498 — EDITION OF MAR 68 IS OBSOLETE — * GPO [illegible]

SUPPLEMENTAL WORK UNIT INFORMATION FOR THE PROJECT ENTITLED "A CLINICAL STUDY OF THE EFFICACY OF LOW-DOSE DOPAMINE THERAPY IN HOSPITALIZED BURN PATIENTS"

Subrecord/Linking Accession Number: Not applicable.

Search Control Data: W6K26L/W6K33L, 9 February 1990.

Product Identification: For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report* for fiscal years 1990-1.

Unclassified Special Categories: Volunteers: Adults; RA II.

ABSTRACT

PROJECT NUMBER: 3M162787A874, 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 1990 - 30 September 1991

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]

Low-dose dopamine therapy is occasionally uti ized in burn patients when the fluid resuscitation requirements exceed the predicted rates of infusion; however, the efficacy of this therapy in burn patients has never been documented. It is uncertain if the effects of low-dose dopamine are the same in burn patients who have an associated hypermetabolic response and elevated levels of aldosterone and antidiuretic hormone as in nonburned patients. The purpose of this study is to document the effects of low-dose dopamine therapy on effective renal plasma flow, glomerular filtration rate, solute excretion, and free-water clearance in burn patients and to compare those with previously documented effects seen in various other clinical situations.

Six patients with thermal injury and 6 control subjects were enrolled in this study during this reporting period. Preliminary results of this study indicate that burn patients have significantly higher glomerular filtration rates, effective renal plasma flows, and lower free water clearance than controls, findings which have been previously documented in the literature. As expected, low-dose dopamine significantly increased glomerular filtration rate, effective renal plasma flow, sodium excretion, fractional excretion of sodium, osmolar clearance, and urine output in the control population. Only fractional excretion of sodium and cardiac index were significantly increased in all burn patients.

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).

Dopamine excretion has a half-life of 1-2 min when administered parenterally. Monoamine oxidase rapidly metabolizes dopamine to sulfates and glucuronides by conjugation. This rapid elimination necessitates that dopamine be administered via a continuous infusion, using a metered pump for strict control of rate of flow. In addition, the dose-related pharmacological effects of dopamine occur gradually and the clinical response is variable from patient to patient. Therefore, careful monitoring of blood pressure, cardiac output, and urine flow is mandatory (5).

Side effects are rarely seen when low-dose dopamine infusion is administered. The renal clearance of other drugs which are eliminated primarily by glomerular filtration is increased and dosage adjustment may be required. The clearance of those drugs eliminated by hepatic degradation is likewise augmented secondary to mesenteric vasodilation and increased hepatic flow. Therefore, drugs eliminated by this route must also be closely monitored. Extravasation of dopamine into the subcutaneous tissue can lead to an intense local vasoconstriction and subsequent necrosis. Therefore, dopamine should be 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 effective renal plasma flow (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 will be measured utilizing radiolabeled tracers, both before and during a continuous intravenous dopamine infusion between 1 and 30 days after injury.

The radiopharmaceuticals to be administered in this study include ^{99m}Tc-diethylenetriamine penta-acetic acid (Tc-DTPA) and ^{131}I-hippuran (I-HIP), used to measure GFR and ERPF, respectively. These radiopharmaceuticals will be administered in a loading dose and continuous infusion, delivering minimal radiation exposure (less than a standard chest roentgenogram) and allowing for precise quantitation by gamma counting. The dose of Tc-DTPA and I-HIP will not exceed 4 mCi and 0.35 mCi, respectively. Clearance will be calculated by measurement of both plasma and urine levels of each radiopharmaceutical.

The patients will receive a priming bolus injection and sustaining infusion of each radiopharmaceutical which will be estimated based upon body size and renal function. The loading dose will be estimated to yield (after distribution) plasma levels of < 40,000 cpm/min/ml for the Tc-DPTA and < 1,000 cpm/min/ml for the I-HIP. The patient will then be begun on a continuous infusion of the two compounds to sustain these serum levels. After a 1-h equilibration period (during which distribution and adjustment in serum tracer levels are expected occur), the infusion will then be

continued for an 8-h study period (T=0-8 h). Blood will be 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 will be separated and a measured volume will be counted in a well-type gamma counter for Tc-DTPA and I-HIP simultaneously within a few hours of collection. Timed urine samples, 1 h each, will be collected for 8 h. Aliquots of the urine samples will be counted for radioactivity in volumes and tubes equivalent to those used for plasma. Diluted proportions of the injectate will be counted to determine the dose actually given and to permit correction of spillover of counts for the ^{131}I channel into the ^{99m}Tc channel of the detector. A special computer program has been written to correct for physical decay. From blood and urine samples, we will determine GFR, ERPF, clearances of Na+, total osmolytes, H_2O, and creatinine, and changes in serum thyrotropin (TSH) and plasma dopamine.

Description of Procedures. Treatment should 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 will be eligible for enrollment in the study. Properly witnessed DA Forms 5303-R, Volunteer Agreement Affidavits, will be obtained from each patient, or their legal guardian, before beginning the study.

1. Male or female patients ≥ 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. 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 old.

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 will be placed in burn patients only, with pulmonary artery catheter advanced to measure cardiac output, central venous pressure, and pulmonary capillary wedge pressure. Control subjects will have a large bore peripheral intravenous catheter placed.

7. A Foley catheter will be placed in each burn patient and hourly urine output before treatment will be recorded. Control subjects will stand to void and no catheter will be placed.

8. The burn patient's and control subject's weight in kilograms will be obtained upon admission.

9. A second intravenous catheter will be placed, at a site distant from the central venous cordis catheter, with a 3-way stopcock in place for blood withdrawal.

10. The patient will be 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 will include the amount of fluid (as D5W) that later (beginning at T=4 h) will contain the dopamine.

11. A 2 ml/min urine flow will be established prior to time 0, at which time the bladder will be flushed with 100 ml of air. This air flush technique will be used to end all urine collections. Control subjects will stand to void and will not have a urinary catheter nor undergo bladder air flush.

12. The urine produced during each hour of the 8-h study will be collected for electrolyte and osmolality determinations and a plasma sample, collected from the midpoint, will likewise be analyzed for electrolyte and osmolality determinations.

13. One hour prior to time 0, the priming bolus of the Tc-DPTA and I-HIP will be injected into the infusion port of the central venous cordis catheter in burn patients and the catheter flushed with 5 ml of saline (T=0). Control subjects will have the same dosages injected into the large peripheral intravenous catheter. The constant infusion of the Tc-DPTA and I-HIP will then begin.

14. Beginning at time 0, 2 ml of blood will be 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 will be taken for determination of serum electrolytes, creatinine, and osmolality. At time 0 and at each hour up to 8 h, a 6-ml blood sample will be taken for determination of serum TSH, a marker of dopamine effect, and plasma dopamine.

Dosage and Administration. At T=240 min, dopamine will be administered at a rate of 3 µg/kg/min by continuous intravenous infusion. It will be infused through a central venous catheter, placed in one of the patient's central veins (femoral, subclavian, or internal jugular). The rate of infusion will be controlled by the use of a metered infusion pump. The weight used to calculate the dosage will be the patient's preburn weight. Control subjects will be weighed immediately prior to participating in the study. The infusion volume of dopamine (in D5W) will replace a portion of the infusion rate of D5W that has been ongoing.

Procedures During the Study

1. Continuous blood pressure monitoring will be performed.

2. Continuous cardiac monitoring will be performed using telemetry.

3. Urine will be collected and the volume recorded each hour, using the bladder air flush technique previously described. Control subjects will stand to void.

4. Cardiac output, central venous pressure, and pulmonary capillary wedge pressure will be measured and recorded each hour in the burn patients.

5. Continuous pulse oximetry will be performed.

6. Dopamine infusion will be continued for 4 h (time 4–8 h), so that GFR, ERPF, all clearances, serum TSH, 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 will be discontinued at T=480 min.

Procedures After the Study

1. Continuous blood pressure monitoring will be continued for 24 h poststudy.

2. Continuous cardiac telemetry will be continued for 24 h poststudy.

3. Hourly urine outputs will be recorded for 24 h poststudy.

4. Cardiac output, central venous pressure, and pulmonary capillary wedge pressure will be measured and recorded each hour for 4 h poststudy.

5. A laboratory profile, including standard chemistry and phosphate level will be obtained immediately poststudy and the following morning.

6. The clinical effectiveness of dopamine infusion will be 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. The results of this study will be evaluated using data tables prepared to compare both burn population and control population in terms of ERPF, GFR, 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.

RESULTS

Six patients with thermal injury and 6 control subjects were enrolled in the study during this reporting period. The mean age of burn patients and control subjects was 28.6 ± 3.9 and 20.2 ± 0.49 yr, respectively. The mean total body surface area burn size of the burn patients was 49.2 ± 9.7%. One patient became overtly septic on the day of study and thus was excluded from further analyses, leaving 5 burn patients and 6 control subjects for analyses. Intravenous fluid administration was tailored in the patients to yield a urine output of approximately 2 ml/kg/h, resulting in a mean fluid administration rate of 310 ml/h. Control subjects received a mean 240 ml/h with a urine output that did not differ significantly from the burn patients. The baseline renal function before dopamine infusion for both groups is depicted in Table 1. Burn patients had significantly higher GFR and ERPF renal plasma flows and lower free water clearances than the control population.

Figure 1 contains the urine output data for each individual before and after dopamine infusion. The administration of dopamine resulted in a significant increase in urine output in the control population. Although all burn patients experienced an increase in urine output, the difference was not statistically different using a paired t test.

The effect of dopamine on GFR is depicted in Figure 2. All control subjects experienced a small but significant increase in the GFR with the administration of dopamine. Four of the 5 burn patients had an increase in GFR, although 1 patient had a significant decrease in GFR after dopamine infusion.

The effect of dopamine therapy on renal plasma flow is depicted in Figure 3. All control subjects demonstrated a marked and significant increase in renal plasma flow. Four of the 5 burn patients also demonstrated a marked increase in renal plasma flow. One patient, who had a baseline renal plasma flow > 900 ml/min/1.73 m^2, had no appreciable change in renal plasma flow after the administration of dopamine.

To define whether the alterations in renal plasma flow in the patient population were secondary to the inotropic effect of low-dose dopamine, cardiac index was measured in the patient population. Figure 4 depicts the change of cardiac output for patients before and after the administration of 3 μg/kg/min of dopamine. All patients demonstrated a significant increase in

TABLE 1. Baseline Renal Function (Mean ± SEM)

Group	Glomerular Filtration Rate (ml/min/1.73 m^2)	Effective Renal Plasma Flow (ml/min/1.73 m^2)	Urine Output (ml/min/1.73 m^2)	Osmolar Clearance (osmol/min/1.73 m^2)	Free Water Clearance (ml/min/1.73 m^2)
Burn Patients	147 ± 5.6*	675 ± 76*	157 ± 32	4.0 ± 0.5	-1.4 ± 0.7*
Control subjects	118 ± 4.5*	511 ± 24	203 ± 29	2.9 ± 0.4	0.48 ± .21

*$P < 0.05$ vs control subjects (student's t test).

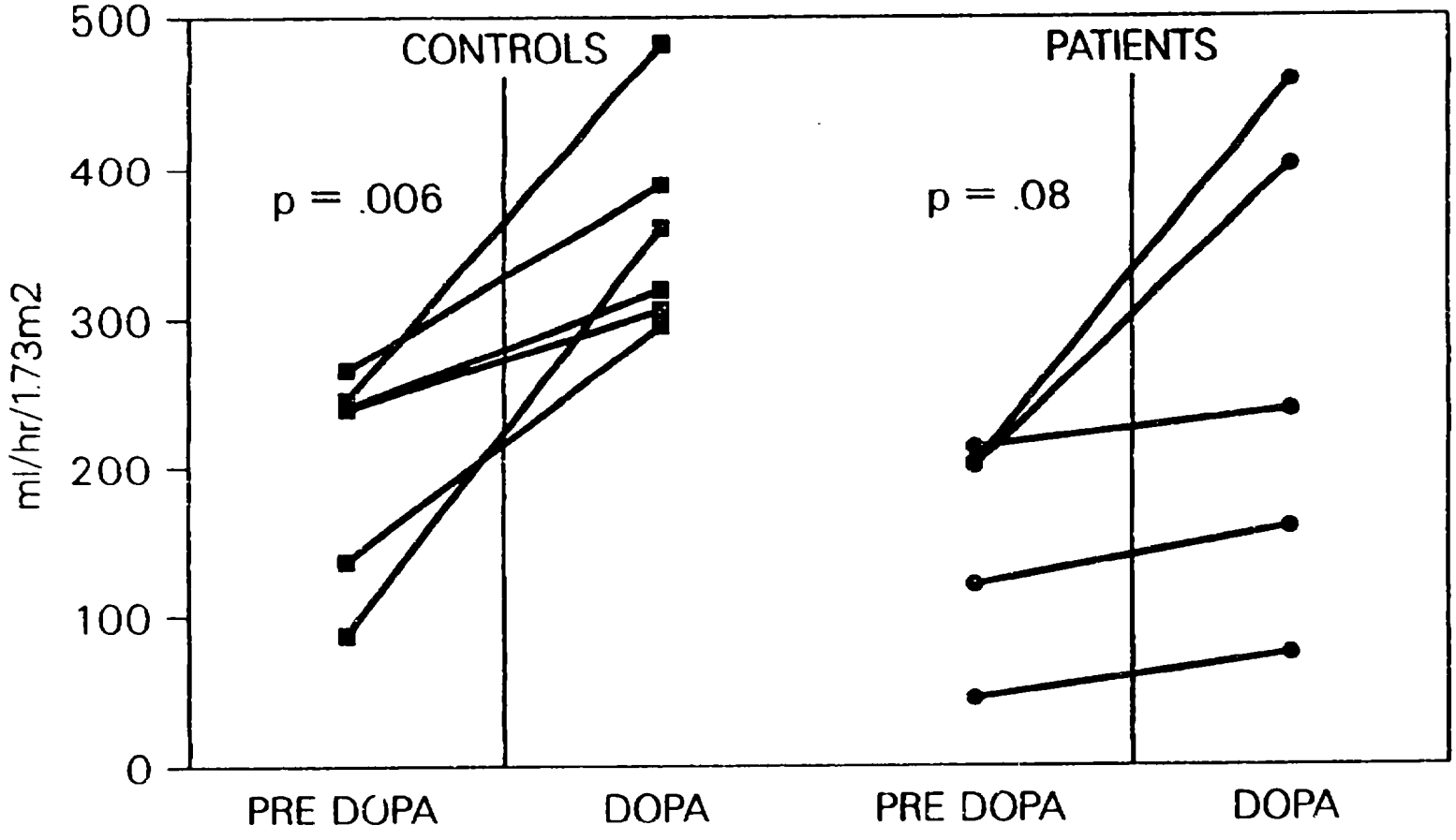

FIGURE 1. Urine output (ml/h/1.73 m^2) for controls and patients before and during dopamine infusion. A paired t test was used to compare predopamine values with dopamine infusion values in each group.

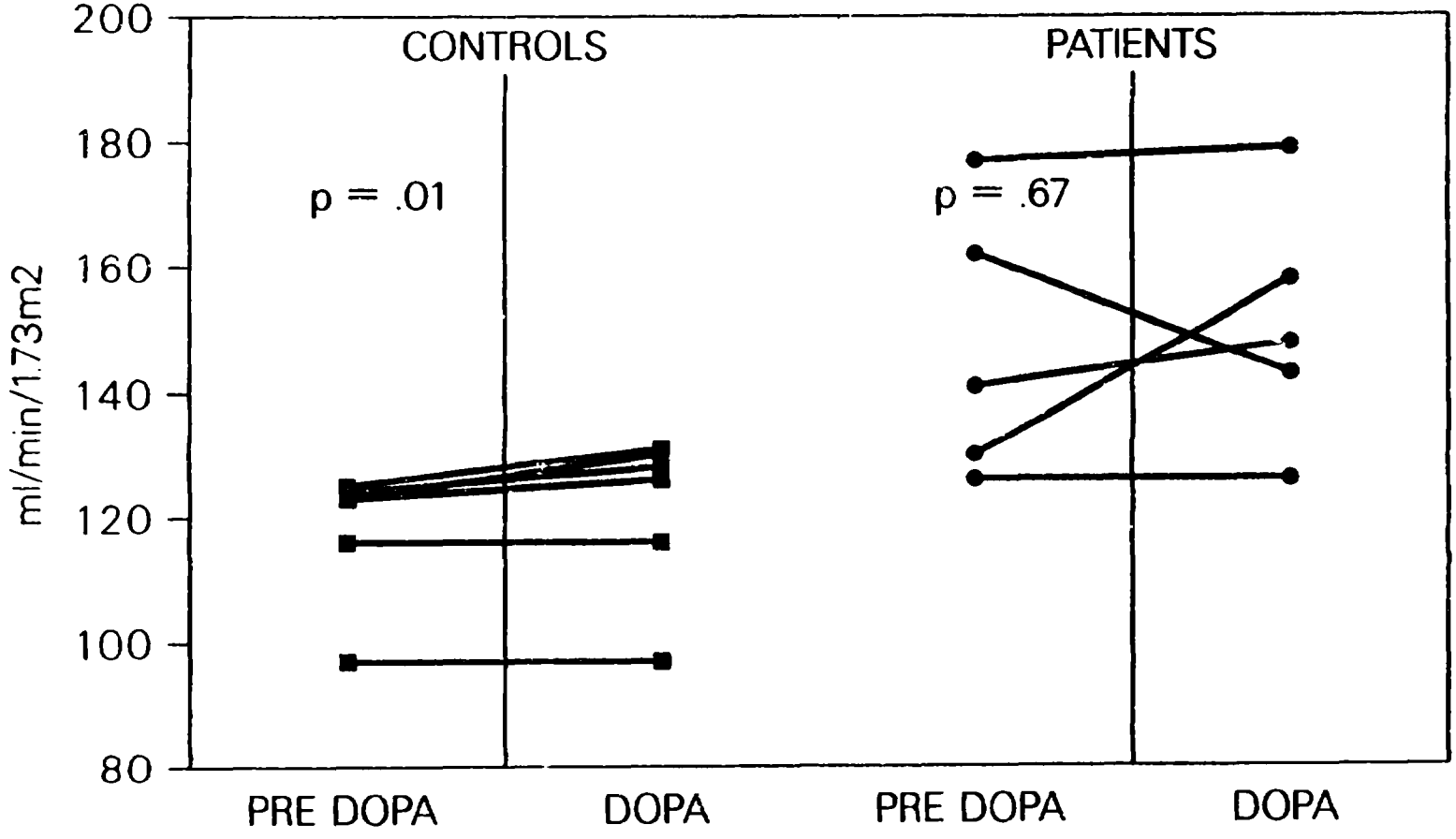

FIGURE 2. Glomerular filtration rate (ml/h/1.73 m^2) before and during dopamine infusion. All control patients demonstrated a small but significant increase in glomerular filtration rate with dopamine infusion. The response in the patients was mixed, with one patient demonstrating a marked decline in function.

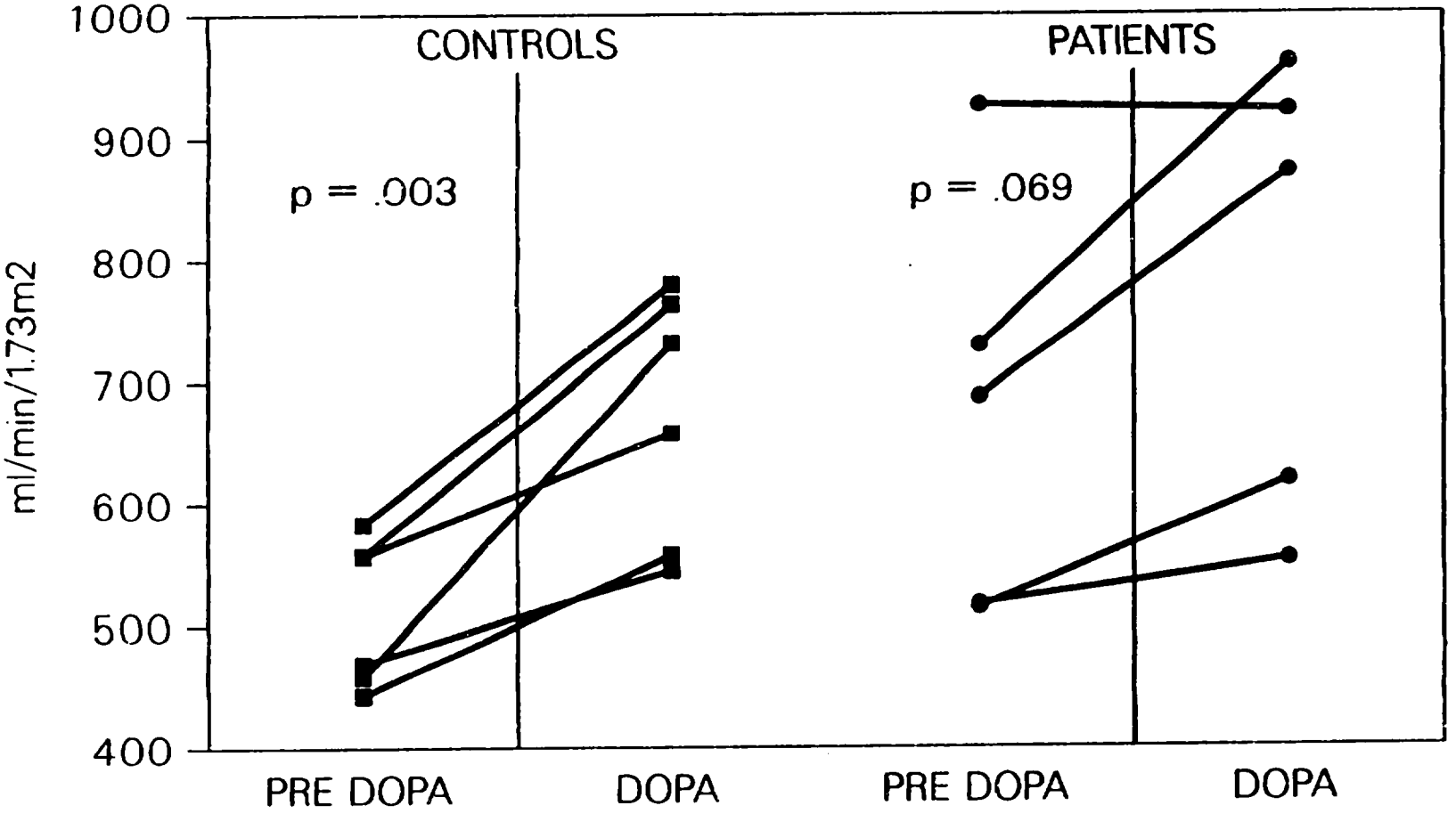

FIGURE 3. Renal plasma flow (%) before and during dopamine infusion. All controls demonstrated a significant increase in renal plasma flow with dopamine. Four of five patients had an increase, while one patient had a small decline in effective renal plasma flow during dopamine infusion.

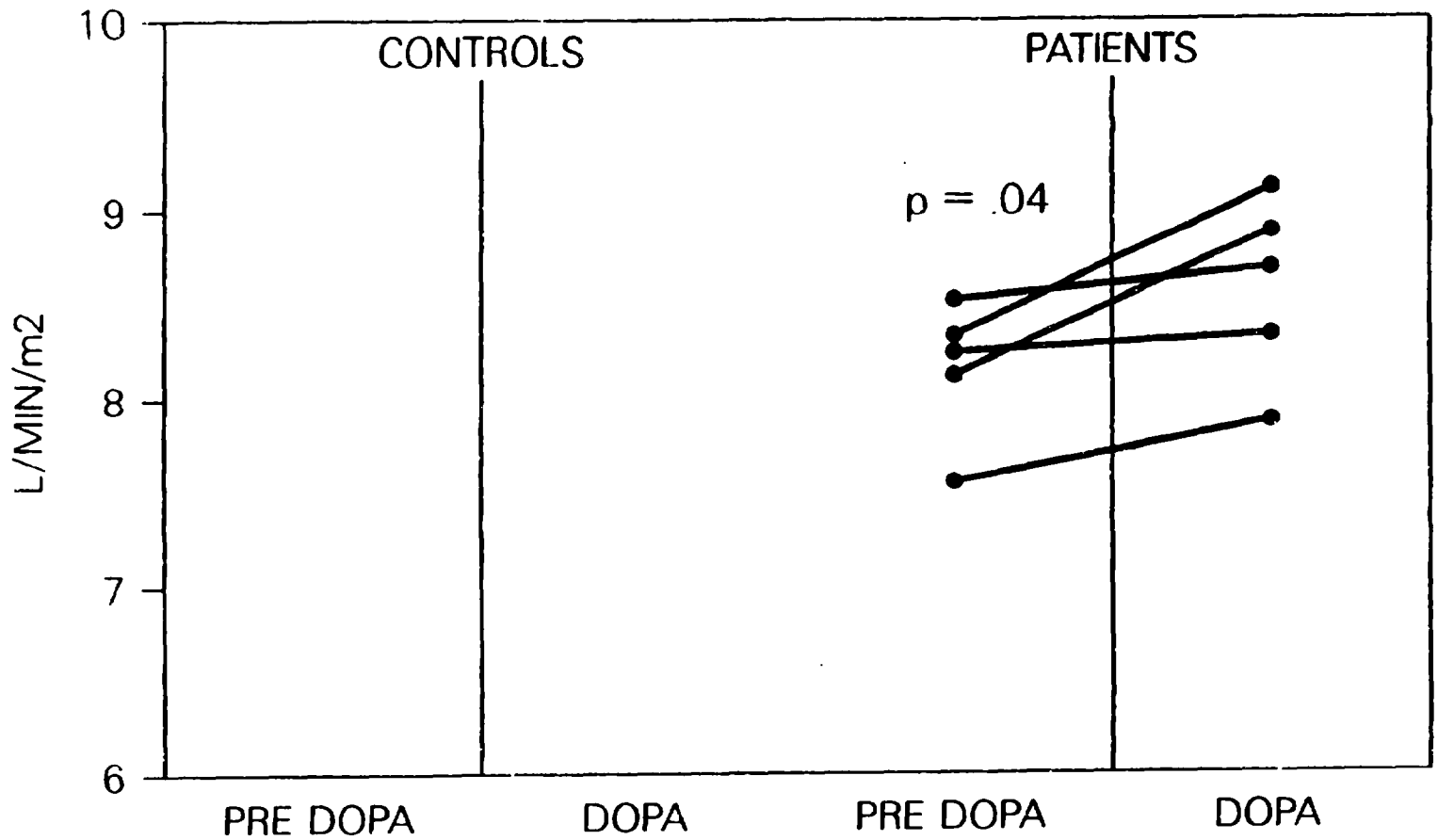

FIGURE 4. Cardiac index (l/min/m^2) before and during dopamine infusion. Cardiac output was not measured in the control population. All patients demonstrated a significant increase in cardiac output with the infusion of low-dose dopamine at 3 μg/kg/min.

cardiac index after dopamine infusion. To define whether or not the increase in renal plasma flow seen in 4 of 5 burn patients was secondary to the increase in cardiac output or to selective renal arterial vasodilatation, the renal plasma flow was indexed to cardiac index. Figure 5 shows that the percent of cardiac output represented by renal plasma flow was not different before and after the administration of low-dose dopamine, indicating that the increase in renal plasma flow was secondary to increase in cardiac output.

To investigate the effect of low-dose dopamine therapy on sodium homeostasis, sodium excretion and fractional excretion of sodium were measured (figs 6 and 7). Fractional excretion of sodium and sodium excretion were significantly increased in the control population by dopamine therapy. These two indices were also increased in 4 of 5 patients. The nonresponder was an individual with an 88% total body surface area burn size whose urine sodium was 2 mEq/l before and after dopamine infusion.

Figure 8 represents the effects of low-dose dopamine therapy on free water clearance in both study populations. Dopamine had no effect on free clearance in either population. Figure 9 depicts the effect of dopamine infusion on osmolar clearance. Osmolar clearance was significantly increased in all control subjects and was increased in 4 of 5 patients by dopamine infusion. The nonresponder was an individual with a 30% total body surface area burn size who also did not have augmentation of renal plasma flow and glomerular filtration rate after dopamine infusion.

DISCUSSION

Preliminary results of this study indicate that burn patients have significantly higher GFRs, ERPFs, and lower free water clearance than controls, findings which have been previously documented in the literature. As expected, low-dose dopamine significantly increased GFR, ERPF, sodium excretion, fractional excretion of sodium, osmolar clearance, and urine output in the control population. Only fractional excretion of sodium and cardiac index were significantly increased in all burn patients. Exclusion of one of the burn patients who did not respond to renal dose dopamine with an increase in cardiac output results in significant increases in renal plasma flow, sodium excretion, osmolar clearance and urine output for the remaining patients. Why this individual with a 30% total body surface area burn failed to respond is not clear.

The augmentation of renal plasma flow in the patients appeared to be secondary to a significant cardiac effect of 3 μg/kg/min of dopamine in the burn patient population. In normals, this level of dopamine infusion has previously been thought to result in little beta-adrenergic receptor stimulation. It appears, from our findings, that the dose-response curve for beta receptors has been

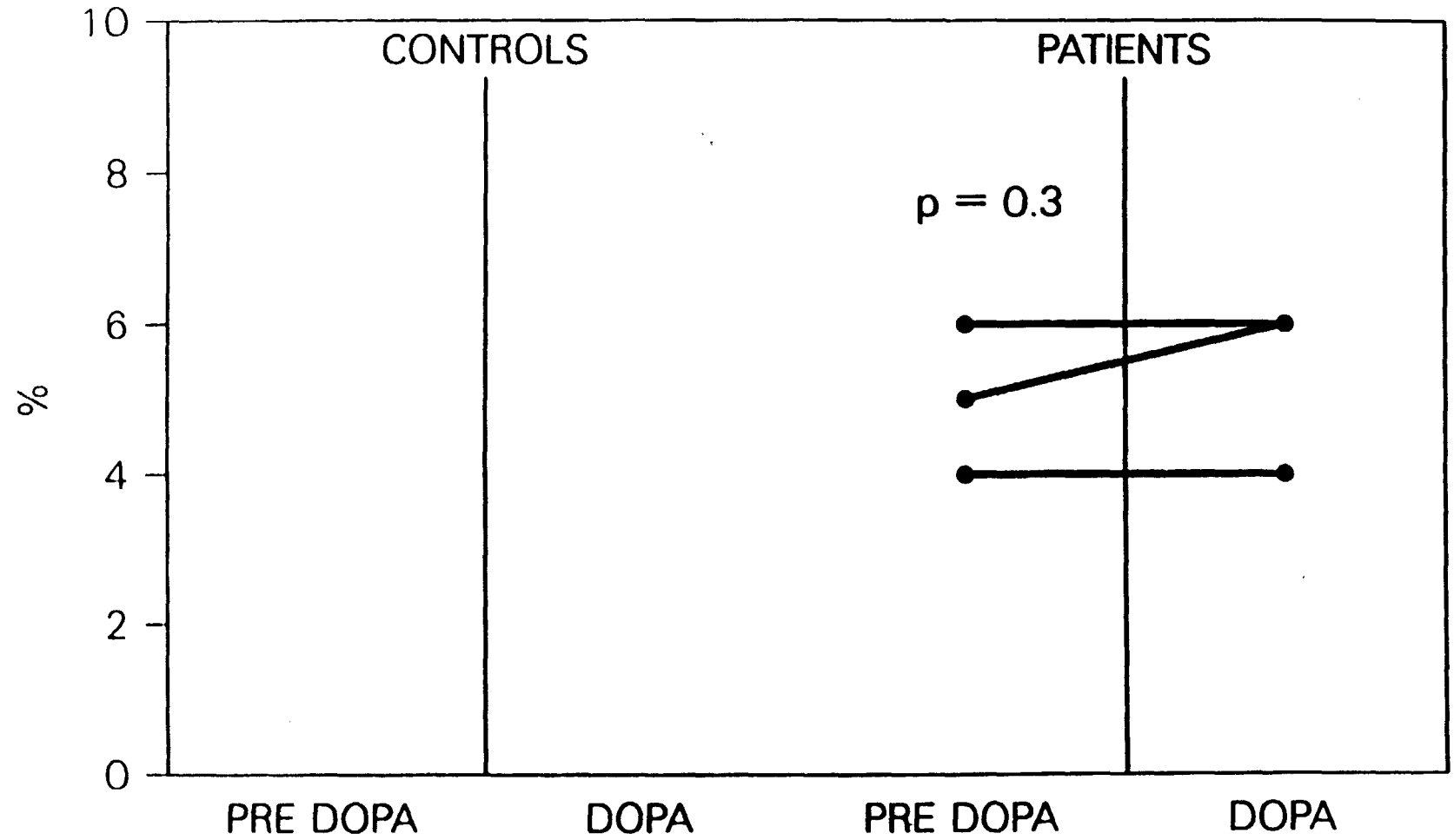

FIGURE 5. Renal plasma flow/cardiac output (ml/min/1.73 m^2) before and during dopamine infusion. In one patient did renal plasma flow increase significantly in proportion to the increase in cardiac output. In all other patients, this ratio did not change, indicating that the increase in effective renal plasma flow was secondary to the increase in cardiac output.

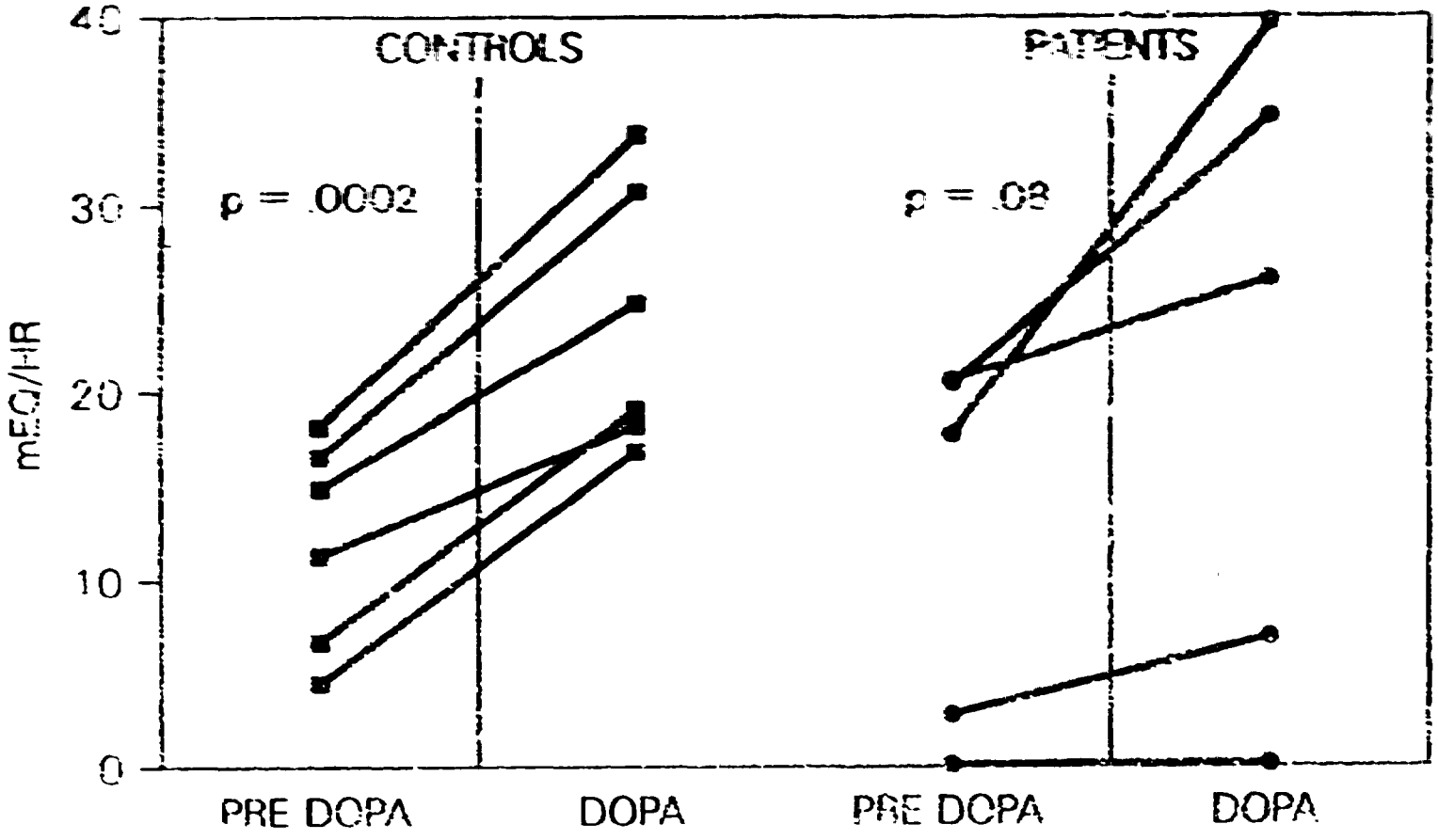

FIGURE 6. Sodium excretion (mEq/h) before and during dopamine infusion. Sodium excretion increased in all control subjects during dopamine infusion. Four of five patients demonstrated a marked increase in sodium excretion, while one patient exhibited no response. This patient excreted < 0.2 mEq/h of sodium, a rate which was not increased by dopamine.

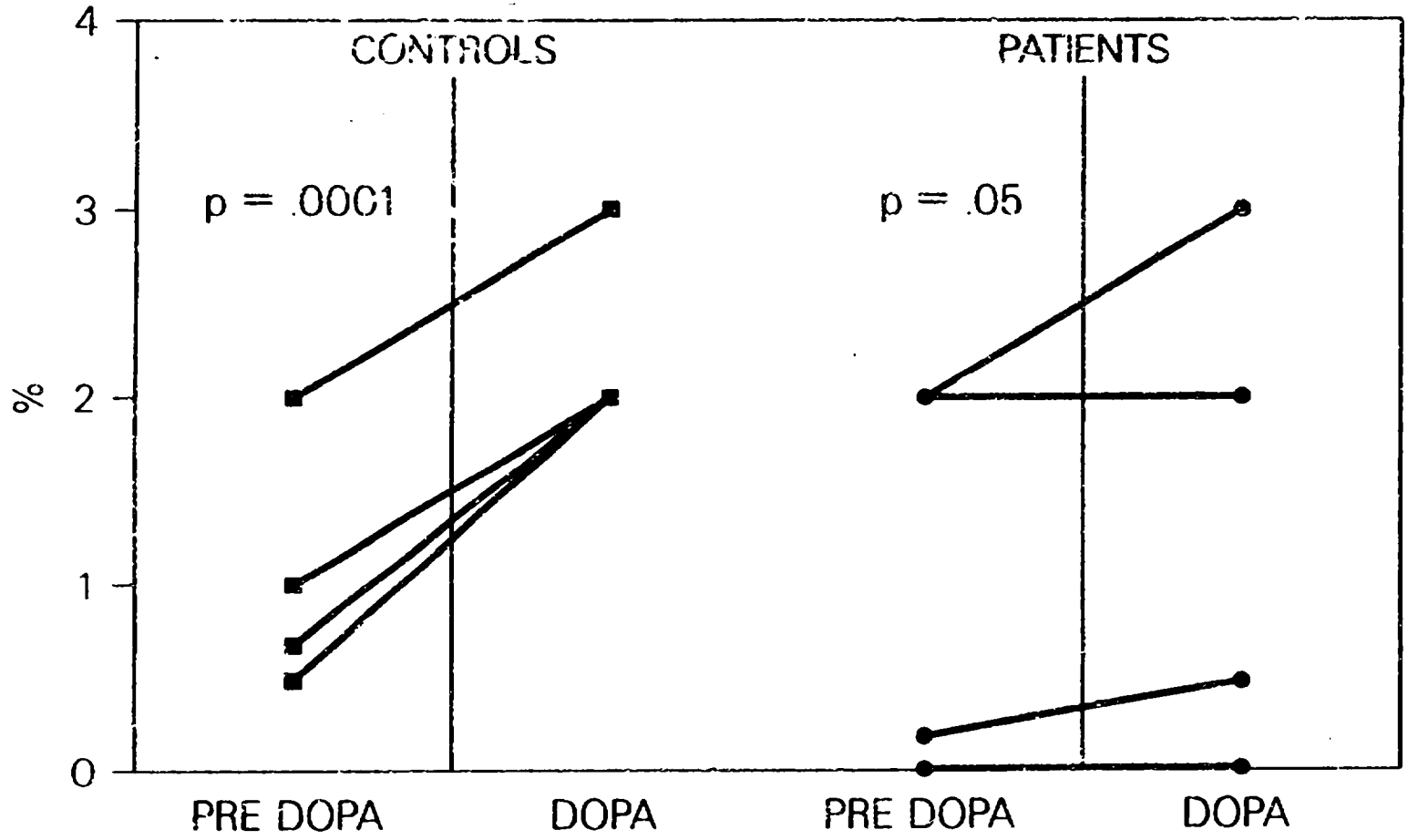

FIGURE 7. Fractional excretion of sodium (%) before and during dopamine infusion. Fractional excretion of sodium before and during dopamine infusion was similar to sodium excretion. In 1 patient, the fractional excretion of sodium was < 0.11%, a rate did not change with dopamine infusion.

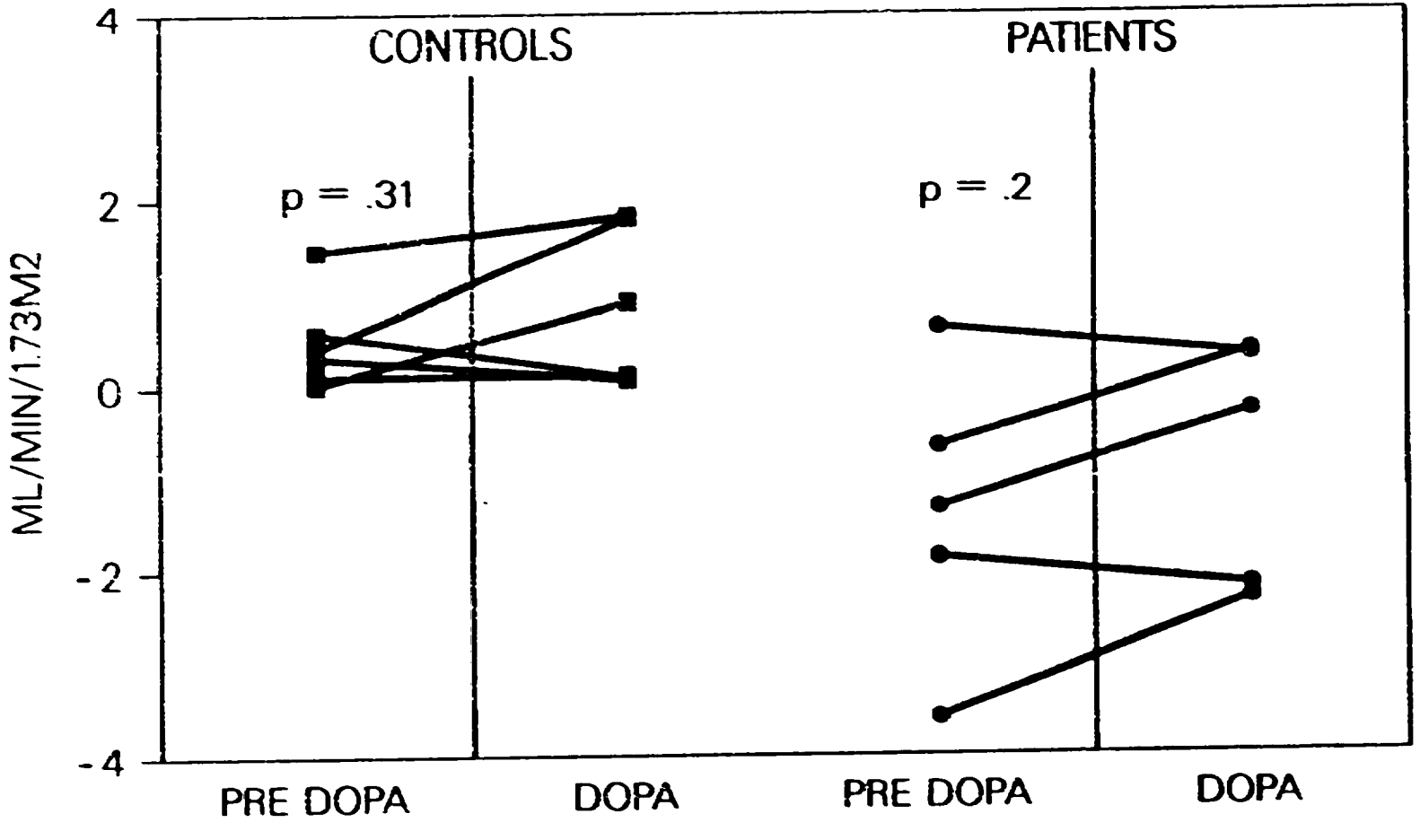

FIGURE 8. Free water clearance (ml/min/1.73 m^2) before and during dopamine infusion. Free water clearance did not change significantly in either population with dopamine infusion.

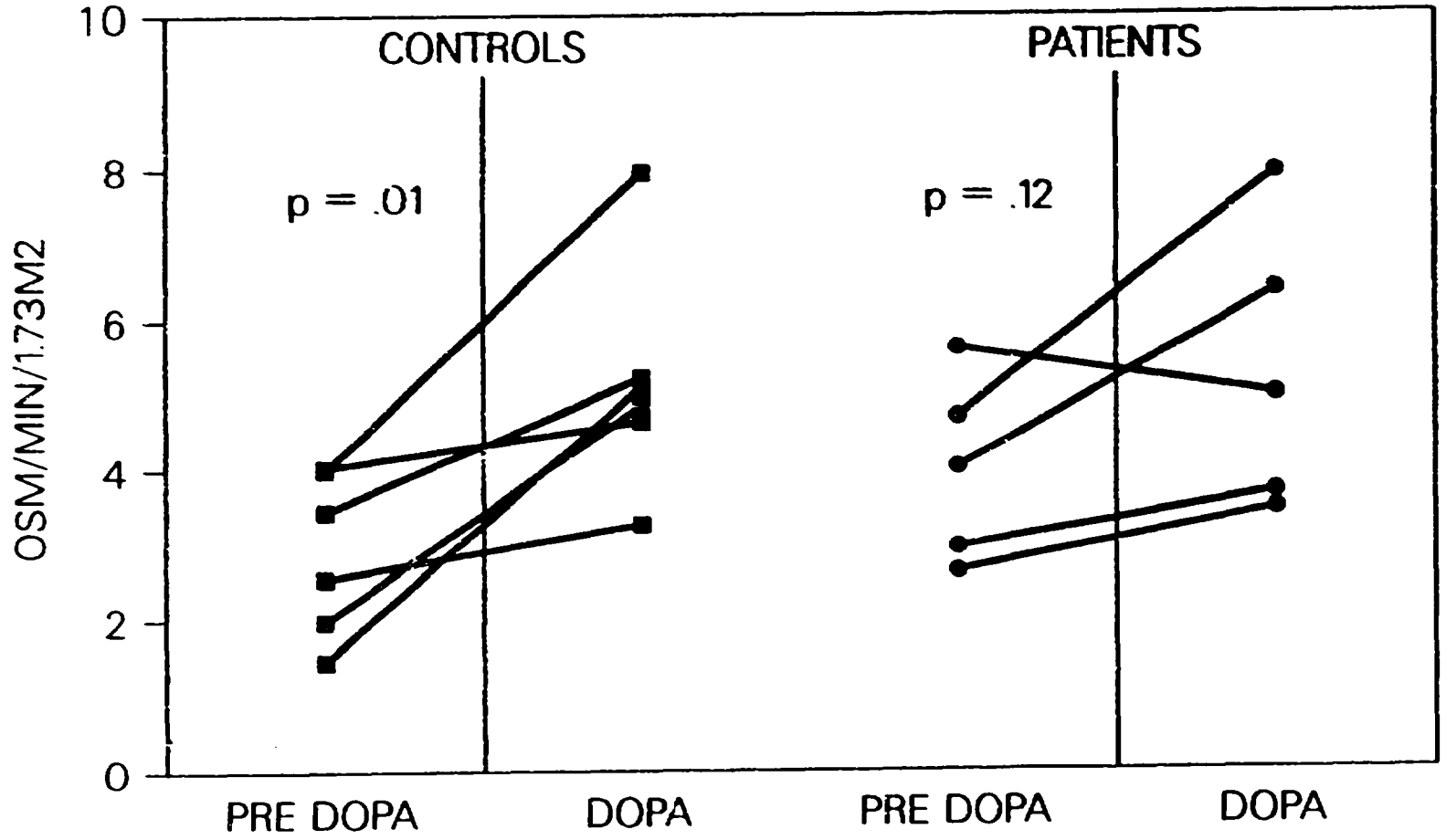

FIGURE 9. Osmolar clearance (osm/min/1.73 m^2) before and during dopamine infusion. Osmolar clearance increased significantly in all control subjects. Four of five patients demonstrated a marked increase in osmolar clearance with dopamine infusion.

shifted to the left, indicating an increase in sensitivity to dopamine. More importantly, it appears that this level of dopamine infusion had a significant effect on the renal tubular handling of sodium in both groups. This indicates that dopamine may play a role as a treatment modality to offload the large sodium load which is administered during the acute resuscitation of thermally injured patients. Completion of the full cohort of patients will be necessary to define in which patients this therapy may be of benefit.

PRESENTATIONS/PUBLICATIONS

Graves TA, Cioffi WG, and Vaughan GM: The effect of low-dose dopamine in thermally injured patients. Presented at the Walter Reed Army Medical Center Surgical Research Conference, Washington, DC, 16 October 1991.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

3 DATE PREP SUMMARY	4 KIND OF SUMMARY	5 SUMMARY SCTY	6 WORK SECURITY	7 REGRADING	8 DISSEMINATION	9
NONE	A. New	U	U		OK	

10 NO CODES — PROGRAM ELEMENT | PROJECT NUMBER | TASK AREA NUMBER | WORK UNIT NUMBER

a PRIMARY 6278 A | 3M162787A878 | SB | 016

b CONTRIBUTING

c CONTRIBUTING

11 TITLE (Precede with Security Classification Code) (U) A Study to Evaluate the Effects of Heparin on the Patency of Arterial Pressure Monitoring Lines

12 SUBJECT AREAS

0605 Medicine and Medical Research 0615 Pharmacology

13 START DATE	14 ESTIMATED COMPLETION DATE	15 FUNDING ORGANIZATION	16 PERFORMANCE METHOD
9105	9209	DA	C. In-House

17 CONTRACT GRANT [redacted] RESOURCES ESTIMATE

a DATE EFFECTIVE	b FISCAL YEARS	c PROFESSIONAL MAN-YEARS	d FUNDS (In thousands)
b. CONTRACT/GRANT NUMBER			
e. TYPE / f. AMOUNT	91	0.1	20
g. KIND OF AWARD / h. CUM TOTAL	92	0.1	20

18 RESPONSIBLE DOD ORGANIZATION

a NAME: US Army Institute of Surgical Research

b ADDRESS (Include zip code): Fort Sam Houston, San Antonio, Texas 78234-5012

c NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A

d TELEPHONE NUMBER (Include area code): 512-221-2720

19 GENERAL USE: FINA

MILITARY/CIVILIAN APPLICATION: M

20 PERFORMING ORGANIZATION

a NAME: US Army Institute of Surgical Research

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

c NAME OF PRINCIPAL INVESTIGATOR: DUNCAN, D J

d TELEPHONE NUMBER (Include area code): 512-221-2482

e NAME OF ASSOCIATE INVESTIGATOR (If applicable): DRAGOO, J M

f NAME OF ASSOCIATE INVESTIGATOR (If applicable): MC HAHUE, W T

22 KEYWORDS (Precede EACH with Security Classification Code) (U) Burns (Injuries); (U) Heparin; (U) Sodium Chloride; (U) Catheters

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

23/24. (U) The objectives of this work are to determine 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. 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.

25. (U) 9105 - 9109. This study was approved by the USAISR Research Council and the US Army Institute of Surgical Research Human Use Committee during the third quarter of fiscal year 1991. Equipment and supplies have been ordered and work will be initiated shortly.

DD FORM 1498 83 MAR — EDITION OF MAR 80 IS OBSOLETE

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION: DA335883	2. DATE OF SUMMARY: 01 Oct 91	REPORT CONTROL SYMBOL (DD-DR&E(AR) 636)

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISS'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
20 May 91	D. Chg	U	U		CX	

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62787A	3M162787A87A	AZ	178
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code) (U) A Study to Evaluate the Effects of Heparin on the Patency of Arterial Pressure Monitoring Lines

12. SUBJECT AREAS
0605 Medicine and Medical Research 0615 Pharmacology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
9105	9209	DA	C. In-House

17. CONTRACT/GRANT MILITARY RELEVANCY CERTIFIED

a. DATE EFFECTIVE	APPROVED [illegible]	d. RESOURCES ESTIMATE		
b. CONTRACT/GRANT NUMBER		a. FISCAL YEARS	b. PROFESSIONAL WORK YEARS	c. FUNDS (In thousands)
c. TYPE	d. AMOUNT	91	0.2	10
e. KIND OF AWARD	f. CUM/TOTAL	92	0.5	23

18. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME: US Army Institute of Surgical Research	a. NAME: US Army Institute of Surgical Research
b. ADDRESS (Include zip code): Fort Sam Houston, San Antonio, Texas 78234-5012	b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-5012
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR: DRISCOLL, D M
d. TELEPHONE NUMBER (Include area code): 512-221-2720	d. TELEPHONE NUMBER (Include area code): 512-221-8482
21. GENERAL USE: FIC	e. NAME OF ASSOCIATE INVESTIGATOR (If available): MC MANUS, W F
MILITARY/CIVILIAN APPLICATION: M	f. NAME OF ASSOCIATE INVESTIGATOR (If available): WARREN, N D

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Burns (Injuries); (U) Heparin; (U) Sodium Chloride; (U) Catheters

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

23/24. (U) The objectives of this work are to determine 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. 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.

25. (U) 9105 - 9109. This study was approved by the USAISR Research Council and the US Army Institute of Surgical Research Human Use Committee during the third quarter of fiscal year 1991. Six patients were enrolled in the study during this reporting period. Upon completion of enrollment, data will be analyzed as indicated.

DD FORM 83 MAR 1498 EDITION OF MAR 88 IS OBSOLETE

SUPPLEMENTAL WORK UNIT INFORMATION FOR THE PROJECT ENTITLED "A STUDY TO EVALUATE THE EFFECTS OF HEPARINIZED VERSUS NONHEPARINIZED FLUSH SOLUTIONS ON THE PATENCY OF ARTERIAL PRESSURE MONITORING LINES"

Subrecord/Linking Accession Number: Not applicable.

Search Control Data: W6L54M/W6L56K, 13 March 1991.

Product Identification: For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1991.*

Unclassified Special Categories: Volunteers: Adults; RA II.

ABSTRACT

PROJECT NUMBER: 3M162787A874, 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: 20 May 1991 - 30 September 1991

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.

Six patients have been enrolled in this study to date. 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 complication 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 old. Female patients must have been surgically sterilized, be postmenopausal (> 45 yr old 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 old.

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

Six patients have been enrolled in this study to date. There have been no complications associated with the patency of arterial lines in these patients.

DISCUSSION

The small number of patients limits the validity of statistical manipulation of the data at this point.

PRESENTATIONS/PUBLICATIONS

None.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION	2. DATE OF SUMMARY 28 JUN 91	REPORT CONTROL SYMBOL DD-DRA&IAR-636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISPOSITION	9. LEVEL OF SUM & WORK UNIT
NONE	A. New	U	U		DM	

10. NO. CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62787A	3M162787A87A	DA	179
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code) (U) A Clinical Study of the Safety and Efficacy of DermaGraft Dermal Replacement

12. SUBJECT AREAS
2605 Medicine and Medical Research

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
9106	9209	DA	C. In-House

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

18. RESOURCES ESTIMATE

FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In Thousands)
91	0.3	27
92	0.5	35

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME: US Army Institute of Surgical Research	a. NAME: US Army Institute of Surgical Research
b. ADDRESS (Include ZIP code): Fort Sam Houston, San Antonio, Texas 78234-5012	b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-5012
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR: WAGUESPACK, R L
d. TELEPHONE NUMBER (Include area code): 512-221-2720	d. TELEPHONE NUMBER (Include area code): 512-221-8440
21. GENERAL USE: PIC MILITARY/CIVILIAN APPLICATION: M	e. NAME OF ASSOCIATE INVESTIGATOR (If available): CIOFFI, W G g. NAME OF ASSOCIATE INVESTIGATOR (If available): DRISCOLL, D M

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

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

23/24. (U) The objective of this work is to assess the short- and long-term usefulness of DermaGraft™ dermal replacement on burn patients. Thirty consecutive patients will be enrolled in the study as part of a multicenter study. Each patient's wounds will be excised in the operating room and one treatment wound site and one control site anatomically comparable as to location and of approximate equal dimensions will be identified. A biopsy for quantitative microbial culture and histology will be taken from each excised wound bed prior to application of grafts. The DermaGraft™ will then be placed onto the chosen site. Split-thickness autograft will then be placed over the treatment and control sites and secured and dressed using standard surgical techniques. Treatment and control wounds will be examined and photographed on postoperative days 5, 7, and 14 and at the time of discharge. Four-millimeter full-thickness punch biopsies will be taken from the center of the treatment and control sites on postoperative day 14. The wounds will be assessed for percent take, pigmentation, vascularity, and pliability and patients will be queried about pain and itching.

25. (U) 9106 - 9109. 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. Patients will be enrolled in the study as they become available.

DD FORM 33 MAR 1498 EDITION OF MAR 88 IS OBSOLETE. USGPO 1991-003/50329

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
				DA335924	01 Oct 91	DD-DR&E(AR) 636
3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISP'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
25 Jun 91	D. Chg	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62787A	3M162787A874	DA	179
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code) (U) A Clinical Study of the Safety and Efficacy of DermaGraft Dermal Replacement

12. SUBJECT AREAS
0605 Medicine and Medical Research

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
9106	9209	DA	C. In-House

17. CONTRACT/GRANT MILITARY RELEVANCY CERTIFIED APPROVED BY Paul A. Brenneman

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

18. RESOURCES ESTIMATE

a. FISCAL YEARS	b. PROFESSIONAL WORKYEARS	c. FUNDS (In thousands)
91	0.2	27
92	0.5	35

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME: US Army Institute of Surgical Research	a. NAME: US Army Institute of Surgical Research
b. ADDRESS (Include zip code): Fort Sam Houston, San Antonio, Texas 78234-5012	b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-5012
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR: WAGUESPACK, R L
d. TELEPHONE NUMBER (Include area code): 512-221-2720	d. TELEPHONE NUMBER (Include area code): 512-221-8440
21. GENERAL USE: FIC MILITARY/CIVILIAN APPLICATION: M	e. NAME OF ASSOCIATE INVESTIGATOR (If available): CIOFFI, W G g. NAME OF ASSOCIATE INVESTIGATOR (If available): DRISCOLL, D M

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

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

23/24. (U) The objective of this work is to assess the short- and long-term usefulness of DermaGraft™ dermal replacement on burn patients. Thirty consecutive patients will be enrolled in the study as part of a multicenter study. Each patient's wounds will be excised in the operating room and one treatment wound site and one control site anatomically comparable as to location and of approximate equal dimensions will be identified. A biopsy for quantitative microbial culture and histology will be taken from each excised wound bed prior to application of grafts. The DermaGraft™ will then be placed onto the chosen site. Split-thickness autograft will then be placed over the treatment and control sites and secured and dressed using standard surgical techniques. Treatment and control wounds will be examined and photographed on postoperative days 5, 7, and 14 and at the time of discharge. Four-millimeter full-thickness punch biopsies will be taken from the center of the treatment and control sites on postoperative day 14. The wounds will be assessed for percent take, pigmentation, vascularity, and pliability and patients will be queried about pain and itching.

25. (U) 9106 - 9109. 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. Equipment and supplies have been ordered and work will be initiated shortly.

DD FORM 83 MAR 1498 EDITION OF MAR 88 IS OBSOLETE

SUPPLEMENTAL WORK UNIT INFORMATION FOR THE PROJECT ENTITLED "A CLINICAL STUDY OF THE SAFETY AND EFFICACY OF DermaGraft™ DERMAL REPLACEMENT"

Subrecord/Linking Accession Number: Not applicable.

Search Control Data: W6031J/W6032J, 15 April 1991.

Product Identification: For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1991.*

Unclassified Special Categories: Volunteers: Adults; RA II.

ABSTRACT

PROJECT NUMBER: 3M162787A874, 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: 25 June 1991 - 30 September 1991

INVESTIGATORS: Robert L. Waguespack, MD, Captain, MC
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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. Supplies have been ordered and work will be initiated shortly.

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 successfully replace thermally injured dermis could result in a better functional result as compared to standard skin grafting techniques. The purpose of this study is 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 will be enrolled in the study as part of a multicenter study. After informed consent is obtained, each patient's wounds will be excised in the operating room and one treatment wound site and one control site will be identified.

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

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

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

2. Patients with donor sites that have not been previously harvested and are sufficiently large for coverage of treatment and control areas.

3. Patients with full-thickness wounds after excision which are 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 is medically indicated for treatment of wound sites.

5. Patients whose wounds can 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 are excluded. The back may also be included.

7. Patients who will be available for the 1-yr follow-up period.

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

1. Patients < 18 yr of age.

2. Patients who are 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 have 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 will undergo a baseline evaluation prior to beginning the study. The following information will be 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 will be archived for future serum or WBC studies. A pregnancy test will be performed on all child-bearing age women.

After full written informed consent is obtained, the patient will be scheduled for excisional treatment within 14 days postburn. After the excision is complete, treatment and control sites will be chosen using a randomization scheme provided by Marrow-Tech Incorporated (Table 1). Treatment and control sites will be of approximately equal dimensions and anatomically comparable as to their location. They may be adjacent. A biopsy for quantitative microbial culture and histology will be taken from each excised wound bed prior to application of the graft. Also, wound bed photographs will be taken prior to application of grafts.

DermaGraft™ will then be prepared. It will be supplied frozen in 4" X 6" sheets enclosed in a Teflon bag for transport. Prior to grafting, the sheets will be thawed (within 2 h of application), rinsed, and removed from the Teflon™ bags. The DermaGraft™ will then be placed onto the chosen site. Split-thickness autograft measuring between 3 and 12/1000s of an inch meshed 1.5:1 or greater will then be prepared using standard surgical techniques. Graft will be placed over the treatment and control areas and secured and dressed using standard surgical techniques.

Treatment and control wounds will then be examined on postoperative days 5, 7, and 14 and at the time of discharge. They will be assessed for percent take, pigmentation, vascularity, and pliability. The patients will be queried about pain and itching. Photographs will be obtained on postoperative days 5, 7, and 14 and at the time of discharge. Four-millimeter full-thickness punch

TABLE 1. Randomization Scheme

Patient	Treatment Site A	Assignment Site B	Order Assignment Site A	Site B	Choice of Placement, If Available								
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.

biopsies will be taken from the center of the treatment and control sites on postoperative day 14.

Determination of Number of Subjects Required. Thirty patients will be enrolled in the study as part of a multicenter effort.

Data Collection. Safety and efficacy data collected during this study will include percent of graft take, percent of epithelization, pigmentation, vascularity, pliability, pain, itching, and overall outcome. All these parameters will be measured at multiple time periods.

Data Analysis Plan. Comparisons of wound healing rates between treatment and control sites will be 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 will be made using categorical log-linear models. Pliability, vascularity, pain, itching, and overall outcome will be measured on scales with a natural ordering. Comparisons between treatment and control sites for these parameters will be made using categorical log-linear models with ordinal (ordered) responses (1).

Time comparisons for pigmentation, vascularity, pliability, scar height, pain, itching, and overall outcome will also be made. If there are responses for all these parameters at all time periods, then the repeated measures analysis will be incorporated into the categorical log-linear analyses (2). If there are missing data, then separate analyses will be made for each time period and a Bonferroni correction will be applied to correct for multiple testing.

RESULTS

This project was approved by the USAISR Research Council and US Army Institute of Surgical Research Human Use Committee during the third quarter of fiscal year 1991. Supplies have been ordered and work will be initiated shortly.

DISCUSSION

When the projected total of 30 patients have completed the study, the data will be analyzed as to the short- and long-term usefulness of the DermaGraft™ dermal replacement on burn patients.

PRESENTATIONS/PUBLICATIONS

None.

REFERENCES

1. Agresti A: *Analysis of Ordinal Categorical Data.* New York: Wiley, 1984.

2. Koch GG, Landis JR, Freeman JL, et al: A general methodology for the analysis of experiments with repeated measurement of categorical data. *Biometrics* 33:133-58, 1977.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION	2. DATE OF SUMMARY 23 Jan 91	REPORT CONTROL SYMBOL DD-DRA&A(AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISPOSITION	9. LEVEL OF SUM A. WORK UNIT
NONE	A. New	U	U		DK	

10. NO. CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	627872	3M162787A874	DA	130
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Provide with Security Classification Code) (U) A Study of the Effects of Weak Direct Current (DC) on Donor Site Healing in the Thermally Injured Patient

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

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
9106	9309	DA	C. In-House

17. CONTRACT/GRANT MILITARY RELEVANCY CERTIFIED

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

18. RESOURCES ESTIMATE

a. FISCAL YEARS	b. PROFESSIONAL WORKYEARS	c. FUNDS (In thousands)
91	0.1	53
92	0.1	123

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME: US Army Institute of Surgical Research	a. NAME: US Army Institute of Surgical Research
b. ADDRESS (Include zip code): Fort Sam Houston, San Antonio, Texas 78234-5012	b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-5012
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR: SHIRANI, K Z
d. TELEPHONE NUMBER (Include area code): 512-221-2720	d. TELEPHONE NUMBER (Include area code): 512-221-4652
21. GENERAL USE: PIC	e. NAME OF ASSOCIATE INVESTIGATOR (If available): CHU, C-S
MILITARY/CIVILIAN APPLICATION: M	f. NAME OF ASSOCIATE INVESTIGATOR (If available): MC MANUS, A T

22. KEYWORDS (Provide EACH with Security Classification Code) (U) Burns (Injuries); (U) Direct Current; (U) Low Voltage; (U) Healing; (U) Skin Grafts

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

23/24. (U) 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. The 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.

25. (U) 9106 - 9109. 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. Nine patients were enrolled in the study during this reporting period. Upon completion of enrollment, the data will be analyzed as indicated.

DD FORM 83 MAR 1498 EDITION OF MAR 88 IS OBSOLETE

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1 AGENCY ACCESSION DA335923	2 DATE OF SUMMARY 01 Oct 91	REPORT CONTROL SYMBOL DD-DR&E(AR) 636

3 DATE PREV SUM'RY	4 KIND OF SUMMARY	5 SUM'ARY SCTY	6 WORK SECURITY	7 REGRADING	8 O.SB'N INSTR'N	9 LEVEL OF SUM A WORK UNIT
20 Jun 91	D. Chg	U	U		CX	

10 NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62787A	3M162787A874	DA	180
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE *(Precede with Security Classification Code)* (U) A Study of the Effects of Weak Direct Current (DC) on Donor Site Healing in the Thermally Injured Patient

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

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
9106	9309	DA	C. In-House

17. CONTRACT/GRANT MILITARY RELEVANCY CERTIFIED
APPROVED BY Paul D. Brooks

a. DATE EFFECTIVE	b. RESOURCES ESTIMATE		
c. CONTRACT/GRANT NUMBER	FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In Thousands)
c. TYPE / d. AMOUNT	91	0.1	53
e. KIND OF AWARD / f. CUM/TOTAL	92	0.1	123

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME: US Army Institute of Surgical Research	a. NAME: US Army Institute of Surgical Research
b. ADDRESS *(Include zip code)*: Fort Sam Houston, San Antonio, Texas 78234-5012	b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-5012
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR: SHIRANI, K Z
d. TELEPHONE NUMBER *(Include area code)*: 512-221-2720	d. TELEPHONE NUMBER *(Include area code)*: 512-221-4652
21. GENERAL USE: FIC	e. NAME OF ASSOCIATE INVESTIGATOR *(If available)*: CHU, C-S
MILITARY/CIVILIAN APPLICATION: N	g. NAME OF ASSOCIATE INVESTIGATOR *(If available)*: MC MANUS, A T

22. KEYWORDS *(Precede EACH with Security Classification Code)* (U) Burns (Injuries); (U) Direct Current; (U) Low Voltage; (U) Healing; (U) Skin Grafts

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

23/24. (U) The objective of this work is to evaluate the effects of weak DC on the healing of partial-thickness skin graft donor sites in patients with thermal injury. The 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.

25. (U) 9106 - 9109. 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. Equipment supplies have been ordered and work will be initiated shortly.

DD FORM 1498 30 MAR — EDITION OF MAR 88 IS OBSOLETE — USAPD [illegible] —91-003/50329

SUPPLEMENTAL WORK UNIT INFORMATION FOR THE PROJECT ENTITLED "A STUDY OF THE EFFECTS OF WEAK DIRECT CURRENT (DC) ON DONOR SITE HEALING IN THE THERMALLY INJURED PATIENT"

Subrecord/Linking Accession Number: Not applicable.

Search Control Data: W6P35E/W6P37F, 4 March 1991.

Product Identification: For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1991.*

Unclassified Special Categories: Volunteers: Adults; RA II.

ABSTRACT

PROJECT NUMBER: 3M162787A874, 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: 20 June 1991 - 30 September 1991

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In the extensively burned patient, the lack of unburned skin is a predominant obstacle to successful wound closure. One solution to the disturbing disparity between skin demand and skin availability in patients with massive burns is to reduce donor site healing time so that available donor skin can be harvested on multiple occasions without sacrificing graft quality. Animal research at the Institute has shown that the application of weak DC to a partial-thickness donor site improves circulation and accelerates healing so that the treated donor site can be used multiple times over a relatively short period of time. If these results are borne out in clinical application, the technique of electric current application to promote wound healing could revolutionize wound care of patients with large burns.

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.

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 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 constitutes 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 2 weeks' 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 an 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 10% 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 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 prior to beginning the study.

Patient Inclusion. Male or female patients ≥ 18 yr old 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 old.

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

This project was approved by the USAISR Research Council, the US Army Institute of Surgical Research Human Use Committee, and The Surgeon General's Human Subjects Research Review Board during the third quarter of fiscal year 1991. Equipment and supplies have been ordered and work will be initiated shortly.

DISCUSSION

When the projected total of 40 patients have completed the study, the data will be analyzed as to the effects of weak DC on the healing of partial-thickness skin graft donor sites in patients with thermal injury.

PRESENTATIONS/PUBLICATIONS

None.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION: DAOG6972	2. DATE OF SUMMARY: 01 Oct 91	REPORT CONTROL SYMBOL: DD-DR&E(AR) 636
3. DATE PREV SUM'RY: 01 Oct 90	4. KIND OF SUMMARY: D. Chg	5. SUMMARY SCTY: U	6. WORK SECURITY: U	7. REGRADING	8. DISP'N INSTR'N: CX	9. LEVEL OF SUM A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS14	FB	301
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code) (U) Studies of Infection and Microbiologic Surveillance of Troops with Thermal Injuries

12. SUBJECT AREAS
0605 Medicine and Medical Research

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

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

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

18. RESOURCES ESTIMATE

FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
91	2.8	105
92	2.8	111

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME: US Army Institute of Surgical Research	a. NAME: US Army Institute of Surgical Research
b. ADDRESS (Include zip code): Fort Sam Houston, San Antonio, Texas 78234-5012	b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-5012
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR: MC MANUS, A T
d. TELEPHONE NUMBER (Include area code): 512-221-2720	d. TELEPHONE NUMBER (Include area code): 512-221-3411
21. GENERAL USE: FIC MILITARY/CIVILIAN APPLICATION: M	f. NAME OF ASSOCIATE INVESTIGATOR (If available) g. NAME OF ASSOCIATE INVESTIGATOR (If available)

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Burns (Injuries); (U) Virulence; (U) Pseudomonas; (U) Klebsiella; (U) Staphylococcus; (U) Septicemia; (U) Chemotherapy; (U) Chemotherapeutic Agents; (U) Antibiotics

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

23/24. (U) The objective of this work is to perform epidemiologic studies, study the response of significant species to topical chemotherapy modalities, and determine the relationship of antibiotic usage to sepsis control. Cultures of human wounds, tissues, and body fluids are carried out with precise strain speciation and differentiation being employed. Virulence is assessed in burn wound models which are also used to assess effectiveness of experimental drugs, both topical and systemic.

25. (U) 9010 - 9109. During calendar year 1990, microbiologic surveillance was carried out on 214 of the 216 admitted and discharged burn patients. More than 9,425 isolates were identified from 11,819 specimens. Gram-negative organisms represented < 39% of isolates. *Klebsiella pneumoniae* was the most common Gram-negative isolate. The most common blood isolate was *Staphylococcus aureus*. *Pseudomonas aeruginosa* was recovered from 3 patients. No *Pseudomonas aeruginosa* wound infections were identified.

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

SUPPLEMENTAL WORK UNIT INFORMATION FOR THE PROJECT ENTITLED "STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE OF TROOPS WITH THERMAL INJURY"

Subrecord/Linking Accession Number: Not applicable.

Search Control Data: W6L05[illegible], W6L09M, 19 October 1989.

Product Identification: For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report* for fiscal years 1977-1991.

Unclassified Special Categories: Volunteers: Adults; Children; Lab Animals: Rats; Mice; RA II.

ABSTRACT

PROJECT NUMBER: 3M161102BS14, 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 1990 - 31 December 1990

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During calendar year 1990, 214 burned patients were cultured and 9,425 isolates were identified. A relatively low colonization frequency (< 39%) with Gram-negative organisms has continued for the seventh reporting period. This was also reflected in an increase in Gram-positive organisms in blood cultures. *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Staphylococcus saprophyticus* represented 27.1% 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 1990. 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 > 1,800 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 1990 antibiotic testing panels are presented in Table 1. Bacterial organisms were tested by agar overlay disc diffusion. Broth dilution minimal inhibitory concentrations and minimal bactericidal concentrations were available upon specific request. The protocol for selecting organisms for in vitro sensitivities was isolation from blood cultures, predominant organisms in biopsy cultures, predominant Gram-negative organisms in sputum and urine cultures with > 10^5 cfu/ml, *Staphylococcus aureus* isolates, *Pseudomonas aeruginosa* isolates, and other organisms as requested.

MICROBIAL SURVEILLANCE

The microbial surveillance protocol established during fiscal year 1983 was continued during calendar year 1990 (1). Cultures were obtained from the wound, sputum, urine, and rectum of each patient upon admission. Thereafter, sputum and urine were cultured three times per week and stools and wound surfaces two times per week. Patients transferred to the convalescent ward and hospitalized > 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 214 patients admitted during 1990 were cultured. Species isolated and number of patients yielding each species are presented in Table 2. Because of the decreased host resistance of the patient population, no organism is considered "normal" flora

TABLE 1. In vitro Sensitivity Panels (1990)

Enteric Organisms	Nonenteric Gram-Negative Organisms	Gram-Positive Organisms
1. Amikacin[a,b]	1. Amikacin[a,b]	1. Amikacin[a,b]
2. Gentamicin[a,b]	2. Gentamicin[a,b]	2. Gentamicin[a,b]
3. Ticarcillin[a]	3. Tobramycin	3. Tobramycin
4. Mezlocillin[a,b]	4. Ticarcillin[a]	4. Mezlocillin[b]
5. Piperacillin[a,b]	5. Mezlocillin[a,b]	5. Piperacillin[a,b]
6. Cefotaxime[a]	6. Piperacillin[a,b]	6. Moxalactam[b]
7. Cefoperazone	7. Moxalactam[a,b]	7. Cefotaxime
8. Sulfadiazine	8. Cefotaxime[a]	8. Cefoperazone
9. Netilmicin[a]	9. Cefoperazone[a]	9. Sulfadiazine
10. Kanamycin	10. Colistin	10. Oxacillin[a]
11. Chloramphenicol	11. Sulfadiazine[a]	11. Cephalothin[a]
12. Tetracycline	12. Netilmicin	12. Vancomycin[a,b]
13. Cefoxitin[a]	13. Kanamycin	13. Chloramphenicol[a]
14. Cefamandole[a]	14. Chloramphenicol	14. Tetracycline[a]
15. Ampicillin[a]	15. Tetracycline	15. Ampicillin
16. Trimethoprim	16. Imipenem-cilastatin[b]	16. Imipenem-cilastatin[b]
17. Trimeth and sulfa	17. Azlocillin[a]	17. Clindamycin[a]
18. Nalidixic acid	18. Norfloxacin	18. Penicillin[a]
19. Imipenem-cilastatin[b]	19. Aztreonam	19. Erythromycin[a]
20. Streptomycin	20. Timentin	20. Streptomycin
21. Aztreonam	21. Ceftazidime[a,b]	21. Ceftazidime[a,b]
22. Norfloxacin	22. Ceftriaxone	22. Ceftriaxone

[a]Reported on daily clinical microbiology report (hard copy).
[b]Reported on computer screen from patient data base.

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 67% 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 4,950 organisms were recovered from respiratory system specimens. The majority of these were sputum cultures collected in the surveillance program. The 10 most frequent species are presented in Table 4, which represents 79.5% of the

TABLE 2. Distribution by Organism (1990)

Organism	Number of Isolates	Number of Patients Colonized	Organism	Number of Isolates	Number of Patients Colonized
Acinetobacter anitratus	53	18	*Proteus mirabilis*	269	34
Acinetobacter lwoffii	15	14	*Proteus vulgaris*	11	4
Aeromonas hydrophila	6	3	*Providencia rettgeri*	4	2
Alcaligenes faecalis	16	7	*Providencia stuartii*	1	1
Aspergillus fumigatus	61	12	*Pseudomonas aeruginosa*	927	69
Aspergillus terreus	11	9	*Pseudomonas cepacia*	1	1
Bacillus	123	71	*Pseudomonas maltophila*	30	5
Bacillus megaterium	1	1	*Pseudomonas putrifaciens*	3	1
Branhamella catarrhalis	2	2	*Pseudomonas putida*	13	7
Candida albicans	277	47	*Salmonella*, serogroup C-2	2	1
Candida tropicalis	6	4	*Serratia marcescens*	506	39
Citrobacter freundii	7	3	*Staphylococcus aureus*	1,684	125
Citrobacter diversus	48	18	*Staphylococcus epidermidis*	682	167
Citrobacter species	1	1	*Staphylococcus saprophyticus*	294	76
Corynebacterium species	1	1	Alpha *Streptococcus*	91	42
Enterobacter aerogenes	135	34	Beta hemolytic *Streptococcus*	1	1
Enterobacter agglomerans	40	21	Beta *Streptococcus*, Not Group A, B, or D	93	39
Enterobacter cloacae	188	41	Group A nonhemolytic beta *Streptococcus*	2	2
Escherichia coli	331	68	Group B *Streptococcus*	9	3
Flavobacterium IIB	2	1	Group C *Streptococcus*	5	3
Flavobacterium species	1	1	Group D *Streptococcus*, not *Enterococcus*	170	87
Haemophilus influenzae	3	3	Group D *Enterococcus*	409	66
Klebsiella oxytoca	30	10	Nonhemolytic *Streptococcus*	3	3
Klebsiella ozaenae	1	1	Nonhemolytic *Streptococcus*, not Group D	439	144
Klebsiella pneumoniae	728	81	*Streptococcus pneumoniae*	*6*	*6*
Morganella morganii	54	16	*Streptococcus viridans*	1,098	175
Neisseria gonorrhocae	1	1	True fungi species (other)	312	59
Neisseria lactamica	2	2	Yeast species (other)	16	10
Neisseria mucosa	4	4			
Neisseria sicca	183	72			
Neisseria species	8	7			
Neisseria subflava	2	2			
Propionibacterium acnes	3	3			

Total Number of Isolates = 9,425

Total Number of Cultured Patients = 214

TABLE 3. Ten Most Frequent Isolates (1990)

Organism	Number of Patients Colonized	% Patients	Number of Isolates	% Total Isolates
Streptococcus viridans	175	81.8	1,098	11.6
Staphylococcus epidermidis	167	78.0	682	7.2
Nonhemolytic *Streptococcus*, not Group D	144	67.3	439	4.7
Staphylococcus aureus	125	58.4	1,684	17.9
Group D *Streptococcus*, not *Enterococcus*	87	40.7	170	1.8
Klebsiella pneumoniae	81	37.9	728	7.7
Staphlococcus saprophyticus	76	35.5	294	3.1
Neisseria sicca	72	33.6	183	1.9
Bacillus	71	33.2	123	1.3
Pseudomonas aeruginosa	69	32.2	927	9.8

Total Number of Patients Cultured = 214
Total Number of Isolates = 9,425

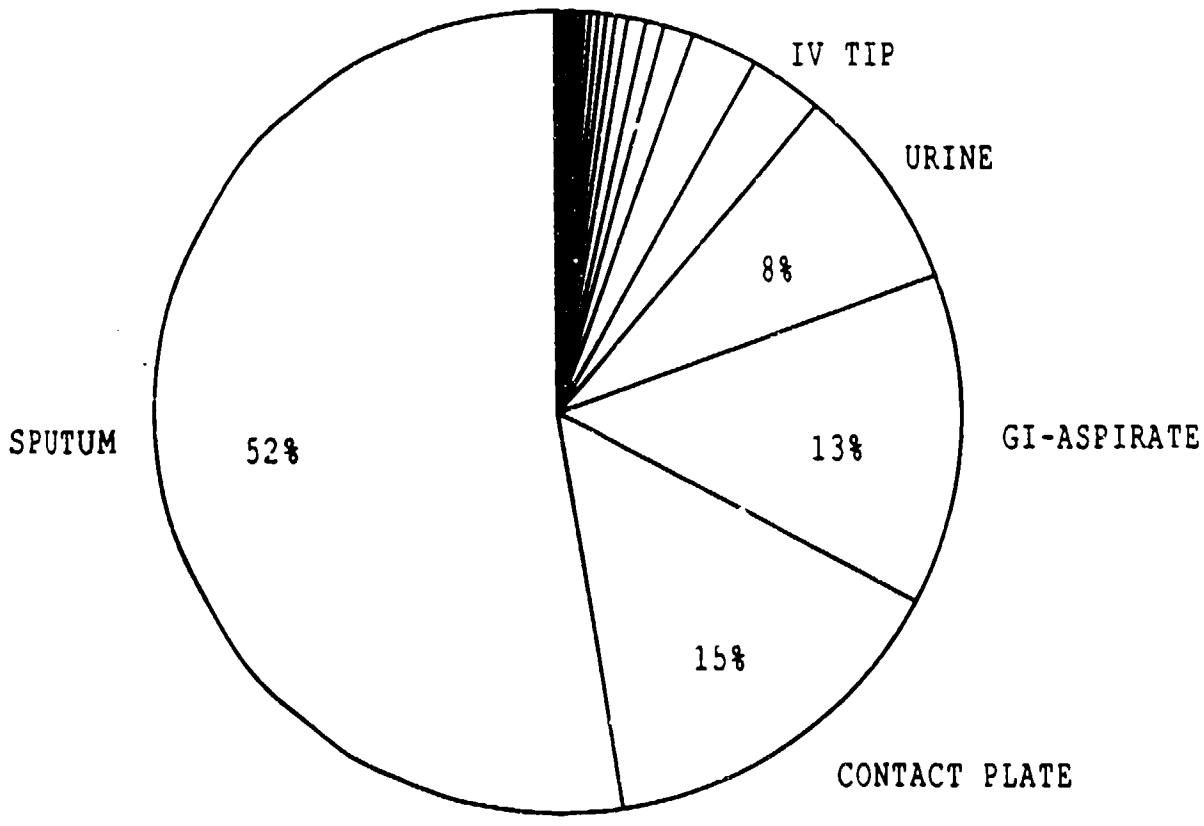


FIGURE 1. Display of the relative frequency of specimen sources yielding isolates in 1990.

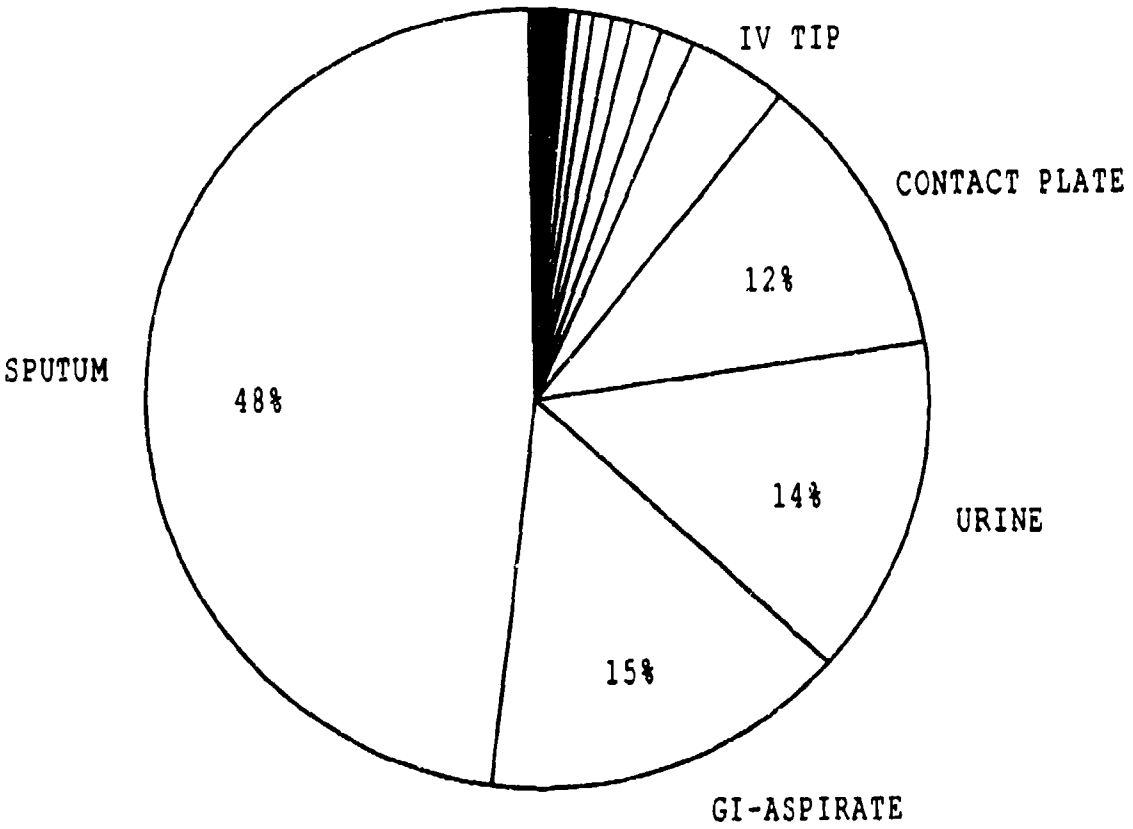


FIGURE 2A. Display of the relative frequency of specimen sources yielding Gram-negative organisms.

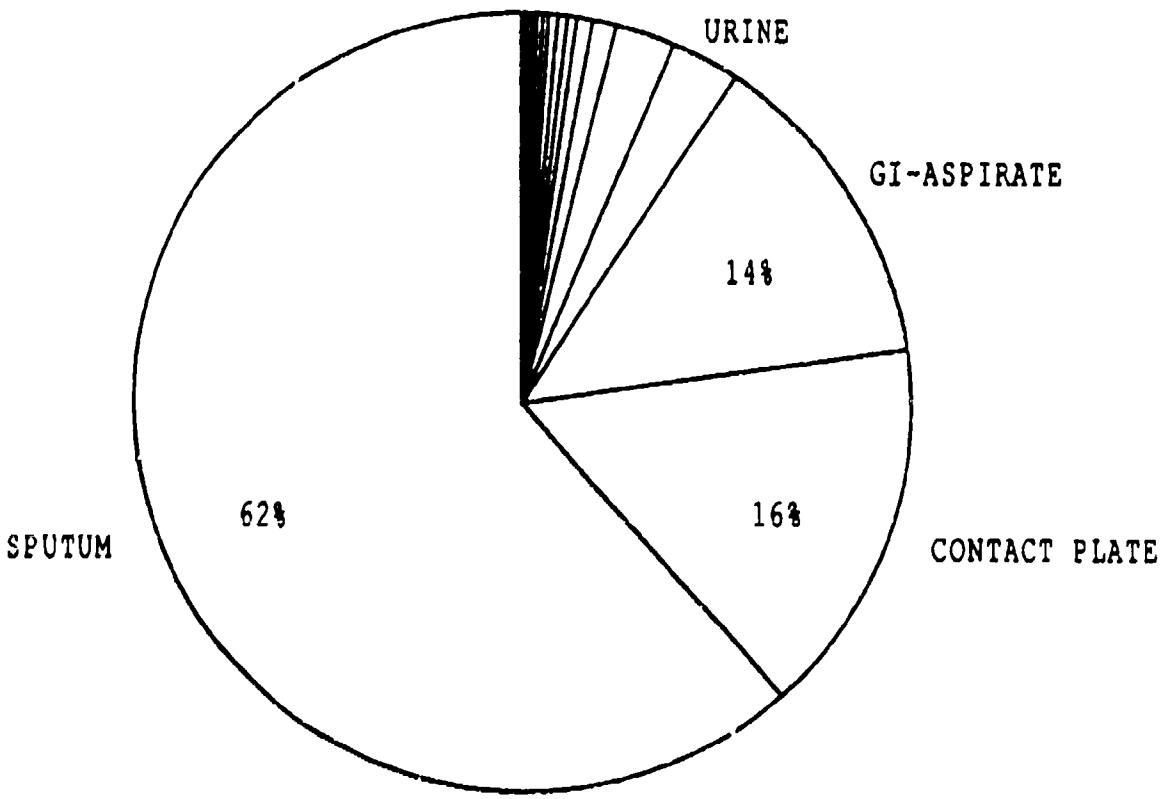


FIGURE 2B. Display of the relative frequency of specimen sources yielding Gram-positive organisms.

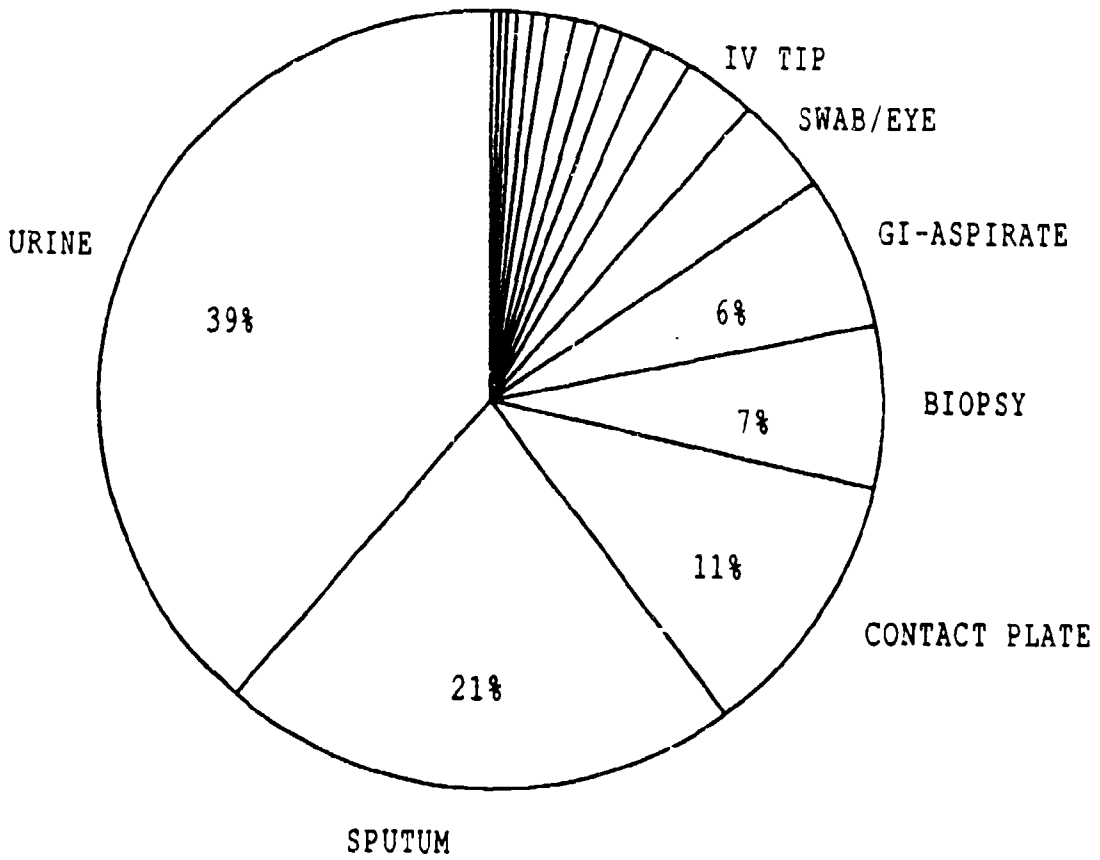


FIGURE 2C. Display of the relative frequency of specimen sources yielding yeast-like organisms.

TABLE 4. Ten Most Frequent Isolates from Respiratory Sources (1990)

Organism	Number of Patients Colonized	% Patients	Number of Isolates	% Total Isolates
Streptococcus viridans	168	94.9	929	18.8
Nonhemolytic *Streptococcus*, not Group D	132	74.6	364	7.4
Staphylococcus epidermidis	101	57.1	275	5.6
Staphylococcus aureus	89	50.3	1,032	20.8
Group D *Streptococcus*, not *Enterococcus*	79	44.6	146	2.9
Neisseria sicca	68	38.4	157	3.2
Klebsiella pneumoniae	39	22.0	326	6.6
Alpha hemolytic *Streptococcus*	38	21.5	74	1.5
Staphylococcus saprophyticus	37	20.9	144	2.9
Pseudomonas aeruginosa	34	19.2	483	9.8

Total Number of Patients Cultured = 177
Total Number of Isolates = 4,950

respiratory isolates. Of particular note is the continued decline of Gram-negative isolates. *Pseudomonas aeruginosa* colonized 34 of the 177 patients with an isolate. This was a significant increase ($P > 0.05$) from previous years.

FLORA RECOVERED FROM WOUND SURFACE SPECIMENS

A total of 2,047 contact plate surface cultures were taken and 1,392 isolates were made. Relative frequencies of isolated species are presented in Figure 3. Subsurface flora, as measured by biopsy specimens, was measured in 955 biopsies taken from 134 patients. Organisms were recovered from 72 of the biopsied patients. The 10 most common organisms are presented in Table 5. Filamentous fungi remained the principal isolate with Aspergillus being the most common fungal genus. *Pseudomonas aeruginosa* was recovered from 33 biopsies taken from 14 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 205 patients yielded 773 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 112 patients for a total of 964 cultures. The principal organisms recovered are listed in Table 8. Positive cultures were obtained from 31 patients and 80 isolates were made from 76 positive cultures. Forty-eight 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 76 patients. Isolations were made from 56 patients and 284 isolates were made. Data are presented in Table 9. These data show an unexpectedly high incidence of contamination.

SUMMARY OF ANTIBIOTIC TESTING

A total of 4,836 bacterial isolates were tested for in vitro sensitivity to antibiotics. A comparison of sources of tested strains is presented in Figure 4. The relative frequency of tested organisms is presented in Figure 5.

Gentamicin resistance was again used as a plasmid surveillance marker. Testing was done on 4,396 isolates. Figure 6 displays the

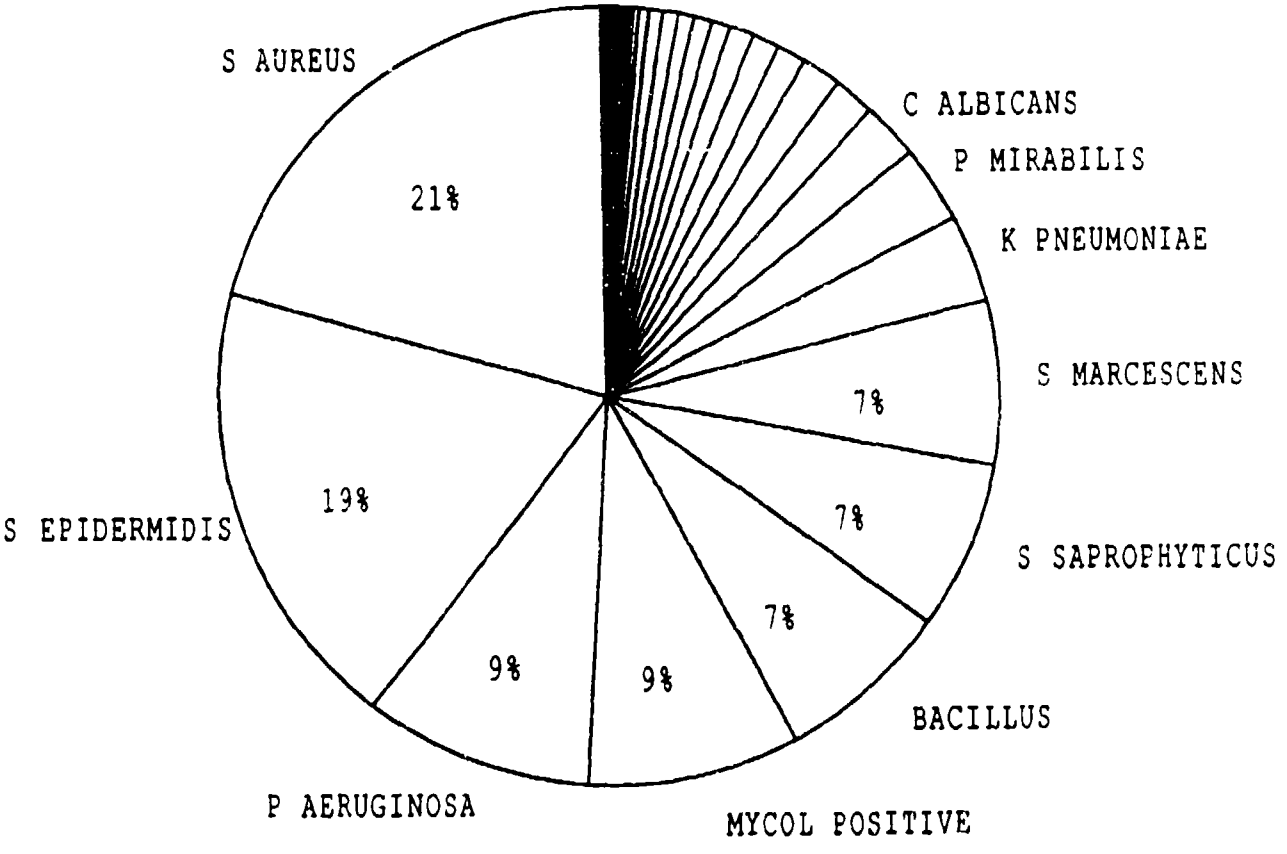


FIGURE 3. Display of the relative frequency of organism types isolated from surface wound cultures.

TABLE 5. Principal Organisms Recovered in Biopsy Specimens (1990)

Organism	Number of Patients Colonized	% Patients	Number of Isolates	% Total Isolates
Filamentous fungi	42	31.3	245	56.8
Staphylococcus aureus	19	14.2	33	7.7
Pseudomonas aeruginosa	14	10.7	33	7.7
Candida albicans	13	9.7	28	6.5
Staphylococcus epidermidis	8	6.0	8	1.9
Group D *Enterococcus*	7	5.2	10	2.3
Klebsiella pneumonia	6	4.5	13	3.0
Staphylococcus saprophyticus	6	4.5	8	1.9
Streptococcus viridans	6	4.5	6	1.3
Escherichia coli	5	3.7	11	2.6

Total Number of Patients Biopsied = 134
Total Number of Isolates = 431
Biopsies Taken = 955

TABLE 6. Ten Most Frequent Organisms from Urinary Specimens (1990)

Organism	Number of Patients Colonized	% Patients	Number of Isolates	% Total Isolates
Klebsiella pneumoniae	48	23.4	107	13.8
Escherichia coli	39	19.0	115	14.9
Pseudomonas aeruginosa	32	15.6	117	15.1
Group D *Enterococcus*	27	13.2	60	7.8
Candida albicans	25	12.2	101	13.1
Proteus mirabilis	21	10.2	53	6.9
Streptococcus epidermidis	20	9.8	29	3.8
Staphylococcus aureus	15	7.3	20	2.6
Enterobacter cloacae	14	6.8	21	2.7
Streptococcus viridans	13	6.3	17	2.2

Total Number of Patients Cultured = 205
Total Number of Isolates = 773

TABLE 7. Ten Most Frequent Organisms from Urinary Specimens with ≥ 10^5 cfu (1990)

Organism	Number of Patients Colonized	% Patients	Number of Isolates	% Total Isolates
Escherichia coli	23	32.4	56	14.5
Klebsiella pneumoniae	23	32.4	48	12.4
Pseudomonas aeruginosa	18	25.4	65	16.8
Proteus mirabilis	15	21.1	28	7.3
Candida albicans	13	18.3	40	10.4
Group D *Enterococcus*	13	18.3	28	7.3
Staphylococcus epidermidis	11	15.5	16	4.1
Enterobacter cloacae	7	9.6	10	2.6
Staphylococcus aureus	6	8.5	8	2.1
Serratia marcescens	5	7.0	30	7.8

Total Number of Patients Cultured = 71
Total Number of Isolates = 386

TABLE 8. Principal Organisms Found in Blood Cultures (1990)

Organism	Number of Patients	% Patients Cultured	% Cases	Number of Isolates	% Total Isolates
Klebsiella pneumoniae	8	7.1	16.7	12	15.0
Staphylococcus aureus	7	6.3	14.6	10	12.5
Staphylococcus epidermidis	5	4.5	10.4	5	6.3
Escherichia coli	3	2.7	6.3	3	3.8
Propionibacterium acnes	3	2.7	6.3	3	3.8
Pseudomonas aeruginosa	3	2.7	6.3	21	26.3
Serratia marcescens	3	2.7	6.3	7	8.8
Candida albicans	2	1.9	4.2	4	5.0
Enterobacter cloacae	2	1.9	4.2	3	3.8

Total Number of Patients Cultured = 112
Total Number of Isolates = 80
Total Number of Cases = 48*

Total Number of Cultures = 964
Total Number of Patient Positives = 31

*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 (1990)

Organism	Number of Patients Colonized	% Patients	Number of Isolates	% Total Isolates
Group D *Enterococcus*	22	28.9	39	13.7
Staphylococcus aureus	20	26.3	32	11.3
Staphylococcus epidermidis	20	26.3	31	10.9
Klebsiella pneumoniae	18	23.7	38	13.4
Pseudomonas aeruginosa	15	19.7	46	16.2
Escherichia coli	10	13.2	8	2.8
Serratia marcescens	9	11.8	28	9.9
Staphylococcus saprophyticus	7	9.2	8	2.8
Proteus mirabilis	6	7.9	8	2.8
Candida albicans	5	6.6	9	3.2

Total Number of Patients Cultured = 76
Total Number of Isolates = 284

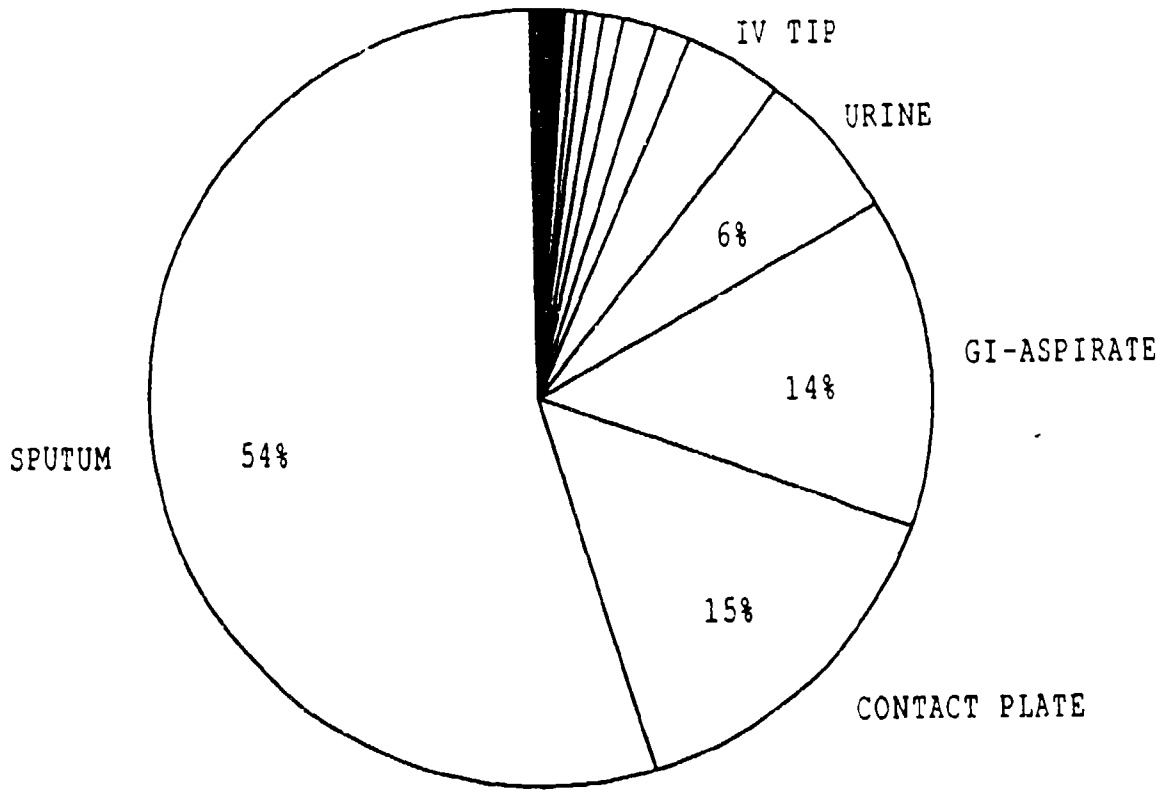


FIGURE 4. Display of the relative frequency of sources yielding organisms tested for in vitro sensitivity to antibiotics in 1990.

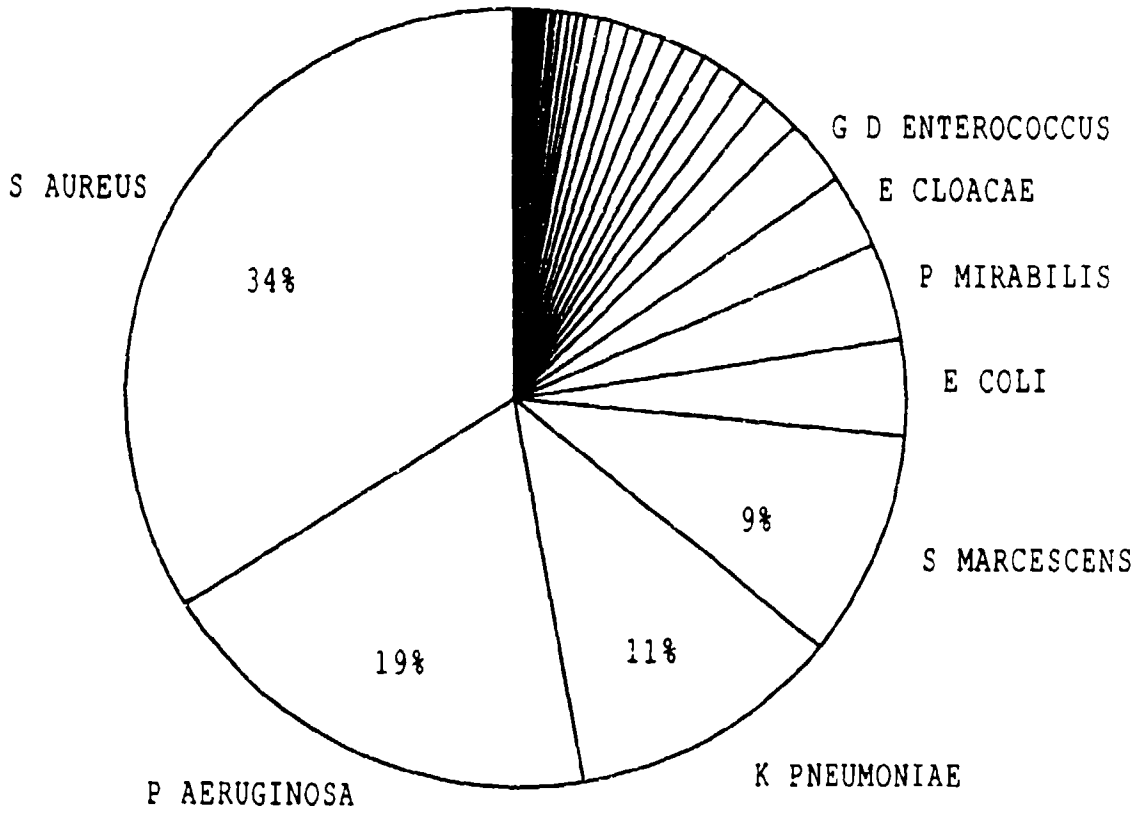


FIGURE 5. Display of the relative frequency of organisms tested for in vitro sensitivity to antibiotics in 1990.

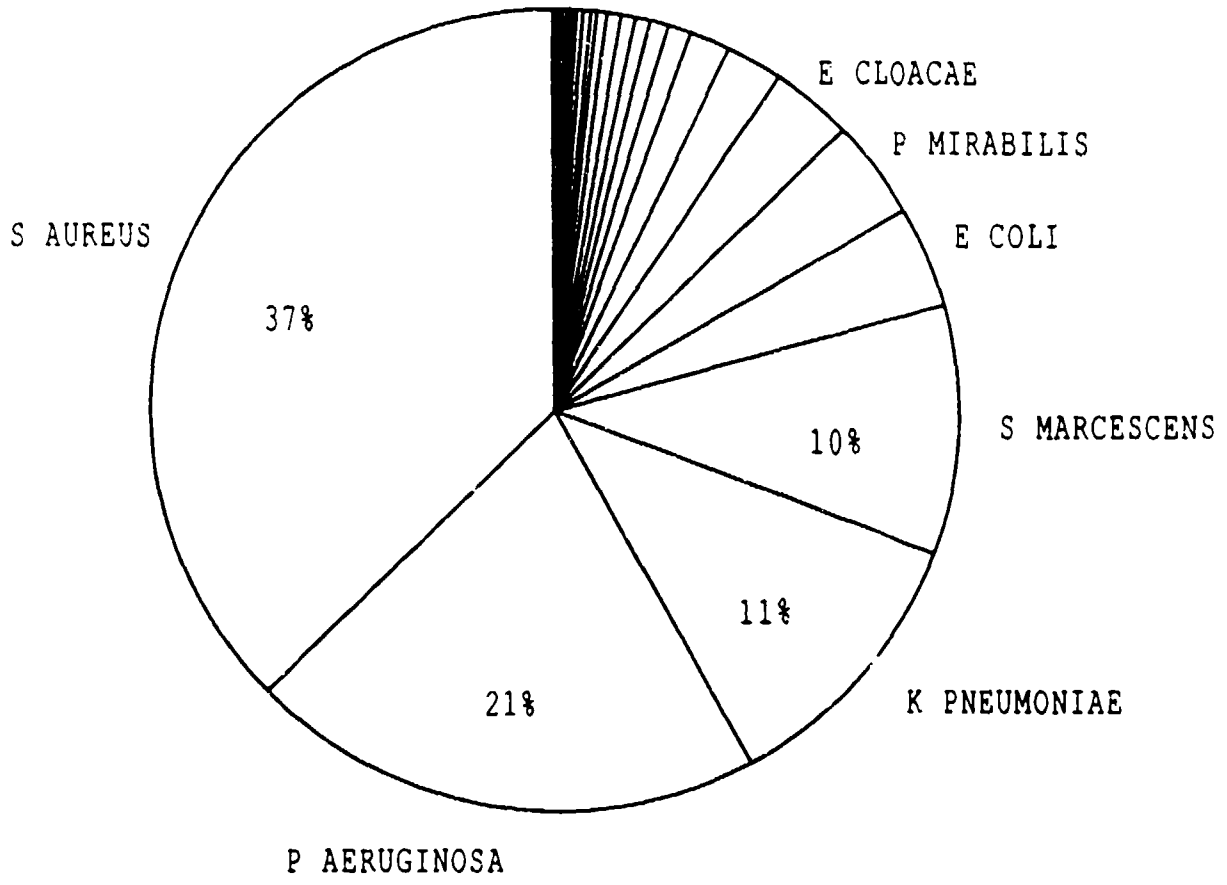


FIGURE 6. Display of the relative frequency of organisms tested for in vitro sensitivity to gentamicin in 1990.

relative frequency of tested organisms. Figure 7 displays the frequency of resistant species. *Staphylococcus aureus* represented 65% of the gentamicin-resistant isolates. Only 582 Gram-negative isolates of 2,789 strains tested were resistant to gentamicin (20.9%). 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* was 34% of isolates and these strains were isolated from 57 patients. The resistant strains are multiply resistant, with expression of gentamicin, erythromycin, oxacillin, and streptomycin resistance. Multiply resistant *Staphylococcus aureus* and gentamicin-sensitive strains are displayed separately in Table 10 and histograms are shown in Figure 9.

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

Klebsiella pneumoniae. A total of 496 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.

Serratia marcenscens. 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 selecced antibiotics are presented in Figure 17.

Escherichia coli. 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.

PRESENTATIONS

McManus AT: *Klebsiella pneumoniae* in burned patients: relationship of colonization and infection to severity of injury.

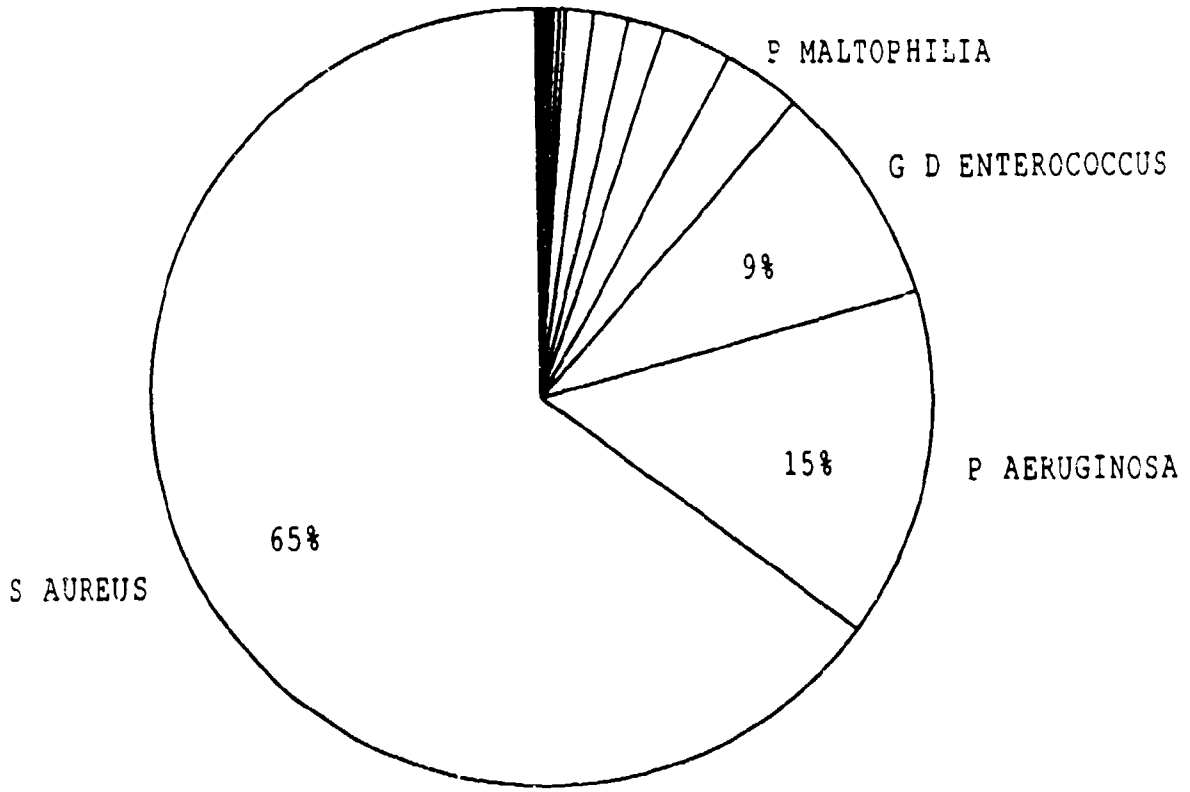


FIGURE 7. Display of the relative frequency of gentamicin-resistant organisms isolated in 1990.

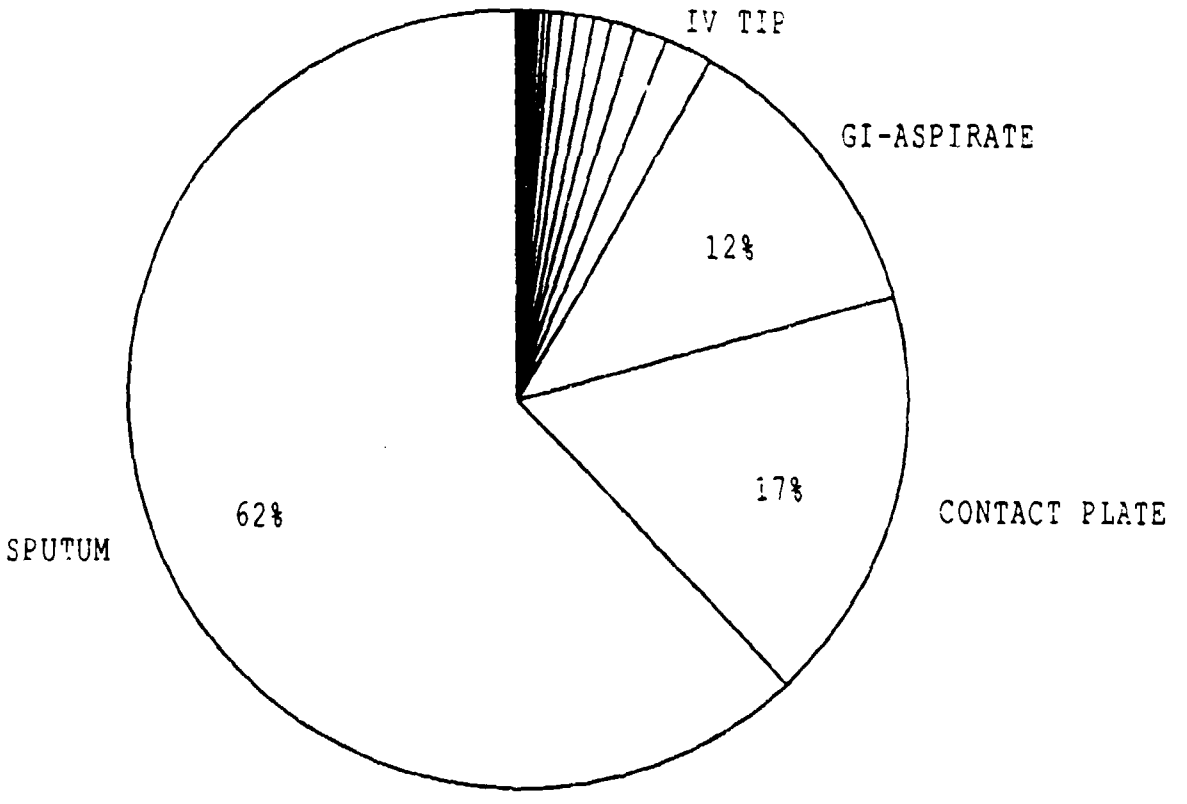


FIGURE 8. Display of the relative frequency of sources yielding *Staphylococcus aureus* tested for in vitro sensitivity to antibiotics in 1990.

TABLE 10A. Antibiotic Sensitivity Data for *Staphylococcus aureus* Sensitive to Gentamicin (1990)

Antibiotic	RESISTANT		INTERMEDIATE		SENSITIVE		Total
	%	Number	%	Number	%	Number	Number
Amikacin	23.53	253	4.47	48	72.00	774	1,075
Ampicillin	27.77	298	2.98	32	69.25	743	1,073
Cefoperazone	18.14	195	9.77	105	72.09	775	1,075
Cefotaxime	25.67	269	2.67	28	71.66	751	1,048
Cefsulodin	50.00	1	-	-	50.00	1	2
Ceftazidime	27.16	292	4.00	43	68.84	740	1,075
Ceftriaxone	-	-	-	-	100.00	1,072	1,072
Cephalothin	13.43	144	2.33	25	84.24	903	1,072
Chloramphenicol	0.37	4	0.09	1	99.53	1,070	1,075
Clindamycin	27.53	296	0.37	4	72.09	775	1,075
Erythromycin	36.47	392	0.09	1	63.44	682	1,075
Gentamicin	-	-	-	-	100.00	1,075	1,075
Imipenem-cilastatin sodium	14.14	152	0.74	8	85.12	915	1,075
Mezlocillin	27.63	297	14.42	155	57.95	623	1,075
Moxalactam	28.28	304	18.98	204	52.74	567	1,075
Oxacillin	27.84	299	0.56	6	71.60	769	1,074
Penicillin	80.82	868	8.66	93	10.52	113	1,074
Piperacillin	28.09	302	15.72	169	56.19	604	1,075
Streptomycin	24.16	259	14.46	155	61.38	658	1,072
Sulfadiazine	26.72	287	4.75	51	68.53	736	1,074
Tetracycline	25.49	274	6.88	74	67.63	727	1,075
Tobramycin	28.74	309	1.02	11	70.23	755	1,075
Vancomycin	0.09	1	-	-	99.91	1,074	1,075

TABLE 10B. Antibiotic Sensitivity Data for *Staphylococcus aureus* Resistant to Gentamicin (1990)

Antibiotic	RESISTANT		INTERMEDIATE		SENSITIVE		Total
	%	Number	%	Number	%	Number	Number
Amikacin	50.80	285	24.24	136	24.96	14	561
Ampicillin	64.95	365	14.23	80	20.82	117	562
Cefoperazone	48.75	274	31.49	177	19.75	111	562
Cefotaxime	52.70	293	34.53	192	12.77	71	556
Cefsulodin	66.67	2	-	-	33.33	1	3
Ceftazidime	79.00	444	9.61	54	11.39	64	562
Ceftriaxone	-	-	-	-	100.00	561	561
Cephalothin	33.69	189	3.21	18	63.10	354	561
Chloramphenicol	1.96	11	0.36	2	97.69	549	562
Clindamycin	44.84	252	0.53	3	54.63	307	562
Erythromycin	55.34	311	0.71	4	43.95	247	562
Gentamicin	98.04	551	1.96	11	-	-	562
Imipenem-cilastatin sodium	23.53	132	6.06	34	70.41	395	561
Mezlocillin	76.33	429	10.32	58	13.35	75	562
Moxalactam	88.06	494	3.21	18	8.73	49	561
Oxacillin	78.79	442	1.43	8	19.79	111	561
Penicillin	90.55	508	1.78	10	7.66	43	561
Piperacillin	75.09	422	11.03	62	13.88	78	562
Streptomycin	58.54	329	6.76	38	34.70	195	562
Sulfadiazine	86.30	485	6.41	36	7.30	41	562
Tetracycline	23.31	131	11.57	65	65.12	366	562
Tobramycin	99.11	557	0.53	3	0.36	2	562
Vancomycin	-	-	-	-	100.00	562	562

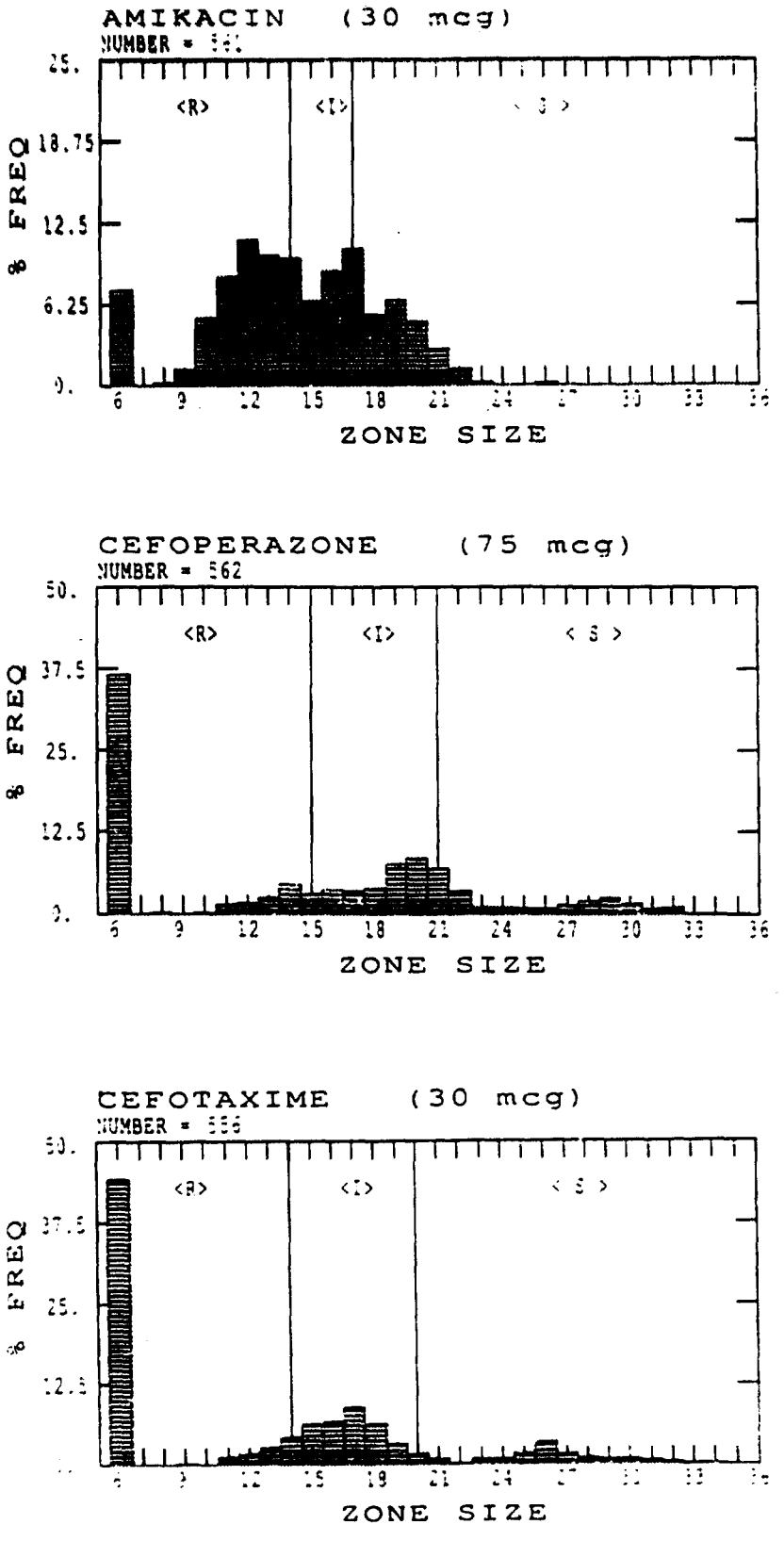


FIGURE 9A. Histogram display of the distribution of zones of inhibition of growth of multiply-resistant *Staphylococcus aureus.*

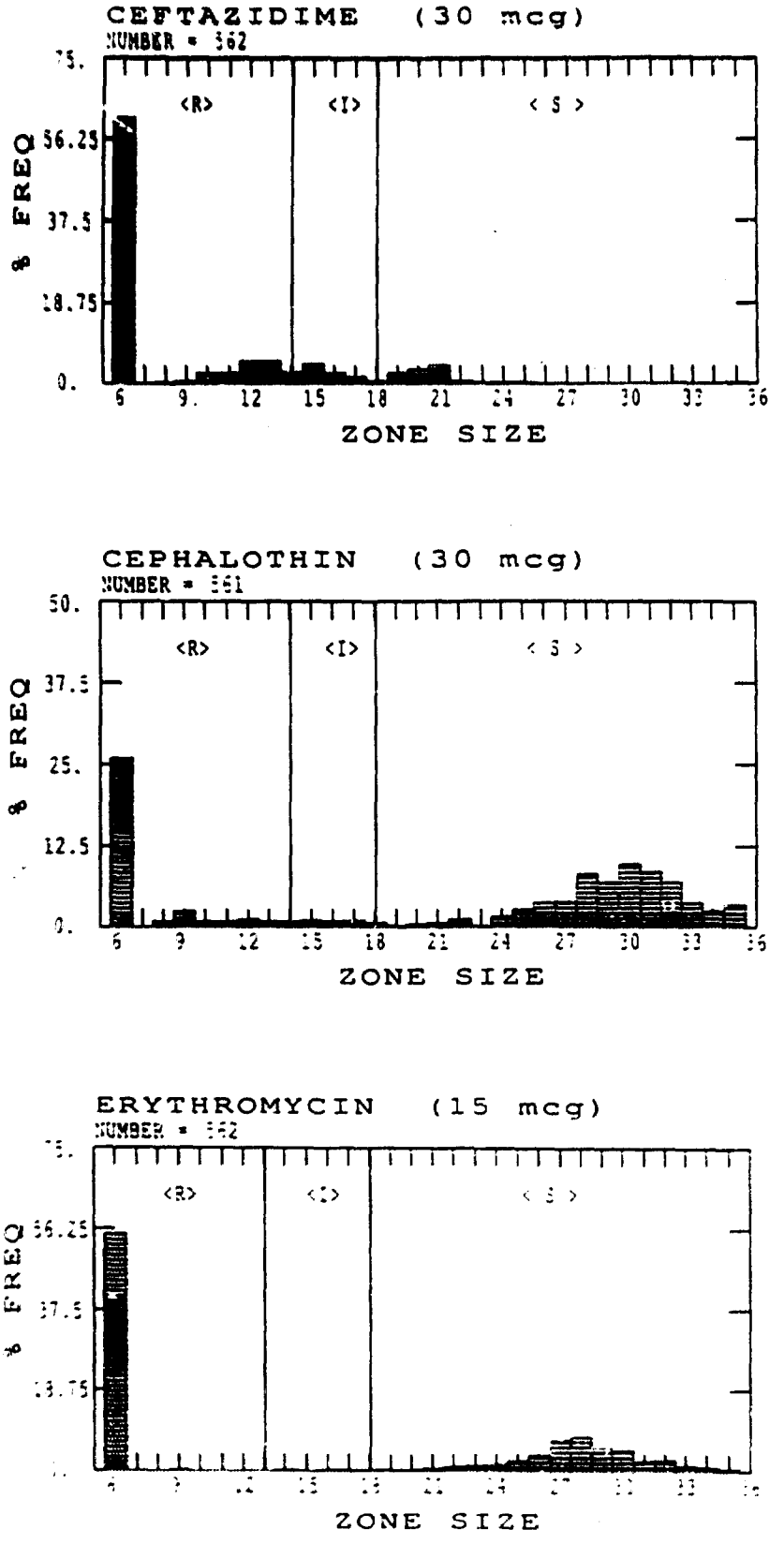
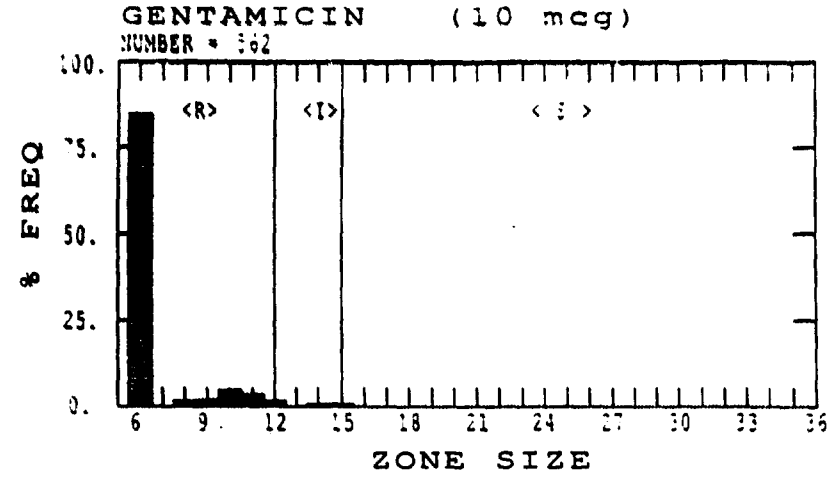
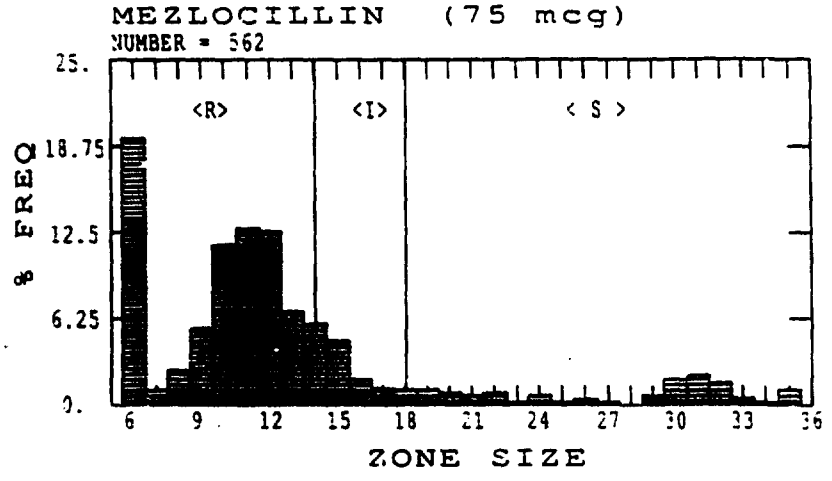


FIGURE 9A. Histogram display of the distribution of zones of inhibition of growth of multiply-resistant *Staphylococcus aureus* (continued).





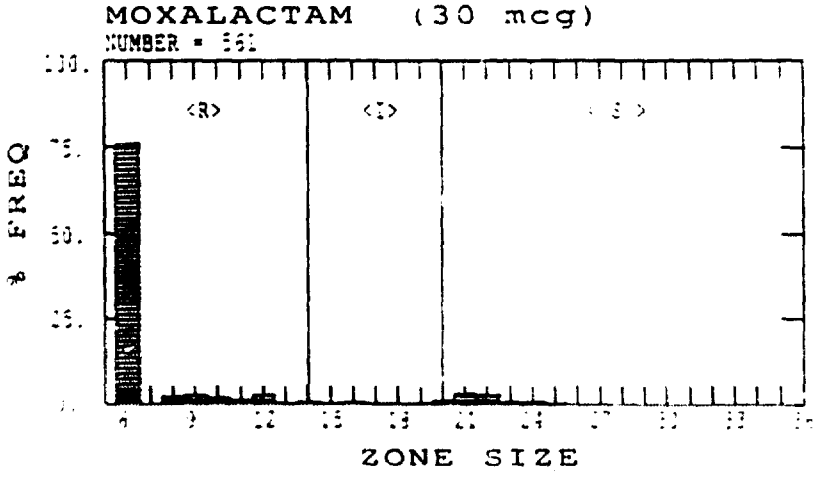


FIGURE 9A. Histogram display of the distribution of zones of inhibition of growth of multiply-resistant *Staphylococcus aureus* (continued).

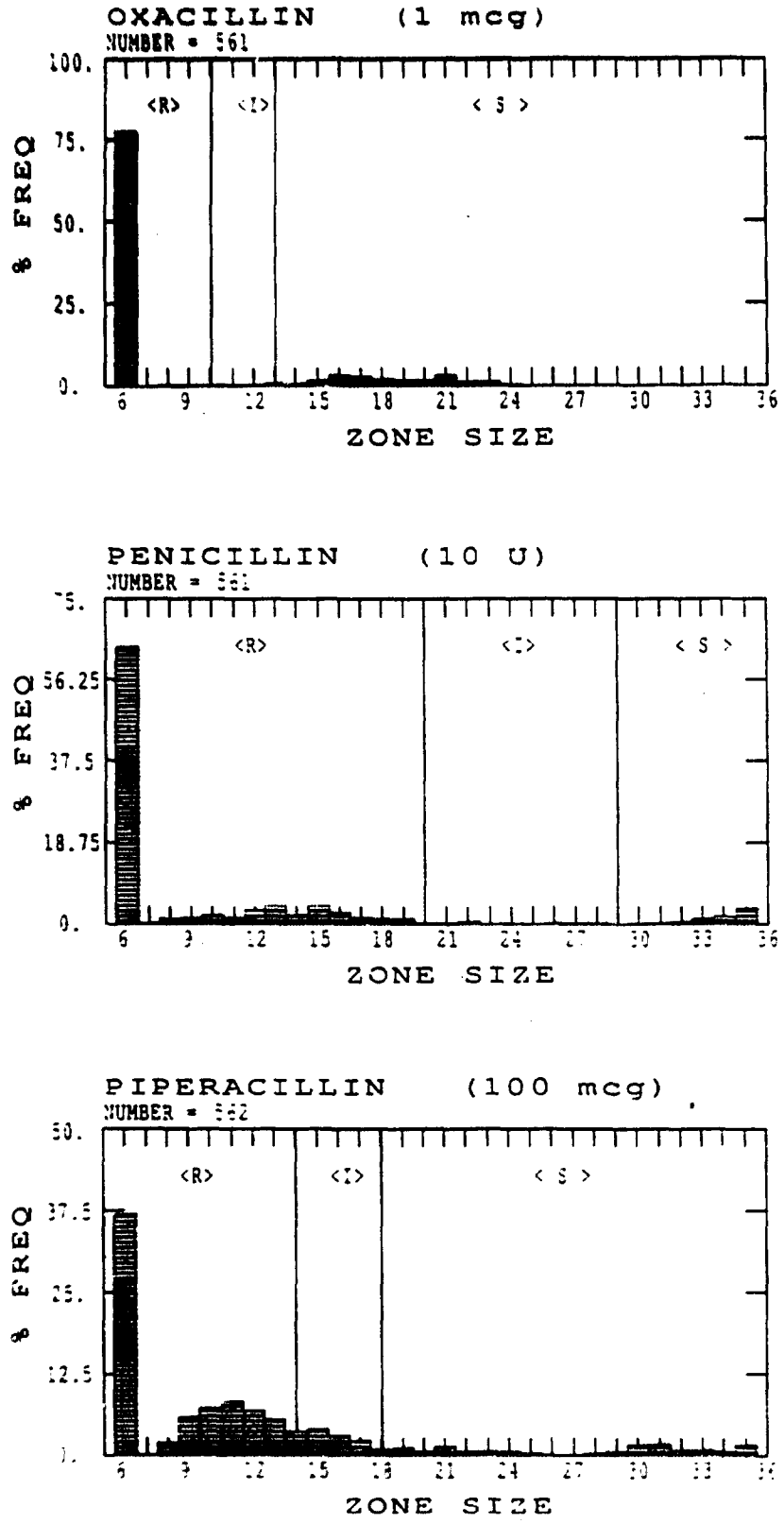


FIGURE 9A. Histogram display of the distribution of zones of inhibition of growth of multiply-resistant *Staphylococcus aureus* (continued).

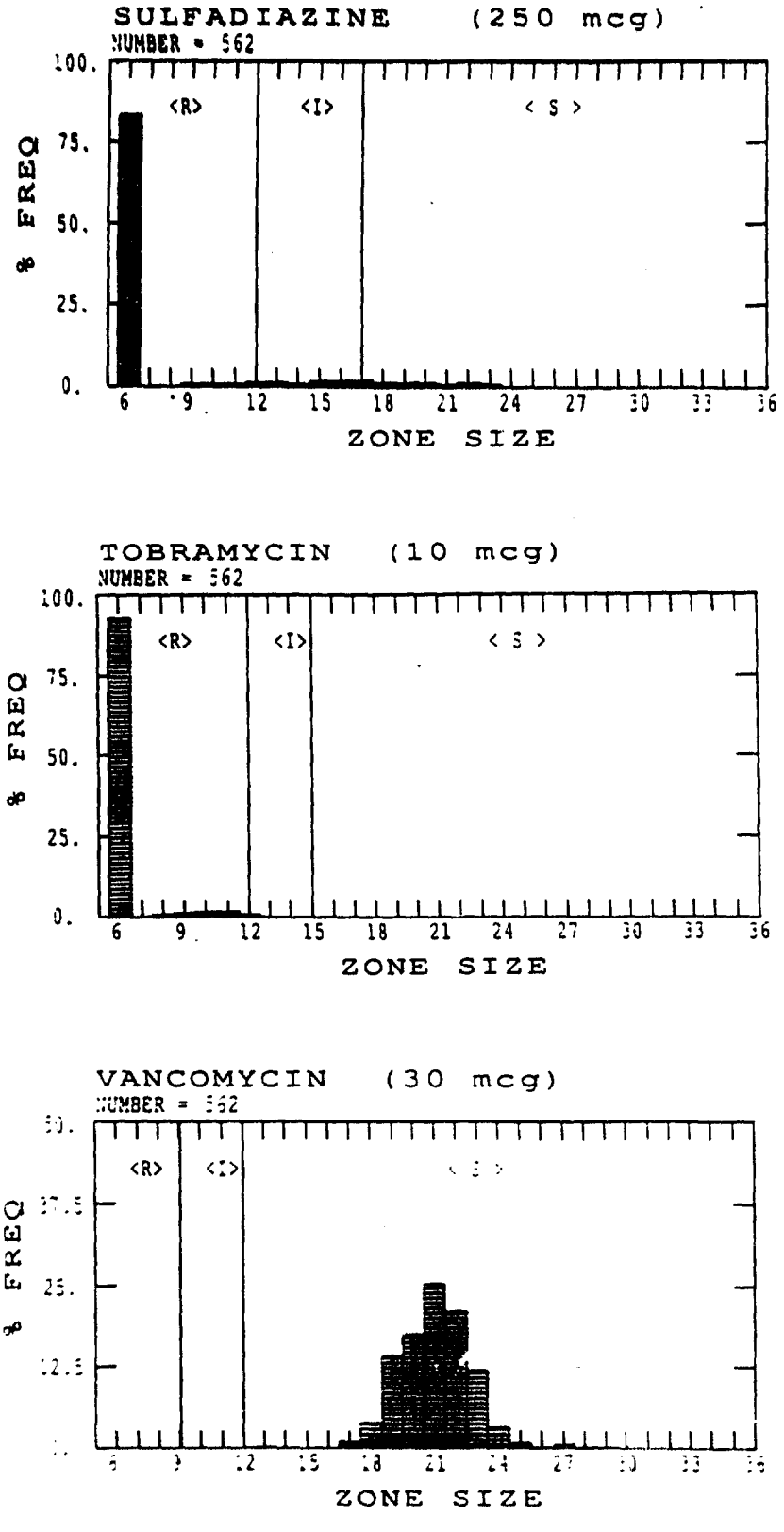


FIGURE 9A. Histogram display of the distribution of zones of inhibition of growth of multiply-resistant *Staphylococcus aureus* (continued).

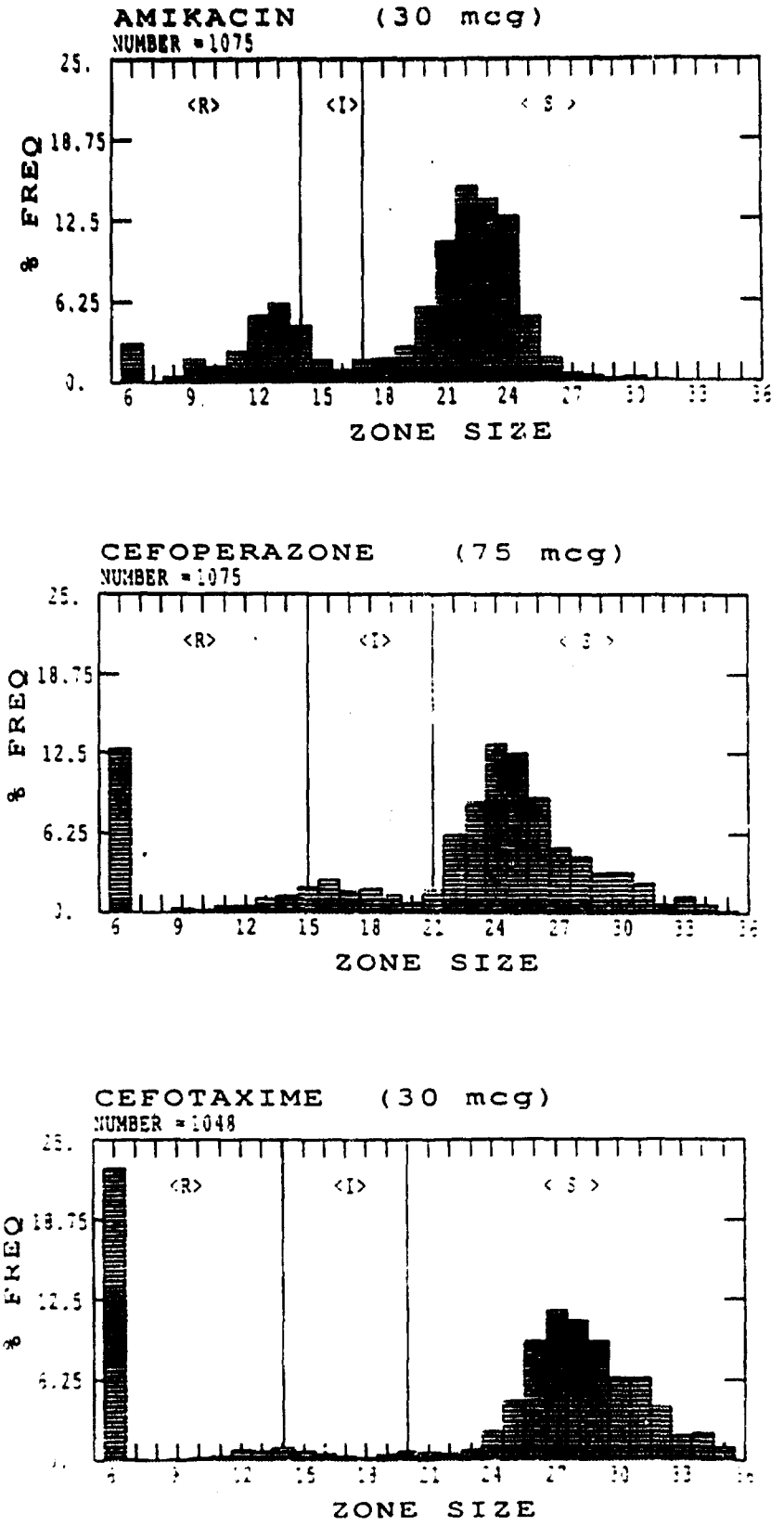
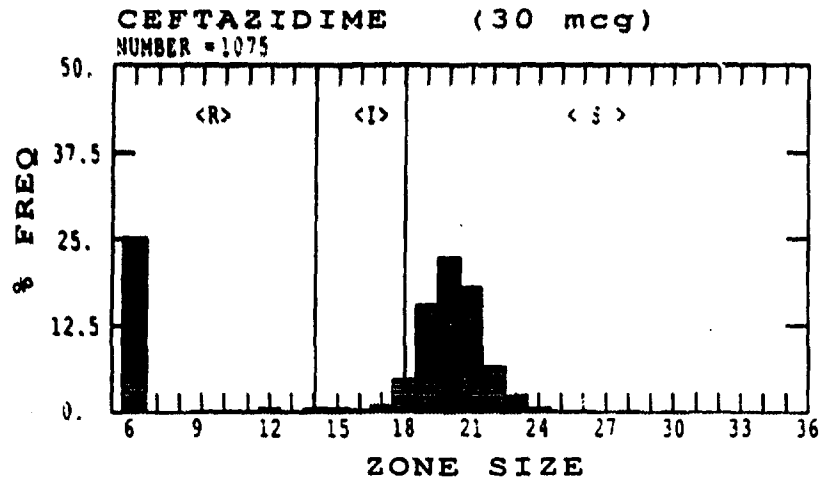
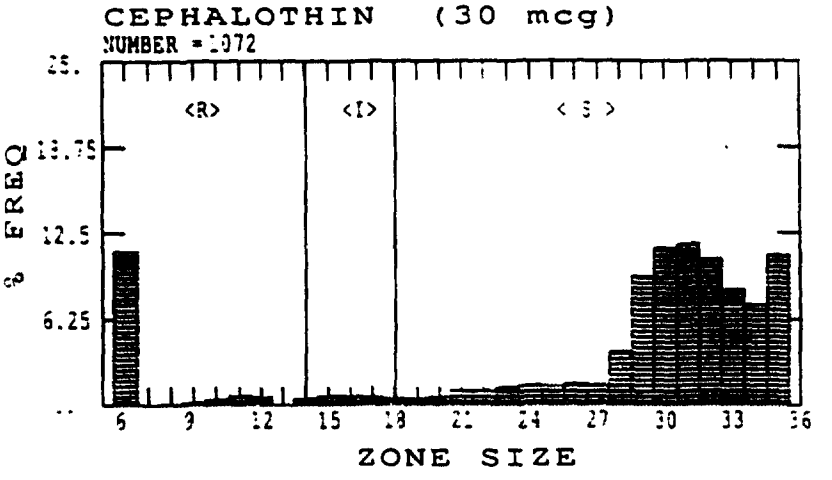


FIGURE 9B. Histogram display of the distribution of zones of inhibition of growth of multiply-sensitive *Staphylococcus aureus*.





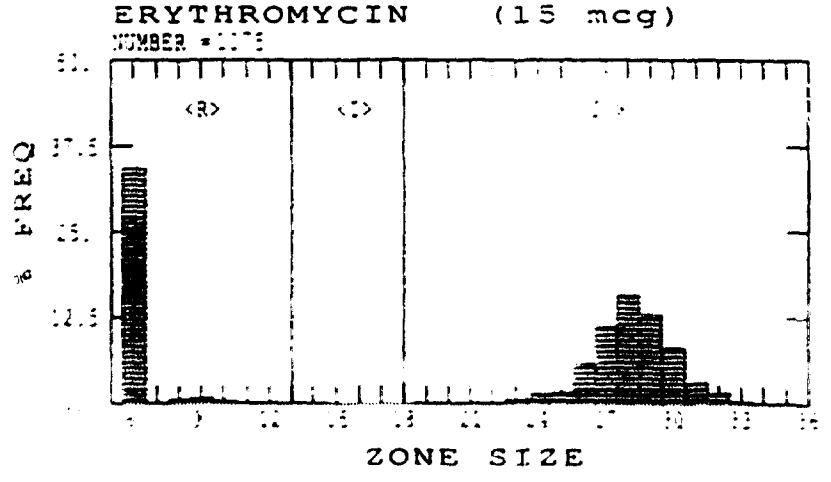


FIGURE 9B. Histogram display of the distribution of zones of inhibition of growth of multiply-sensitive *Staphylococcus aureus* (continued).

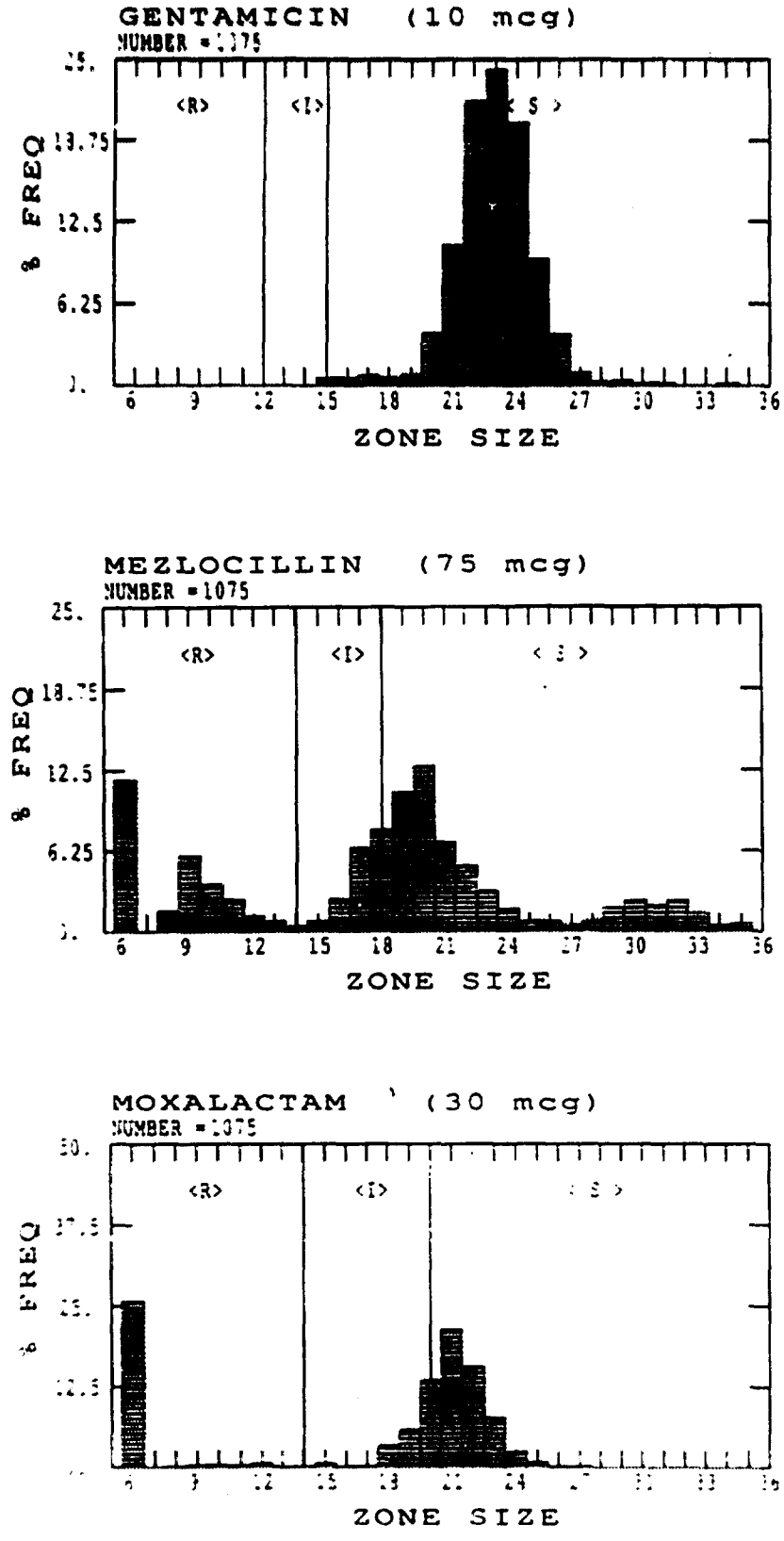


FIGURE 9B. Histogram display of the distribution of zones of inhibition of growth of multiply-sensitive *Staphylococcus aureus* (continued).

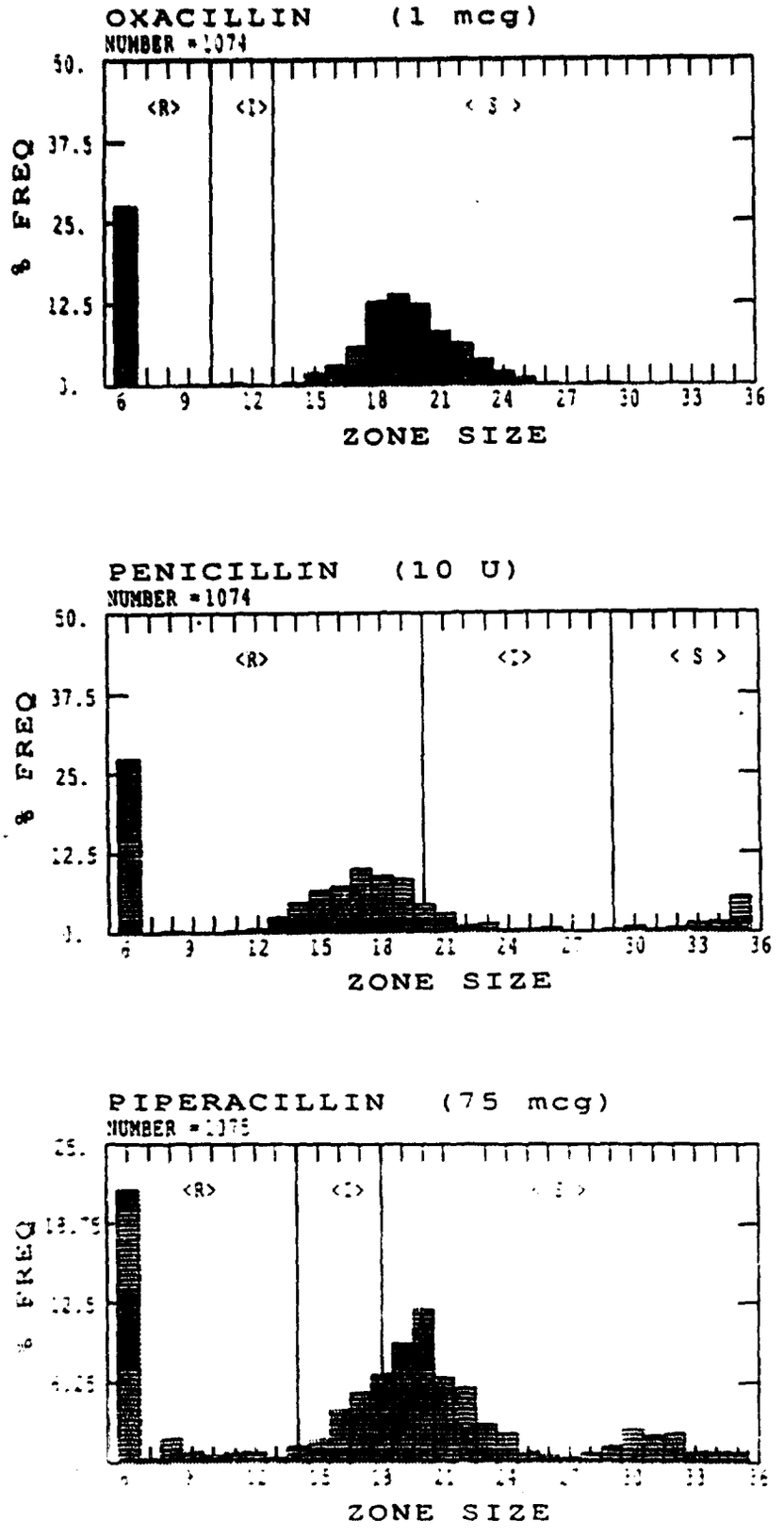
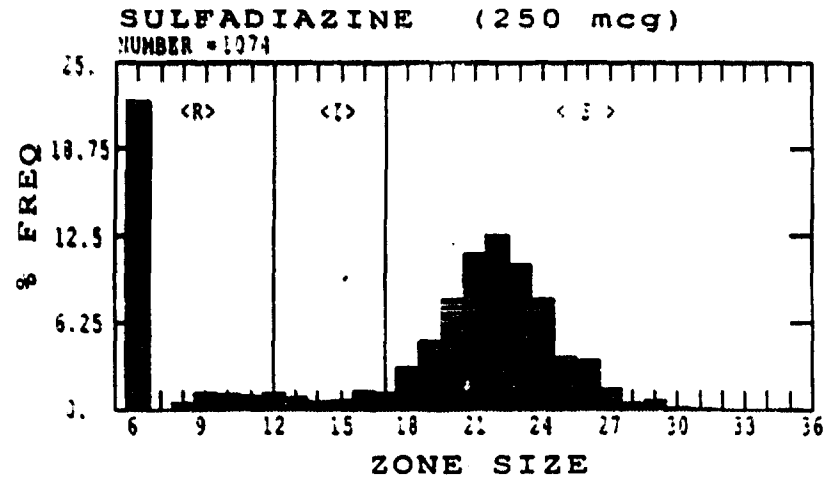
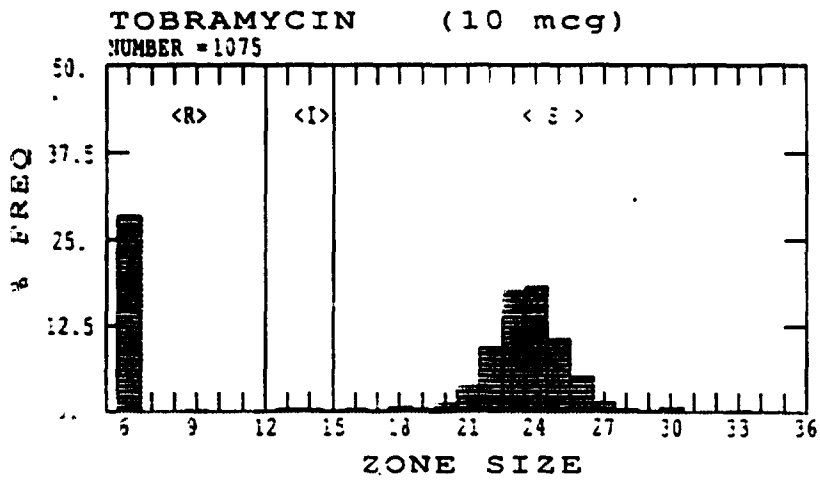


FIGURE 9B. Histogram display of the distribution of zones of inhibition of growth of multiply-sensitive *Staphylococcus aureus* (continued).





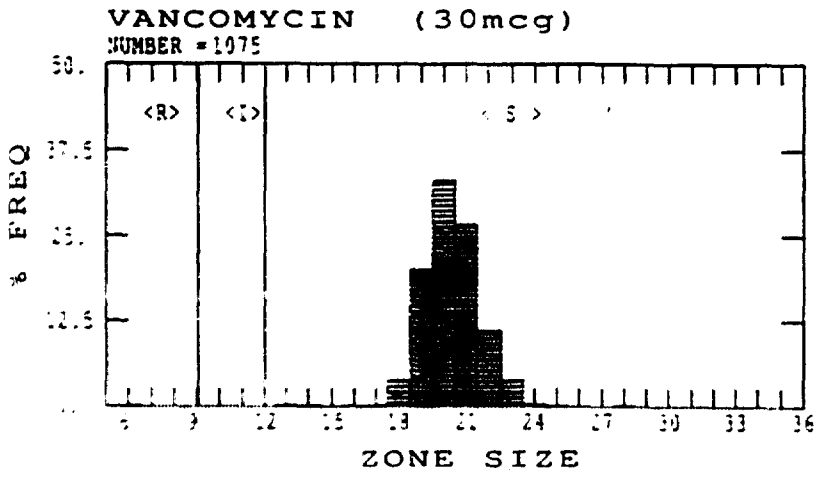


FIGURE 9B. Histogram display of the distribution of zones of inhibition of growth of multiply-sensitive *Staphylococcus aureus* (continued).

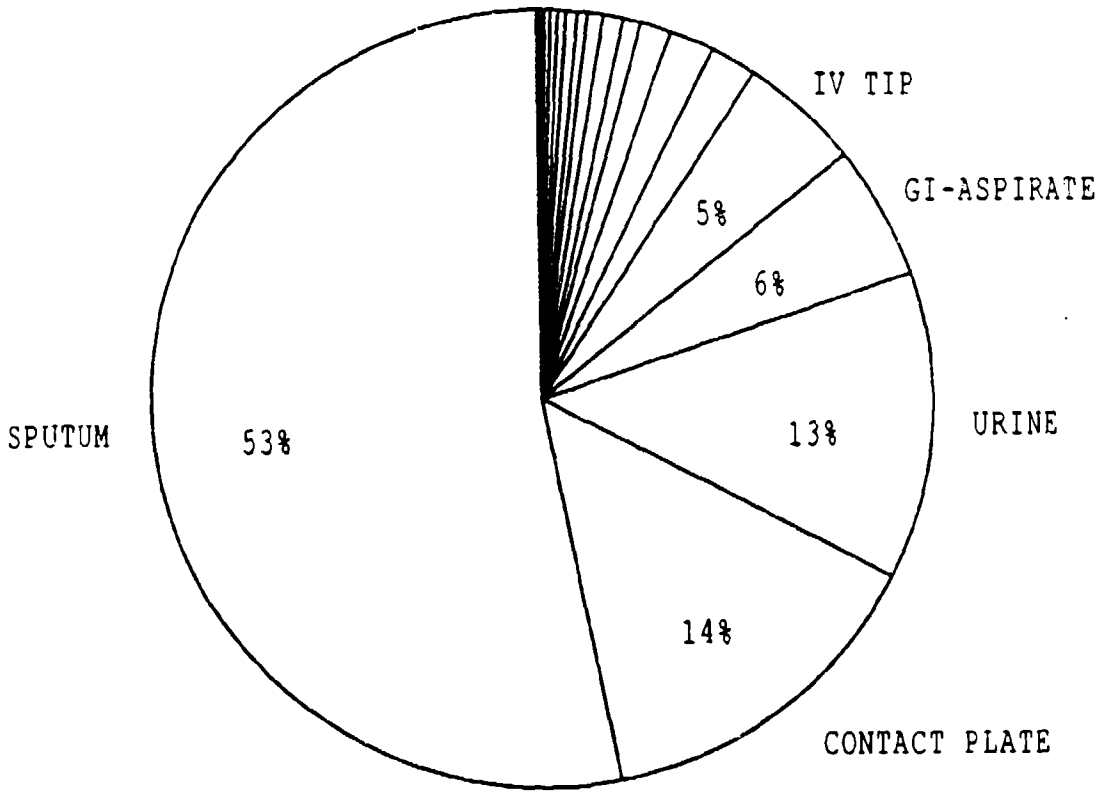


FIGURE 10. Display of the relative frequency of sources yielding *Pseudomonas aeruginosa* tested for in vitro sensitivity to antibiotics in 1990.

TABLE 11. Antibiotic Sensitivity Data for *Pseudomonas aeruginosa* (1990)

Antibiotic	RESISTANT		INTERMEDIATE		SENSITIVE		Total
	%	Number	%	Number	%	Number	Number
Amikacin	22.85	207	11.81	107	65.34	592	906
Azlocillin	27.46	248	6.87	62	65.67	593	903
Aztreonam	20.93	189	46.51	420	32.56	294	903
Cefoperazone	21.37	193	25.91	234	52.71	476	903
Cefotaxime	57.84	524	40.40	366	1.77	16	906
Cefsulodin	50.00	1	-	-	50.00	1	2
Ceftazidime	13.15	119	8.	78	78.23	708	905
Ceftriaxone	-	-	-	-	100.00	903	903
Chloramphenicol	97.95	859	2.0	18	-	-	877
Colistin	0.78	7	0.22	2	99.00	893	902
Gentamicin	13.70	124	22.98	208	63.31	573	905
Imipenem-cilastatin sodium	15.91	144	1.88	17	82.21	744	905
Kanamycin	98.12	888	1.33	12	-	-	900
Mezlocillin	36.19	325	31.63	284	32.18	289	898
Moxalactam	36.88	333	52.27	472	10.85	98	903
Netilmicin	9.72	88	10.39	94	79.89	723	905
Norfloxacin	8.40	76	25.64	232	65.97	597	905
Piperacillin	20.86	189	7.51	68	71.63	649	906
Sulfadiazine	38.30	347	22.96	208	38.74	351	906
Tetracycline	83.00	752	15.78	143	1.21	11	906
Ticarcillin	24.56	221	9.78	88	65.67	591	900
TIM-85	26.00	234	8.89	80	65.11	586	900
Tobramycin	8.64	78	0.66	6	90.70	819	903

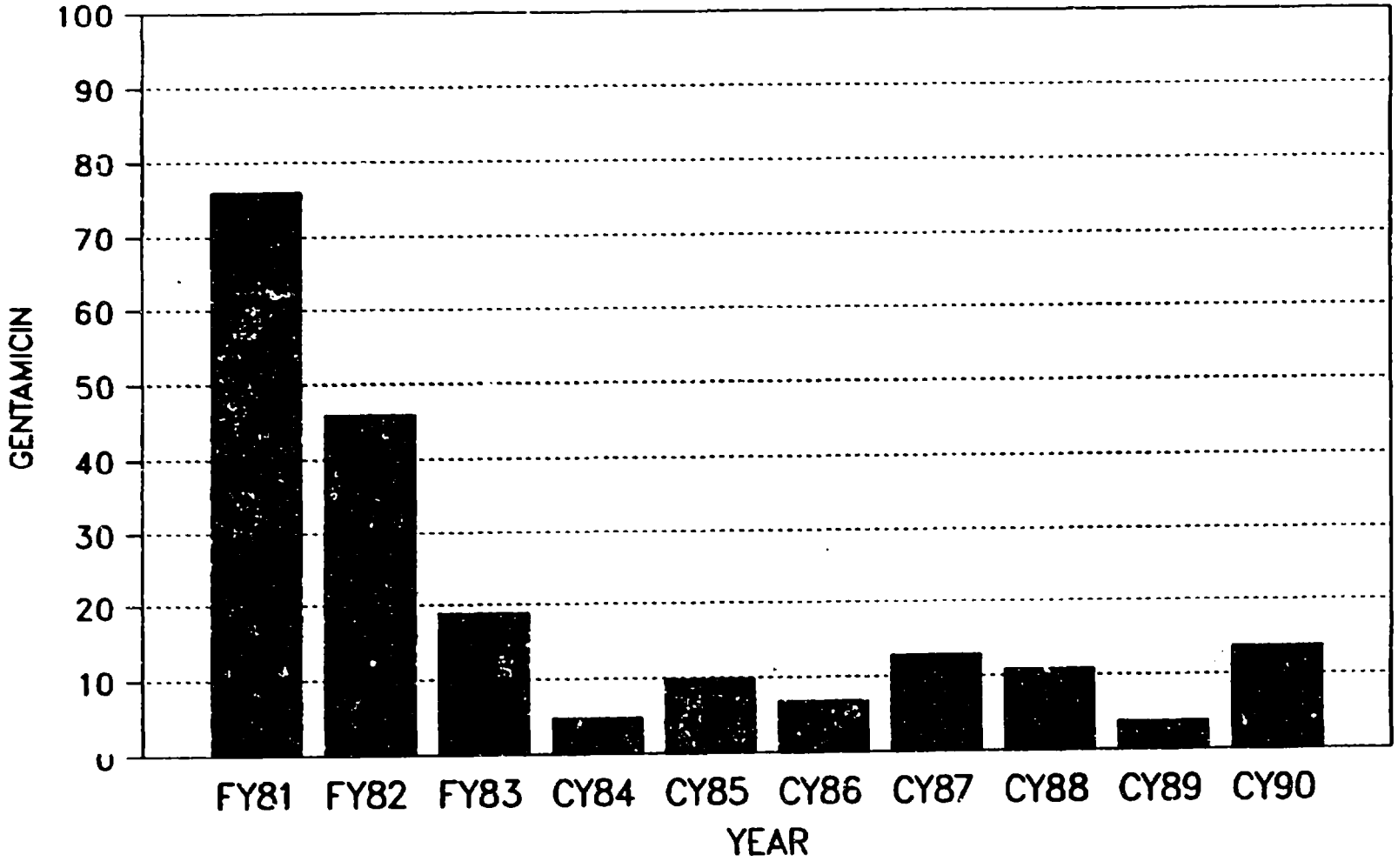


FIGURE 11. Relative frequency (%) of *Pseudomonas aeruginosa* resistant to gentamicin for fiscal years 1981-3 and calendar years 1984-90.

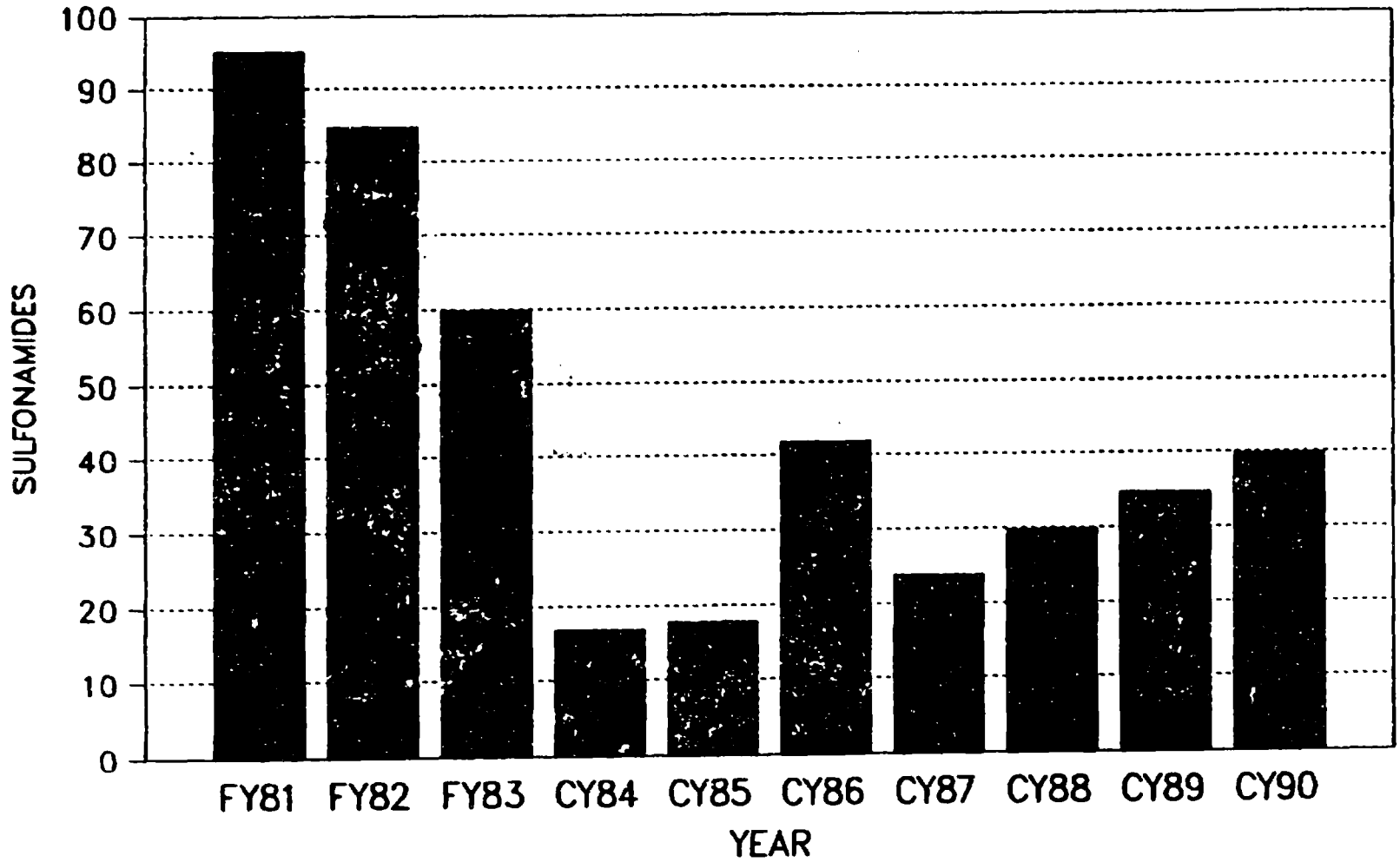


FIGURE 12. Relative frequency (%) of *Pseudomonas aeruginosa* resistance to sulfonamides for fiscal years 1981-3 and calendar years 1984-90.

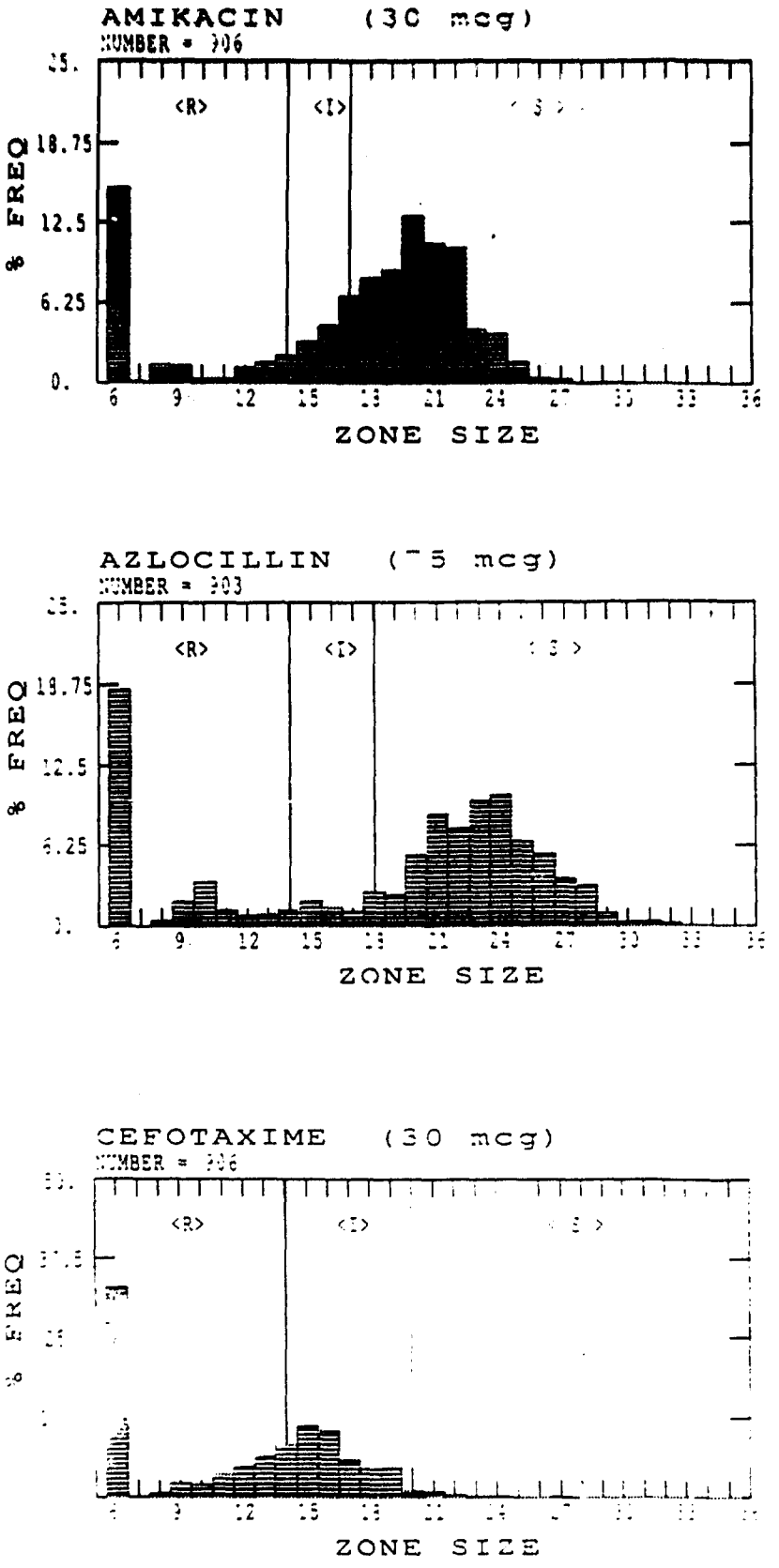
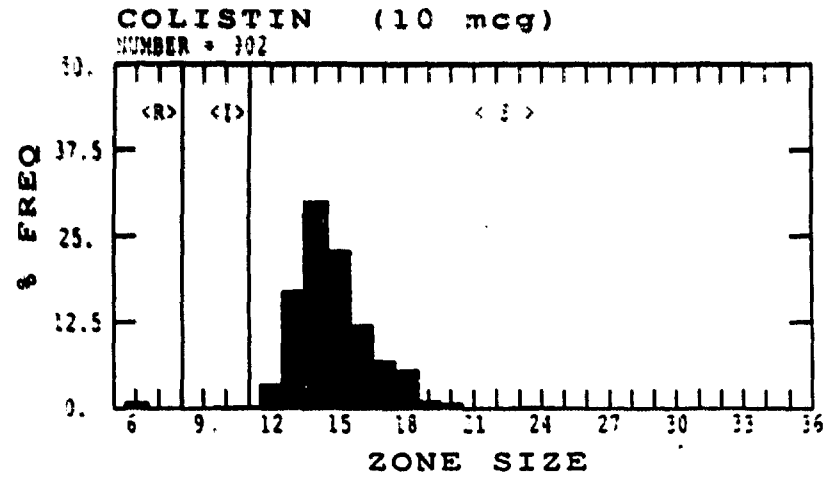
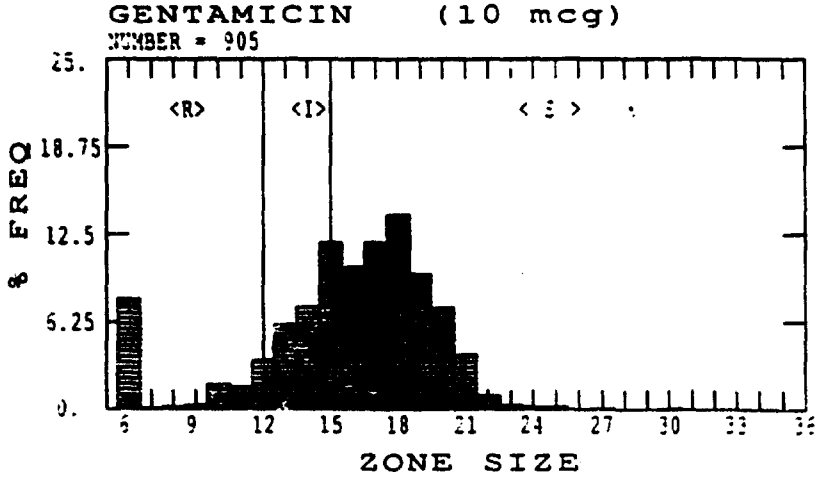


FIGURE 13. Histogram display of the distribution of zones of inhibition of growth of *Pseudomonas aeruginosa*.





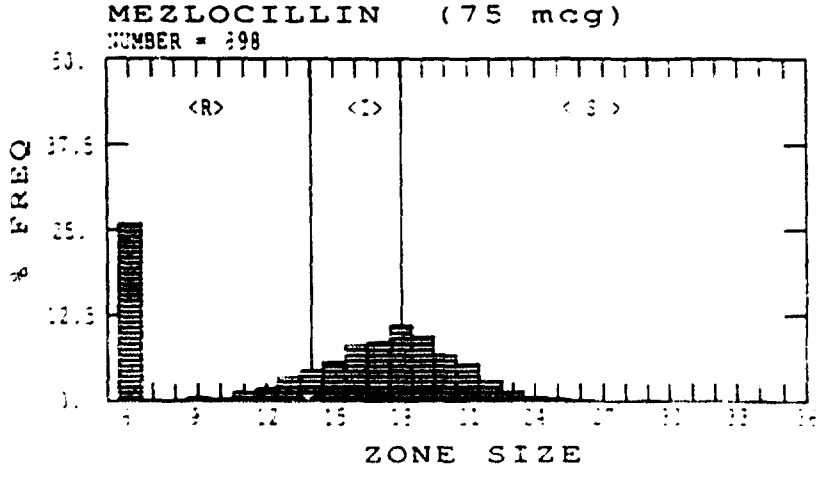
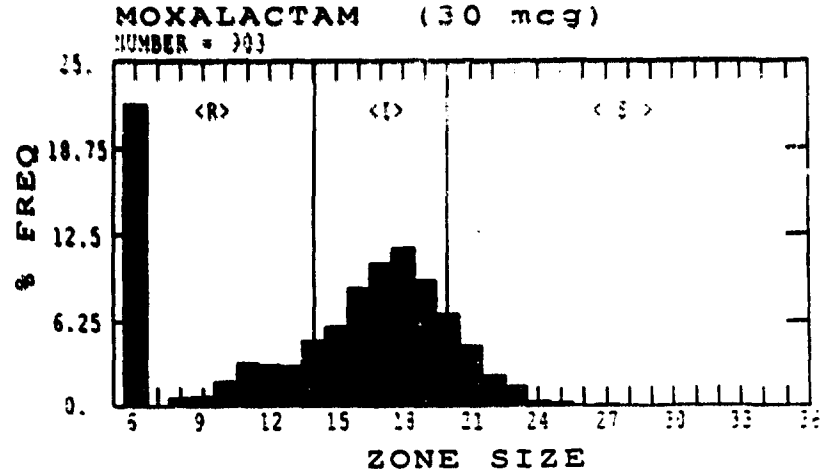
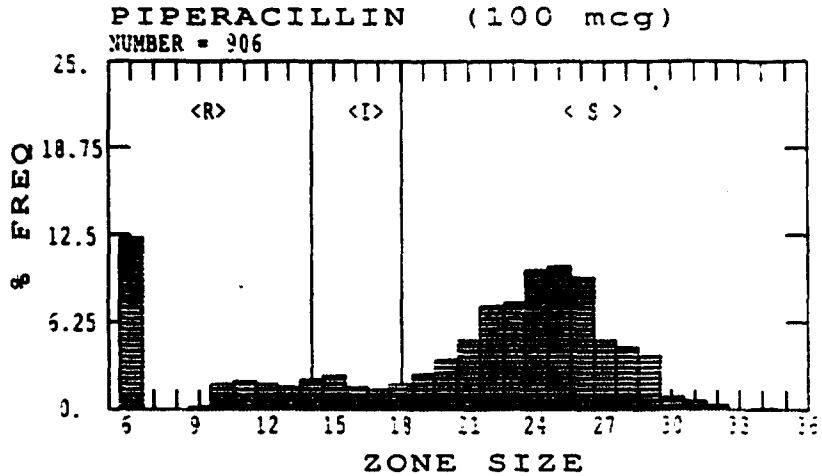


FIGURE 13. Histogram display of the distribution of zones of inhibition of growth of *Pseudomonas aeruginosa* (continued).





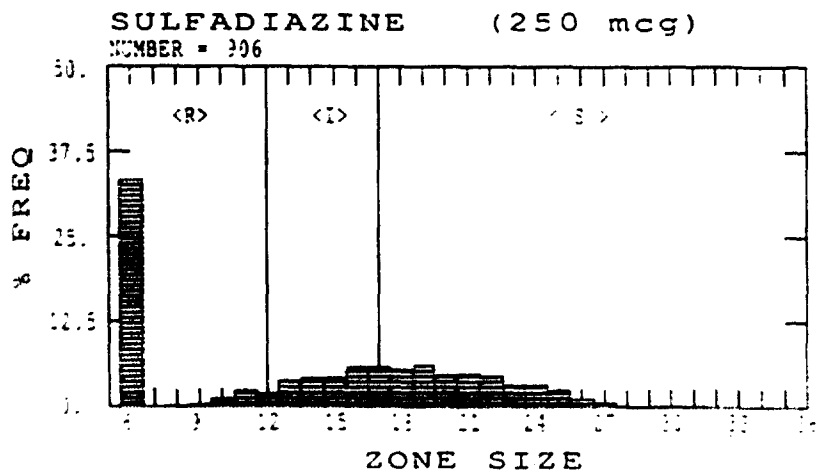
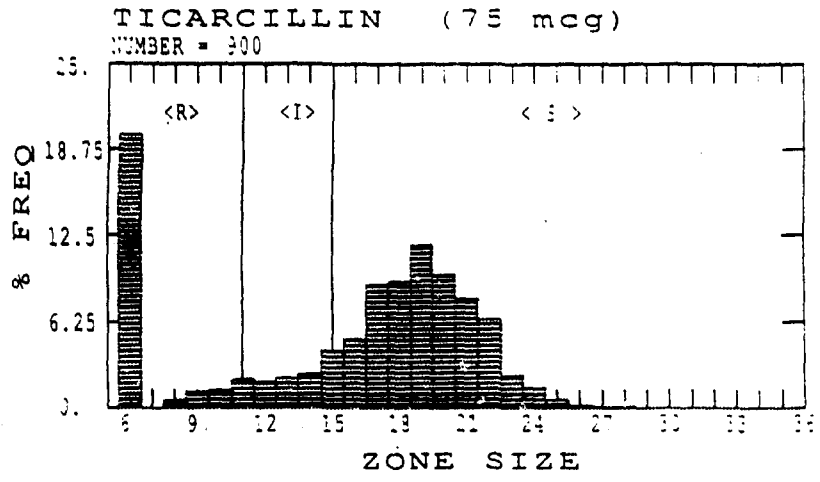


FIGURE 13. Histogram display of the distribution of zones of inhibition of growth of *Pseudomonas aeruginosa* (continued).



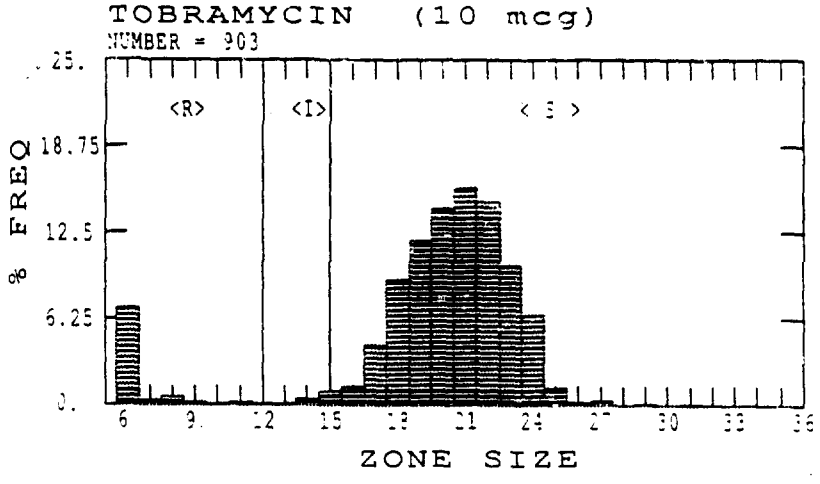


FIGURE 13. Histogram display of the distribution of zones of inhibition of growth of *Pseudomonas aeruginosa* (continued).

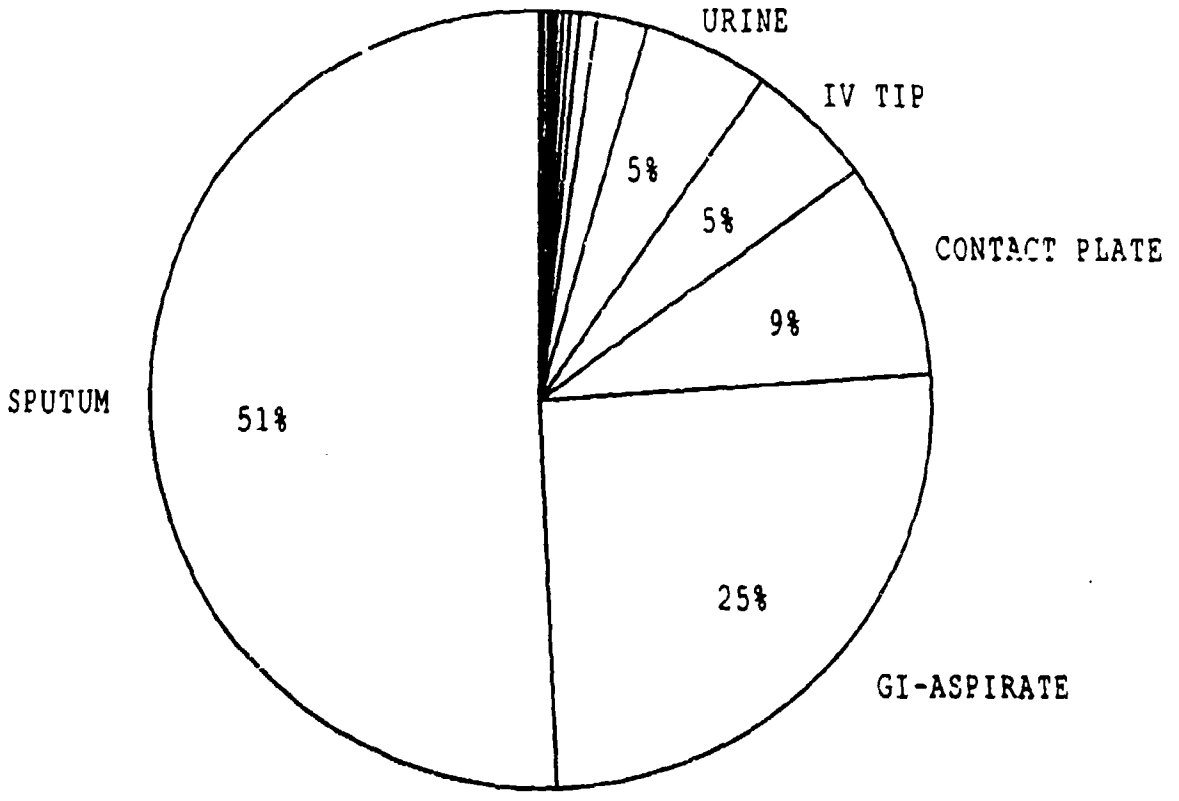
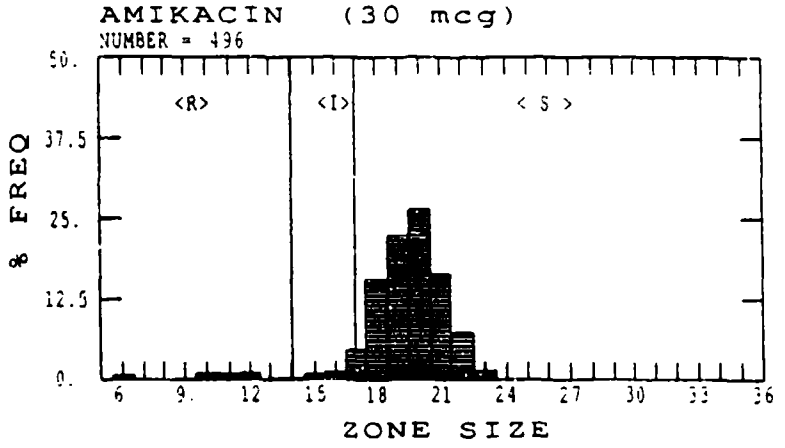
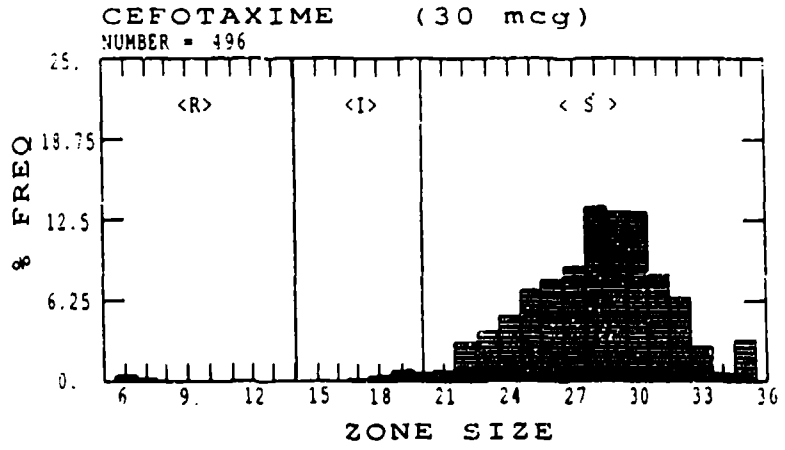


FIGURE 14. Display of the relative frequency of sources yielding *Klebsiella pneumoniae* tested for in vitro sensitivity to antibiotics in 1990.

TABLE 12. Antibiotic Sensitivity Data for *Klebsiella pneumoniae* (1990)

Antibiotic	RESISTANT		INTERMEDIATE		SENSITIVE		Total
	%	Number	%	Number	%	Number	Number
Amikacin	3.83	19	4.84	24	91.33	453	496
Ampicillin	88.51	439	6.05	30	5.44	27	496
Aztreonam	1.21	6	3.63	18	95.16	472	496
Cefamandole	15.93	79	19.96	99	64.11	318	496
Cefoperazone	7.71	38	27.38	135	64.91	320	493
Cefotaxime	0.60	3	1.61	8	97.78	485	496
Cefoxitin	2.62	13	8.87	44	88.51	439	496
Ceftazidime	18.99	94	7.68	38	73.33	363	495
Ceftriaxone	-	-	-	-	100.00	496	496
Chloramphenicol	2.87	13	0.44	2	96.69	438	453
Gentamicin	2.02	10	2.42	12	95.56	474	496
Imipenem-cilastatin sodium	-	-	-	-	100.00	496	496
Kanamycin	4.44	22	26.41	131	69.15	343	496
Mezlocillin	42.34	210	7.06	35	50.60	251	496
Nalidixic acid	2.42	12	4.24	21	93.33	462	495
Netilimicin	0.20	1	-	-	99.80	494	495
Norfloxacin	-	-	1.61	8	98.39	488	496
Piperacillin	40.32	200	5.24	26	54.44	270	496
Streptomycin	48.19	239	18.75	93	33.06	164	496
Sulfadiazine	69.56	345	8.47	42	21.98	109	496
Tetracycline	45.77	227	2.42	12	51.81	257	496
Ticarcillin	77.17	382	12.73	63	10.10	50	495
Trimethoprim	6.45	32	0.40	2	93.15	462	496
Trimeth & sulfa	7.07	35	6.46	32	86.46	428	495





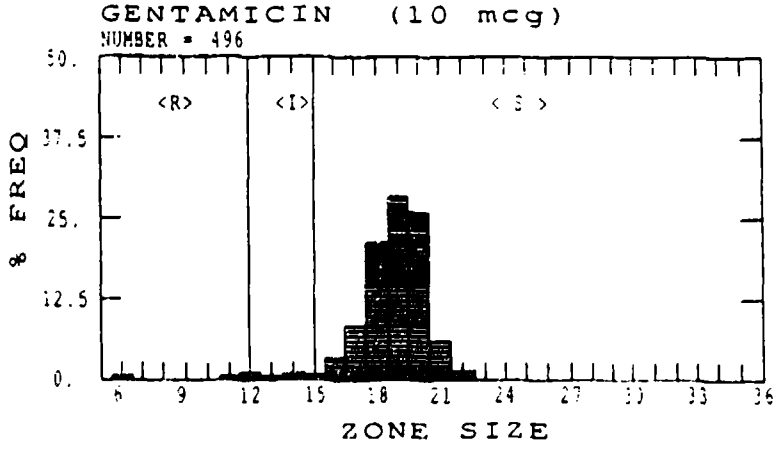


FIGURE 15. Histogram display of the distribution of zones of inhibition of growth of *Klebsiella pneumoniae*.

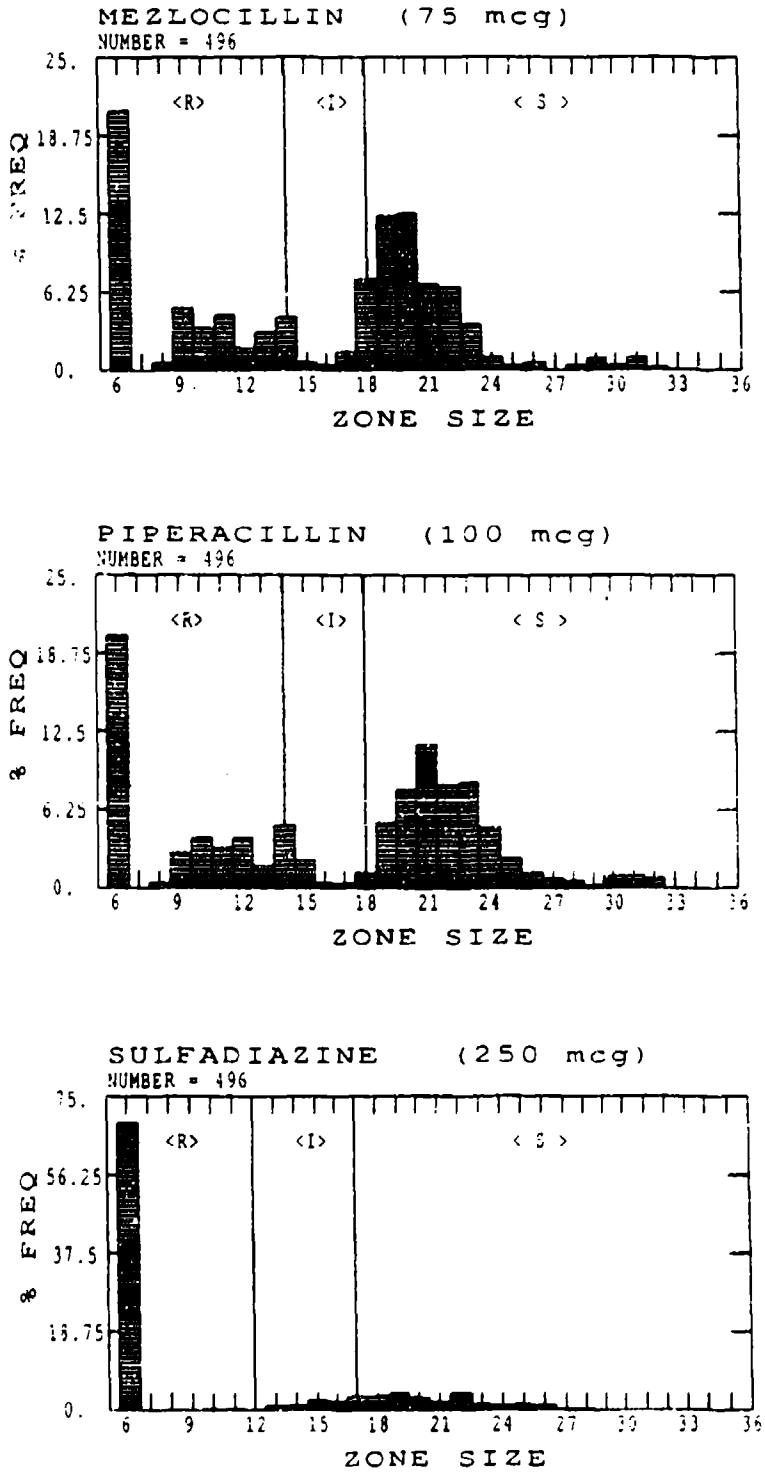


FIGURE 15. Histogram display of the distribution of zones of inhibition of growth of *Klebsiella pneumoniae* (continued).

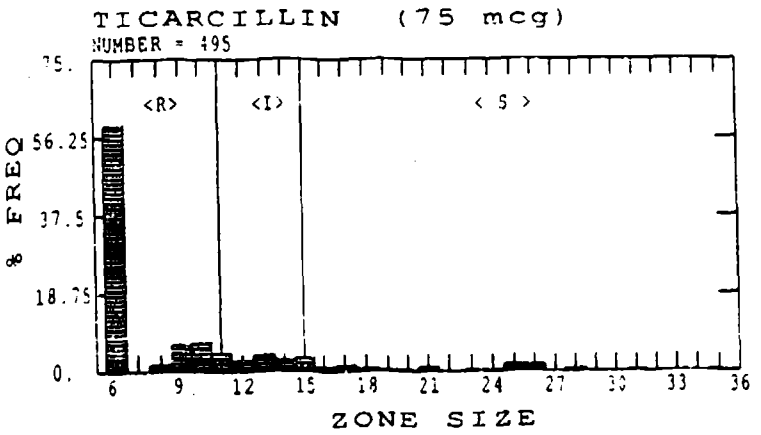


FIGURE 15. Histogram display of the distribution of zones of inhibition of growth of *Klebsiella pneumoniae* (continued).

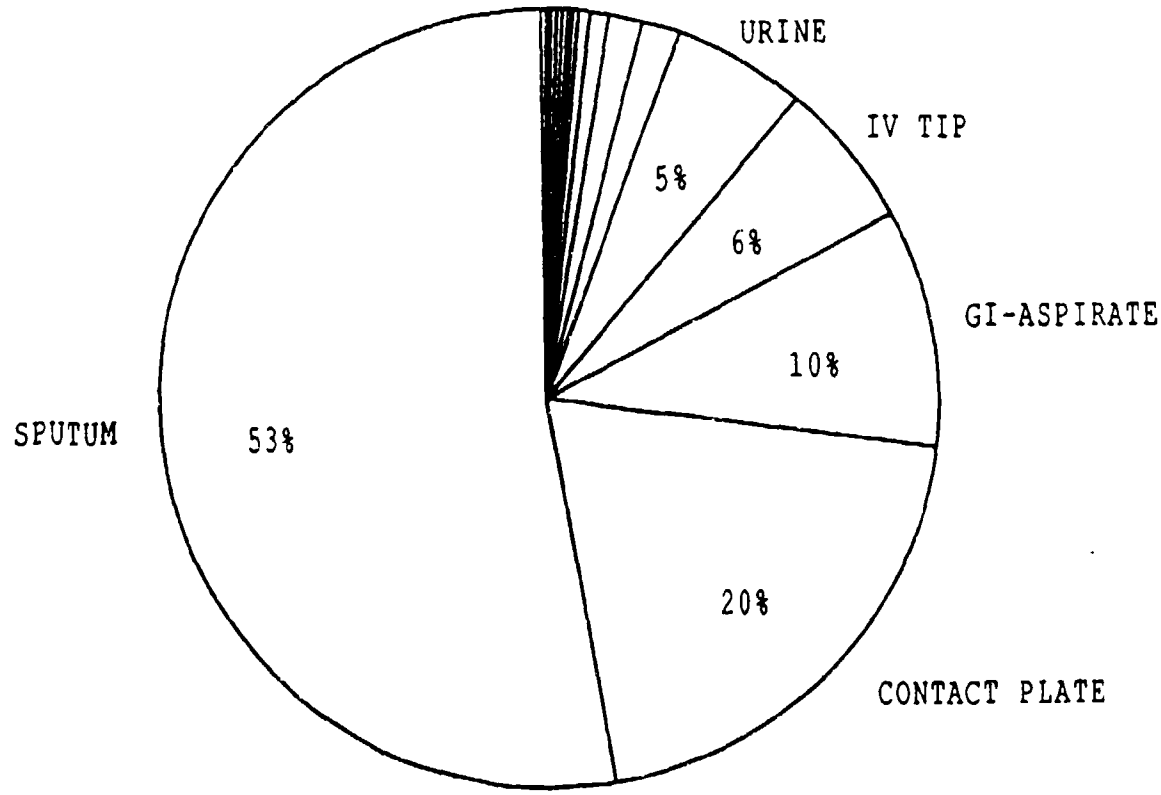


FIGURE 16. Display of the relative frequency of sources yielding *Serratia marcescens* tested for in vitro sensitivity to antibiotics in 1990.

TABLE 13. Antibiotic Sensitivity Data for *Serratia marcescens* (1990)

Antibiotic	RESISTANT		INTERMEDIATE		SENSITIVE		Total
	%	Number	%	Number	%	Number	Number
Amikacin	32.96	146	12.19	54	54.85	243	443
Ampicillin	99.77	441	-	-	0.23	1	442
Aztreonam	1.35	6	5.42	24	93.23	413	443
Cefamandole	99.77	441	-	-	0.23	1	442
Cefoperazone	1.59	7	19.09	84	79.32	349	440
Cefotaxime	0.23	1	5.42	24	94.36	418	443
Cefoxitin	40.86	181	57.79	256	1.35	6	443
Ceftazidime	0.23	1	0.45	2	99.32	440	443
Ceftriaxone	-	-	-	-	100.00	440	440
Chloramphenicol	2.27	9	65.40	259	32.32	128	396
Gentamicin	0.45	2	0.68	3	98.87	438	443
Imipenem-cilastatin sodium	0.23	1	0.45	2	99.32	440	443
Kanamycin	37.25	165	18.74	83	44.02	195	443
Mezlocillin	1.58	7	7.00	31	91.42	405	443
Nalidixic acid	1.13	5	0.68	3	98.19	435	443
Netilimicin	33.79	149	13.15	58	53.06	234	441
Norfloxacin	0.23	1	-	-	99.77	441	442
Piperacillin	0.68	3	1.13	5	98.19	435	443
Streptomycin	94.81	420	2.03	9	3.16	14	443
Sulfadiazine	54.18	240	23.25	103	22.57	100	443
Tetracycline	93.91	416	5.87	26	0.23	1	443
Ticarcillin	0.23	1	0.90	4	98.87	438	443
Trimethoprim	0.90	4	2.93	13	96.16	426	443
Trimeth & sulfa	1.13	5	6.32	28	92.55	410	443

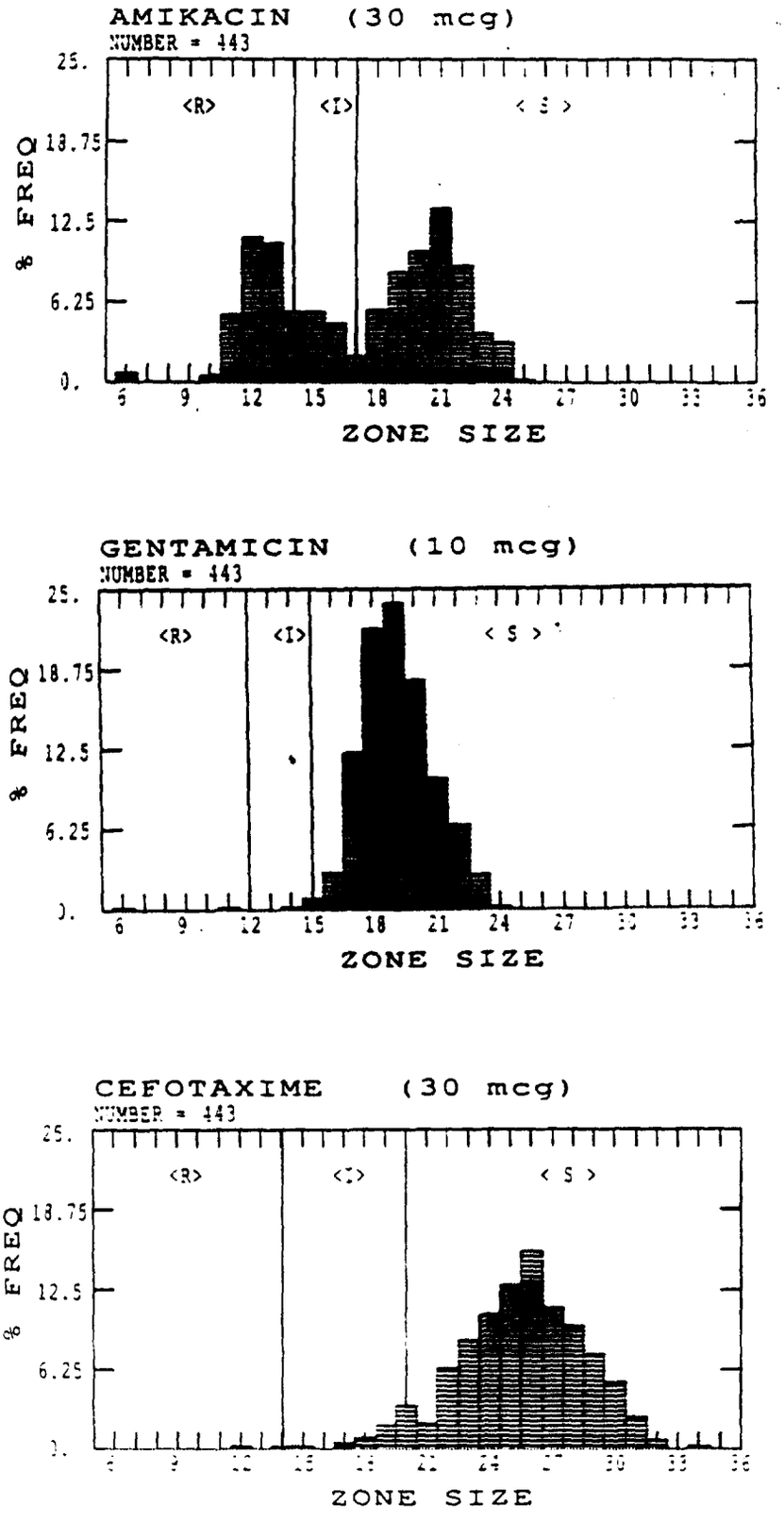
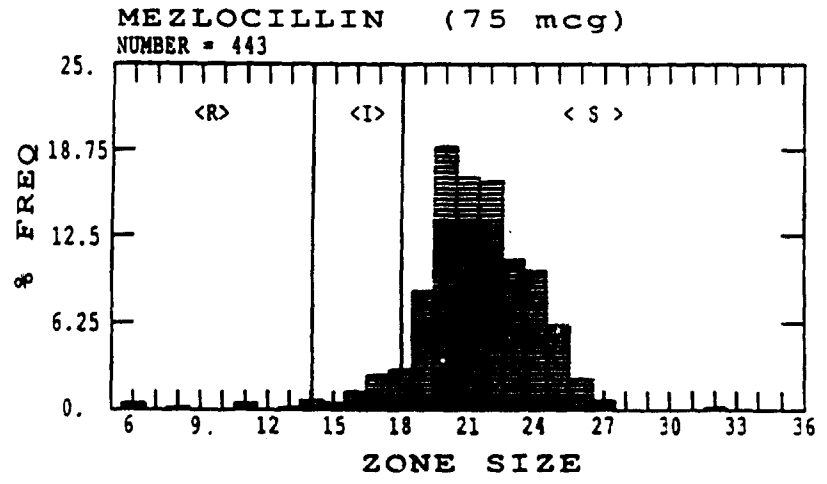
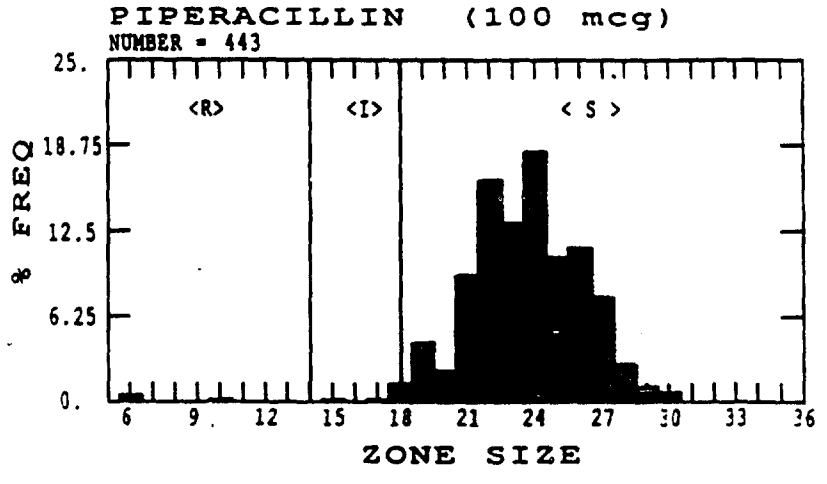


FIGURE 17. Histogram display of the distribution of zones of inhibition of growth of *Serratia marcescens*.





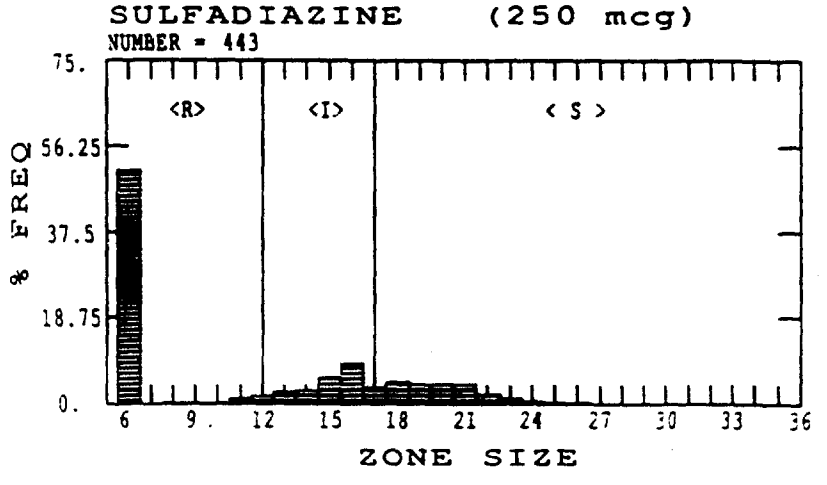


FIGURE 17. Histogram display of the distribution of zones of inhibition of growth of *Serratia marcescens* (continued).

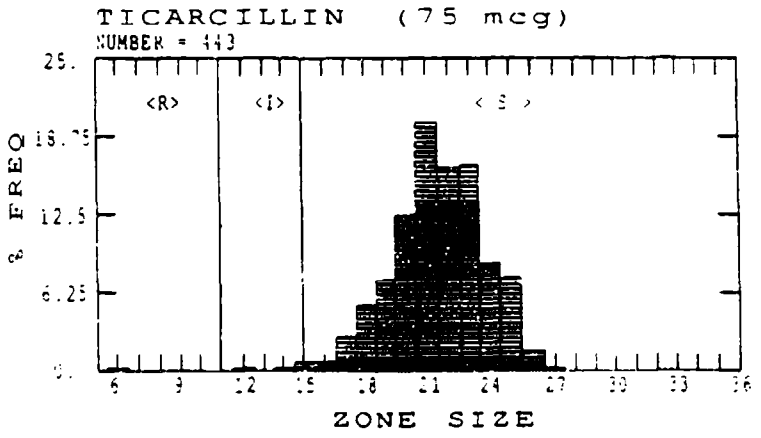


FIGURE 17. Histogram display of the distribution of zones of inhibition of growth of *Serratia marcescens* (continued).

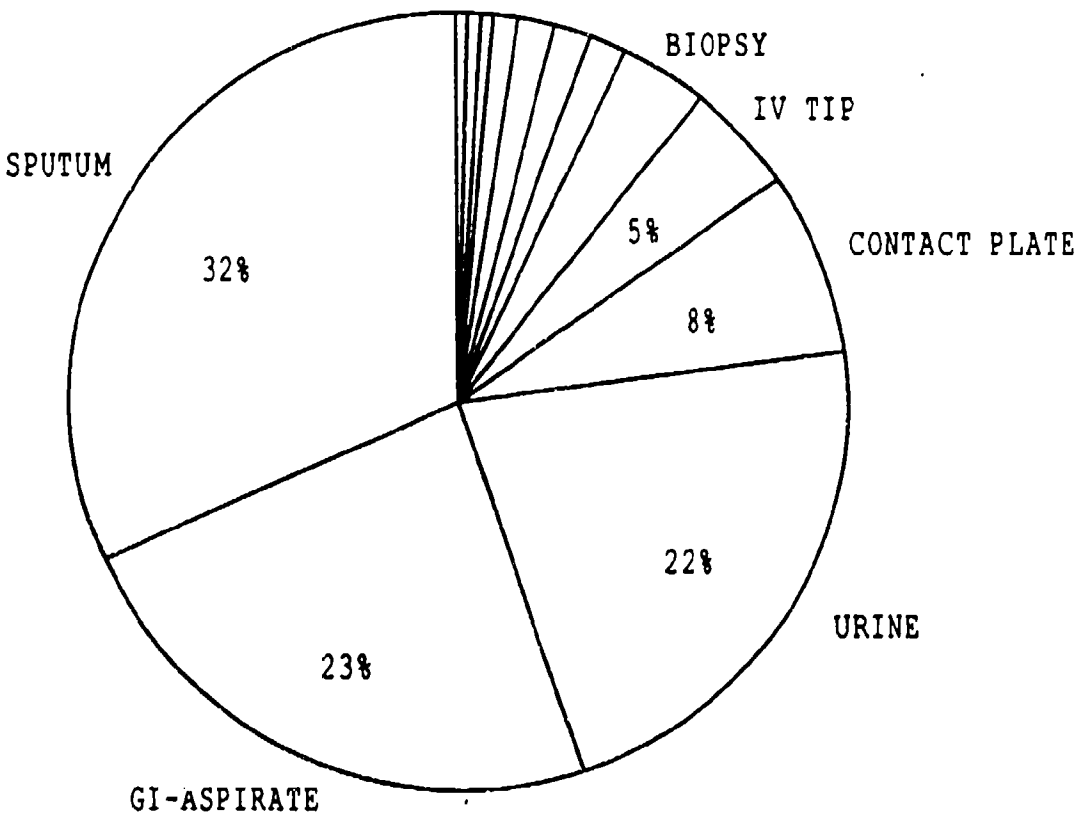


FIGURE 18. Display of the relative frequency of sources yielding *Escherichia coli* tested for in vitro sensitivity to antibiotics in 1990.

TABLE 14. Antibiotic Sensitivity Data for *Escherichia coli* (1990)

Antibiotic	RESISTANT		INTERMEDIATE		SENSITIVE		Total
	%	Number	%	Number	%	Number	Number
Amikacin	3.37	6	2.81	5	93.82	167	178
Ampicillin	33.71	60	1.69	3	64.61	115	178
Aztreonam	-	-	-	-	100.00	178	178
Cefamandole	1.12	2	12.36	22	86.52	154	178
Cefoperazone	0.56	1	8.99	16	90.45	161	178
Cefotaxime	-	-	0.56	1	99.44	177	178
Cefoxitin	0.56	1	0.56	1	98.88	176	178
Ceftazidime	-	-	-	-	100.00	177	177
Ceftriaxone	-	-	-	-	100.00	174	174
Chloramphenicol	7.89	12	6.58	10	85.53	130	152
Gentamicin	-	-	5.62	10	94.38	168	178
Imipenem-cilastatin sodium	-	-	-	-	100.00	177	177
Mezlocillin	16.85	30	12.92	23	70.22	125	178
Moxalactam	-	-	-	-	100.00	1	1
Nalidixic acid	2.81	5	-	-	97.19	173	178
Netilmicin	-	-	1.69	3	98.31	175	178
Norfloxacin	-	-	-	-	100.00	177	177
Piperacillin	16.85	30	13.48	24	69.66	124	178
Streptomycin	43.26	77	32.58	58	24.16	43	178
Sulfadiazine	63.48	113	9.55	17	26.97	48	178
Tetracycline	34.27	61	3.93	7	61.80	110	178
Ticarcillin	33.71	60	-	-	66.29	118	178
Tobramycin	-	-	-	-	100.00	1	1
Trimethoprim	8.99	16	-	-	91.01	162	178
Trimethoprim & sulfa	8.99	16	2.25	4	88.76	158	178

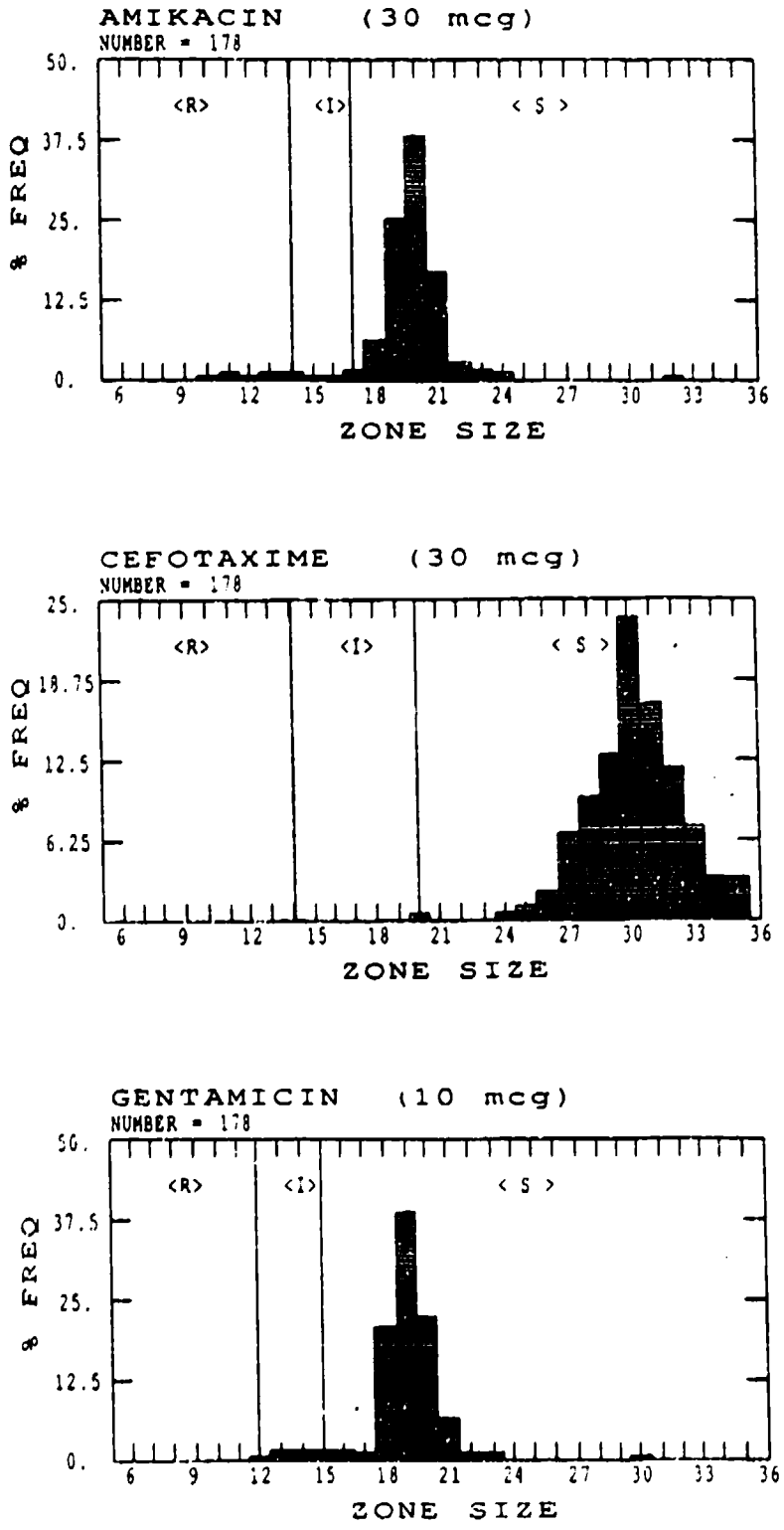
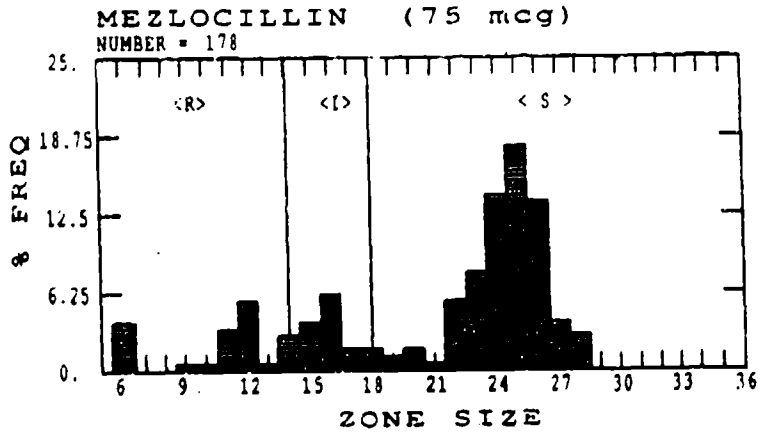
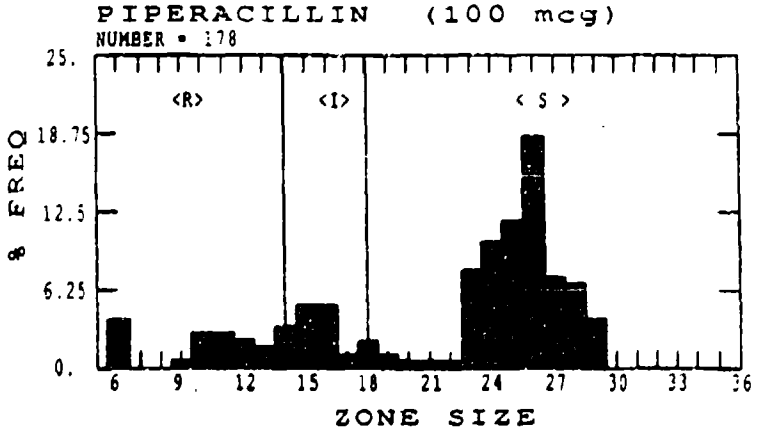


FIGURE 19. Histogram display of the distribution of zones of inhibition of growth of *Escherichia coli*.





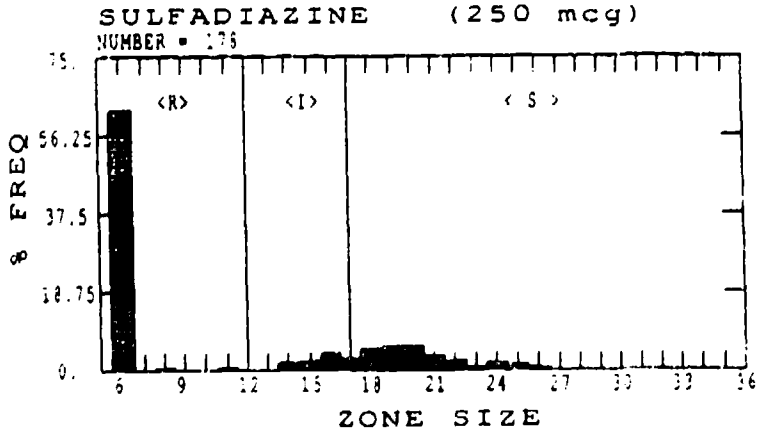


FIGURE 19. Histogram display of the distribution of zones of inhibition of growth of *Escherichia coli* (continued).

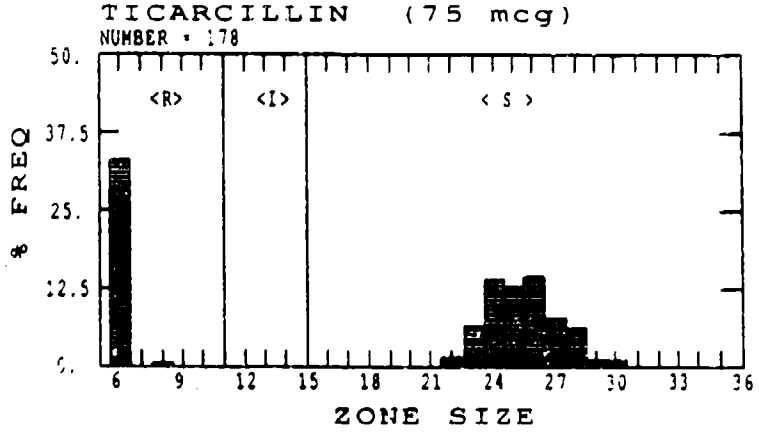


FIGURE 19. Histogram display of the distribution of zones of inhibition of growth of *Escherichia coli* (continued).

Presented at the 8th International Congress on Burn Injuries, New Delhi, India, 16 November 1990.

McManus AT: A survey of blood culture isolates collected from 49 North American units with 8642 admissions. Presented at the 9th International Congress on Burn Injuries, New Delhi, India, 16 November 1990.

PUBLICATIONS

Mason AD Jr, McManus AT, and Hollan E: Microbiologist's notebook: controlling infection in a burn unit. In Creager JG, Black JG, Davison VE (eds): Microbiology Principles and Applications. New Jersey: Prentice Hall, 1990, pp 420-3.

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1. McManus AT, Henderson JR, Lawson TJ, et al: Studies of Infection and Microbiologic Surveillance of Infection in Troops with Thermal Injury. In *US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1985*, c1987, pp 146-194.

ABSTRACT

PROJECT NUMBER: 3M161102BS14, Research

PROJECT TITLE: STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE OF TROOPS WITH THERMAL INJURY: Evaluation of Imipenem-Cilastatin Sodium (Primaxin®) for Prophylactic Activity Against Bacterial Pneumonias in Burned Patients with Inhalation Injury: A Prospective Randomized Trial

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

PERIOD COVERED IN THIS REPORT: 1 October 1990 - 31 March 1991

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

This study was designed to evaluate the efficacy of imipenem-cilastatin sodium for prophylactic activity against bacterial pneumonias in burned patients with inhalation injury. Patients enrolled in the study would have received standard appropriate therapy for their burns and would have been randomized in pairs to receive or not receive prophylaxis with imipenem-cilastatin sodium. Patient pairs would have then been evaluated for development of pneumonia within the first 10 days (during antibiotic administration), development of pneumonia within 30 days postburn, and death or discharge from the hospital.

Because the population for this study conflicted with another study, no patients were enrolled in this study. This study was terminated effective 31 March 1991.

EVALUATION OF IMIPENEM-CILASTATIN SODIUM (PRIMAXIN®) FOR PROPHYLACTIC ACTIVITY AGAINST BACTERIAL PNEUMONIAS IN BURNED PATIENTS WITH INHALATION INJURY: A PROSPECTIVE RANDOMIZED TRIAL

Inhalation injury is an important comorbid factor in burn patients. It increases mortality above that related to age and burn size. In the most recent review, inhalation injury was found in 35% of patients (1). Inhalation injury also seems to predispose to the development of bacterial pneumonia, with 45.8% of patients with severe inhalation injury developing pneumonia. In this analysis, patients with inhalation injury who developed pneumonia more commonly showed the pneumonia in the first week after injury

Unfortunately, there is no specifically effective treatment of inhalation injury. Patients are supported according to their clinical status with appropriate fluid administration, maintenance of airway patency, adequate amounts of oxygen, and mechanical ventilation as necessary. Some patients tolerate the insult well and recover rapidly; however, others develop pneumonia, initially with *Staphylococcus aureus* and later with Gram-negative organisms, which can lead to progressive respiratory failure and death.

Prophylactic treatment against the sequelae of inhalation injury may be useful as there is no other therapy available to prevent bacterial pneumonia. Levine et al (2) evaluated the effect of prophylactic aerosolized gentamicin in patients with inhalation injury and found no difference in mortality, time of death, or pulmonary or septic complications. Gram-negative pneumonias were the most common pulmonary infections in the patients in their study, but more recently, Gram-positive pneumonia has become more common.

Imipenem-cilastatin sodium (Primaxin®), a thienamycin antibiotic which has been recently released, could be of use in the prevention of this problem. This antibiotic has a wide spectrum, being active against Gram-positive, Gram-negative, and anaerobic bacteria. It is bactericidal even against aminoglycoside-resistant and beta-lactamase-producing organisms. It is effective against strains of *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Only strains of *Pseudomonas maltophilia*, *Pseudomonas cepacia*, *Streptococcus faecium*, flavobacteria, and diptheroids have been found to be resistant to imipenem-cilastatin sodium. Minimal toxicity has been attributed to the drug, which is excreted by the kidneys; dosage reductions to one-third are required in anuretic patients. Therefore, it is a good choice for prophylactic therapy for inhalation injury.

Prophylactic treatment in the past has been principally directed toward wound infection and utilized antibiotics that were more inherently toxic without the wide spectrum of

imipenem-cilastatin sodium. Antibiotics have also been administered into the tracheobronchial tree without beneficial effects. The regimen proposed offers the advantage of an intravenous agent with good penetration of lung tissue, minimal toxicity, and a wide spectrum of activity against the organisms most often involved in the pneumonias associated with inhalation injury. Randomization will be employed to compare this new prophylactic regimen with the usual expectant treatment.

The purpose of this study was to evaluate prophylactic treatment of inhalation injury and correlate with the clinical prevention of pneumonia in such patients.

MATERIALS AND METHODS

Number of Patients. Up to 200 patients were to be enrolled in this study, with an early cutoff by closed-end sequential analysis possible.

Criteria for Admission to the Study. Patients admitted to the US Army Institute of Surgical Research with evidence of inhalation injury were to be offered the opportunity to participate in this study. Properly signed and witnessed DA Forms 5303-R, Volunteer Agreeement Affidavit, were to be obtained prior to initation of the study. Patients meeting the following criteria were considered eligible for enrollment in the study:

1. A history of inhalation of smoke and/or flames.

2. A history of burn occurring in a closed space with the physical findings of burns to the face, lips, nose, or mouth or singeing of facial or nasal hair.

3. Carbonaceous sputum production.

4. Stridor, hoarseness, or airway obstruction.

5. Dyspnea, wheezing, or rhonchi.

6. Xenon scan showing trapping of gas in a pattern consistent with inhalation injury.

Patient Inclusion. Male and female patients meeting the following criteria were eligible for participation in the study:

1. Patient ≥ 18 yr old.

2. Patients with a diagnosis of inhalation injury confirmed by bronchoscopic examination.

3. Patients with an expected probability of survival of 20-80% based on an age and burn size predictor model.

4. Patients not subject to any preexisting disease which contraindicated administration of imipenem-cilastatin sodium.

5. Patients who could begin treatment by 72 h postburn.

Patient Exclusion. Patients with any of the following characteristics were ineligible for participation in this study:

1. Patients < 18 yr old.

2. Patients who were pregnant or nursing.

3. Patients who had a prior history of renal dysfunction.

4. Patients who were hypersensitive to thienamycin or who had an anaphylactic reaction to any of the beta-lactam groups of antibiotics, including cephalosporins, oxacephalosporins, penicillins, or cephamycins.

5. Patients with prosthetic valve endocarditis.

6. Patients in danger of or in hepatic coma.

Study Design. Patients would have received appropriate standard therapy for their burn and would have been randomized in pairs to receive or not receive prophylaxis with imipenem-cilastatin sodium. Imipenem-cilastatin sodium at 500 mg every 6 h would have been started between 36 and 72 h postburn and administered for 240 h (10 days). No other modifications were to be made in the care of either group of patients. Sputum cultures and other cultures would have been collected according to the usual protocol for all patients. Respiratory status would have been carefully monitored according to the usual procedures in all patients and documented appropriately.

Patients in both groups would have been carefully watched for the development of pulmonary infection. Tracheobronchitis and pneumonia would have been diagnosed by the usual criteria (Table 1) and were to be treated with antibiotics when judged appropriate by the primary physician. Cellulitis diagnosed by clinical criteria and treated with penicillin would not have been grounds for exclusion from the study. Patient pairs were to be excluded from the study if either patient developed a deep tissue infection requiring treatment with antibiotics or succumbed without pneumonia in the first 10 days. Patient pairs were to be evaluated for the development of pneumonia within the first 10 days (during antibiotic administration), pneumonia developing within 30 days postburn, and death or discharge from hospital.

Patients would have been enrolled in the study as sequential pairs with treatment randomly allocated between the paired patients. This would have allowed sequential analysis with a

TABLE 1. Diagnosis of Infection

Pneumonia
1. Clinical findings consistent with pneumonia, i.e., pleuritic chest pain, fever, purulent sputum, or other signs of sepsis.
2. A significant number (> 25) of PMLs on methylene blue stain of endotracheal secretions with < 25 squamous epithelial cells per 100X field.
3. Roentgenographic findings consistent with pneumonia.
4. Positive sputum culture (confirmatory, but not essential for diagnosis).

Tracheobronchitis
1. Clinical findings consistent with the diagnosis, i.e., fever, purulent sputum, sepsis, or significant findings on bronchoscopy.
2. A significant number (> 25) of PMLs on methylene blue stain of endotracheal secretions with < 25 squamous epithelial cells per 100X field.
3. Roentgenographic findings <u>not</u> consistent with pneumonia.
4. Positive sputum culture (confirmatory, but not essential for diagnosis).

closed-end statistical model (3). Based on the probability of the untreated group experiencing pneumonia (45% from past clinical experience), a significance level of 0.05, a power of 0.75, and an improvement with therapy to half the untreated value, the maximum number of patients theoretically required was estimated to be 72 patients per group (fig 1). If imipenem-cilastatin sodium therapy proved to be either very effective or detrimental, the sequential analysis would have allowed the study to have been completed with significantly fewer patients.

RESULTS

Because the population for this study conflicted with another study, no patients were enrolled in this study.

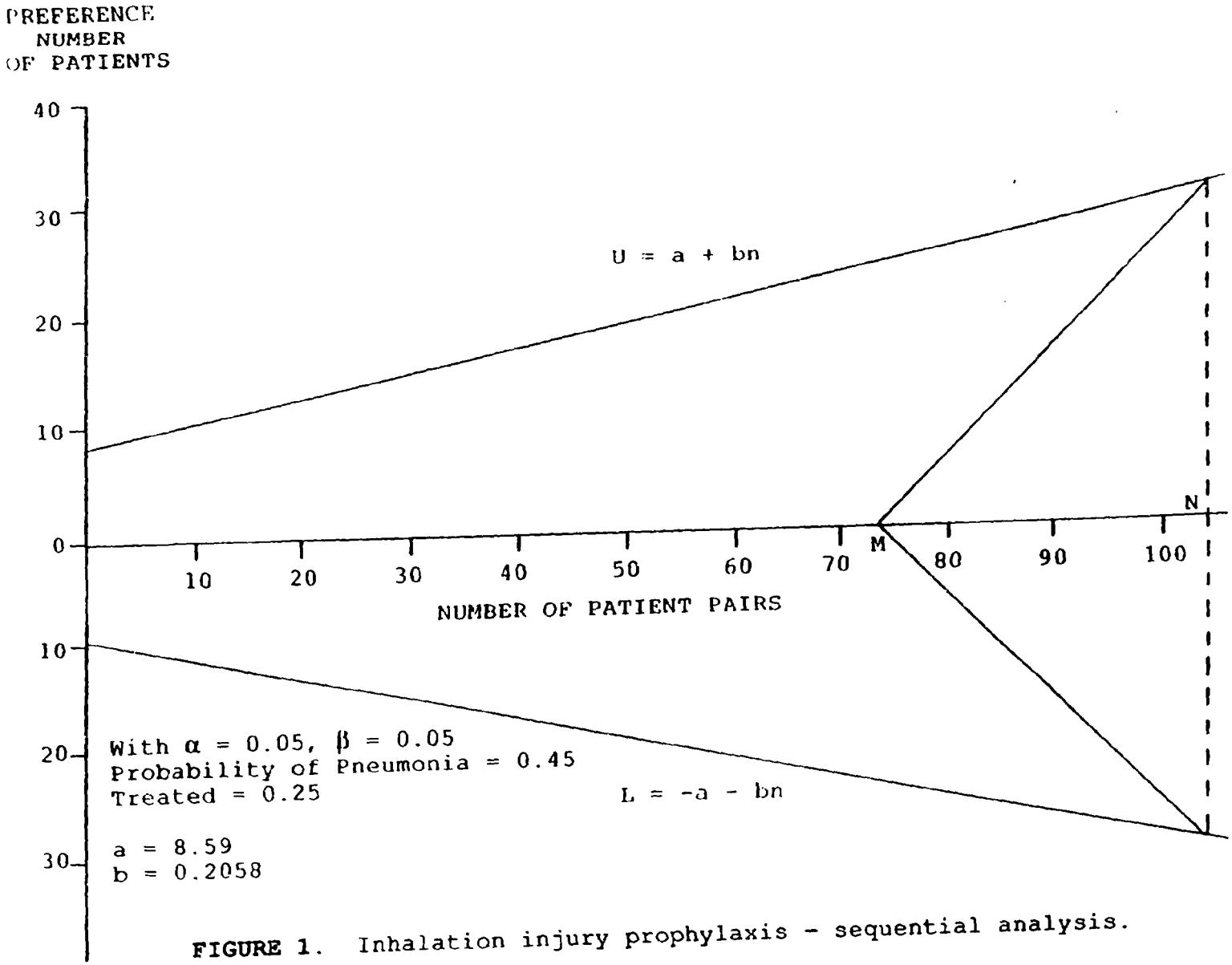

FIGURE 1. Inhalation injury prophylaxis - sequential analysis.

DISCUSSION

This study was terminated effective 31 March 1991.

PRESENTATIONS/PUBLICATIONS

None.

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3. Armitage P: *Sequential Medical Trials*. Springfield: CC Thomas Publishers, 1960.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
					10 Oct 90	DD-DR&E(AR) 636
3. DATE PREV SUMMARY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISP'N INSTR'N	9. LEVEL OF SUM
NONE	A, New	U	U		CX	A. WORK UNIT

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS14	CH	302
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code) (U) In vitro Antigen-Presenting Capacity of Macrophages and in vivo Assessment of Immunological Consequences

12. SUBJECT AREAS
0601 Biochemistry 0605 Medicine and Medical Research

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
9010	9209	DA	C. In-House

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

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

18. RESOURCES ESTIMATE

a. FISCAL YEARS	b. PROFESSIONAL WORK YEARS	c. FUNDS (In thousands)
90	0.0	0
91	0.1	114

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME: US Army Institute of Surgical Research	a. NAME: US Army Institute of Surgical Research
b. ADDRESS (Include zip code): Fort Sam Houston, San Antonio, Texas 78234-5012	b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-5012
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR: BURLESON, D G
d. TELEPHONE NUMBER (Include area code): 512-221-2720	d. TELEPHONE NUMBER (Include area code): 512-221-7138
21. GENERAL USE: FINA MILITARY/CIVILIAN APPLICATION: M	e. NAME OF ASSOCIATE INVESTIGATOR (If available): KOPPENHEFFER, T L g. NAME OF ASSOCIATE INVESTIGATOR (If available): MC MANUS, A T

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

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

23/24. (U) The objectives of this work are to 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. 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 cell) and pooled BN splenocytes (responder cells). Cultures will be maintained for 3 days, after which time ^{3}H-thymidine will be added to the cultures 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 stimulation of a murine CTL line and IL2R expression by flow cytometry. Splenic macrophages will also be isolated from burned and sham-burned Lewis rats and individual unburned BN rats will be given intravenous inoculations 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.

25. (U) 8810 - 8909. Not applicable.

(U) 8910 - 9010. Not applicable.

DD FORM 1498 83 MAR — EDITION OF MAR 68 IS OBSOLETE

CONTINUATION OF DD FORM 1498 FOR THE PROJECT ENTITLED "*IN VITRO* ANTIGEN-PRESENTING CAPACITY OF MACROPHAGES AND IN VIVO ASSESSMENT OF IMMUNOLOGICAL CONSEQUENCES"

(U) 9010 - 9109. This project 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 1991. Equipment and supplies have been ordered and work will be initiated shortly.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION: DA335673	2. DATE OF SUMMARY: 01 Oct 91	REPORT CONTROL SYMBOL: DD-DR&E(AR) 636
3. DATE PREV SUM'RY: 10 Oct 90	4. KIND OF SUMMARY: D. Chg	5. SUMMARY SCTY: U	6. NOR SECURITY: U	7. REGRADING	8. DISP'N INSTR'N: CX	9. LEVEL OF SUM'RY WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS14	CH	302
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code) (U) In vitro Antigen-Presenting Capacity of Macrophages and in vivo Assessment of Immunological Consequences

12. SUBJECT AREAS
0601 Biochemistry 0605 Medicine and Medical Research

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
9010	9209	DA	C. In-House

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

18. RESOURCES ESTIMATE

FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
91	1.9	109
92	1.9	112

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME: US Army Institute of Surgical Research	a. NAME: US Army Institute of Surgical Research
b. ADDRESS (Include ZIP code): Fort Sam Houston, San Antonio, Texas 78234-5012	b. ADDRESS: Fort Sam Houston, San Antonio, Texas 73234-5012
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR: BURLESON, D G
d. TELEPHONE NUMBER (Include area code): 512-221-2720	d. TELEPHONE NUMBER (Include area code): 512-221-4858
21. GENERAL USE: FIC	e. NAME OF ASSOCIATE INVESTIGATOR (If available): KOPPENHEFFER, T L
MILITARY/CIVILIAN APPLICATION: M	g. NAME OF ASSOCIATE INVESTIGATOR (If available): MC MANUS, A T

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

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

23/24. (U) The objectives of this work are to 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. 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 cultures 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 stimulation of a murine CTL line and IL2R expression by flow cytometry. Splenic macrophages will also be isolated from burned and sham-burned Lewis rats and individual unburned BN rats will be given intravenous inoculations 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.

25. (U) 9010 - 9109. This project 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 1991. Approximately 30

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

MLR studies have been performed. When a technique is translated across species, assay parameters must be frequently optimized for the new species. Initial MLR results gave a low signal to noise ratio and frequently were inconsistent. Additionally, when rat macrophages were isolated by the classical pathway of plastic adherence, they appeared to preactivate and give anomalously high results. Currently, we are optimizing MLR conditions for the rat and developing a macrophage purification technique that does not preactivate the macrophages.

SUPPLEMENTAL WORK UNIT INFORMATION FOR THE PROJECT ENTITLED "IN VITRO ANTIGEN-PRESENTING CAPACITY OF MACROPHAGES AND IN VIVO ASSESSMENT OF IMMUNOLOGICAL CONSEQUENCES"

Subrecord/Linking Accession Number: Not applicable.

Search Control Data: W6R38K/W6R39J, 9 January 1990.

Product Identification: For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1991.*

Unclassified Special Categories: Lab Animals: Rats; RA II.

ABSTRACT

PROJECT NUMBER: 3M161102BS14, 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: 10 October 1990 - 30 September 1991

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

We have initiated a study of the role of macrophages in the response of burned rats to infection challenge. Initial studies have centered on development of the rat MLR as an assay of antigen-presenting capacity. Rat spleen contained cells that suppressed the MLR. Efforts to evaluate the response of purified rat macrophages were frustrated by the suppressive action of these spleen cells. Rat RBCs or rat hemoglobin reversed the inhibition of the MLR in a dose-dependent manner. Further studies will use natural splenic red cells to reduce the suppression of the MLR during the measurement of the antigen-presenting capacity of splenic macrophages.

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 burn patients. One of the noteworthy changes is found in the function of antigen-presenting cells (APCs). Since the macrophage plays a central role in the immune response to infection, it is important to characterize more completely the functional capacity of macrophages after burn injury and determine its relationship to infection susceptibility.

Recent studies by others have also begun to illuminate the critical role played by APCs in T-lymphocyte activation. Under circumstances which lead to a specific immune response, APCs release IL1 and other costimulatory signals upon interaction with T lymphocytes (1). This leads to IL2R expression and IL2 production by T lymphocytes, thereby establishing a positive self-generating stimulus and subsequent clonal proliferation. It is becoming apparent that the delivery of appropriate costimulatory signals by APCs is critical to the eventual functional status of T cells and other lymphocytes (2). For example, Jenkins and Schwartz (3) demonstrated that mouse T-cell clones are induced into a state of proliferative unresponsiveness in vitro when antigen is presented by chemically fixed macrophages. Apparently, the absence of costimulatory signals which are normally delivered by accessory cells at the time of antigen presentation leads to intracellular ionic changes that shunt T cells into an anergic state (4). A similar phenomenon has recently been observed in vivo (5) where intravenous injection of allogeneic, nonantigen-presenting, Ia-bearing cells in mice induces a state of unresponsiveness to "immunizing" antigens. In this system, spleen cells from normal mice produce a vigorous proliferative response in the MLR. In this system, prior exposure to Ia-like antigens on cells that are incapable of delivering costimulatory signals induces an anergic state in T cells. Analysis of anergized T cells at the molecular level has demonstrated that antigen presentation results in the expression of IL2R, but these cells do not produce IL2 and are therefore unable to proliferate (6).

Defects have 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 (7), defective antigen-presenting capacity of macrophages that can be partly restored by exogenous IL1 in mice (8), and reduced MLR stimulatory capacity of monocytes from human burn victims (9). In the latter studies, both humans (9) and animals (10) had variably reduced MLR responses after burn injury, whereas the inability of mononuclear cells to provide stimulation

was completely lost during the first 9 days postburn and partially restored thereafter.

If macrophage function is altered after burn injury, it may contribute to infection susceptibility. Understanding how macrophage function changes after protective vaccination may suggest several potential therapies for enhancing infection resistance by modifying macrophage activity after burn injury. The objective of this study is to analyze macrophage function after thermal injury in the Walker-Mason burned rat infection model before and after burn injury. Our hypothesis is that macrophages from burned animals are poor stimulators in MLRs because they do not deliver appropriate costimulatory signals which would normally induce IL2 production and result in subsequent proliferation of responder cells.

MATERIALS AND METHODS

Cell Preparations. Lewis and Brown-Norway rats were anesthetized with sodium pentobarbital (35 mg/kg IP) and exsanguinated by bleeding from the portal vein. The spleens and lymph nodes were removed aseptically. The spleens were minced with sterile forceps and passed through 60-mesh wire screens. The 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 then separated by adherence to plastic Petri dishes (37°C for 30 min) or by gradient purification (lympholyte-rat, Cedarlane Laboratories Limited, Hornby, Ontario, Canada). 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 an histoincompatible rat. Cultures were maintained for 3 days at 37°C in an humidified 5% CO_2 environment, after which time ^{3}H-thymidine was added to the 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.

Addition of RBCs or Hemoglobin. In certain studies, rat RBCs or rat hemoglobin was added to the MLR culture. Heparinized blood drawn during exsanguination was diluted 1:2 in isotonic saline, spun at 3,000 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 three times in cold saline. Hemoglobin was prepared by freezing packed RBCs in an acetone-dry ice bath. The cell preparation was frozen and thawed 4X (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.

RESULTS

The classical MLR was developed using leukocytes from mouse and human sources. Adaption of the reaction to rat cells has not been straight forward. Our first trials showed little or no stimulation using rat spleen and lymph node cells. A typical example is shown in Table 1. Stimulation of Brown-Norway lymph node responder cells by density-purified splenic mononuclear cells resulted in only minimal uptake of ^{3}H-thymidine. Many combinations of cellular density and ratio of stimulators to responders were attempted. Literature reports on rat MLRs revealed that when spleen cells were used successfully in an MLR, they either were not separated from splenic RBCs or they were used after adherent cells were removed from the cultures. The presence of "suppressive" adherent cells in spleen had been postulated (12). Lewis spleen stimulator cells were lysed to remove RBCs and compared to spleen stimulator cells that were not lysed before culture. The results shown in Table 2 indicate that leaving the RBCs in the culture enhanced the mitogenic response, confirming the observation from the literature.

TABLE 1. Rat MLR Mitogenic Response Using Purified Spleen Cells

Stimulator[a]	Responder[b]	S:R Ratio[c]	^{3}Thymidine Uptake
None	Brown-Norway	-	6,631
Brown-Norway	Brown-Norway	1:2	9,828
Brown-Norway	Brown-Norway	1:1	5,684
Brown-Norway	Brown-Norway	2:1	3,957
Lewis	Brown-Norway	1:2	9,168
Lewis	Brown-Norway	1:1	12,968
Lewis	Brown-Norway	2:1	13,294

[a]Lewis or Brown-Norway splenic mononuclear cells were purified from RBCs and granulocytes by gradient density centrifugation, treated with mitomycin C, and cultured as stimulator cells at the appropriate concentration to give the ratio indicated.
[b]Brown-Norway responder lymph node cells were cultured at 4×10^{5} per well.
[c]Ratio of stimulator cells to responder cells.

Since our experimental design required that we use the adherent cells as stimulators, we further investigated the nature of this phenomenon to find possible ways to circumvent it. Rat MLRs consisting of gradient-purified mononuclear spleen cells as stimulator cells were cultured in the presence of various

TABLE 2. Rat MLR Mitogenic Response with RBC-Lysed and -Unlysed Splenocytes

Stimulator[a]	Responder[b]	S:R Ratio[c]	Lysis	^{3}Thymidine Uptake
None	Brown-Norway	–	–	961
Lewis	Brown-Norway	1:2	–	3,904
Lewis	Brown-Norway	1:1	–	8,186
Lewis	Brown-Norway	2:1	–	11,547
None	Brown-Norway	–	+	415
Lewis	Brown-Norway	1:2	+	971
Lewis	Brown-Norway	1:1	+	728
Lewis	Brown-Norway	2:1	+	1,091

[a]Lewis spleen cells were used as stimulator cells, either without purification or with lysis with ammonium chloride.
[b]Brown-Norway responder lymph node cells were cultured at 2×10^5 per well.
[c]Ratio of stimulator cells to responder cells.

concentrations of allogeneic RBCs and hemoglobin. Figure 1 depicts the MLR response of Lewis spleen stimulator cells and Brown-Norway responder cells in the presence of Lewis RBCs and hemoglobin from Lewis RBCs. The MLR response increased in a dose-dependent manner as increasing concentrations of RBCs were added. Hemoglobin added in approximately equivalent amounts to that contained in the RBCs was even more effective in enhancing DNA synthesis in the responder cells. There is a slight enhancement of the spontaneous DNA synthesis of the Brown-Norway responder cells alone when either Lewis RBCs or hemoglobin was added to the culture. In order to eliminate the possibility that allogeneic antigens on the RBCs or hemoglobin from Lewis rats was causing the enhanced response, autologous Brown-Norway RBCs and hemoglobin were added to MLR cultures. The results of these assays are illustrated in Figure 2. Essentially the same enhancement is seen with the autologous Brown-Norway RBCs and hemoglobin as with allogeneic Lewis RBCs. Cells autologous to the responder cells were as effective as nonautologous cells in enhancing the MLR DNA synthetic response. Thus, allogeneic antigen must not have been involved. Since hemoglobin was as effective or more effective than the RBCs, the effect must have been due to hemoglobin. The conclusion from these studies is that rat spleen cells contain cells that suppress the MLR mitogenic response and that the suppression can at least partly be reversed by hemoglobin.

DISCUSSION

Rat cells seem to be unique in having splenic adherent cells that suppress the MLR response. In fact, several authors have used the phenomenon as a model for suppression (12,15). The effect of

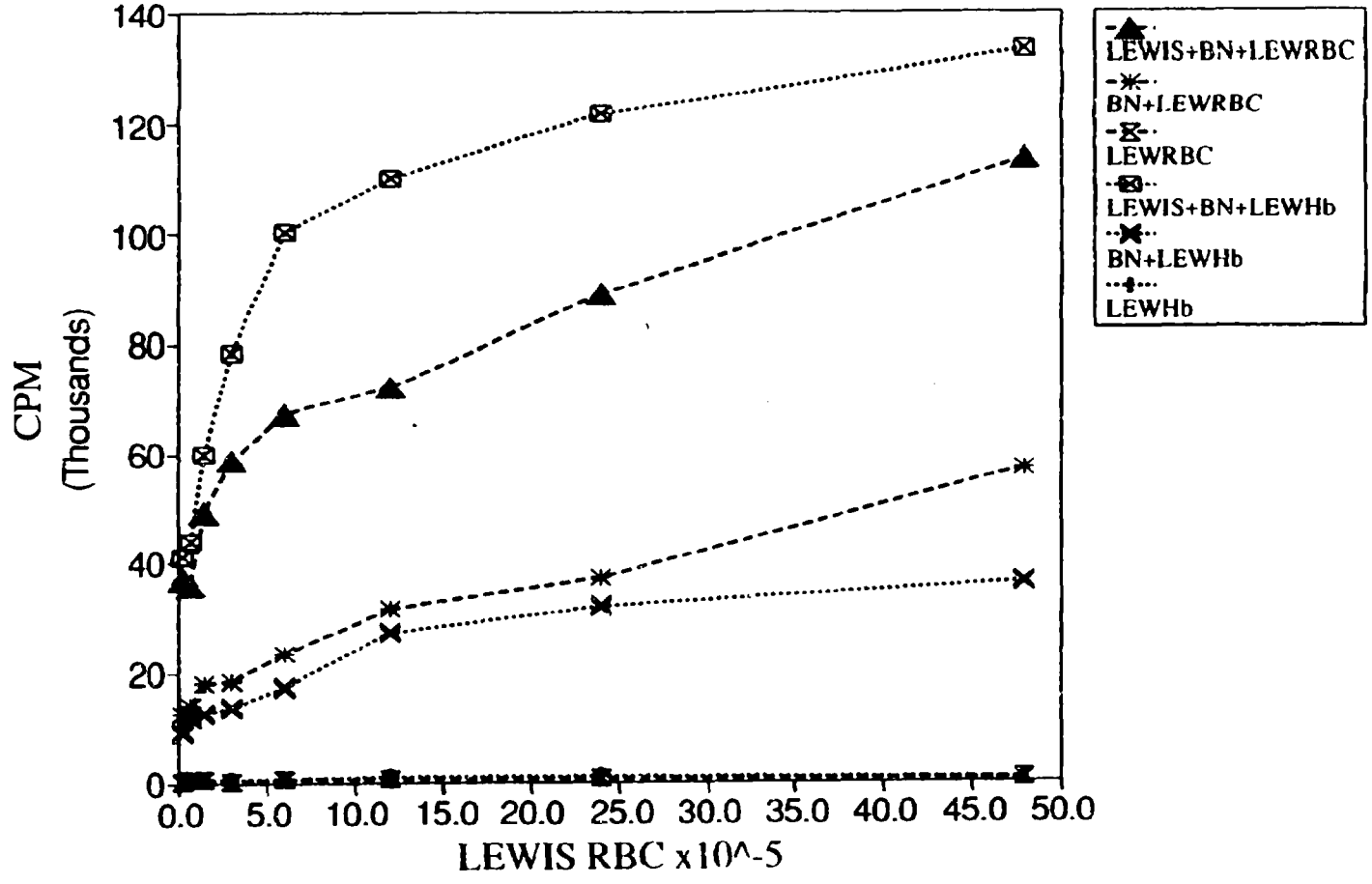

FIGURE 1. Rat MLR versus increasing concentration of Lewis RBCs or hemaglobin.

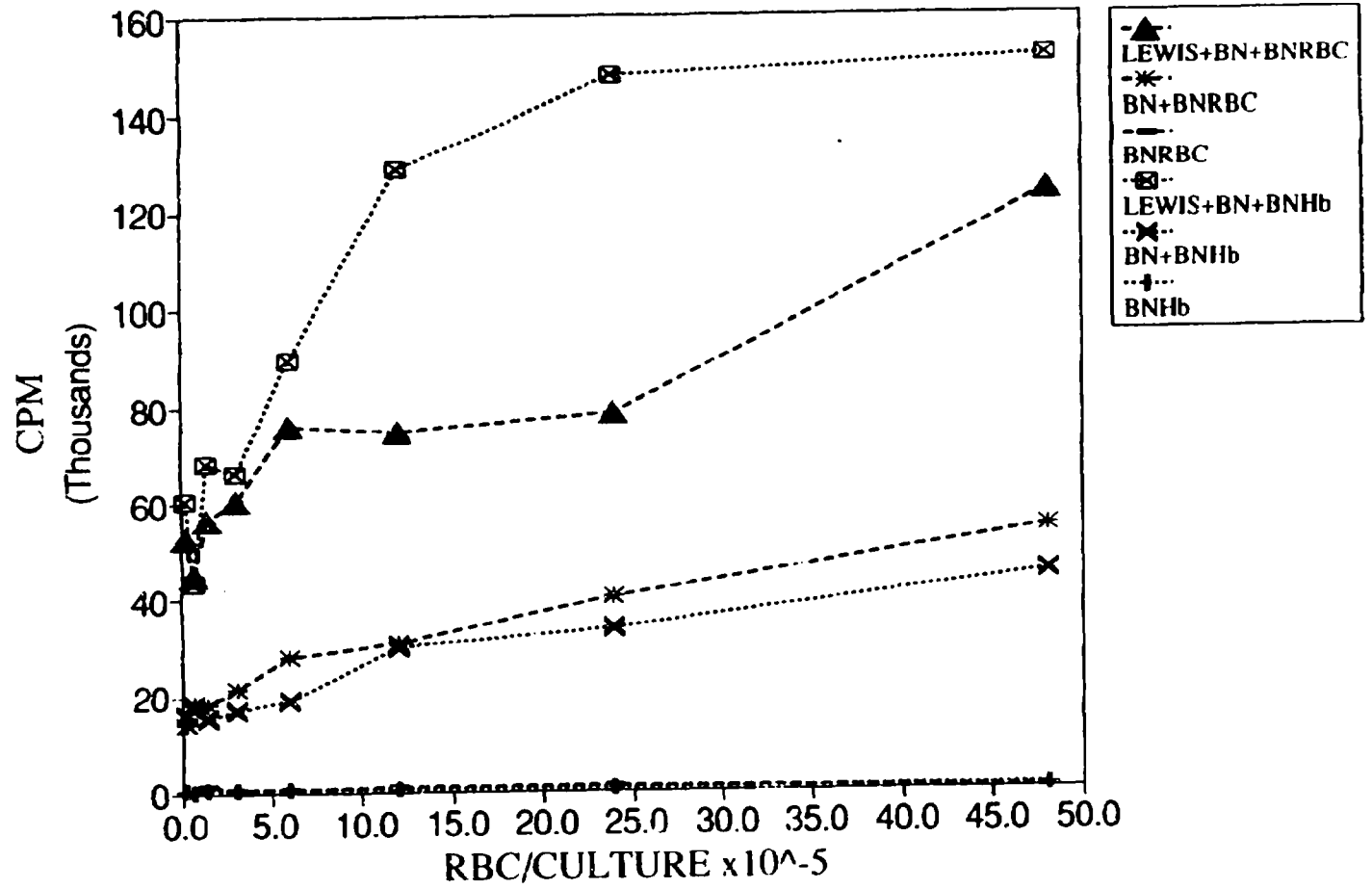

FIGURE 2. Rat MLR versus increasing concentrations of Brown-Norway RBCs or hemaglobin.

hemoglobin is intriguing and provides valuable insight into the mechanism of suppression. Langrehr et al (14,15) have proposed that rat spleen cells suppress MLR responses through a nitric oxide mechanism. Activated macrophages can produce nitric oxide from L-arginine. In support of that mechanism, Shirnomiya et al (16) have induced suppression of MLRs consisting of unpurified rat spleen cells with rat liver arginase and L-arginine. Increased proliferation of ConA-stimulated rat spleen cells after addition of hemoglobin was reported by Albina and Henry (17) and they attributed the enhanced response to the binding of nitric oxide by hemoglobin. Additionally, Webb et al (18) have described suppressor cells in tumor-bearing rats with their suppression of ConA stimulation reversed by the addition of RBCs.

If activation of macrophage arginase and nitric oxide production are the mechanism for rat spleen cell suppression, then N-monomethyl-L-arginine, a competitive inhibitor of nitric oxide production, should reverse the suppression and facilitate the MLR mitogenic response. Studies to clarify that possibility are in progress. If N-monomethyl-L-arginine can effectively reduce the suppression, it may allow the evaluation of antigen presentation function in the rat MLR.

PRESENTATIONS/PUBLICATIONS

None.

REFERENCES

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2. Nossal GJV: Immunologic tolerance: collaboration between antigen and lymphokines. *Science* 245:147-53, 1989.

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4. Jenkins MK, Pardoll DM, Mizuguchi J, et al: Molecular events in the induction of a nonresponsive state in interleukin 2-producing helper T-lymphocyte clones. *Proc Natl Acad Sci USA* 84:5409-13, 1987.

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

9. Sakai H, Daniels JC, Beathard GA, et al: Mixed lymphocyte culture reaction in patients with acute thermal burns. *J Trauma* 14:53-7, 1974.

10. Kupper TS, Green DR: Immunoregulation after thermal injury: sequential appearance of I-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				1. AGENCY ACCESSION DAOG6968	2. DATE OF SUMMARY 01 Oct 91	REPORT CONTROL SYMBOL DD-DR&E(AR) 636
3. DATE PREV SUM'RY 01 Oct 90	4. KIND OF SUMMARY D. Chg	5. SUMMARY SCTY U	6. WORK SECURITY U	7. REGRADING	8. DISP'N INSTR'N CX	9. LEVEL OF SUM A. WORK UNIT
10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER	WORK UNIT NUMBER	
a. PRIMARY	61102A	3M161102BS14		FB	303	
b. CONTRIBUTING						
c. CONTRIBUTING						

11. TITLE *(Precede with Security Classification Code)*
(U) Alteration of Host Resistance in Burned Soldiers

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

13. START DATE	14. ESTIMATED COMPLETION DATE	16. FUNDING ORGANIZATION	18. PERFORMANCE METHOD
7610	9909	DA	C. In-House

17. CONTRACT/GRANT MILITARY RELEVANCY CERTIFIED APPROVED BY

15. RESOURCES ESTIMATE

a. DATE EFFECTIVE	b. CONTRACT/GRANT NUMBER	c. TYPE	d. AMOUNT	e. KIND OF AWARD	f. CUM/TOTAL	a. FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
						91	3.0	200
						92	3.0	208

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME: US Army Institute of Surgical Research	a. NAME: US Army Institute of Surgical Research
b. ADDRESS *(Include zip code)*: Fort Sam Houston, San Antonio, Texas 78234-5012	b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-5012
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR: MC MANUS, A T
d. TELEPHONE NUMBER *(Include area code)*: 512-221-2720	d. TELEPHONE NUMBER *(Include area code)*: 512-221-3411
21. GENERAL USE: FIC MILITARY/CIVILIAN APPLICATION: M	e. NAME OF ASSOCIATE INVESTIGATOR *(If available)* g. NAME OF ASSOCIATE INVESTIGATOR *(If available)*

22. KEYWORDS *(Precede EACH with Security Classification Code)* (U) Burns (Injuries); (U) Infectious Disease Transmission; (U) Immunity; (U) Virulence; (U) Antibiotics

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

23/24. (U) The objective of this work is to define the microbial basis of opportunistic infection in susceptible burned patients, identify specific mechanisms of decreased host resistance that are targeted by opportunistic pathogens, and develop and evaluate countermeasures. The effect of in vitro alterations of specific microbial characteristics on infection will be investigated. Specific antimicrobial and immunostimulatory therapies will be examined.

25. (U) 9010 - 9109. A novel mafenide acetate compound, mafenide phosphanilate (ISR-55), has been synthesized. The compound was designed to expand the antimicrobial spectrum and potency of mafenide acetate and has been shown *in vivo* to have at least five times the potency of mafenide acetate. In addition, a model of surface burn-dependent susceptability to *Pseudomonas aeruginosa* bronchopneumonia has been developed.

DD FORM 1498 83 MAR — EDITION OF MAR 83 IS OBSOLETE — * U.S.G.P.O. 1986-491-003/50329

SUPPLEMENTAL WORK UNIT INFORMATION FOR THE PROJECT ENTITLED "ALTERATION OF HOST RESISTANCE IN BURNED SOLDIERS"

Subrecord/Linking Accession Number: Not applicable.

Search Control Data: W6K56A/W6K56F, 20 October 1989.

Product Identification: For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report* for fiscal years 1976-91.

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

ABSTRACT

PROJECT NUMBER: 3M161102BS14, 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 1990 - 30 September 1991

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A novel mafenide acetate compound, mafenide phosphanilate (ISR-55), has been synthesized. The compound was designed to expand the antimicrobial spectrum and potency of mafenide acetate and has been shown in vivo to have at least five times the potency of mafenide acetate. In addition, a model of surface burn-dependent susceptability to *Pseudomonas aeruginosa* bronchopneumonia has been developed.

New Antibiotics in Clinical Use. The parenteral antibiotic agents, ceftazidime, ceftriaxone sodium, and aztreonam, were used clinically for the second year. The fluoroquinolone agents, ciprofloxacin and norfloxacin, were introduced. Results of in vitro testing are presented in Table 1.

TABLE 1. Activity of Newly Released Antibiotics for Fiscal Years 1986-91

Fiscal Year	1986	1987	1988	1989	1990	1991
			Aztreonam[a]			
Resistant	162 (14.5)	119 (10.5)	540 (25.3)	602 (36.8)	669 (30.4)	966 (27.7)
Sensitive	955	1,009	1,593	1,035	1,529	2,518
			Ceftazidime[b]			
Resistant	165 (16.6)	248 (12.5)	480 (15.4)	683 (22.3)	531 (15.9)	764 (22.6)
Sensitive	829	1,731	2,633	2,374	2,805	2,610
			Ceftriaxone Sodium[b]			
Resistant	287 (29.7)	267 (13.5)	843 (27.0)	786 (25.8)	885 (28.2)	1,497 (44.2)
Sensitive	678	1,708	2,279	2,265	2,449	1,892
			Norfloxacin[b]			
Resistant	108 (8.3)	24 (1.6)	41 (1.9)	198 (12.1)	129 (5.9)	416 (17.2)
Sensitive	1,187	1,472	2,158	1,439	2,070	2,000

( ) = 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 43 burned patients. Agar dilution minimal inhibitory concentration

(MIC) assays were completed on 158 strains. The median MIC was 0.156 g/100 ml, a decrease from the Fiscal Year 1990 value of 0.312 g/100 ml. Data comparing the past three reporting periods are presented in Table 2 and the cumulative display of MIC for Fiscal Year 1991 is presented at Figure 1.

TABLE 2. Minimal Inhibitory Concentration for *Pseudomonas aeruginosa* Strains to Mafenide Acetate for Fiscal Years 88-91

Mafenide Acetate Concentration (g/100 ml)	Number of Strains			
	1988	1989	1990	1991
0.019	10	24	2	13
0.039	16	11	18	24
0.078	36	26	15	31
0.156	39	50	38	51
0.312	42	59	61	32
0.625	13	23	36	7
1.250	2	-	6	-
Total Number of Strains	**158**	**193**	**176**	**158**

Three experimental compounds were tested for topical antipseudomonal chemotherapeutic activity. Compounds were tested in the Walker-Mason scalded rat model with *Pseudomonas aerguinosa* (Strain 59-1244) as the test organism. All compounds were initiated 24 h after burn inoculation. Table 3 shows the results of a silver chloride titanium dioxide product (JMAC®, Johnson-Matthey Company), Table 4 shows the results of a product called Ultra-Kleen® (Ridgely Products Corportion), and Table 5 shows the results of a topical preparation containing myeloperoxidase (ExOxEmis Corporation). These products did not demonstrate chemotherapeutic activity.

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

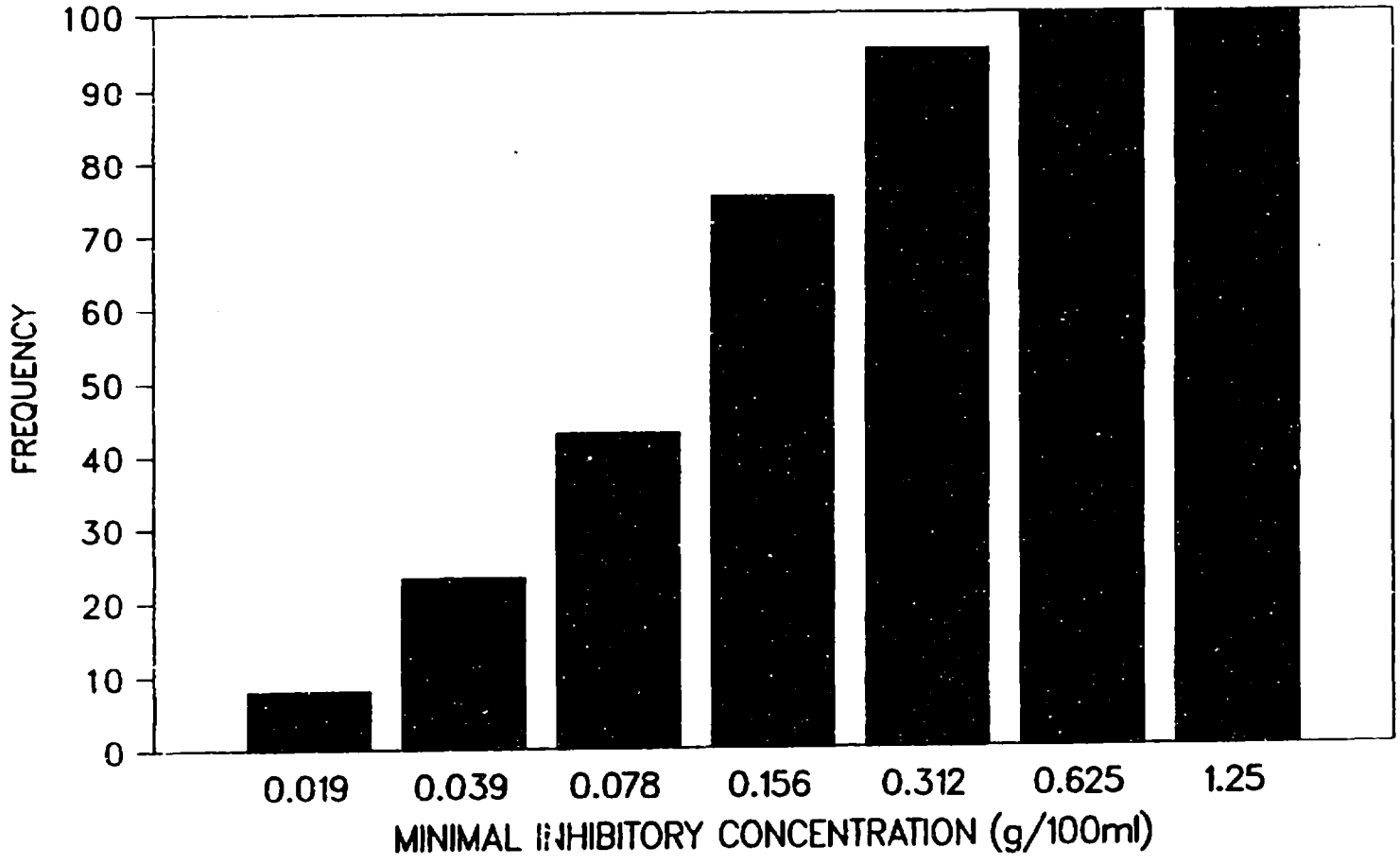

FIGURE 1. Cumulative distribution of MIC for mafenide acetate against *Pseudomonas aeruginosa*.

TABLE 3. Examination of Silver Chloride Titanium Dioxide (JMAC®) in *Pseudomonas aeruginosa*-Infected Rats

Group	Number of Animals Total	Dead	Mortality (%)
Control	30	29	96.7
Silver sulfadiazine	30	1	3.3
Mafenide acetate	10	7	70.0
JMAC® carrier	20	20	100.0
2% JMAC®	20	19	95.0
5% JMAC®	20	19	95.0
5% JMAC®/Unibase®	9	9	100.0

TABLE 4. Examination of Ultra-Kleen® in *Pseudomonas aeruginosa*-Infected Rats

Group	Number of Animals Total	Dead	Mortality (%)
Control	9	9	100.0
Silver sulfadiazine	9	1	11.1
2% Ultra-Kleen®	10	10	100.0
5% Ultra-Kleen®	10	9	90.0
5% Ultra-Kleen® mist	10	10	100.0

patients with each serotype and total number of isolates per serotype.

PRESENTATIONS/PUBLICATIONS

McManus AT: Demonstrated value of infection control in burns. Presented to the Society of Plastic Surgeons of Taiwan, Taiwan, China, 19 November 1990.

TABLE 5. Examination of a Topical Preparation Containing Myeloperoxide in *Pseudomonas aeruginosa*-Infected Rats

Group	Number of Animals		Mortality (%)
	Total	Dead	
Control	20	20	100.0
Silver sulfadiazine	20	6	30.0
Mafenide acetate	9	7	77.8
Myeloperoxidase	20	20	100.0
Myeloperoxide in nonionic carrier	10	10	100.0

McManus AT: Demonstrated value of infection control in burns. Presented to the Department of Plastic Surgery, National Defense Medical Center and Tri-Service General Hospital, Taipei, China, 21 November 1990.

McManus AT: Infection control in burns. Presented to the Department of Microbiology, Chinese University of Hong Kong Medical School, Victoria, Hong Kong, 23 November 1990.

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 CH: 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.

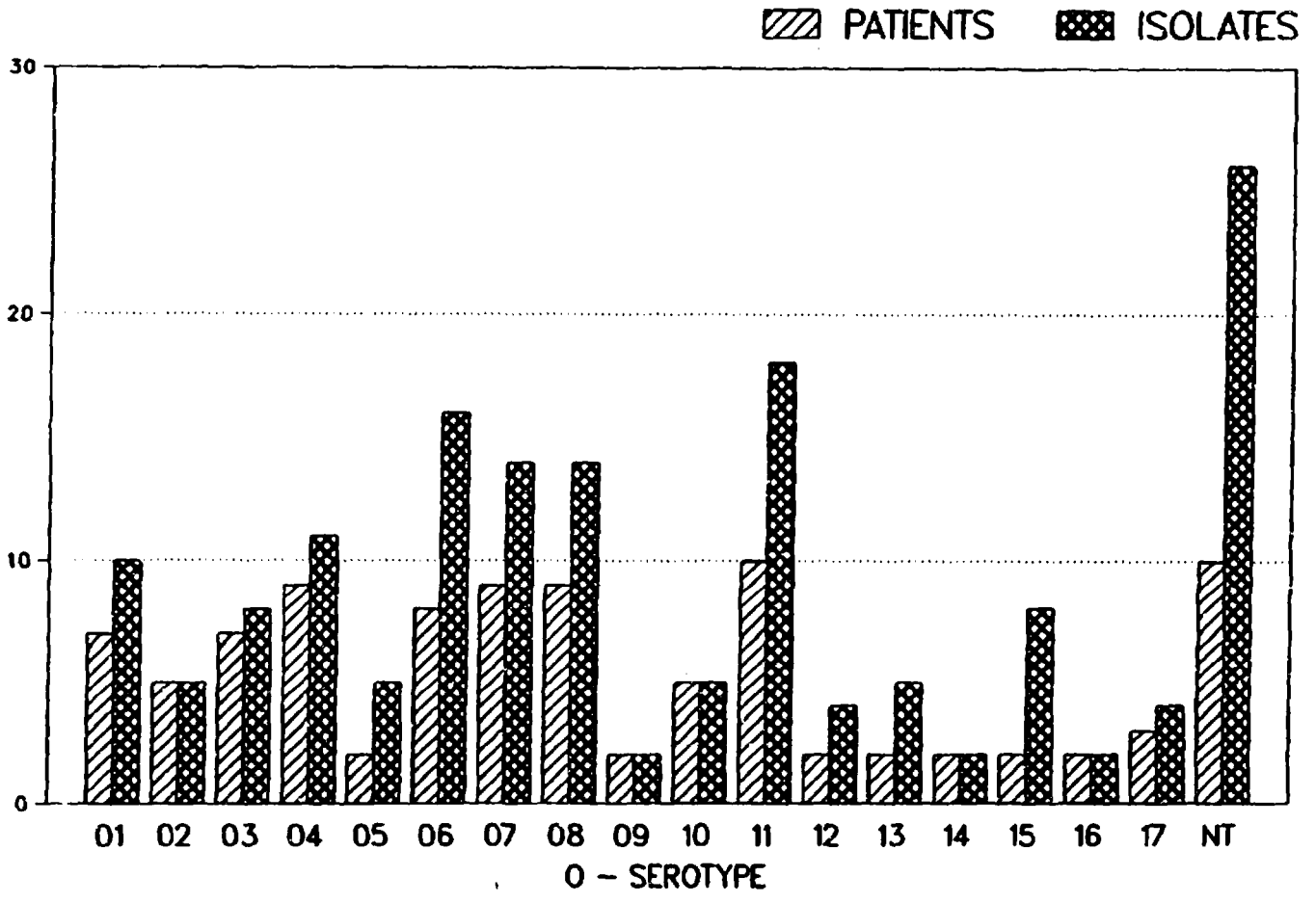

FIGURE 2. Histogram display of the frequency of *Pseudomonas aeruginosa* O-serotypes (Difco International Typing Sera™) by number of patients and isolates. NT indicates all strains not typeable by the standard 17 typing sera.

ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3M161102BS14, 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 1990 - 30 September 1991

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Previous studies have indicated that the presence of neopterin in serum might be useful as an early indicator of infection in patients with thermal injury. Neopterin levels were measured in a series of burn patient urine and sera. The amount of neopterin present was quantified by reverse-phase HPLC. Sample integrity was verified by monitoring creatinine clearance. Urine neopterin levels in burn patients were about equal to those of healthy control subjects during the first week postburn and gradually reached 400% to 500% of control by the end of 8 weeks. Serum levels of neopterin were within the range of healthy control subjects during the first week postburn, gradually increased 3- to 4-fold by postburn week 5, and returned to more normal levels by postburn week 8. There was no correlation of neopterin levels with burn size. A preliminary analysis of samples from patients with bactermia, pneumonia, and wound infections revealed that serum levels of neopterin were increased in bacteremic patients and decreased in patients with pneumonia and wound infection compared to patients without infection; however, with the current number of patients, none of these changes were statistically significant. Urine levels were decreased in all infected patients compared to those of uninfected patients. Though unexpected, the lower levels of neopterin found in urine may have some utility in the diagnosis of infection. Collection and analyses of patient samples and evaluation of the results continues.

CHARACTERIZATION OF BIOCHEMICAL INDICATORS OF INFECTION IN THE THERMALLY INJURED

Abnormal levels of hormones (1,2), acute-phase proteins (3,4), and fluorescent substances (5) in blood and plasma occur in the presence of inflammation and/or infection in burned humans and animals. Their presence in blood partly reflect a metabolic response to both trauma and infection. An easily measured biochemical metabolite in serum or blood specific to infection that would permit monitoring of the response to infection separate from the response to trauma might have diagnostic utility. We have previously reported the presence of increased levels of neopterin in the sera of burn patients compared to healthy control subjects (6).

Neopterin is secreted by activated cultures of mononuclear leukocytes and is excreted in increased amounts by humans whose immune systems are responding to viral infection (7), including HIV (8,9), tuberculosis (10), and sepsis (11-13). Neopterin has been used as a prognostic indicator for certain kinds of cancer (14) and as a measure of transplant rejection (15). Despite its widespread presence, no physiological role for neopterin has been found. Its precursor, dihydroneopterin triphosphate, is also a precursor of tetrahydrobiopterin which is a cofactor in hydroxylation reactions, particularly the hydroxylation of phenylalanine to form tyrosine, the precursor of serotonin and the catecholamines.

The presence of elevated levels of neopterin in burn patients and the demonstrated utility of neopterin levels in other clinical settings led us to investigate further 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 632 serum and urine samples drawn for clinical purposes were used in 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 89 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 concentration (ng/ml) or as nanograms neopterin per milligram percent creatinine to compensate for variations in the renal 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 (16).

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 20,000 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. The chromatograph was 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). 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 Company, Waltham, MA).

RESULTS

Previous studies have used serum creatinine or urine creatinine values to correct for variations in the concentration of neopterin due to variations in renal excretion rates (17,18). Neopterin is normally removed from the blood rapidly by the kidneys. When glomerular filtration rates are decreased during renal insufficiency, neopterin can build to high levels. To monitor for this condition, the serum and urine samples used in this report were analyzed for creatinine and neopterin. Creatinine clearance was calculated from the ratio of urine to serum creatinine and urine volume. The effect of low glomerular flow on the level of neopterin measured is illustrated in Figure 1. Serum neopterin concentrations were averaged for samples with creatinine clearance rates falling within consecutive 4 ml/min ranges and the resulting means were plotted against the creatinine clearance ranges. When clearance rates fell below 50 ml/min, serum neopterin values increased sharply.

The limitation of using serum creatinine values to correct for decreased excretion can be deduced from Figure 2. Creatinine clearance values for all patient samples were averaged for samples with serum creatinine concentrations falling within consecutive 0.25 mg% ranges. In order for creatinine values to be useful for correcting for large variations in creatinine clearance, the serum creatinine values must vary in a predictable way with creatinine clearance. As can be seen in Figure 2, serum creatinine varies

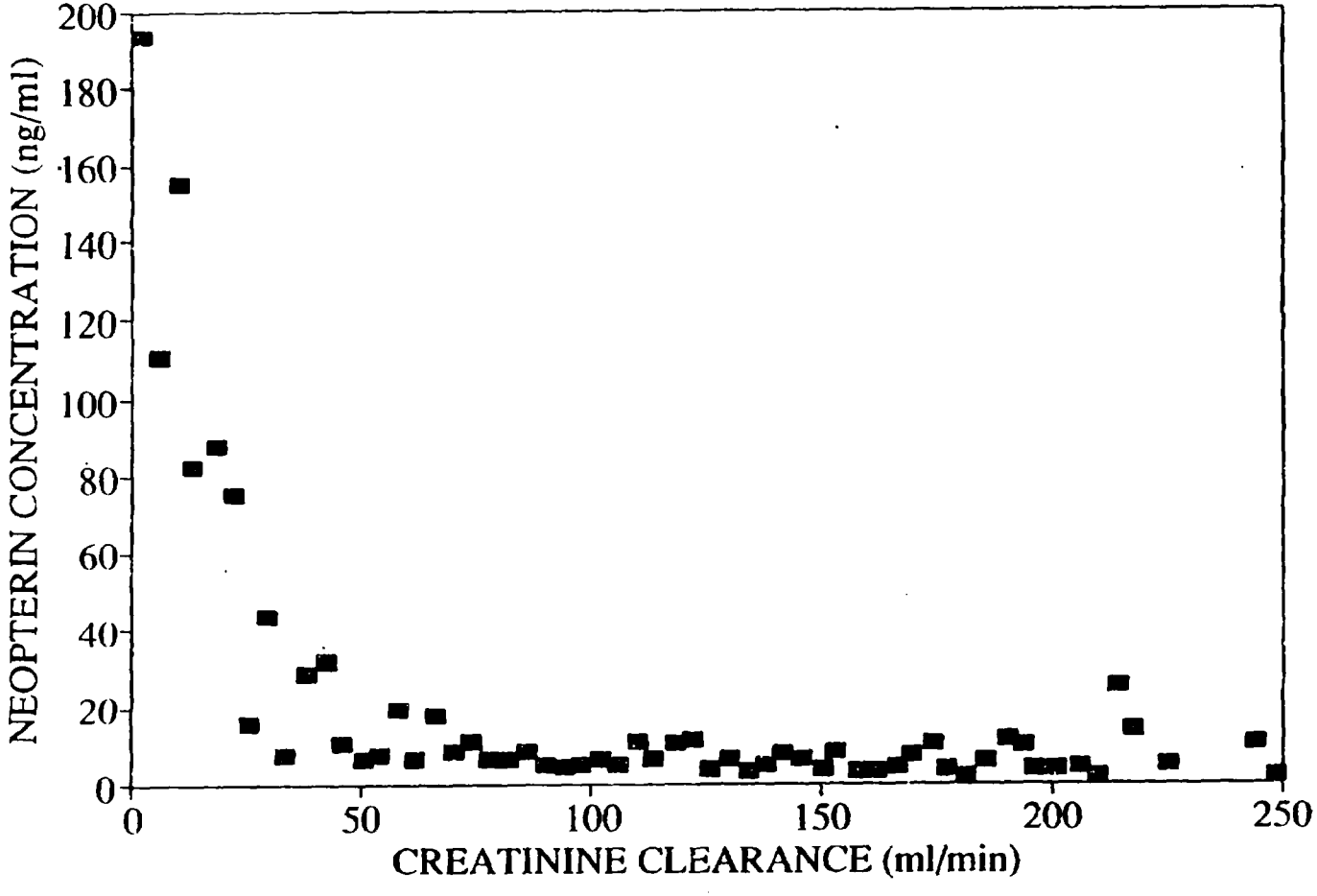

FIGURE 1. Mean serum neopterin levels versus creatinine clearance in burn patients. Each point represents mean neopterin values for samples whose creatinine clearance values fall within a particular 4 ml/min range. Beginning at 1, the mean values for each succeeding range, i.e., 1-4.99, 5-8.99, etc., are plotted against the creatinine clearance value for each range, i.e., 1, 5, etc. Blank spaces occur where there were no values within a range.

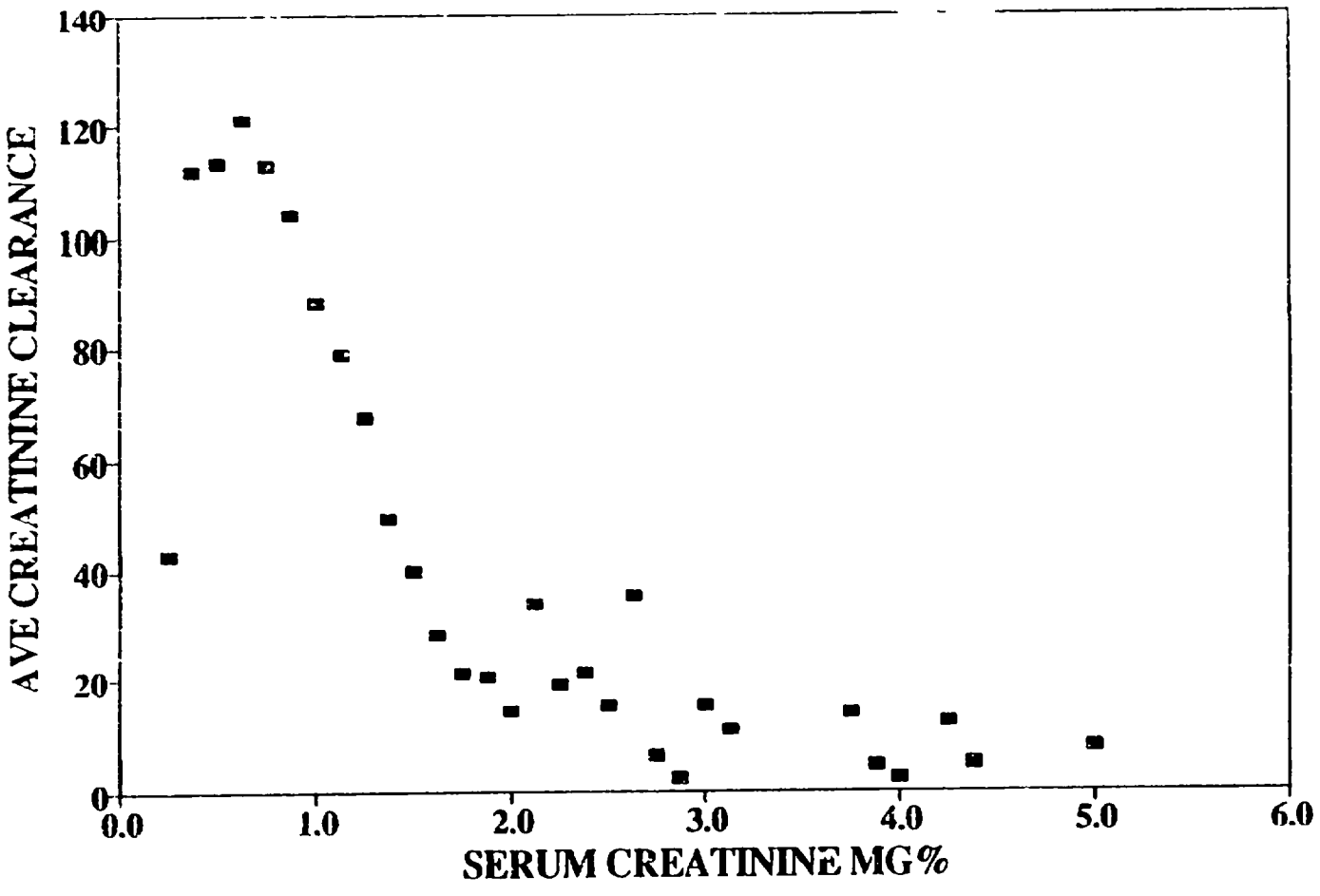

FIGURE 2. Mean creatinine clearance versus serum creatinine in burn patients. Each point represents mean creatinine clearance values for samples whose serum creatinine values fell within a particular 0.25 mg% range. Beginning at 0.25, the mean clearance values for each succeeding range, i.e., 0.25-0.49, etc., are plotted against the serum creatinine range value, i.e, 0.25, etc.

predictably with creatinine clearance over a relatively short range of 0.5 to 2. Thus, serum creatinine values would be ineffective in correcting for variations in excretion rates outside these ranges. In fact, creatinine clearance rates fall below 50 ml/min at serum creatinine values of about 1.5 mg%. Since the measurement of serum neopterin levels reflects mainly renal insufficiency below that point (fig 1), serum creatinine would only be useful as a correction factor over the range of 0.5 to 1.5 mg%.

The potential problems in data interpretation revealed by the pattern of neopterin and creatinine excretion at various creatinine clearance rates were prevented 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 should better protect sample integrity and data interpretation. Urine values of neopterin were reported per milligram percent creatinine to conform to literature convention and for ease of comparison to serum values. As a practical matter, there was no difference in the statistical analysis using simple concentration or creatinine normalized values.

Statistical Analysis. Preliminary analysis of the data from the patient samples was performed. Figure 3 is a depiction of the variation of neopterin concentrations in serum and urine as a function of time postburn. Urine neopterin values are normal immediately following burn injury and steadily increase to 4-5 times that of the levels found in controls by 8 weeks postburn. Serum values are at normal levels immediately following injury and increase 3- to 4-fold above control by 5 weeks postburn and return to more normal ranges by 8 weeks postburn.

Neopterin concentration was also analyzed for correlation with burn size. Figure 4 depicts mean serum and neopterin levels as a function of burn size. Neopterin values for patients falling within successive 10% increments in total body surface area burn size were averaged for each increment. There was no apparent correlation of neopterin levels in serum or urine with burn size.

A preliminary analysis of samples from patients diagnosed for pneumonia, bacteremia, and wound infection is depicted in Figure 5. There was a slight numerical increase in serum neopterin levels in bacteremic patients and a decrease in patients who had pneumonia and wound infections compared to patients who had no infections; however, with the number of patients currently studied, none of these changes were statistically significant. Increased levels of serum neopterin in bacteremic patients have been previously reported for a group of patients in an earlier study (6). Neopterin concentrations in urine samples, however, were sharply decreased for all three groups compared to patients who remained uninfected.

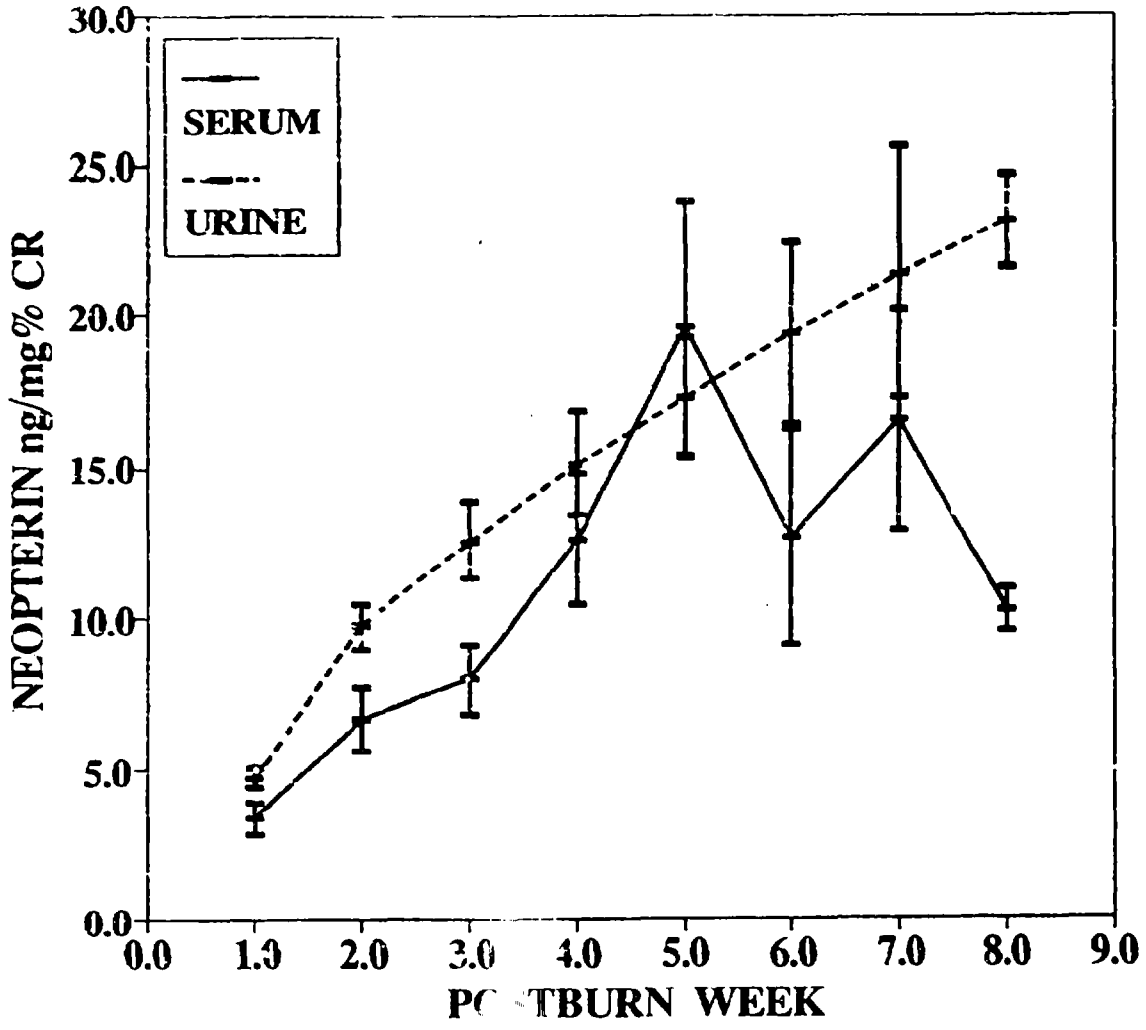

FIGURE 3. Mean serum and urine neopterin levels in burn patients versus postburn week. Mean neopterin values for all serum and urine samples taken during each week after injury are plotted against the postburn week. Neopterin values are expressed as neopterin per milligram percent creatinine. Error bars represent mean ± SEM.

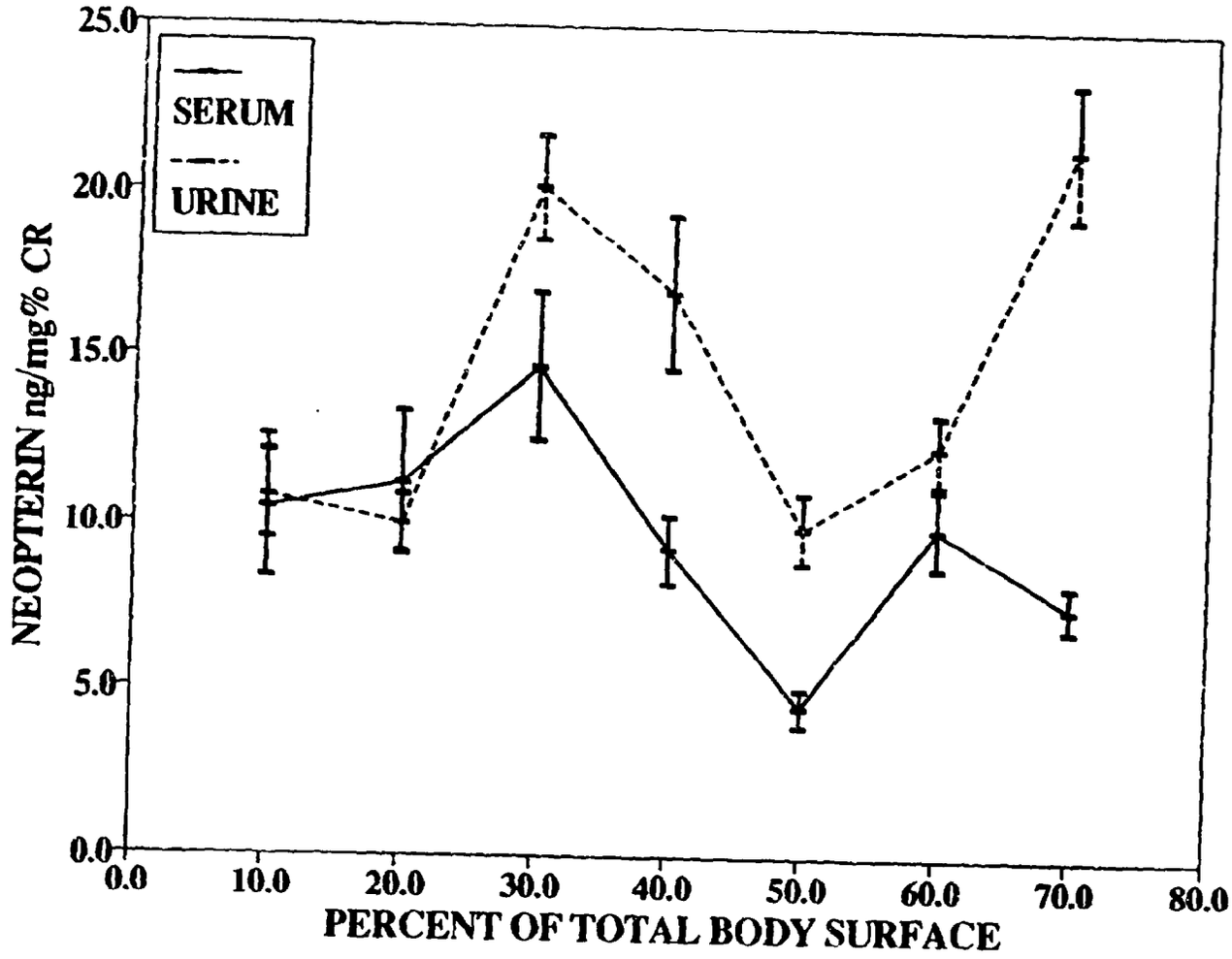

FIGURE 4. Mean serum and urine neopterin levels in burn patients versus total body surface area burn size. Mean neopterin values for all serum and urine patient samples within a particular range of burn sizes are plotted against the range of burn sizes, e.g., 10 = burn size range from 10-19%. Neopterin values are expressed as naograms neopterin per mg% creatinine. Error bars represent mean ± SEM.

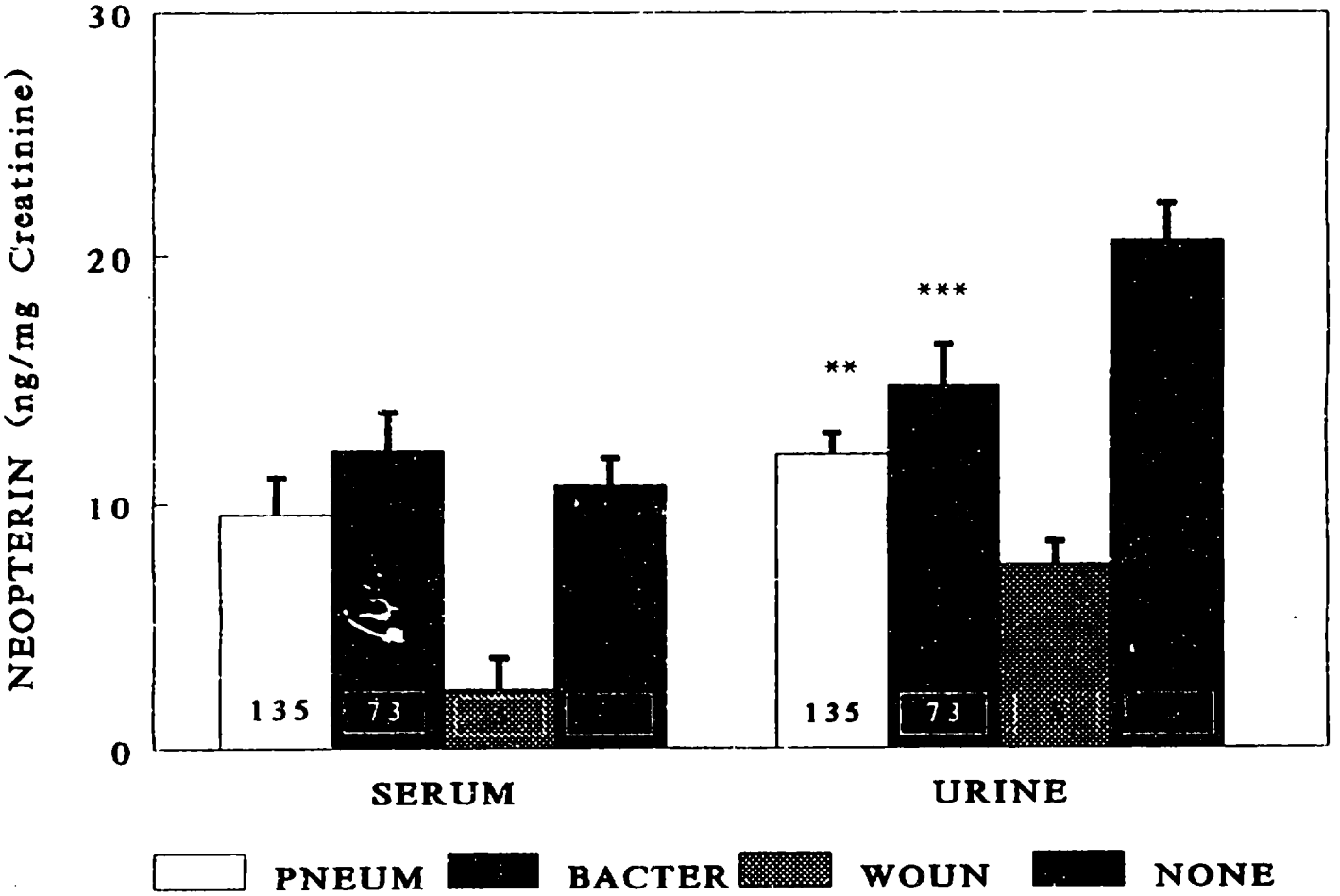

FIGURE 5. Mean serum neopterin concentrations for sera and urine from patients with various types of infection or no infection. The bars represent the mean values taken for all samples from patients experiencing a particular infection regardless of time of infection diagnosis. The upper bar represents mean ± SEM. ** = $P < 0.01$; *** = $P < 0.05$.

DISCUSSION

The utility of neopterin in the clinical diagnosis of infection depends on the specificity of the response and its clear association with episodes of infection. The specificity of neopterin is derived from its excretion from gamma interferon-activated macrophages during an immune response (19). This specificity can be compromised in serum by the complication of impaired renal function. A recent paper by Godai et al (20) demonstrated that serum neopterin was closely related to glomerular filtration rates in patients with chronic renal disease. Renal dysfunction is common in trauma patients. Several reports in the literature have related increased levels of serum neopterin to patients who are usually referred to as "septic" (11-13). Since serum creatinine levels, and not creatinine clearance, were used to screen data from the patients in these studies, it is possible that the presence of renal insufficiency may not have been adequately identified in those patient populations. In the current study, urine neopterin levels did not appear to be as sensitive to renal function as serum levels and urine may be better suited for evaluation of neopterin production in the burn patient.

Further analysis of the data and the addition of more patients may shed more light on the efficacy of urine or serum neopterin concentrations as an aid to infection diagnosis.

PRESENTATIONS/PUBLICATIONS

None.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION: DAOG1842	2. DATE OF SUMMARY: 01 Oct 91	REPORT CONTROL SYMBOL: DD-DR&E(AR) 636
3. DATE PREV SUM'RY: 01 Oct 90	4. KIND OF SUMMARY: D. Chg	5. SUMMARY SCTY: U	6. WORK SECURITY: U	7. REGRADING:	8. DISP'N INSTR'N: CX	9. LEVEL OF SUM A. WORK UNIT:

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS14	CJ	304
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)
(U) Role of Thyroid Hormones in Burn Pathophysiology

12. SUBJECT AREAS
0601 Biochemistry 0605 Medicine and Medical Research

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
7908	9909	DA	C. In-House

17. CONTRACT/GRANT — MILITARY RELEVANCY CERTIFIED — APPROVED BY David Pruitt

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

18. RESOURCES ESTIMATE

FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
91	1.5	99
92	1.5	104

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

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Burns (Injuries); (U) Thyroid Hormones; (U) Thyroxine; (U) Thyrotropin

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

23/24. (U) The objective of this work is to assess postburn alterations in thyroid function and develop treatment to improve survival in injured soldiers. Alterations in thyroid axis control will be characterized in a burned rat model.

25. (U) 9010 - 9109. Recovery and specificity of an immunoprecipitation method (IMPPT) to determine the amount of radioactive thyroid hormone (T_4 or T_3) tracer in a serum sample have been elucidated. IMPPT (logistic projection of precipitated tracer at infinite antibody from reaction at four concentrations of a monoclonal antibody) often recovers < 100% of the total tracer counts. For T_4, IMPPT analysis of samples spiked with various sources of tracer T_4 then treated (or not) with either of two anion exchange resins revealed that apparent recovery declined biexponentially, reaching 63% by 130 days after tracer production, and that the tracer recovered in a given IMPPT reaction represents at least about 99% of tracer T_4. Radioactive contaminants from catastrophic decay of some of the T_4 into moieties that both do and do not bind to serum proteins appear not to interfere. For T_3, recovery in samples with some tracer preparations declined to as low as 92%, though 100% recovery could be obtained by using new tracer T_3. Thus, IMPPT recovery for T_3 also appears to be complete. Specificity was assessed by including an heterologous tracer in the reaction. For T_4 IMPPT, either tracer T_3 or reverse T_3 (rT_3) crossreacted, but when present in the amounts e..pected after injection of tracer T_4 in kinetics studies would produce an expected error in the T_4 IMPPT results of < 0.5%. For T_3 IMPPT, no crossreactivity of tracer

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T_4 or T_3 could be detected when present in amounts approximately equal to that of tracer T_3. The recovery and practical specificity (both near 100%) of this method suggest that IMPT may represent a substantial improvement over other tracer detection methods and may thus provide a means to study T_4 and T_3 kinetics in burned rats and humans.

SUPPLEMENTAL WORK UNIT INFORMATION FOR THE PROJECT ENTITLED "ROLE OF THYROID HORMONES IN BURN PATHOPHYSIOLOGY"

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ABSTRACT

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PROJECT TITLE: ROLE OF THYROID HORMONES IN BURN PATHOPHYSIOLOGY: Immunoprecipitation (IMPT) Recovery and Specificity for Tracer Hormone Determination

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

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Reaction of aliquots of a serum sample containing tracer T_4 or T_3 with zero and three or four concentrations of homologous monoclonal antibody to T_4 or T_3 yields precipitated (bound) counts that vary with the amount of antibody present and with the sample type. However, in this IMPT procedure, the logistic projection of the maximally bound (Bmax) at infinite antibody concentrations did not vary with sample type, whether endogenous amounts of iodothyronine were normal, reduced, or removed. For T_4 and T_3, the logistic IMPT Bmax represented essentially complete recovery of the iodothyronine present. Crossreactivity of T_4 antibody with T_3 and rT_3 was not of a magnitude that would interfere with serum tracer T_4 determination in T_4 kinetic studies. No crossreaction of T_3 antibody with tracer T_4 or rT_3 was detected. This method is simpler than previously described techniques used for the same purpose (quantitation of tracer T_4 or T_3 after introduction of the respective compound into the circulation), and gives the advantage of virtually complete recovery.

ROLE OF THYROID HORMONES IN BURN PATHOPHYSIOLOGY: IMMUNOPRECIPITATION (IMPT) RECOVERY AND SPECIFICITY FOR TRACER HORMONE DETERMINATION

Kinetic parameters of thyroid hormone economy in burn injury have not been adequately determined. Single-pulse intravenous injection of tracer amounts of thyroxine (T_4), triiodothyronine (T_3), or reverse triiodothyronine (rT_3) labeled with radioactive iodine ordinarily allows the determination of some of the particular hormone's kinetic parameters, such as volume of distribution, plasma clearance rate, disposal and secretion (turnover) rate, and average resident time, all from the serum decay pattern of the injected iodothyronine (1-13). Further, such kinetics of any two iodothyronine hormones can be investigated simultaneously, if each is labeled with a different isotope (^{125}I or ^{131}I) and the labels in serum can be separated in a dual-channel gamma scintillation detector. Whether one tracer is injected alone or simultaneously with another one, if the only labeled iodothyronines targeted for determination in the circulation are the parent compounds that were injected, then the kinetic parameters derived are those of the injected tracers, without further elucidation of conversion rate parameters for the formation of specific daughter iodothyronines. In this situation, analysis can be performed adequately if the serum radioactivity present in iodothyronine moieties can be chemically isolated from radioactive iodide and iodoprotein formed from metabolism of the injected iodothyronine hormone, because it has been thought that other metabolites (less iodinated daughter iodothyronines) do not accumulate sufficiently to affect the results. This separation of iodothyronine from serum, where it is avidly bound by serum carrier proteins, and where labeled iodide and iodoprotein also accumulate, is usually accomplished by one of several general methods.

Trichloroacetic Acid (TCA)/Alcohol. TCA precipitation of protein-bound iodothyronine and of iodoprotein eliminates most of the iodide and allows alcohol extraction of iodothyronine (1-7). The alcohol is usually ethanol or acidified butanol. This method is problematic in that the TCA may precipitate up to 20% of the iodide, depending on the relative volume of TCA and whether more TCA is used to wash the precipitate. Further, use of more TCA may remove some of the iodothyronine from the precipitate. Thus, the extraction of the precipitate with alcohol (though removing iodothyronine very efficiently) may recover labeled iodothyronine poorly (a result sustained to minimize contamination with labeled iodide after more TCA), or may recover labeled iodothyronine well but include erroneous iodide radioactivity after less TCA (14). Many reports with this method have not specified the recovery or the error from iodide contamination, though one (1) claims 100% recovery of labeled T_3 without giving details of the methods and calculations for recovery, another (3) specifies a recovery of 90% for T_4 and 85% for T_3, and another (7) cites 90% recovery of T_3.

Though contamination by labeled iodoprotein is usually considered negligible with this method, labeled iodide contamination may be a factor, particularly with greater recovery and may confound interpretation of reported recovery. In contrast, the following methods are not associated with significant contamination by either iodide or iodoprotein radioactivity. Consequently, the most relevant factors are their labeled iodothyronine recovery, complexity, and usefulness for additional daughter iodothyronine separation or elimination.

Washed Matrix. Extraction of labeled iodothyronine onto a solid matrix allows iodide and iodoprotein to be washed free (8-11). Examples of recovery have been 55% for T_4 and 58% with a resin column (8), "virtually all" (? 100%) for T_4 with PD-10 columns but without details given (9), and 85% (10) and 79% (11) for rT_3 with C_{18} Sep-Pak™ cartridges. Extraction of serum over a Sephadex™ gel column in alkaline medium and buffer elution of iodide and iodoprotein allow subsequent use of whole normal serum to elute iodothyronines for 99% recovery of T_4 and T_3 (33, citation discussed again below).

Ethyl Acetate/Butanol (EAB) Extraction. Extraction of acidified samples with 9:1 ethyl acetate/butanol leaves the iodide and iodoprotein in the aqueous layer. Recoveries have been 57% for T_4, 55% for T_3 (12), 75% for T_4, and 68% for T_3 (13).

Gel Separation with Antibody Elution (GSAE). Application of serum to a Sephadex™ gel column in an alkaline (NaOH) medium allows the iodothyronines to dissociate from binding proteins and adhere to the gel, whereupon the iodide, iodoprotein, and binding proteins are washed away. A lower pH buffer containing specific antibody then elutes off the iodothyronine in question with a much lower volume than if the buffer does not contain antibody (14-20). Adequacy of recovery might be influenced by the amount of nonradioactive target iodothyronine present, since only one concentration of antibody can be used. Though one report (14) claimed 98% recovery of labeled T_3 with normal or elevated endogenous T_3, another (16) cited only 92% recovery of labeled T_3 from samples with normal total T_3, and another (17) cited only 50% to 90% for a number of iodothyronines including T_4, T_3, and rT_3. Others (15,18,19) did not mention recovery values. A perceived advantage of this method is that the use of an antibody adds some specificity to the recovered activity, diminishing the contamination of the obtained counts by metabolic daughter products of the injected material. The extent of effectiveness in this regard is not entirely clear. Theoretically, it may contribute some if the target iodothyronine for measurement is the originally injected one (since daughter product label accumulates to lesser levels in serum in a kinetic study) and if antibody crossreactivities with daughters are low. However, if the target labeled compound is a daughter iodothyronine in the presence of the much larger amounts of the labeled parent compound, this method

would become even more questionable and has not been demonstrated to be of use. The higher the (one) concentration of antibody that is used, the better the recovery but the more nonspecific is the method (20).

IMPT. Reaction of a serum sample with specific antibody is said to recover 70% to 85% of T_3 or rT_3 (21) or 90% of T_3 (7). Since only one concentration of antibody was used, there was a great risk of influence on recovery by the amount of endogenous target iodothyronine present (20), though this was not usually explored.

Another report (22) utilized this approach in combination with a complicated preliminary EAB extraction (and concentration) and further resin column extraction of label from urine. Daughter T_3 label together with injected labeled parent T_4 appears in urine with the T_3 enriched relative to T_4, compared to the very small amounts of T_3 relative to parent T_4 simultaneously present in plasma. T_3 antibody was used to immunoprecipitate the labeled T_3 from extracted and concentrated total urinary iodothyronine, with an unreported IMPT recovery for T_3 and an overall (extraction + IMPT) recovery of only 18%. Again, the single dose of antibody exposes the result to variation of IMPT recovery based on the amount of endogenous T_3 present. With the advantage in urine of the relatively large amount of T_3 label in proportion to the (though still somewhat larger) amount of T_4 parent label, it was implied (but not shown) that no labeled T_4 was determined as T_3. Further, it is possible that labeled T_3 and/or rT_3 daughter products were not adequately excluded from the labeled T_3 measurement. These products are more concentrated (relative to parent compounds) in urine than in plasma. The relevance or utility of the complicated extraction/concentration procedure prior to IMPT, particularly if use of serum samples is contemplated, is not clear. The extremely poor recovery is likely due to this preparatory phase and might mitigate the advantage of concentration from larger sample volumes.

Another IMPT procedure (23) was used to analyze normal human serum samples spiked with tracers of various iodothyronines and their products, but this was also complex and potentially problematic. After gel column adsorption of iodide, the eluate was treated sequentially with one dose of single antibody to rT_3 and then to T_3 to remove rT_3, then T_3, as precipitates, and the supernatant was cycled three times on a resin column to absorb T_4 for subsequent elution of iodoprotein. The recoveries were reported as 98% for T_4, 95% for T_3, and 88% for rT_3. However, the T_4 result was contaminated by 1.8% (iodide), 9.1% (rT_3), 3.8% (T_3), and 3.1% (iodoprotein) of the other elements; and potential daughters of T_4 (T_3 and rT_3) were contaminated by 1.2% and 2.7% (respectively) of the T_4 present. It was claimed that the latter contamination (which might represent as many or more counts than those in the daughters after tracer T_4 injection in a kinetic

study) could be corrected out by use of the separately determined respective nonspecific binding ratios. Though such a procedure might be used to study the kinetics of injected tracer T_4, T_3 or rT_3, its utility for studying the appearance of daughters from injected T_4 label is highly questionable. The use of only one concentration of antibody adds further uncertainty to recoveries and specificities among samples, despite the specified addition of nonradioactive extra T_4.

Extraction Followed by Chromatography (EC). After resin column extraction of serum (24), or after ethyl acetate extraction and concentration of iodothyronines from urine with further resin column extraction (25), paper chromatography of the eluate has been used to separate iodothyronines, usually T_3 from T_4. In the former case (24), recoveries are given: 46% to 63% for T_4 and 17% to 32% for T_3. Resin extraction of serum followed by thin layer chromatography (26) was said to recover 91% of T_4 and 94% of T_3. However, 94% of the rT_3, 5.6% of "other intermediates", and 3% of the iodide appeared as T_4; and 83% of the "other intermediates" appeared as T_3. HPLC of the acid-ethanol supernatant of plasma extraction has been used to separate T_3 from T_4, though recoveries that included extraction were not given (27). Further, separation of rT_3 from T_4 and T_3 was less than adequate and the behavior of other daughter products was not described.

Usually, before HPLC isolation of an analyte, more extensive treatment of the serum is required to remove substances which interfere with column function and final detection of analyte. Accordingly, after gel column extraction, elution with an albumin solution, further extraction by Sep-Pak™ C_{18} columns or by EAB, final concentration by evaporation, and preparation in HPLC solvent, the material from serum was separated by HPLC (28). Complete separation among T_4, T_3, and rT_3 was achieved, and two principal representatives of further daughter products (T_2's) eluted together but well separated from the above-mentioned iodothyronines. Recoveries of T_4 and T_3 were in the 85 to 90% range. It appears that among all the methods discussed so far, extensive preliminary preparation of the samples and HPLC analysis are required for adequate separation of T_4 and daughter iodothyronines. While such separation of T_4 from its daughters might not be necessary to study the disappearance kinetics of T_4, T_3, or rT_3, it is necessary for the study of the appearance of daughters from an injected parent tracer (such as in the isotopic determination of the peripheral conversion of T_4 to T_3).

Sephadex™ Fractionation. It has been found that Sephadex™ G-25 columns in an alkaline medium very efficiently separate iodothyronines from iodides and iodoprotein in serum, and can be used at the same time to separate the iodothyronines. Because of the lack of prior extraction of the samples and from the results of rechromatography studies, it appears that these column procedures yield 98 to 100% recovery for iodothyronines such as T_4 and T_3.

Sephadex™ G-25 super-fine columns have been used to separate the daughter iodothyronines. When relatively small (1 X 20 cm) columns were used with relatively short total elution times (4-5 h), T_4 was separated from T_3 and its daughters, but not T_3 from its daughters; and though rT_3 was separated from its daughters, the separation of rT_3 from T_4 (and/or T_3) was not clear (29-31). Use of larger columns (2.5 X 35 cm) and longer total elution times (13 h) allowed probably adequate separation of daughters of T_3 and rT_3 (so-called "pre-T_3") from T_3, separation of rT_3 from T_3 and pre-T_3, but not rT_3 from T_4 (32). With use of even larger columns (1.5 X 115 cm) running for what appears to be about 42 h, better separation of pre-T_3 from T_3 was achieved, and rT_3 appears to have been separated from T_4 (33,34). Besides column volumes and total elution times, other factors such as slight variations in sample additives and in elution buffers were also different among the various reports cited above. Even for shorter elution times, such procedures are made difficult by the needs for column preparations and for the generation, handling, counting, and analysis of many fractions, all for the assessment of one given sample. Furthermore, because the activities of interest are partitioned into many fractions, large (up to 5 ml) original samples are required for relevant fractions to have sufficient activity.

Our goal was to improve IMPT as a means of determining the amount of tracer T_4 or T_3 in serum samples, initially without assessment of the ability to separate daughters of T_3 and rT_3 from T_3. Such a procedure would allow kinetic studies of T_4 and T_3 and should represent an improvement over the first five procedures outlined above.

MATERIALS AND METHODS

T_4, T_3, and rT_3, each mono-labeled on the phenolic ring with ^{125}I, were purchased from DuPont/New England Nuclear (Wilmington, DE). Respective specific activities were 150, 3300, and 1200 Ci/g, and initial radiochemical purities were > 96%, > 98%, and > 99% as determined by the manufacturer on HPLC. Monoclonal anti-T_4 antibody (MAB086-156/7), monoclonal anti-T_3 antibody (MAB097/1), goat anti-mouse gammaglobulin (AB26), normal mouse serum (S25), rabbit polyclonal anti-rT_3 antibody (AB113), normal rabbit serum, (S20), and normal human charcoal-thyronine-stripped serum (CTSS) were obtained from Chemicon International, Inc. (Temecula, CA). Goat anti-rabbit gammaglobulin (R0881) was purchased from Sigma Chemical Company (St. Louis, MO). Iobeads resin (T15-0150) was obtained from Technicon Instruments Corporation (Tarrytown, NY). Dowex™ 2-X8 anion exchange resin, 20-50 mesh, sphericity > 85%, Cl^- ionic form, was obtained from the Baker Chemical Company (Phillipsburg, NJ).

Tracer quantities of labels were added to serum samples from different sources (charcoal-thyronine-stripped serum, CTSS; normal human serum, NHS; burned human serum; normal rat serum; and burned

rat serum). Previous studies in this laboratory had indicated that the procedure and conditions outlined in Table 1 resulted in near-optimal binding and precipitation of tracer by homologous antibody. This binding was detected by precipitation of bound tracer by a second antibody system targeting the antibody to iodothyronine along with larger amounts of target carrier gammaglobulin. The reactions were carried out in 12 X 75-mm glass tubes. The bound radioactivity was counted in a gamma scintillation counter to 2% error or better, except for those tubes containing small amounts of radioactivity approaching that seen in tubes containing no antibody, which were counted with greater error. All precipitated cpm were corrected for background cpm. These procedures focused on tracer T_4 and T_3 IMPT with respective homologous monoclonal antibodies. However, because rT_3 tracer was also used in cross-reactivity studies, rT_3 IMPT with a polyclonal rT_3 antibody was also used to estimate the amount of rT_3 present in those studies. For the rT_3 IMPT, the procedure was the same as in Table 1, except that the first antibody was rT_3 antibody (polyclonal from rabbit), normal rabbit serum (2%) was substituted for the normal mouse serum, and goat anti-rabbit gammaglobulin (1:5 dilution) was substituted for the goat anti-mouse gammaglobulin.

TABLE 1. IMPT to Determine Tracer Hormone Radioactivity ($*T_4$ or $*T_3$) in Serum

$*T_4$- or $*T_3$-spiked serum sample	0.05 ml
0.125% 8-analino-1-naphthalenesulfonic acid	0.025 ml in buffer 1
First antibody (monoclonal anti-T_4 or -T_3) Incubate 1 h at 37°C	0.05 ml in buffer 1
2% normal mouse serum	0.05 ml in buffer 2
1:7.5 goat anti-mouse gamma globulin	0.05 ml in buffer 1
6% polyethylene glycol 8000 Incubate 15 min at room temperature	0.4 ml in buffer 1
6% polyethylene glycol 8000 Centrifuge 2000 g, 4°C, 17 min Decant Count precipitate (bound) radioactivity	0.3 ml in buffer 1

Buffer 1 = 0.075 M barbiturate, 0.1% gelatin, 0.05% NaN_3, pH 7.85.
Buffer 2 = 0.1 M EDTA, 0.05% NaN_3, pH 7.85 mixed 1:1 with buffer 1.

Reactions were carried out on different aliquots of tracer-spiked serum sample, such that for some replicates, buffer 1 was used instead of antibody (zero antibody) and for other replicates different dilutions of antibody (in buffer 1) were used. The antibody dilutions were expressed as the dilution that was added to the reaction mixture, i.e., not accounting for the additional dilution provided by the IMPT reaction mixture itself. Duplicate aliquots were usually taken for each antibody concentration or dose (dilution), unless otherwise noted. Zero antibody plus three or four antibody doses (at serial twofold dilution) were usually employed, unless otherwise noted. Finally, aliquots of a sample were counted without any IMPT reaction to determine the amount of tracer added to the IMPT tubes (total counts or radioactivity). This latter quantity was usually used as a denominator to normalize the bound counts as percent bound.

The bound label (dependent variable) as counts per minute (cpm) or as percent bound (PB) was related to the independent variable (dose of antibody, as AQI) by logistic regression to provide the logistic parameters A, B, C, and D in the formula shown in Figure 1. Any such regression included the zero-antibody tubes. If the bound label was expressed as PB, then logistic parameter B was also termed "PBmax" and represented the apparent recovery achieved by the procedure: the percent of total tracer counts that would be bound at infinite homologous antibody concentration, assuming no interference from deficiency of second antibody at very high doses of first antibody. Figure 1 shows that in the T_4 IMPT system, T_4 antibody dilutions stronger than 1:100 provided T_4 antibody in excess of the amount of mouse gammaglobulin (including carrier) that can be precipitated by the constant amount of second antibody directed against the gammaglobulin. The amount of normal mouse serum present (providing the carrier gammaglobulin for the T_4 antibody) is sufficient to prevent the fall-off in binding from occurring at T_4 antibody doses at or below that represented by the 1:100 dilution. It is also seen that three antibody doses (plus the zero-antibody dose) are sufficient to determine the maximal binding as well as the other logistic parameters. For subsequent tests in any IMPT system, antibody doses were chosen such that they were all well below that which would cause a relative insufficiency of second antibody and such that at least two doses were above the ED_{50} even for serum samples that contained normal amounts of nonradioactive hormones. Logistic regressions were performed using the PAR program of the BMDP software package on the VAX computer. All r^2 were above 0.99.

If the dependent variable was expressed as cpm, then the logistic parameter B was termed "Bmax" and represented the amount of immunoactive tracer present in the sample, the quantity that would be precipitated by an infinite amount of homologous antibody, assuming that no relative deficiency of second antibody would occur in that condition.

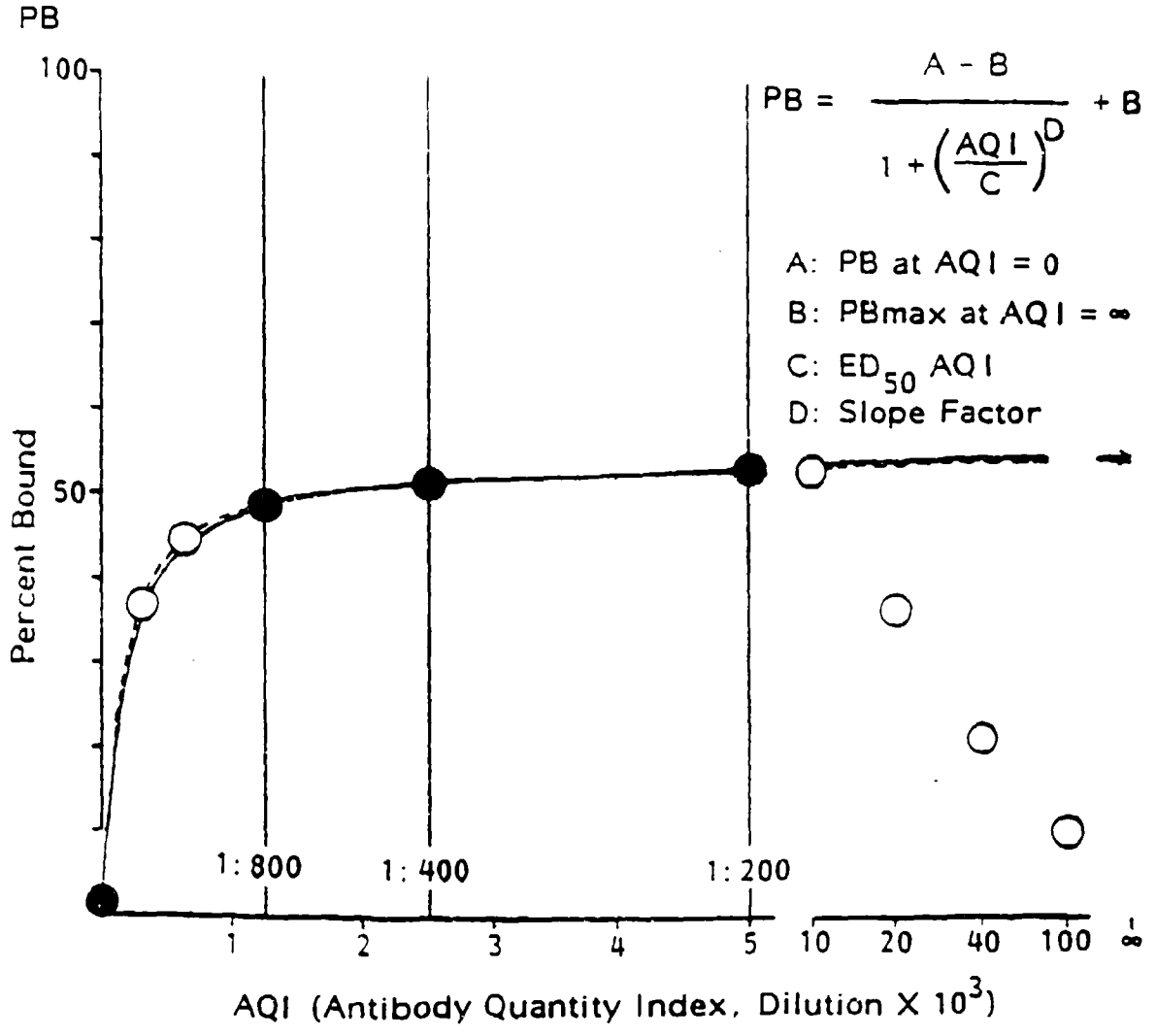

FIGURE 1. T_4 antibody and T_4 tracer - binding profile. Observed binding (% total cpm) for tracer in charcoal-thyronine-stripped serum; nonspecific binding (nonzero PB at zero AQI) not subtracted; logistic fit through data of only the closed circles (continuous line) or of all symbols for AQI 0-10 (broken line). Logistic parameter B, also denoted as "PBmax" (% of total cpm), is the apparent recovery (%) for the test. If the ordinate axis data are expressed as raw bound cpm (not shown), the maximal binding (parameter B or "Bmax") then would represent the quantity of tracer recovered as cpm. Logistic parameter C represents the dose of antibody producing half-maximal effect (ED_{50}) on binding.

Whether the dependent variable (precipitated or bound tracer) is expressed as PB or cpm, the data might be corrected by subtracting an estimate of the nonspecific binding (NSB). One such estimate (NSB_0) is obtained from the cpm in the absence of homologous (first) antibody (zero-antibody tubes). However, for the amount of NSB in the tubes with first antibody present, there is no direct estimate. But, since the NSB comes from the cpm not bound to antibody, a proportion of such cpm (assumed to be equal to the NSB_0 ratio, the NSB_0 cpm over the total cpm) not bound to antibody joins the precipitated bound cpm by such mechanisms as the inability to remove the last traces of supernatant liquid when it is decanted. Thus, similar terms can be used to describe both the overall relationship after decanting (total cpm = specifically bound cpm + NSB cpm + free cpm, where free cpm are those successfully decanted) as well as the NSB_0 ratio [NSB_0 cpm/total cpm = NSB cpm/(NSB cpm + free cpm)]. The latter can thus be expressed as NSB cpm in proportion to those cpm contributing to NSB in tubes containing first antibody. (With more antibody, those cpm contributing to NSB are fewer.) Through algebraic interrelationship between the two above equations, the precipitated cpm in all tubes can be corrected to those cpm specifically bound = (precipitated cpm - NSB_0 cpm)/[1 - (NSB_0 cpm/total cpm)].

It can be seen that with no antibody, the correction involves subtracting the entire NSB_0 cpm, resulting in zero activity. With greater amounts of antibody present, the precipitated cpm are corrected by, in effect, removal of fewer cpm. Thus, if the precipitated cpm approaches the total cpm, the corrected (specifically bound) cpm approaches the precipitated cpm with no effective correction. Correction for NSB in this manner is thus proportional to the cpm not specifically bound and is herein termed proportional correction for NSB. Since these IMPT procedures were designed to include tubes with high antibody binding (and almost no relevant applicable NSB correction) approaching the total homologous tracer hormonal immunoreactive cpm present, which usually represented most of the total cpm present, the projected (regressed) maximally bound cpm is expected to be essentially the same whether precipitate cpm data were proportionally corrected for NSB or uncorrected. This was observed to be the case, with differences in projected maximal binding (correction versus no correction) usually < 1% and less than the variation of the overall method. However, in some studies, e.g., those involving intentional inclusion of relatively large amounts of heterologous (potentially nonantibody-binding) tracer, proportional correction for NSB was employed.

It is to be noted that whether or not the above NSB correction is employed, PB is the bound cpm (corrected or uncorrected) divided by the total cpm present. Thus, if nonimmunoreactive counts are present, the PBmax (apparent recovery) is < 100%. This, however, might represent an actual recovery of 100% if the unreactive counts do not represent target hormone ligand.

RESULTS

T_4 IMPT System. Figure 1 shows that T_4 antibody dilutions could be identified that allowed the observed PB to approach the PBmax, yet did not overbalance the amount of second antibody present. Because the sample used (CTSS) contains little or no nonradioactive iodothyronines, even the lesser antibody doses gave binding near the maximal. The PB max (apparent recovery) was about 50%, indicating that the T_4 tracer source added to the sample contained a large amount of nonimmunoactive label, presumably not T_4. This provides the initial indication that the activity projected as parameter B, i.e., PBmax in Figure 1, may give the total amount of T_4 present, since many nonzero antibody doses in the range below excess (relative to second antibody) gave PB values very near to and steadily increasing toward the PBmax and that this maximal binding parameter may provide specificity against misreading counts not in T_4, such as those that might accumulate as radiochemical purity of the label deteriorates due to catastrophic decay of the tracer during the time elapsed between manufacture and use of the tracer preparation.

Figure 2 shows the results of similar T_4 IMPT runs on 5 samples of different type, each spiked with T_4 tracer from the same preparation, which tracer preparation was different from the one used in Figure 1. Among the serum samples assessed, previous observations indicated that endogenous nontracer iodothyronine concentrations are highest in NHS, very low in CTSS, and intermediate in the other samples. Not surprisingly, the IMPT reaction was markedly influenced in a pattern compatible with that expected from variations of endogenous T_4, with wide variation in ED_{50} which was highest with NHS and lowest with CTSS. There was also wide variation in the slope term which appeared less characteristic of serum type. Nevertheless, despite these profound effects of sample type with varying endogenous T_4 concentration on parameters reflecting different antibody binding at individual antibody doses from sample to sample, the maximal binding parameter projected a nearly constant estimate of the amount of T_4 label. In this case, it represented approximately 80% of the radioactivity present.

In Table 2, with use of a different tracer preparation which was the same for the various samples studied, again there was marked variation in ED_{50} and the slope factor. However, the projected PBmax represented approximately 92% of the radioactivity present as T_4, with no consistent variation by sample type.

Table 3 allows assessment of multiple samples within sample type containing tracer from yet another preparation, the same for all the samples. Though the apparent recovery of tracer T_4 as 85% of the total radioactivity did not vary with sample type (ANOVA), the ED_{50} and slope factor did.

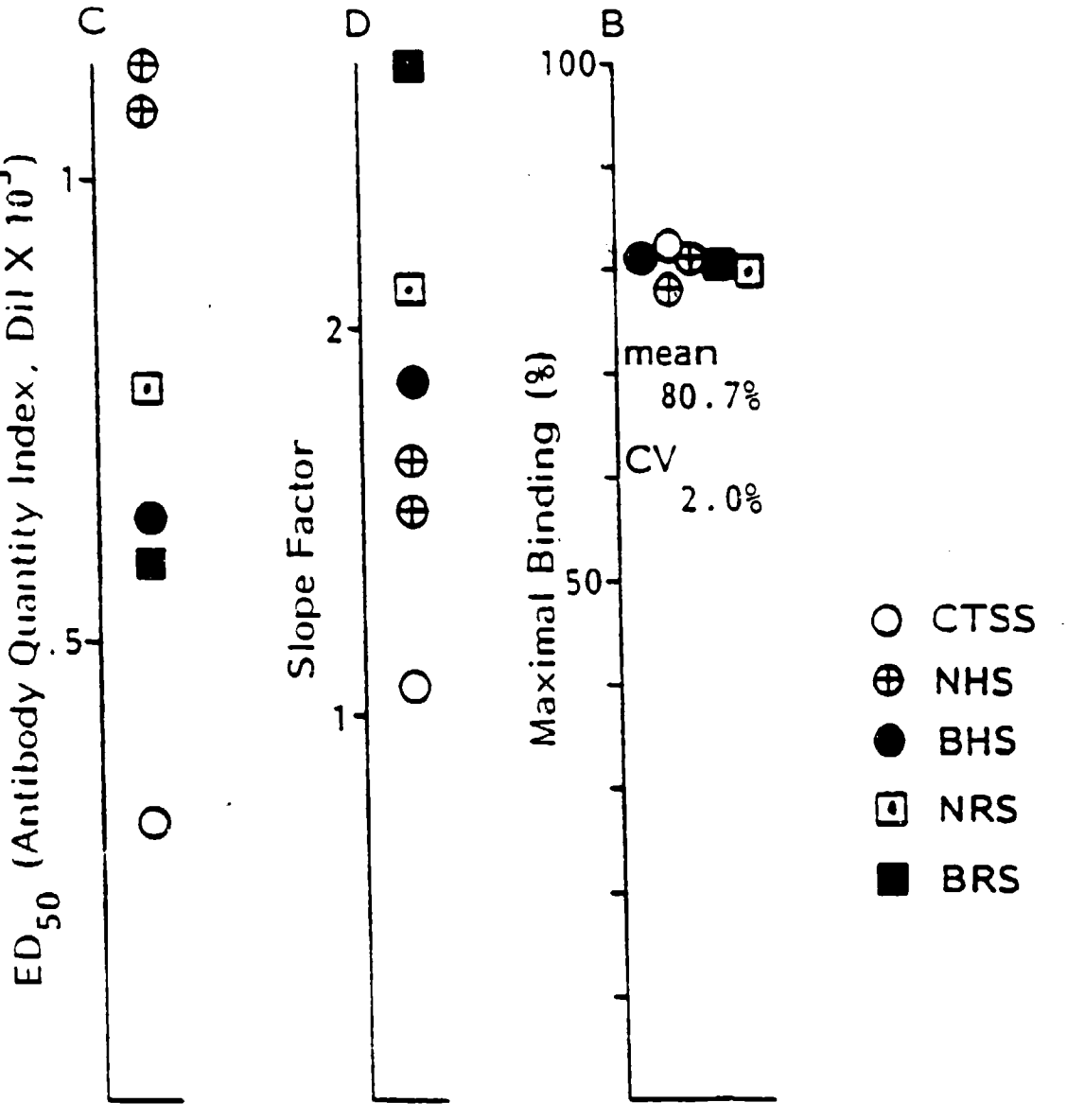

FIGURE 2. T_4 antibody - logistic parameters for samples with T_4 tracer from the same source. C, D, and B atop the ordinate axes are logistic parameters from equations as in Figure 1, with data corrected for nonspecific binding. Antibody dilutions were 0, 1:800, 1:400, and 1:200. CV indicates coefficient of variation; CTSS, charcoal-thyronine-stripped serum; NHS, normal human serum (one sample from one individual, the other from a pool of 12); BHS, burned human serum (pooled from a large number of patients); NRS, normal rat serum (pooled); and BRS, burned rat serum (pooled).

TABLE 2. T_4 Antibody – Logistic Parameters for Samples with T_4 Tracer from One Preparation

Sample	C ED_{50} (AQI)	D Slope Factor	B* PBmax (%)
CTSS	0.22	1.0	93
NHS1	0.89	1.8	91
NHS2	0.51	1.4	91
NHS3	0.63	1.1	95
NHS4	0.84	1.4	93
BHS	0.53	2.0	91
NRS	0.60	2.9	90

*PBmax means are 92.5% (NHS) and 91.9% (overall); coefficient of variation = 2% overall. Sample and column heading terms follow the scheme in figures 1 and 2 (data not corrected for nonspecific binding). Antibody dilutions were 0, 1:800, 1:400, and 1:200. AQI indicates antibody quantity index; CTSS, charcoal-thyronine-stripped serum; NHS, normal human serum from four individuals; BHS, burned human serum from a large pool; and NRS, normal rat serum from one rat.

TABLE 3. T_4 Antibody – Logistic Parameters for Samples with T_4 Tracer from Another Preparation (Mean ± SE)

Sample	n	C ED_{50} (AQI)	D Slope Factor	B* PBmax (%)
CTSS	7	0.347 ± 0.061	1.38 ± 0.32	85.9 ± 1.9
NHS	7	0.990 ± 0.077	1.51 ± 0.11	85.4 ± 2.4
BHS	7	0.573 ± 0.066	2.27 ± 0.37	84.9 ± 2.2

*PBmax overall mean is 85.4% with coefficient of variation = 6% overall. Sample and column heading terms follow the scheme in figures 1 and 2 (data not corrected for nonspecific binding). Antibody dilutions were 0, 1:800, 1:400, and 1:200. AQI indicates antibody quantity index; CTSS, charcoal-thyronine-stripped serum; NHS, normal human serum; and BHS, burned human serum from separate individuals.

Figure 3 assesses CTSS containing a tracer preparation whose labeled T_4 represents about 64% of the total radioactivity according to the IMPT results of untreated tracer-spiked CTSS (left-hand end of the abscissa). Prior exposure of the spiked CTSS with different amounts of resin beads (milligrams dry resin per milliliter CTSS, 20 min at room temperature with vortexing every 5 min) allowed subsequent IMPT to recover a greater proportion of total radioactivity as immunoreactive T_4. This effect was brought about not by raising the precipitated counts (which were affected very little) but by removing portions of the total cpm which were not immunoactive. Since resin at 65 mg/ml appeared to provide a near maximal effect in this regard, this resin concentration was used in subsequent trials. Since the Dowex™ contains moisture, in some cases, it was allowed to dry overnight by air exposure at room temperature before use. This did not appear to alter its effect.

Because we suspected that the variation in apparent recovery of tracer T_4 by IMPT might be explained by the time elapsed before tracer use, we plotted the PBmax from CTSS samples that were spiked and analyzed at various times after the preparation (at the commercial source) of the tracer for several tracer preparations. In most cases, after arrival here, the tracer was placed in stock CTSS for storage and frozen in aliquots for subsequent spiking of different CTSS or other samples for use. In some cases, storage of the tracer in the originally received form (aqueous propanol) yielded the same IMPT result as that stored in CTSS. Figure 4 shows that the apparent recovery (PBmax) falls off with tracer age, indicating accumulating radiochemical impurity presumably from catastrophic decay of the tracer. Labeled iodide might likely have been a product of this process (note the marked improvement in recovery after treatment with anion exchange resins). However, simple deiodination of the T_4 tracer to T_3 or rT_3 is unlikely in that immunoactive T_3 and rT_3 cpm were not detected in such samples using IMPT with antibodies for those compounds (data not shown). As shown in Figure 4, in those samples with apparent recovery reduced to below 70%, resin treatment did not restore recovery completely to 100%. With time, some of the decay products (not removed by resin) appear to be compound(s) with an attraction to serum proteins that is relatively high compared to an attraction to resin. Nevertheless, the patterns of recovery with and without prior resin treatment both project back to 100%, indicating that any label not included in the logistic projection of maximal binding (not detected as immunoactive) is not T_4 but represents other nonspecific material. This indicates that the actual logistic IMPT recovery is approximately 100% and suggests some degree of specificity for the logistic IMPT Bmax in detecting tracer T_4 itself. Further studies of T_4 IMPT employed T_4 tracer with > 80% of the total radioactivity as immunoactive T_4, without resin treatment of the spiked samples.

Specificity of the procedure for T_4 label (vs T_3 and rT_3 labels) was tested by making T_4 IMPT runs, i.e., all with T_4 antibody,

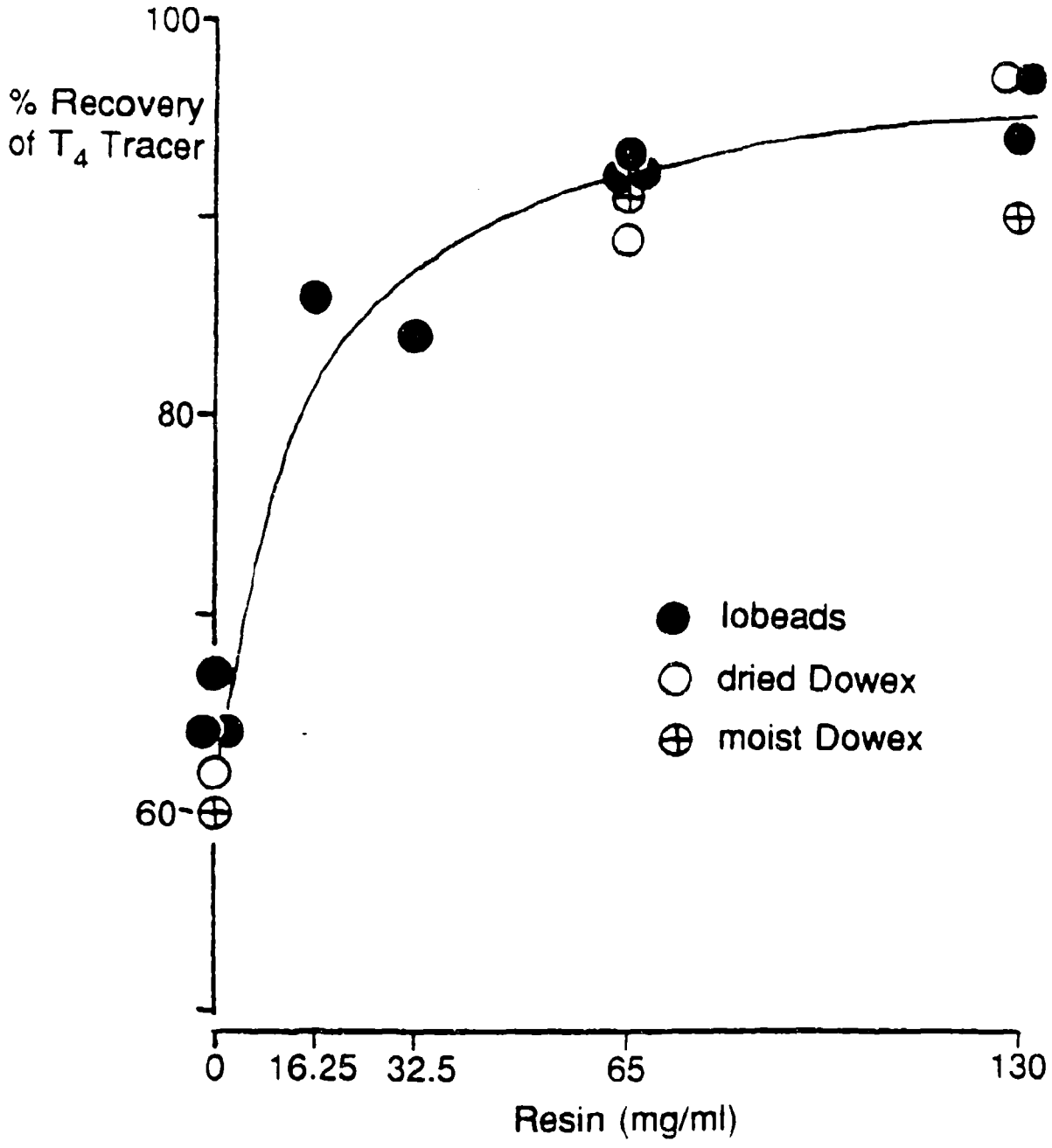

FIGURE 3. Apparent recovery (as PBmax, logistic parameter B) by T_4 antibody (dilutions usually 0, 1:1600, 1:800, 1:400, 1:200) of T_4 tracer in charcoal-thyronine-stripped serum (CTSS). The tracer-spiked samples were treated with various amounts of anion exchange resin (milligrams per milliliter CTSS) prior to IMPT. Moisture was not included in the resin weight. Data were proportionately corrected for nonspecific binding.

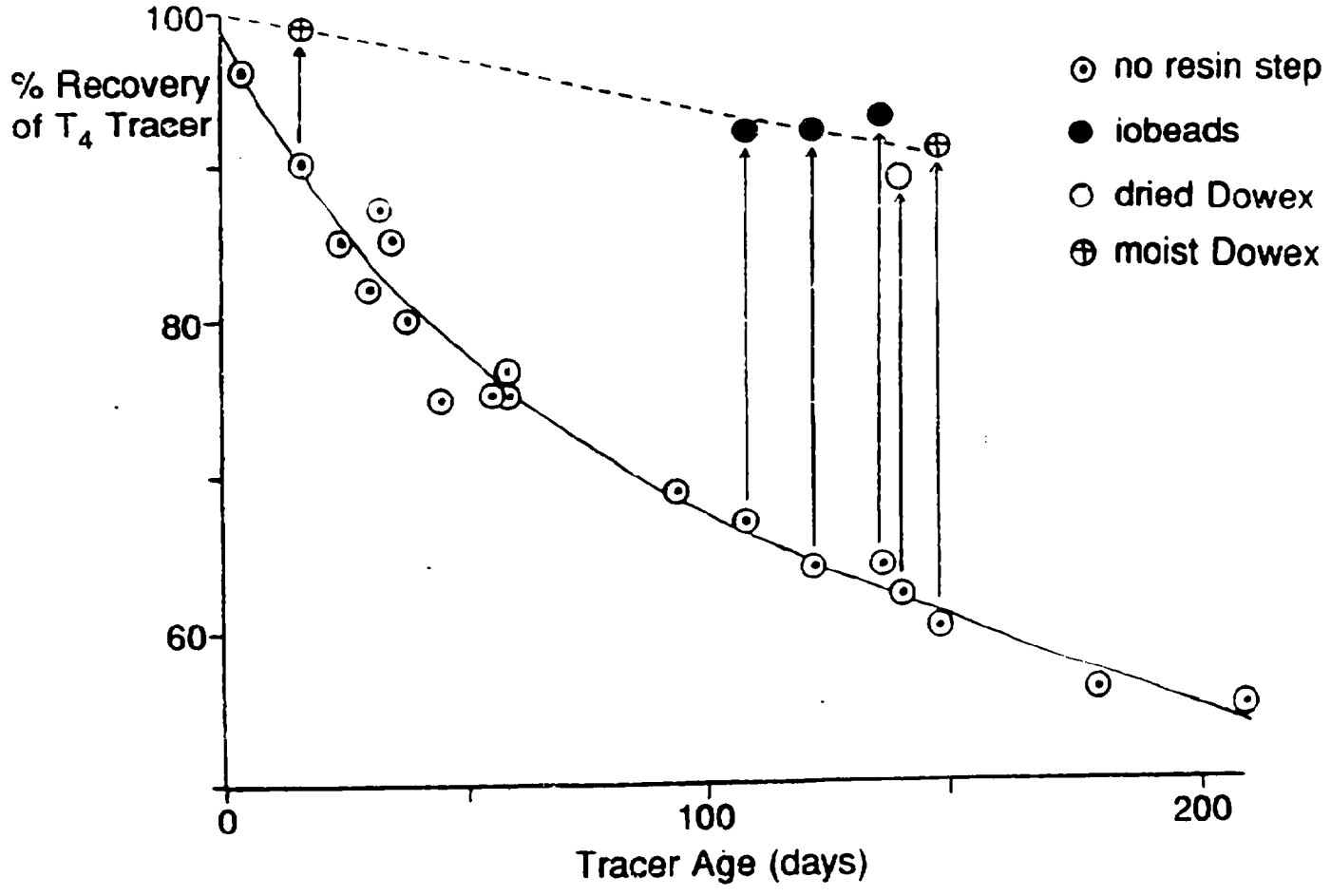

FIGURE 4. Apparent T_4 tracer recovery as a function of tracer age and resin treatment. Apparent recovery (as PBmax, logistic parameter B) by T_4 antibody (dilutions usually 0, 1:1600, 1:800, 1:400, 1:200) of T_4 tracer in charcoal-thyronine-stripped serum (CTSS), according to time since factory formulation of tracer and treatment of tracer-spiked samples with anion exchange resin (65 mg/ml CTSS). Moisture was not included in the resin weight. Data were proportionately corrected for nonspecific binding.

separately with radioactive T_4, T_3, and rT_3 tracers. In Figure 5, the ordinate data (the cpm bound and precipitated by T_4 antibody) are expressed as a percent of the total cpm present (as noted in the figure) for a specific tracer in the absence of the other tracers. This total was determined as the maximally bound cpm (Bmax) in different IMPT runs with the appropriate antibody.

It appeared that the T_4 antibody does bind nonspecifically small proportions of labeled T_3 and rT_3 that might be present. It was therefore necessary to determine whether the presence of heterologous tracer T_3 or rT_3 along with the homologous tracer T_4 would alter the logistic IMPT Bmax in the procedure with T_4 antibody, falsely elevating the estimation of the amount of labeled T_4 present. Table 4 shows the results in four samples, each divided into three aliquots (containing labeled T_4 alone or together with labeled T_3 or rT_3) analyzed with separate T_4 IMPT trials. In samples with more nonradioactive thyronine than is found in plain CTSS, up to 29% of the tracer T_3 and up to 43% of the tracer rT_3 was included in the estimate of the amount of tracer T_4 present. However, if these heterologous labels are each present in a sample as contaminants in an amount that is at or below 1% of the amount of T_4 label present, then the IMPT result would represent a combined error of < 1%, i.e., less than the coefficient of variation of the method, in the estimate of the amount of labeled T_4 present.

T_3 IMPT System. Figure 6 shows that zero antibody and three T_3 antibody concentrations can be used for logistical projection of the amount of radioactivity present as T_3 by determination of Bmax. In this example, the apparent recovery is approximately 100% of the total radioactivity present. Note also that the useful T_3 antibody dilutions represent doses low enough that the second antibody system is not overwhelmed even by T_3 antibody doses far in excess of those needed.

Figure 7 shows the logistic parameters for a series of T_3 IMPT trials on various samples. Despite marked variation of ED_{50} according to sample type (similar to that seen in T_4 IMPT trials) and marked variation in the slope factor, the apparent recovery of T_3 as the PBmax was not different among sample types (CTSS compared with NHS or with all non-CTSS samples by t test). For this T_3 tracer preparation (different from the one related to Figure 6), the overall mean PBmax was 95.5%.

Because of the high apparent recovery for most preparations of T_3 tracer, the effect of removing nonimmunoreactive material with resin was not investigated extensively. One sample with a PBmax of 98% had a PBmax of 100% after resin treatment. In a subsequent study (see fig 10), the mean PBmax was 97.4% of the total cpm in the tracer T_3 source. All but one preparation of T_3 tracer maintained a PBmax of over 94% for several months. The radiochemical purity reported by the commercial source is > 98%

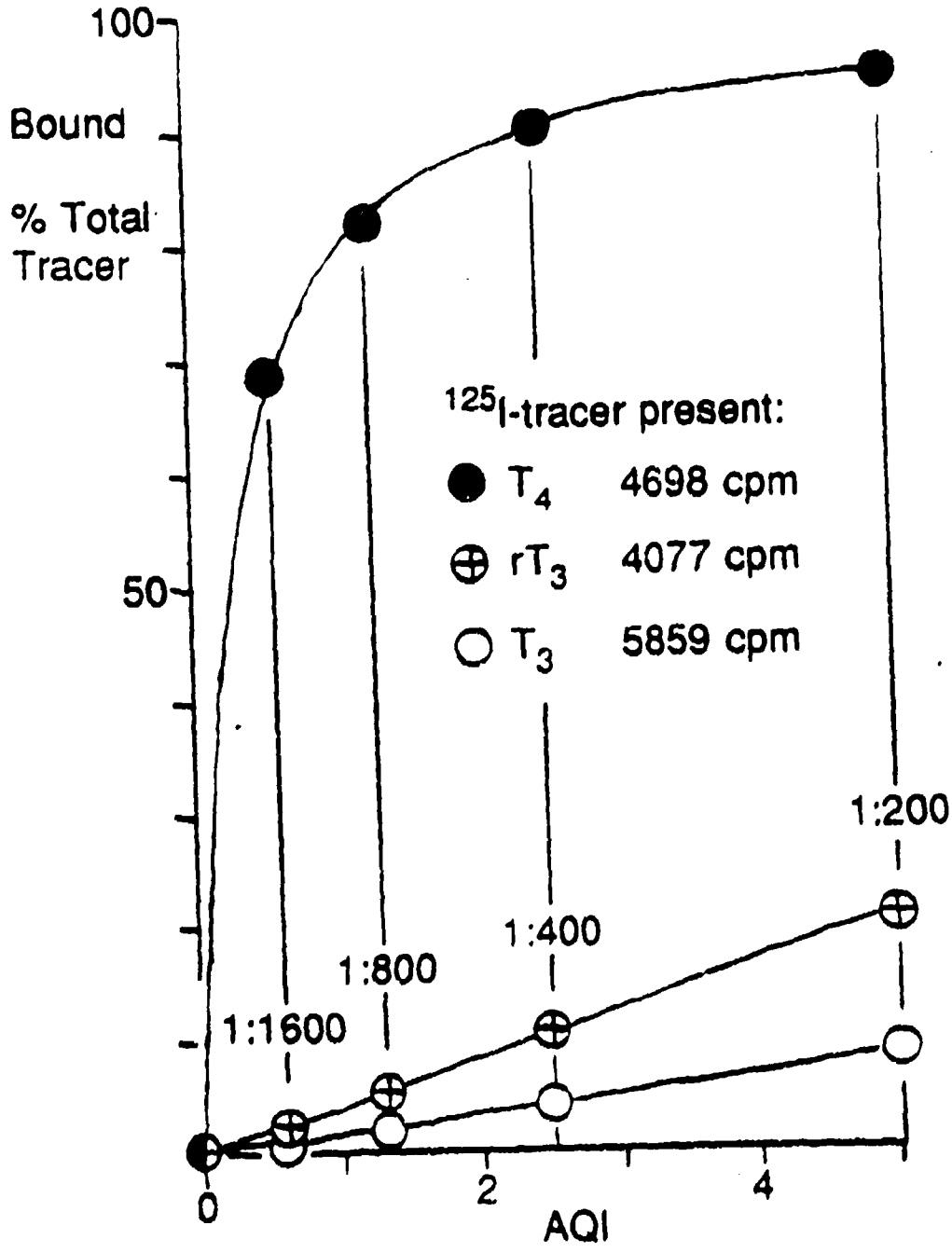

FIGURE 5. T_4 antibody binding profile with separate homologous and heterologous tracer. Percent total tracer cpm in charcoal-thyronine-stripped serum bound by various amounts (AQI, antibody quantity index) of antibody. For each tracer, total cpm were determined as Bmax cpm in separate logistic regressions with tracer-specific antibody (not shown). Data were proportionately corrected for nonspecific binding.

TABLE 4. T_4 Antibody Binding of Heterologous Tracer in the Presence of T_4 Tracer

Sample ---------->	CTSS			[illegible]			Burned Human Serum			Normal Human Serum		
Tracer ---------->	*T_4	*T_4 T_3	*T_4 [illegible]	*T_4	*T_4 [illegible]	*T_4 [illegible]	*T_4	*T_4 T_3	*T_4 rT_3	*T_4	*T_4 T_3	*T_4 rT_3
Bmax (cpm)	3772	4436	[illegible]	[illegible]	[illegible]	2417	3625	3729	3766	1844	2152	2129
Total heterologous cpm	–	1055	[illegible]	–	[illegible]	2176	–	397	329	–	2257	1659
Excess Bmax (cpm)	–	664	889	–	521	915	–	104	141	–	308	285
Excess Bmax (% total heterologous cpm)	–	62.9	76.8	–	28.7	42.1	–	26.2	42.8	–	13.7	17.2
Error (%)	–	0.6	0.8	–	0.3	0.4	–	0.3	0.4	–	0.1	0.2
Antibody dilution	0	1:800	1:400	[illegible]	0	[illegible]	1:800	1:400	0	1:1600	1:800	1:400	1:200	0	1:1600	1:800	1:400	1:200
n	4	4	4	4	4	4	4	4	4	4	4	4	4	2	2	2	2	4
Mean *T4 binding (% Bmax)	0	89.7	95.1	[illegible]	0	79.7	91.0	96.3	0	59.8	89.4	96.9	100.2	0	40.1	62.9	82.3	91.9

* indicates ^{125}I tracer present; Bmax, maximal binding from logistic regression, proportionally corrected for total nonspecific binding; heterologous, referring to *T_3 or *rT_3 tracer; error, that expected in Bmax, for total heterologous cpm as 1% total *T_4 cpm; antibody dilution (initial, as added to tubes), dilution of T_4 antibody used in the trials without and with heterologous tracer; n, number of tubes in each trial at the dilution; and mean *T_4 binding, from the trial without heterologous tracer. Sample entry "CTSS + T_3, rT_3" indicates nontracer hormones added at 100 ng/dl each. T_4 tracer was present in the same quantity (indicated by Bmax in *T_4 columns), whether with or without heterologous tracer present, in a given sample. Total heterologous cpm was determined as maximal binding in separate assays (not shown) with appropriate antibodies.

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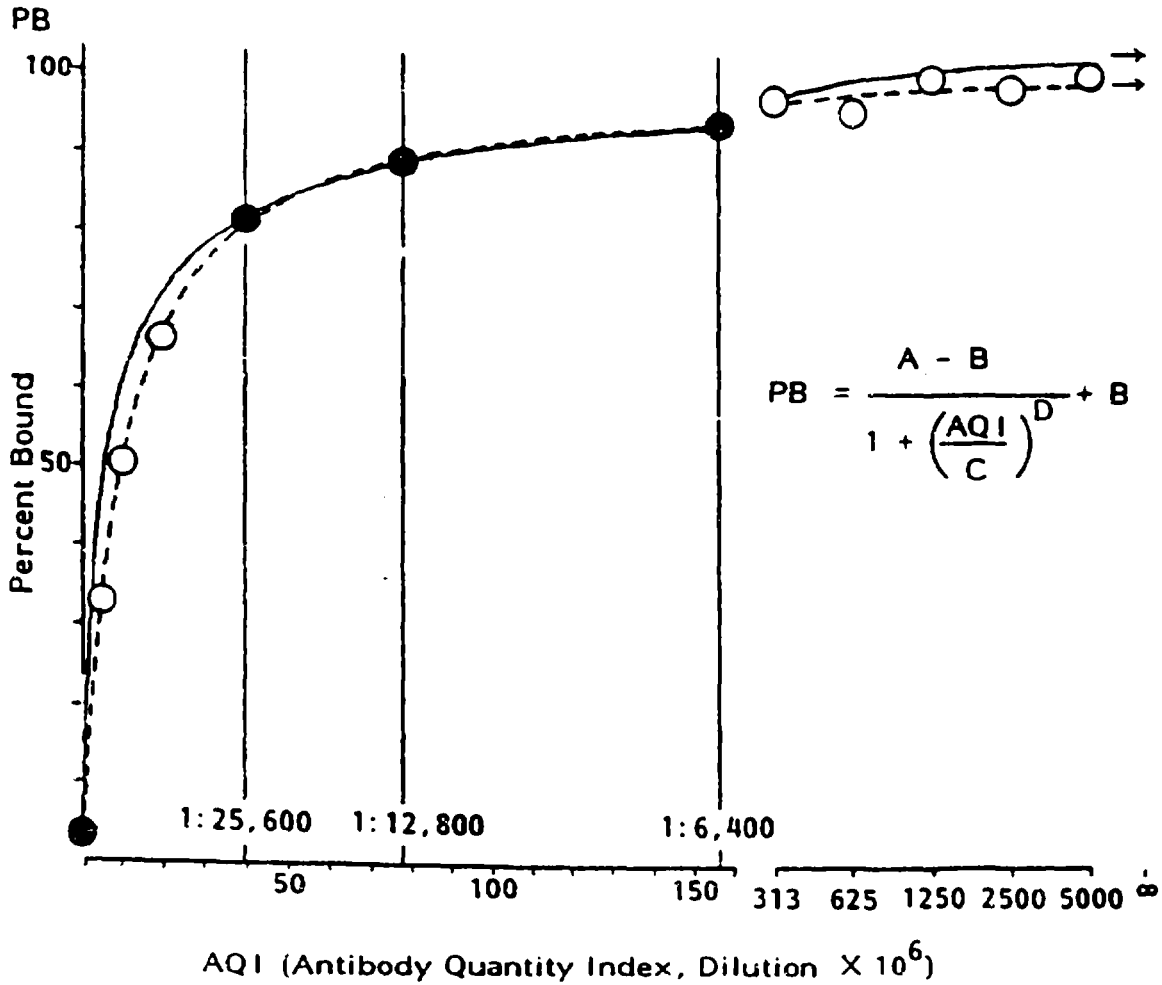

FIGURE 6. T_3 antibody and T_3 tracer - binding profile. Observed binding (% total cpm) for tracer in charcoal-thyronine-stripped serum; nonspecific binding (nonzero PB at zero AQI) not subtracted; logistic fit through data of only the closed circles (continuous line) or of all symbols (broken line). See Figure 1 for interpretation of logistic parameters (A, B, C, D).

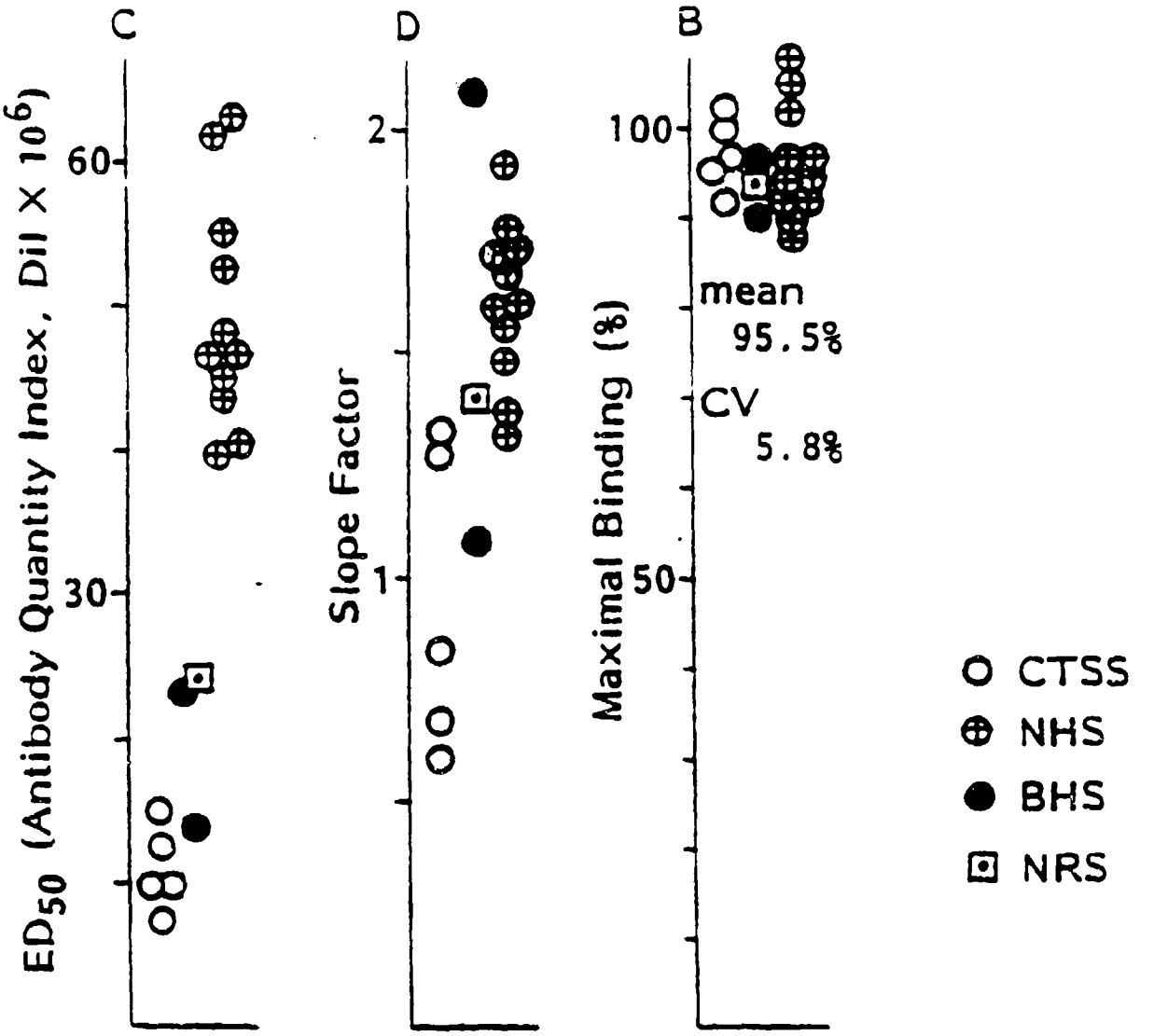

FIGURE 7. T_3 antibody - logistic parameters for samples with T_3 tracer from the same source. C, D, and B atop the ordinate axes are logistic parameters from equations as in Figure 6, with data not corrected for nonspecific binding. Antibody dilutions were 0, 1:25,600, 1:12,800, and 1:6,400. CV indicates coefficient of variation; CTSS, charcoal-thyronine- stripped serum; NHS, normal human serum (from individuals); BHS, burned human serum (from individual patients); and NRS, normal rat serum (pooled).

initially for these preparations. It appears that, as with the T_4 IMPT procedure, that the real T_3 IMPT Bmax recovery is approximately 100%, with any small amount of nonimmunoactive material representing impurities.

Figure 8 represents T_3 antibody binding in separate IMPT trials for CTSS with different radioactive tracers whose total cpm are indicated. There was no detectable binding of heterologous T_4 and rT_3 tracers, with ANOVA detecting no variation of bound cpm with the quantity of antibody.

Table 5 shows the binding profiles in separate T_3 IMPT trials for burned human serum with T_3 tracer alone or with heterologous tracer also present. Note that with the T_3 tracer preparation alone, the apparent recovery was 99% of the total radioactivity present and the activity thus detected (Bmax) was not augmented by the additional presence of relatively high amounts of either T_4 or rT_3 tracer. There was no significant variation of precipitated cpm due to the presence of either of the heterologous tracers after accounting for variation of precipitated cpm with T_3 antibody dilution.

Figure 9 shows the T_3 IMPT binding for CTSS and normal and burned human serum. For each type of serum, data for aliquots (all with tracer T_3) with and without additional heterologous tracer are pooled for the curves and their associated symbols. However, the projected Bmax values are plotted (far right) separately for additional presence of heterologous tracer from their respective T_3 IMPT trials. Though the curves varied greatly with type of sample, the projected amount of tracer T_3 present (Bmax) appeared to vary neither with sample type nor the additional presence of heterologous (contaminant) tracer.

Figure 10 shows two of the logistic parameters from an expanded series of trials similar to those in Figure 9 and including the latter. A two-way ANOVA detected no influence of heterologous tracer or of sample type on PBmax. Though sample type altered the ED_{50}, heterologous tracer did not. These and the previous data indicate that the T_3 IMPT procedure has no detectable nonspecificity with regard to T_4 or rT_3 and has approximately 100% true recovery of T_3 label which is not altered by sample type.

NSB_o. NSB_o is the cpm in the precipitate after reaction with no iodothyronine antibody present, expressed as the mean of replicates (usually duplicates) in terms of cpm or a ratio of the precipitate cpm to the total cpm present during the reaction (NSB_o ratio). As explained in the **MATERIALS AND METHODS** section, this ratio may be used to make a small correction in what is taken as the cpm bound (precipitated) in tubes containing antibody, such that any contribution of nonantibody-bound cpm to the cpm detected by antibody precipitation might be eliminated. This contribution as cpm, though still small, is maximal with the largest possible

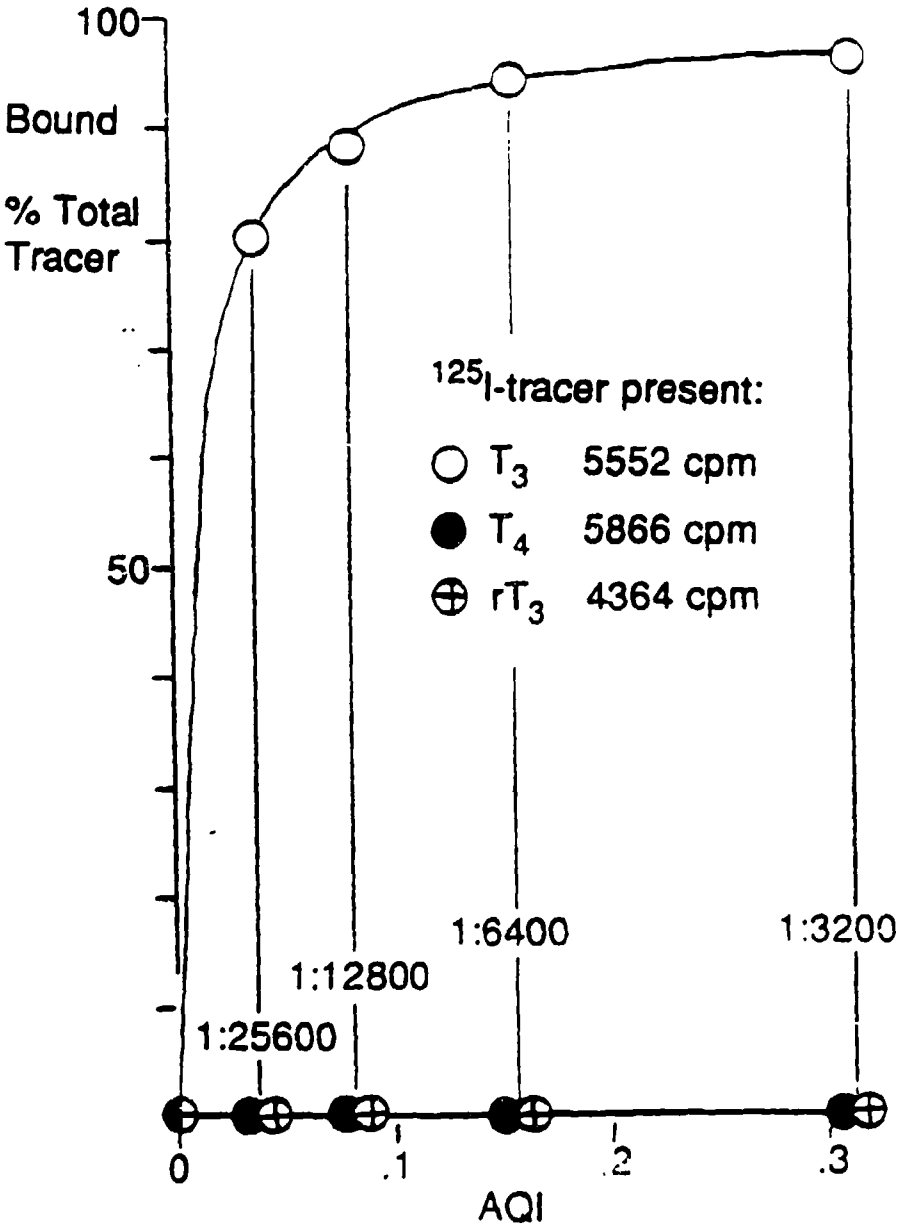

FIGURE 8. T_3 antibody binding profile with separate homologous and heterologous tracer. Percent total tracer cpm in charcoal-thyronine-stripped serum bound by various amounts (AQI, antibody dilution X 1000) of antibody. For each tracer, total cpm were determined as Bmax cpm in separate logistic regressions with tracer-specific antibody (not shown). Data were proportionately corrected for nonspecific binding. For T_4 and rT_3 tracers, each symbol is the mean for six tubes at each AQI. For both these (heterologous) tracers, there was no significant variation of bound cpm with AQI (including cpm with zero antibody).

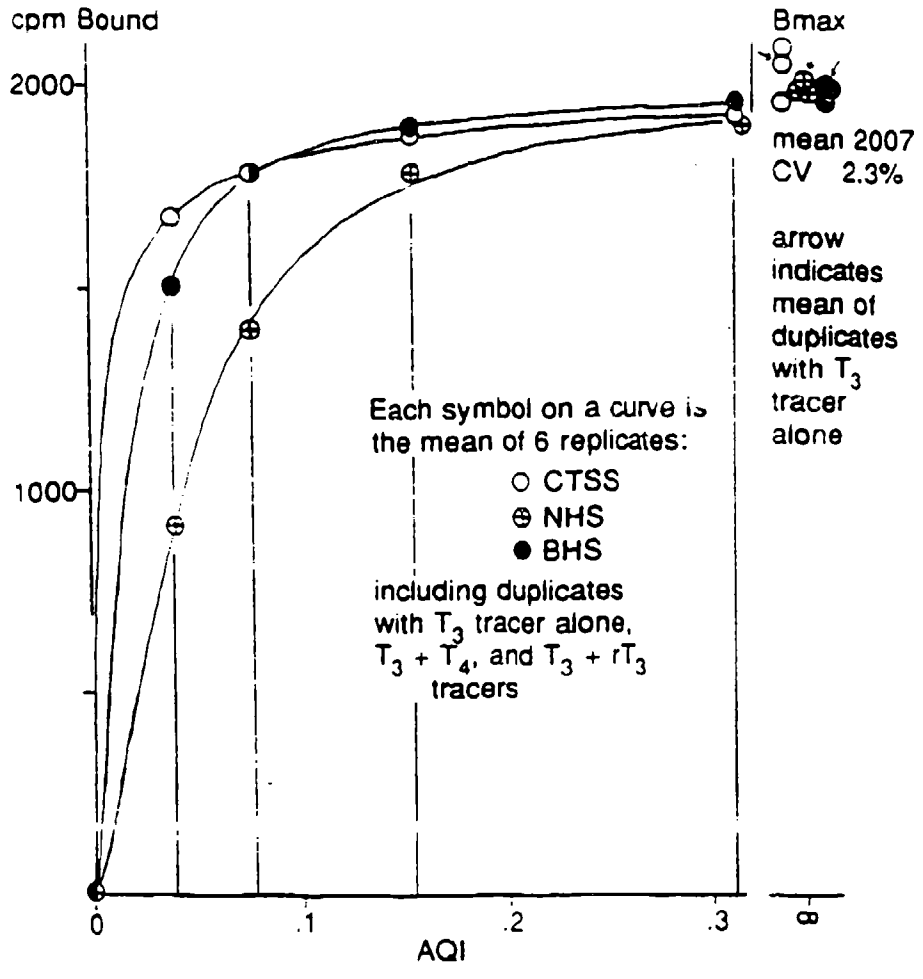

FIGURE 9. T_3 antibody binding profile with combined homologous and heterologous tracer. Observed and fitted binding, proportionately corrected for nonspecific binding, as a function of AQI (antibody dilution X 1000). Duplicate aliquots for the various AQI were run with tracer T_3 alone and with additional T_4 and separately rT_3 tracer, in either charcoal-thyronine-stripped serum (CTSS), normal human serum (NHS) from one individual, or burned human serum (BHS) from a pool. Within each type of serum, the bound cpm did not vary significantly with whether or not heterologous tracer was present. Thus, only single symbols and a single logistic curve for data pooled within each serum are shown. The Bmax, determined from separate logistic regressions for each tracer condition, did not vary significantly among serum types. All aliquots contained 2007 cpm of T_3, or additionally 1567 cpm of T_4 or 1273 cpm of rT_3. The latter were determined as Bmax by separate logistic regressions (not shown) with appropriate antibody.

TABLE 5. T_3 Antibody of Heterologous Tracer in the Presence of T_3 Tracer (Mean ± SEM)

Antibody Dilution	n	$*T_3$	$*T_3*T_4$	$*T_3*rT_3$
0	4	0 ± 6	0 ± 6	0 ± 3
1:25,600	4	2937 ± 23	2959 ± 25	3038 ± 32
1:12,800	4	3681 ± 13	3706 ± 33	3675 ± 31
1:6,400	4	3933 ± 37	4011 ± 38	4007 ± 35
1:3,200	4	4191 ± 32	4110 ± 34	4083 ± 40
Bmax		4260**	4156	4159
Total heterologous cpm		-	7479	7262
Total cpm		4314	13144	13607

*^{125}I tracer present in BHS, **98.7% of total cpm. Entries are in cpm corrected for nonspecific binding (100% of heterologous NSB and proportionately for T3 (NSB). T_3 tracer was present in the same quantity with heterologous tracer (T_4 or rT_3) present or absent. Total heterologous cpm determined as maximal binding in separate assays (not shown) with appropriate antibodies. Two-way ANOVA revealed no differences in cpm (those with ± SE) according to whether $*T_3$ was alone or with a heterologous tracer after accounting for variation with antibody dilution. Bmax indicates maximal binding from logistic regression.

portion of the cpm not immunospecifically bound (antibody concentrations approaching zero), is less with more of the total cpm bound to antibody (less cpm available for nonspecific binding), is minimal when fewest cpm are not bound to antibody ("infinite" antibody concentration), and zero in the latter case if no nonligand cpm is present and all the ligand cpm are bound to antibody. In this sense, NSB refers to any cpm counted in the precipitate area of the tube that remain in that area, but not as a result of antibody binding, whether those cpm are in a ligand potentially able to be bound by the antibody or are in contaminant substances. Thus, NSB does not refer to nonspecificity of those cpm for an antibody being used, but to their presence along with the observed radioactivity that is immunologically bound to antibody. The measurement, NSB_0, represents the maximal amount of NSB cpm (observed in the absence of first antibody).

In 71 measurements made in CTSS, the mean NSB_0 ratio was 2.35% of total counts present, showing no apparent variation with whether

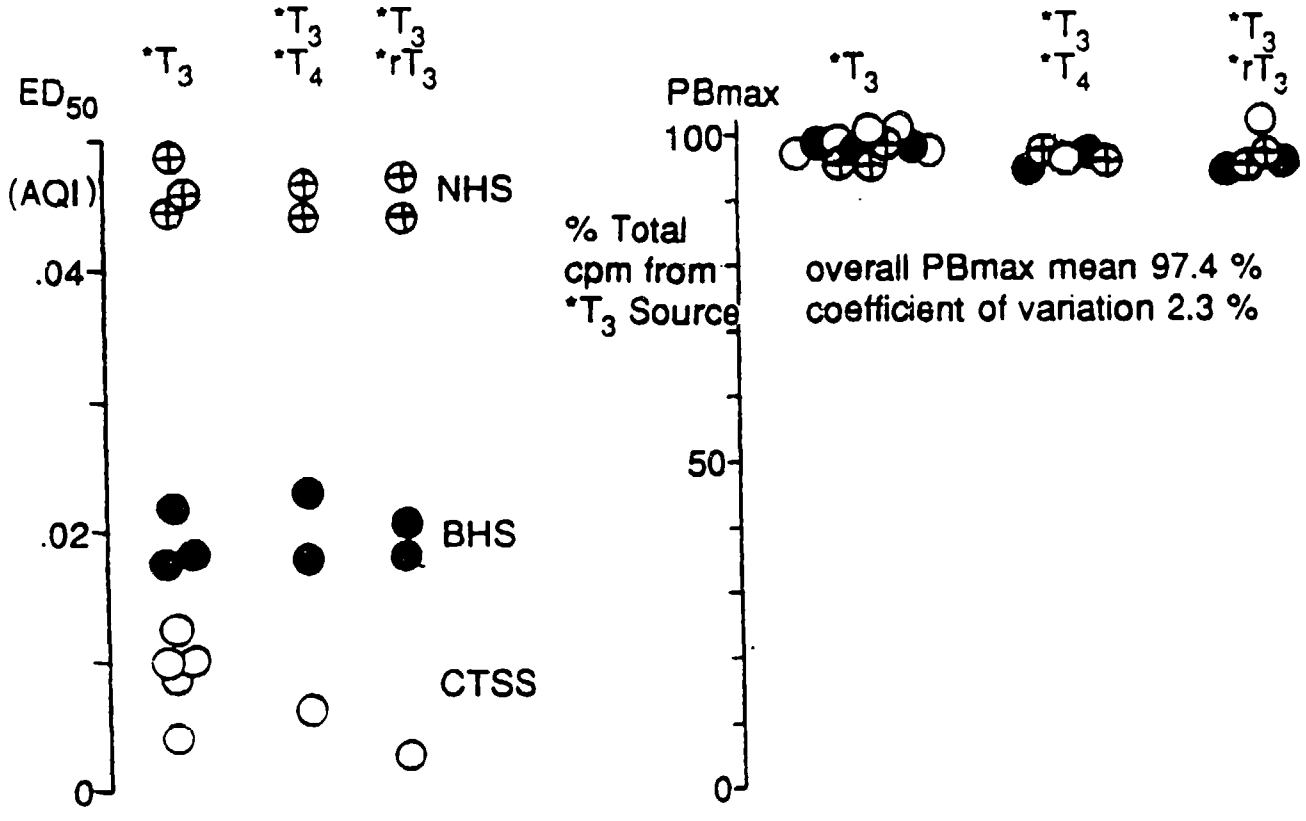

FIGURE 10. ED_{50} and PBmax for T_3 antibody with combined homologous and heterologous tracer. ED_{50} (as AQI, antibody dilution X 1000) and PBmax (% total T_3-source cpm) from Bmax in logistical regressions of cpm bound (proportionately corrected for total nonspecific binding) over 0, 1:25,600, 1:12,800, 1:6,400, and 1:3,200 dilutions of antibody. Tracer T_3 activity ranged 2007-4260 cpm and the other trace cpm (if present) ranged 63%-176% of the T_3 cpm present (their quantity determined by separate logistic project with appropriate antibody). Though ED_{50} varied significantly with serum type, PBmax did not. Neither ED_{50} nor PBmax varied with whether T_3 tracer was or was not accompanied by a heterologous tracer. * indicates ^{125}I tracer present; NHS, normal human serum (from one individual); and BHS, burned human serum (pooled from a large number of patients).

the tracer preparation present was nominally (predominantly) T_4, T_3, rT_3 or a combination.

However, in 34 determinations made in NHS or burned human serum, though no difference appeared between these types of sample,

the NSB_o ratio varied according to whether or not the T_3 tracer preparation was present. No differences in NSB_o ratio could be seen between T_4 and rT_3 tracer preparations present in the samples. Table 6 shows the higher NSB_o ratio for T_3 tracer (column 2 vs column 1) and indicates that this was less pronounced (but still present) when the contribution of T_3 cpm to the total cpm was diluted with other tracer cpm (column 3 vs column 2). In either case (column 2 or 3), the data are consistent with a greater proportion of the tracer T_3 present (then for other tracers) to be nonspecifically attracted to the precipitate from normal and burned human serum. This phenomenon was not seen in precipitates from CTSS.

TABLE 6. NSB_o Ratio (%) in Normal and Burned Human Serum According to Tracer Preparation Present

	*T_4 and/or *rT_3	*T_3	*T_3 and (*T_4 or *rT_3)
Mean ± SE	2.56 ± 0.08	4.60[a] ± 0.29	3.60[a,b] ± 0.09
n	15	8	11

*Tracer preparation present, [a]$P < 0.001$ vs column 1 mean, and [b]$P < 0.05$ vs column 2 mean (Bonferroni-corrected t tests). NSB_o ratio indicates precipitate cpm after decanting/total cpm present before decanting (in the absence of antibody).

Though it is expected that the main protein component of the precipitates is the (carrier mouse or rabbit gamma globulin/second antibody) complex, serum proteins originating in the sample may also be present. Thus, the precipitate might contain proteins that ordinarily bind iodothyronines in human serum. In the reaction and then in the precipitate, they might also, in turn, contribute to NSB. However, the 8-analino-1-naphthalenesulfonic acid added to the reaction is supposed to disrupt such binding, which is otherwise greatest for T_4, least for rT_3, and intermediate for T_3. Perhaps it is possible that this disruption of normal serum protein binding to iodothyronines is less complete for T_3 than for the others or that T_3 participates in a low-grade transfer iodination reaction with a precipitable protein in the mixture. Further, if one of these explanations is the case (for which we have no evidence), prior extraction of human serum with charcoal (for CTSS) may remove or inactivate such a T_3 binding (or iodinatable) protein, or supply a factor that inhibits its action.

In the formula that corrects for NSB in the precipitated counts, immune-bound cpm = (precipitate cpm - NSB_o cpm)/(1 - NSB_o ratio), the numerator contains the NSB_o as the precipitated cpm

observed in the IMPT trial aliquots without first antibody and the denominator contains the NSB_o as the ratio to the total counts. If the reaction mixture contains an unusually large proportion of counts that are not potentially reacting with the antibody along with counts that do potentially react with the antibody, then all of the former contribute equally to the NSB regardless of the amount of antibody; whereas, the latter contribute "proportionately" (less with greater amounts of antibody). In order to correct for all the nonimmunologic NSB along with the proportionate amount of NSB from potentially immunologically bound cpm, one uses for the numerator the usual NSB_o observed in the reaction, but for the denominator, the NSB_o ratio that is from the ratio of the counts for the tracer targeted by the antibody (but present in the precipitate without the antibody) over the counts for the total amount of this tracer itself that is present in the reaction. The elements of this ratio can be directly approximated from the IMPT trial itself if few of the total cpm are present as heterologous material (not targeted by the antibody).

However, a different situation obtains, for example, when the T_3 IMPT is being used to quantitate tracer T_3 in the presence of equal or greater amounts of tracer T_4 or rT_3, as was the case in some of the present studies. In this case, there was no immunologic reaction with T_4 or rT_3. Since the NSB_o ratio for tracer T_3 is different from that of the others (Table 6), the NSB_o ratio in the denominator theoretically should be that for T_3 (the homologous tracer) to get proportional correction for the T_3 component of NSB. With use of the NSB_o ratio from the IMPT trial itself as a standard of comparison, the effect of calculating the corrected bound cpm with the mean denominator NSB_o ratio for T_3 alone (column 2, Table 6), the mean NSB_o ratio from column 1, or the range of NSB_o ratio values contributing to the mean in column 3 was assessed for an IMPT trial of tracer T_3 having an approximate equal amount of T_4 tracer present. The logistic IMPT Bmax varied by only up to approximately 1% from the initial calculation. Therefore, except in one case (Table 5) in which the precipitated cpm were corrected for total heterologous NSB but proportionately for homologous (T_3) cpm (with the denominator homologous NSB_o ratio being that for the T_3 tracer present), the correction used was proportional for total NSB (with the denominator NSB_o ratio from the IMPT run itself) in those trials with added heterologous tracer.

DISCUSSION

Though correction of IMPT counts for nonspecific binding does not protect against error in the final result due to possible nonspecific but real immunoreaction with contaminant material, it does protect against an error due to nonimmunoreactive tracer materials. Because the latter materials may be present, e.g., iodide and iodoprotein in a tracer kinetic study, correction with the NSB_o ratio is recommended for use of the IMPT method.

Our finding of virtually complete recovery in quantifying tracer T_4 and T_3 in serum represents an apparent improvement in recovery over that in the methods (TCA/alcohol, washed matrix, EAB extraction, GSAE, the previously reported IMPT, and EC) which do not rely on Sephadex™ fractionation. Of these, the first five procedures are intended to quantitate in serum a parent iodothyronine tracer that might be injected for determination of its disappearance kinetics. Central to such a use is the intention to quantitate a tracer moiety that is present in far higher concentration than that of other iodothyronines which remain at the end and contain the identical type of detectable tracer radioactivity. This is because all tagged iodothyronines present in the untreated sample are likely included in the final measurement. These methods cannot be supported for use in determining the presence or appearance of tracers, e.g., daughter compounds that are present along with higher levels of other, e.g., parent, compounds with identical tracer radioactivity characteristics. The IMPT procedure described herein is intended for the same purpose as are those methods, to quantitate a tracer iodothyronine (in this case, T_4 or T_3) in the absence of large contamination in the sample from other radioactive iodothyronines.

For T_4 IMPT, a small advantage in this regard occurs because less than half of daughters T_3 and rT_3 tracers are detected as tracer T_4. This would be more than adequate to study tracer T_4 kinetics in which labeled T_3 accumulates to about 1% of tracer T_4 present, and the accumulation of labeled rT_3 is less than that (26,27,33,34). For our T_3 IMPT, specificity is even greater with no cross-detection of T_4 or rT_3 label up to almost 2-fold higher heterologous label concentration than that for tracer T_3. Higher excess contaminant heterologous counts in the presence of T_3 label were not explored. However, detection of no heterologous label when used alone suggests that the limit of (for example) excess T_4 label would be its interference only through NSB "noise". With regard to the T_3 IMPT, such a "noise" due to serum T_4 label in a T_4 kinetic study would be of the same order of magnitude (or greater) as the labeled daughter T_3 "signal". Because of the inherent impropriety of "correcting" out such a large noise component, use of the NSB_c ratio to do so would not be recommended to support a case for the role of our T_3 IMPT in assessing T_3 appearance from injected T_4 label with use of peripheral serum sampling. A case for its use in this way might be explored for sampling of urine in which labelled T_3 is enriched relative to parent label T_4.

Use of our T_3 IMPT might more readily be supported for studying the serum tracer disappearance kinetics of T_3. This method's extreme specificity for T_3 (tested against T_4 and rT_3) may likely provide an additional advantage in that such specificity against its own daughter products is also likely. Though an advantage was claimed for accuracy in determining plasma clearance rate of tracer T_3 by exclusion of activity in T_3 products (32), the need for exclusion of these T_3 daughters in studying parent tracer T_3

disappearance kinetics is not widely accepted, nor is such exclusion usually undertaken. The only currently available methods for such exclusion are highly work-intensive and complex (28,32-34). If our T_3 IMPT procedure does this (further demonstration that it does is still necessary), and if such exclusion of T_3 daughters is important, our procedure has a profound advantage of simplicity for this purpose.

Compared to previous methods using antibody (GSAE and IMPT), our IMPT method has greater recovery, but also offers the notable advantage of lack of susceptibility to variation according to sample type (including variation due to different endogenous nontracer hormone concentration). This is due to our use of multiple concentrations of antibody. The other comparable methods use only one concentration of antibody and thus risk error due to variation in recovery among different samples.

With respect to washed matrix approaches, our IMPT method offers greater recovery, except for one of those approaches (33) in which 99% recovery was obtained after Sephadex™ G25 column absorption and then elution by whole serum. This latter approach is more complex to perform than ours, in that it involves the preparation of columns and the production and handling of eluant fractions. With respect to the TCA/alcohol method, its incomplete recovery and the problem of variable contamination of the results by coextraction of labeled iodide make it undesirable.

With respect to the EAB method, though it is simple in principle, its recovery is notably incomplete. As with any method having incomplete recovery, it is made more complex by the need to append separate procedures to monitor the recovery which may vary in the samples studied.

The potential use for our IMPT method is principally the study of the serum disappearance kinetics of the biologically active iodothyronines, T_4 and T_3. With respect to most other methods previously developed for this purpose, it is simpler and offers the advantage of virtually complete recovery. The few other methods offering such recovery involve column fractionation and are more complex. Further, for the more specific T_3 IMPT, our method has some potential for use in assessing labeled T_3 appearance from parent labeled T_4. The possible circumstances for this use include those in which the samples would be expected to be enriched with daughter T_3 label for the amount of labeled T_4 present. These include in vivo sampling of serum effluent in the venous drainage of an organ, e.g., liver, which supplies a large amount of T_3 to the circulation by conversion of T_4 to T_3, and in vitro assessment of T_4 to T_3 conversion by tissues (5'-monodeiodinase activity). All these potential uses will be explored in our endeavor to elucidate the changes in thyroid hormone economy after burn injury.

PUBLICATIONS/PRESENTATIONS

Vaughan GM: Altered TSH-thyroid axis control in burned rats. Presented at the 8th International Congress on Burn Injuries, New Delhi, India, 14 November 1990.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
					21 Nov 90	DD-DR&E(AR) 636
3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISP'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
NONE	A. New	U	U		CX	

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS14	CA	305
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code) (U) Thermal-Dye Double Indicator Technique for Estimating Extravascular Lung Water - A Comparison Study

12. SUBJECT AREAS
0605 Medicine and Medical Research

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
9011	9209	DA	C. In-House

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

RESOURCES ESTIMATE		
a. FISCAL YEARS	a. PROFESSIONAL WORK YEARS	b. FUNDS (In thousands)
91	0.1	58
92	0.1	61

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

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Burns (Injuries); (U) Lungs; (U) Pulmonary Function; (U) Pulmonary Edema; (U) Gravimetric Analysis

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

23/24. (U) The objective of this work is to evaluate the efficacy of the thermal-dye indicator method for estimating extravascular lung water as compared with gravimetric analysis. This technique will be evaluated over a range of hemodynamic parameters. In addition, measurements of extravascular lung water will be correlated with colloid osmotic pressure and varying doses of smoke inhalation in an attempt to index the severity of inhalation injury.

25. (U) 8810 - 8909. Not applicable.

(U) 8910 - 9009. Not applicable.

(U) 9011 - 9109. This project 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 1991. Equipment and supplies have been ordered and work will be initiated shortly.

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

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION: DA335692	2. DATE OF SUMMARY: 01 Oct 91	REPORT CONTROL SYMBOL: DD-DR&E(AR) 635
3. DATE PREV SUM'RY: 21 Nov 90	4. KIND OF SUMMARY: D. Chg	5. SUMMARY SCTY: U	6. WORK SECURITY: U	7. REGRADING:	8. DISS'N INSTR'N: CX	9. LEVEL OF SUM A. WORK UNIT:

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS14	CA	305
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code) (U) Thermal-Dye Double Indicator Technique for Estimating Extravascular Lung Water - A Comparison Study

12. SUBJECT AREAS
0605 Medicine and Medical Research

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
9011	9209	DA	C. In-House

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

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

18. RESOURCES ESTIMATE

FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
91	0.5	60
92	0.5	63

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME: US Army Institute of Surgical Research	a. NAME: US Army Institute of Surgical Research
b. ADDRESS (include zip code): Fort Sam Houston, San Antonio, Texas 78234-5012	b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-5012
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR: RUE, L W
d. TELEPHONE NUMBER (include area code): 512-221-2720	d. TELEPHONE NUMBER (include area code): 512-221-8440
21. GENERAL USE: FIC MILITARY/CIVILIAN APPLICATION: M	e. NAME OF ASSOCIATE INVESTIGATOR (if available): CIOFFI, W G g. NAME OF ASSOCIATE INVESTIGATOR (if available): BECKER, W K

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Burns (Injuries); (U) Lung; (U) Pulmonary Function; (U) Pulmonary Edema; (U) Gravimetric Analysis

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

23/24. (U) The objective of this work is to evaluate the efficacy of the thermal-dye indicator method for estimating extravascular lung water as compared with gravimetric analysis. This technique will be evaluated over a range of hemodynamic parameters. In addition, measurements of extravascular lung water will be correlated with colloid osmotic pressure and varying doses of smoke inhalation in an attempt to index the severity of inhalation injury.

25. (U) 9011 - 9109. This project 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 1991. Two animals have been studied. Preliminary data has revealed that the double indicator method is highly influenced by variations in cardiac output and may consequently not be an accurate method for determining extravascular lung water under conditions of extremes in hemodynamic performance. Further evaluation of this technique will continue during the next fiscal year.

DD FORM 83 MAR 1498 — EDITION OF MAR 88 IS OBSOLETE. — * U.S.G.P.O. 1988—491-003/50329

SUPPLEMENTAL WORK UNIT INFORMATION FOR THE PROJECT ENTITLED "THERMAL-DYE DOUBLE INDICATOR TECHNIQUE FOR ESTIMATING EXTRAVASCULAR LUNG WATER - A COMPARISON STUDY"

Subrecord/Linking Accession Number: Not applicable.

Search Control Data: W6048E/W6049C, 6 November 1990.

Product Identification: For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1991.*

Unclassified Special Categories: Lab Animals: Sheep, RA II.

ABSTRACT

PROJECT NUMBER: 3M161102BS14, 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: 21 November 1990 - 30 September 1991

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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 have been studied to date. Consequently, it is too early to undertake any meaningful data analysis; however, preliminary review of the data suggests that the double indicator method may be highly influenced by variations in cardiac output.

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 oncctic pressure is 20-35 cmH_2O 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 is to evaluate the efficacy of the thermal-dye indicator method for estimating EVLW as compared with gravimetric analysis. This technique will be evaluated over a range of hemodynamic parameters. In addition, measurements of EVLW will be correlated with colloid osmotic pressure and varying doses of smoke inhalation in an attempt to index the severity of inhalation injury.

MATERIALS AND METHODS

Study Design. Twenty-four male sheep weighing 25-45 kg will be utilized for this study. The study will be divided into three phases.

Phase I. The goal of the first phase of the study is to correlate the thermal-dye double indicator technique with gravimetric estimates of EVLW at varying levels of cardiac output. Phase I will employ 12 animals, 6 of which will be anesthetized, intubated, and, using the femoral vessels, have a side-to-side arteriovenous fistula constructed so as to increase the cardiac output. The animals will be placed in their normal resting positions postoperatively and returned to their cages for a 3-day equilibration period. On postoperative day 4, the animals will again be 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 will be 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 will be made. Three measurements of the thermal-dye double indicator EVLW will then be made. This will consist 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 will be injected into the right atrium and, using a syringe pump, blood will be withdrawn at a constant rate of about 20-30 ml/min through a densitometer, and the femoral thermistor-tipped arterial line will detect temperature changes to provide estimates of mean transit time for both the thermal and dye indicators. A lung water computer will calculate the EVLW. Following completion of these measurements, a partial occluding vascular clamp will be placed across the arteriovenous fistula so as to occlude it and presumably lower cardiac output. Once again, baseline measurements of hemodynamic indices, arterial blood gases, and colloid osmotic pressure will be made. Repeat measurements of the thermal-dye double indicator EVLW will then be made in

triplicate. Following completion of these measurements, the animals will be placed on their backs and the thorax opened rapidly with both pulmonary hila clamped, and then euthanized with potassium chloride. The lungs will be removed, weighed, homogenized with equal amounts of distilled water and a gravimetric estimate of EVLW, as described by Pearce (3), will be performed.

Six additional animals will be studied at baseline hemodynamic states. They will be anesthetized, intubated, and instrumented with a Swan-Ganz catheter and a femoral thermistor-tipped arterial line used for lung water analysis. Using the contralateral femoral vessels, a side-to-side arteriovenous fistula will be constructed and a partial occluding clamp will be placed across the arteriovenous fistula. The sheep will be placed in their normal upright resting positions and baseline measurements of hemodynamic indices, arterial blood gases, and colloid osmotic pressure will be made. Again, three measurements of the thermal-dye double indicator EVLW will be made. Following these measurements, the partial occluding clamp will be removed from the arteriovenous fistula so as to create sudden elevation in cardiac output. Once again, baseline measurements of hemodynamic indices, arterial blood gases, and colloid osmotic pressure will be made and triplicate measurements of the thermal-dye double indicator EVLW will be performed. Following completion of these measurements, the animals will be placed on their backs, euthanized, and the thorax opened. The lungs will be removed, weighed, and homogenized and a gravimetric estimate of EVLW performed.

Phase II. The second phase of this study will examine the influence of colloid osmotic pressure on EVLW accumulation. Six animals will be anesthetized, intubated, and instrumented as described previously. In this group of animals, the Swan-Ganz catheter and the femoral thermistor-tipped arterial line will be brought out through subcutaneous tunnels onto the back of the animal and protected with a Velcro™ pouch. The animals will then be plasmapheresed to colloid osmotic pressure levels of 18 or 12 mmHg. They will then be returned to their cages and allowed food and water ad libitum. Following a 24-h equilibration period, the colloid osmotic pressure will be reassessed and additional plasmapheresis will be performed as necessary (possibly over ensuing days) until the desired colloid osmotic pressure remains constant. The animals will then be anesthetized and intubated and baseline measurements of hemodynamic parameters, arterial blood gases, and colloid osmotic pressure will be made. Three measurements of EVLW using the double indicator technique will be performed. Following the final double indicator measurement, the animal will be euthanized and a gravimetric estimate of lung water will be undertaken.

Phase III. The final phase will examine the influence of smoke inhalation on EVLW accumulation over time. Inhalation injury

will be induced using the standard smoke inhalation model developed at this Institute (12). Three sheep will receive a 1-U smoke injury and 3 sheep will receive a 3-U smoke injury. Following a 24-h equilibration period, the animals will be anesthetized, intubated, and instrumented, again using a Swan-Ganz catheter and a femoral thermistor-tipped arterial line. These devices will be subcutaneously tunnelled to the back of the animal and secured with a Velcro™ pouch. Baseline measurements and double indicator EVLW determinations will be made at this point and again at 48, 72, and 96 h following smoke injury. In the interim period, the animals will be permitted food and water ad libitum. Following completion of all measurements, the animals will be euthanized and a gravimetric estimate of their lung water will be undertaken.

Description of Procedures. Twenty-four male sheep weighing 25-45 kg/kg will be utilized for this study. Each sheep will be housed in a conventional outdoor run and will have access to food and water ad libitum. Sheep will be dewormed 2 weeks prior to use.

Phase I. On the day of the study, 6 animals will be anesthetized with sodium pentobarbital (35 mg/kg IV, Calbiochem, La Jolla, CA) through a 20-ga needle and intubated. They will then be instrumented with a peripheral venous catheter. Lactated Ringer's will be constantly infused at a rate of 1 ml/kg/h and anesthesia will be maintained with sodium pentobarbital. A side-to-side femoral arteriovenous fistula will then be constructed using a 6-0 Proline™ suture. The wounds will be closed and the animals will be placed in the resting upright position and returned to their cage upon awakening. After a 3-day equilibration period, the animals will be 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) which will be placed in the femoral artery by cutdown. The previously constructed femoral arteriovenous fistula will be dissected out so as to permit the application of a partial occluding clamp in the latter phase of this phase. Anesthesia will be maintained with alpha-chloralose and lactated Ringer's will be constantly infused at a rate of 1 ml/kg/h. Animals will then be paralyzed with pancuronium bromide (0.03-0.04 mg/kg, Pavulon®, Organon Pharmaceuticals, West Orange, NJ). After instrumentation, the animals will be positioned in the resting upright position and conventional ventilation will be continued with a volume-limited ventilator (Bear II®, Bear Medical Systems, Inc., Riverside, CA). Ventilator settings will be altered to maintain the pH between 7.4 and 7.5. Animals will be ventilated with an FIO_2 of 0.21 and a PEEP of 0. Central venous and pulmonary artery pressures will be 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 will be obtained. Arterial blood gases and mixed venous blood gases will be drawn and immediately analyzed. A serum sample will be drawn for an estimate of colloid osmotic pressure. The thermal-dye double indicator estimate of EVLW will be 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 will be injected into the right atrium rapidly and the thermal indicator will be 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 will be 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) will be used to calculate cardiac output and EVLW. Following completion of these measurements in triplicate, a partial occluding clamp will be placed on the femoral arteriovenous fistula so as 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 will be obtained. Arterial blood gases and mixed venous blood gases will be drawn and immediately analyzed. Again, thermal-dye double indicator estimates of EVLW will be performed in triplicate. Following completion of these measurements, the animals will be placed on their backs, the chest opened, and vascular clamps placed on the pulmonary hila. The animals will then be euthanized with a potassium chloride bolus (20 ml) through the central venous catheter and the lungs excised. The lungs will be weighed and homogenized in a Waring^{R} blender, to which a measured amount of water will be added. An aliquot of the homogenate will be centrifuged at 10000 g for 30 min. Aliquots of blood (drawn when the organs were removed), lung homogenate, and homogenate supernatant will be weighed before and after drying at 80°C to a constant weight. Standard formulas will then be used to calculate EVLW and dry weight.

To complete this phase of the study, 6 animals will be anesthetized, intubated, and instrumented as described above. After instrumentation, the animals will be positioned in the resting upright position and conventional ventilation will be continued as described above. After a 30-min equilibration period, baseline measurements will be performed. EVLW determinations using the thermal-dye double indicator technique will be performed in triplicate. Following completion of these measurements, the partial occluding clamp will be removed. After a 30-min equilibration period, similar baseline measurements will be undertaken. EVLW measurements will be performed in triplicate and following determination of these measurements, a gravimetric estimate will be performed as previously described.

Phase II. For Phase II, the animals will undergo a similar anesthesia and instrumentation period; however, in these animals,

the catheters will be brought out through subcutaneous tunnels onto the back of the animals, secured, and protected using Velcro™ pouches. The animals will be heparinized and plasmapheresed using an Asahi® plasma separator (Parker Hannifin Corporation, Irvine, CA) after baseline indices are measured. This system utilizes a cellulose acetate hollow fiber core which allows the passage of plasma but not cellular elements. Three animals will be plasmapheresed to a colloid osmotic pressure of 18 mmHg and 3 other animals to a level of 12 mmHg. The animals will then be returned to their cages. Following a 24-h equilibration period, repeat measurements of colloid osmotic pressure will be made. If necessary, repeat plasmapheresis will be performed daily until the desired level of colloid pressure is achieved. The animals will then be reanesthetized and the previously placed devices will be used to measure hemodynamic indices, arterial blood gases, colloid osmotic pressure, EVLW determinations using the double indicator thermal-dye technique as previously described. Following serial measurements, a gravimetric determination of EVLW will be performed as previously described.

Phase III. Phase III will utilize 3 sheep exposed to a 1-U smoke injury, as previously developed by this Institute, and 3 exposed to a 3-U smoke injury. Twenty-four hours after smoke exposure, the animals will be reanesthetized, intubated, and instrumented as previously described. Again, the monitoring catheters will be brought out through subcutaneous tunnels and secured with Velcro™ pouches. Baseline measurements will then be obtained. These measurements will be repeated at 48, 72, and 96 h after smoke exposure without reanesthetizing the animal. Following final data collection, the animal will be euthanized and a gravimetric estimate of EVLW determined as previously described.

Determination of Number of Animals Required. A total of 24 animals will be used for this study. This study has not been undertaken previously, but it is felt that the number of animals to be used will allow one to detect a significant difference between groups with a type I error of 0.05 and a type II error of 0.10.

Data Analysis Plan. A paired t test will be performed to determine the presence of significant differences between groups.

RESULTS

Two animals have been studied to date. Consequently, it is too early to undertake any meaningful data analysis; however, preliminary review of the data suggests that the double indicator method may be highly influenced by variations in cardiac output.

DISCUSSION

Though it is too early to derive any conclusions from this study, the results from the first two animals studied suggest

significant variations in EVLW measurements using the double indicator technique with variations in cardiac output. If this observation is sustained by further animal study, this method may not be an accurate means for determining EVLW under conditions of extremes in hemodynamic performance, such as may be encountered in burn patients. Further evaluation of the technique will continue during the next fiscal year.

PRESENTATIONS/PUBLICATIONS

None.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
				DA311499	01 Oct 91	DD-DRAB(AR) 636
3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISP'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
01 Oct 90	D. Chg	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS14	CK	306
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code) (U) Preliminary Studies on Zinc Homeostatic Control and Immunocompetence in a Burned Animal Model

12. SUBJECT AREAS
0601 Biochemistry 0613 Microbiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
8610	9209	DA	C. In-House

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

18. RESOURCES ESTIMATE

FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
91	0.5	37
92	0.5	40

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME: US Army Institute of Surgical Research	a. NAME: US Army Institute of Surgical Research
b. ADDRESS (Include zip code): Fort Sam Houston, San Antonio, Texas 78234-5012	b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-5012
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR: SHIPPEE, R L
d. TELEPHONE NUMBER (Include area code): 512-221-2720	d. TELEPHONE NUMBER (Include area code): 512-221-4858
21. GENERAL USE: FIC MILITARY/CIVILIAN APPLICATION: M	e. NAME OF ASSOCIATE INVESTIGATOR (If available) g. NAME OF ASSOCIATE INVESTIGATOR (If available)

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Burns (Injuries); (U) Nutrition; (U) Septicemia; (U) Bioassay; (U) Immunosuppression

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

23/24. (U) The objectives of this work are to determine changes in zinc metabolism caused by burn and infection in a murine model as manifested by altered mechanisms at the whole body, organ, and molecular levels, relate the role of these changes to septic complications, and determine optimal levels of zinc supplementation in burned humans. The interaction between zinc nutriture, burn injury, and immunocompetence will be studied.

25. (U) 9010 - 9109. Radial immunodiffusion and hemagglutination techniques were used to further delineate the magnitude and temporal response to both sheep and human RBCs in the burned rat model. Results confirm our earlier observations that the primary humoral response to these antigens given 1, 3, and 5 days postburn results in increased antibody levels in the burned animal. Furthermore, the burned animals responded to dose levels (0.2%) that were below threshold for the nonburned animals. During the year, a new technique (ELISPOT) was developed and refined that will be used to measure the response to hapten-carrier compounds.

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

SUPPLEMENTAL WORK UNIT INFORMATION FOR THE PROJECT ENTITLED "PRELIMINARY STUDIES ON ZINC HOMEOSTATIC CONTROL AND IMMUNOCOMPETENCE IN A BURNED ANIMAL MODEL"

Subrecord/Linking Accession Number: Not applicable.

Search Control Data: W6L28F/W6L29E, 20 October 1989.

Product Identification: For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report* for fiscal years 1987-91.

Unclassified Special Categories: Lab Animals: Rats; RA II.

ABSTRACT

PROJECT NUMBER: 3M161102BS14, Research

PROJECT TITLE: Preliminary Studies on Zinc Homeostatic Control and Immunocompetence in a Burned Animal Model: Jerne Plaque Assay

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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Clinical and animal studies suggest that burn injury induces alterations in zinc metabolism. It also has been established that zinc nutriture can cause alterations in the immune response, but there is a paucity of information concerning the interrelationship between burn injury, zinc nutriture, and the immune response. In the present study, rats were subjected to full-thickness dorsal scald injuries of 30% of the total body surface and then maintained on sufficient or deficient zinc intake. The rats were immunized with sheep RBCs on day 6 postburn and sacrificed 4 days later. The spleens were excised and spleen lymphocytes isolated and used in a Jerne plaque assay to determine the number of plaque-forming cells (PFCs). The burn/zinc-sufficient regimen significantly increased ($P < 0.01$) the PFC response when compared to unburned zinc-sufficient or zinc-deficient control rats. Burned rats that were maintained on a zinc-deficient regimen showed a significant decrease ($P < 0.05$) in PFCs when compared to burned rats maintained on a zinc-sufficient regimen. This study indicates an interaction of zinc in the primary humoral immune response following thermal injury.

PRELIMINARY STUDIES ON ZINC HOMEOSTATIC CONTROL AND IMMUNOCOMPETENCE IN A BURNED ANIMAL MODEL: JERNE PLAQUE ASSAY

A considerable body of evidence exists relating zinc nutriture to immunocompetence in both laboratory animals and humans (1-4). Zinc deficiency in young animals produces abnormalities in thymus morphology which appear to correlate with defective T cell maturation and function (5). Postthymic differentiation of T lymphocytes is also affected by zinc intake as evidenced by impaired delayed hypersensitivity responses observed in zinc-restricted adult animals (6). Thus, thymic-dependent and -independent maturational events of T lymphocytes, which function in both cellular and humoral immune responses, are influenced by zinc nutriture.

In view of the well-documented perturbations in metabolism and immune function in burn victims, we have begun studies on the possible interactions of zinc metabolism and immunocompetence following burn injury. We previously demonstrated that rats receiving 30% total body surface burn injuries and zinc restricted for 10 days postburn exhibited a significant increase in T-suppressor cells (7). The present report describes the results of further studies designed to determine whether zinc nutriture influences the humoral response to a single injection of sheep red blood cells (SRBCs) in rats following burn injury.

MATERIALS AND METHODS

Experimental Animals. A total of 72 male Sprague-Dawley rats (Harlan Sprague-Dawley, Inc., Houston, TX) weighing approximately 350 g were used for these studies. The rats were housed in individual stainless steel cages, given distilled deionized water ad libitum, maintained on a 12/12 light/dark cycle, and fed a semipurified diet (Ziegler, Inc., Gardners, PA) (ingredients as a percentage of diet: egg white solids, 20%; sucrose, 31%; corn starch, 31.2%; cellulose powder, 3%; vitamin premix, 0.5%; mineral premix, 4.0%; choline bitartrate, 0.3%; and corn oil, 10%) designed to meet all the nutrient requirements of the adult rat except for zinc.

All animals were fed the zinc-deficient diet (< 0.5 ppm) ad libitum for 2 weeks and given daily subcutaneous injections of 1 mg Zn/kg as zinc sulfate. Weigand and Kirchgessner (8) have determined the maintenance requirement for the rat to be $9+125W^{0.75}$(kg) µg Zn/day. Accordingly, rats weighing 350 g would be estimated to require 65 µg Zn/day or 0.2 mg Zn/kg/day to meet their maintenance requirement. Five times this requirement was given in this study to provide an additional requirement for the burn injury. This level of supplementation appears not to be excessive based on the fact that we have not seen an increase in

hepatic or intestinal metallothionein levels in control animals (7).

After a 2-week equilibration period, the animals were weighed and assigned to one of the following regimens in a manner that equalized mean body weight among the treatment groups (n=6):

Burn Sufficient (BZS). Animals were subjected to a 30% total body surface area burn, injected with 1 mg Zn/kg daily, and fed the semipurified diet ad libitum.

Burn Deficient (BZD). Animals were subjected to a 30% total body surface area burn, injected with saline, and fed the semipurified diet ad libitum.

Control Sufficient (CZS). Animals were injected with 1 mg Zn/kg daily and fed the semipurified diet ad libitum.

Control Deficient (CZD). Animals were injected with saline and fed the semipurified diet ad libitum.

Prior to burn injury, all animals were anesthetized with sodium pentobarbital (35 mg/kg IP). The 30% total thickness burn injury was effected by exposure of the shaved dorsal area to 90°C water bath for 10 sec. Six days postburn, all animals were immunized by injection of 0.2 ml of a 20% solution of SRBCs into a tail vein. Four days after immunization, the rats were anesthetized with sodium pentobarbital (35 mg/kg IP) and sacrificed by exsanguination.

Spleens were placed in disposable Petri dishes and teased apart with stainless steel spatulas in HBSS. The cells were aspirated into 15-ml tubes and washed twice with HBSS. Cells were then pipetted onto 4 ml of a Ficoll-Paque™ solution and centrifuged at 450 g for 30 min. The cells so isolated were enumerated using a Coulter™ counter (Model Zm, Coulter Electronics, Ltd, Beds, England) and diluted to a concentration of 1 X 10^6 cells/ml.

Hemolytic Plaque Assay. Tubes containing 0.05 ml SRBCs (14% in minimum essential media (MEM)) and 0.40 ml agarose were maintained at 42°C in a water bath. These tubes were removed from the water bath and 0.1 ml of the cell suspension added. This mixture was spread on a 75 X 50-mm glass slide with a stirring rod and allowed to solidify at ambient temperature for 2 min. The slides were incubated in MEM for 1 h at 37°C in a 5% CO_2 atmosphere. After incubation, the slides were rinsed with physiological saline and incubated with fresh MEM-guinea pig complement mixture (1:30). After 45 min incubation, the slides were rinsed in saline and fixed in acetone and ethanol. Splenic plaque-forming cells (PFCs) were counted using a dissecting microscope.

which 10 ml of a solution containing 21.5% perchloric acid, 7.0% sulfuric acid, and 71.5% nitric acid was added. After concentrating on a hot plate to approximately 2 ml, the solution was diluted to 25 ml with distilled deionized water and aspirated directly into an atomic absorption spectrophotometer (Model 5000 AAS, Perkin-Elmer, Norwalk, CT) for determination of zinc concentration.

One milliliter of 20% trichloroacetic acid was added to 1-ml samples of plasma. These mixtures were vortexed and then centrifuged for 20 min at 500 g. Zinc concentrations of the supernatants were also determined by atomic absorption spectrophotometry.

Statistical Analysis. Descriptive statistics and one-way ANOVA of body weight change, plasma zinc, and hepatic zinc were performed (Version 5.0, Statistical Analysis System, SAS Institute, Cary, NC). When the F statistic was found to be significant ($P < 0.05$), a Duncan's multiple range test was used to test for differences among the treatment means.

Due to the nature of the data, a different approach was used to analyze the PFC data. Analysis of the PFC results as described by Daniel (9) showed that the data did not follow a normal distribution; a logarithmic transformation, however, normalized the distribution. To adjust for possible differences in assay conditions, the PFC data were expressed as percentages of the control (nonburned, zinc-sufficient) mean for that particular repetition. A factorial ANOVA was used to test for effects of treatments and replication. Means were compared using a Duncan's multiple range test.

RESULTS

The weight changes of the rats from burn day until day of sacrifice are shown in Table 1. CZS animals gained a mean of +13 g over the 10-day period, as opposed to +10, +3, and -2 g for the CZD, BZS, and BZD regimens, respectively. Zinc-deficient animals showed significantly lower mean plasma zinc concentrations than the supplemented groups ($P < 0.05$, Table 1). A significant increase in hepatic zinc concentration was noted in the BZS rats ($P < 0.05$). Also shown in Table 1 are plasma and hepatic zinc concentrations from 5 sham-burned animals fed Purina™ laboratory chow ad libitum. These concentrations were not significantly different than the sham-burned, zinc-sufficient animals fed the purified ration ($P < 0.05$).

A summary of preliminary studies to determine the kinetics of the PFC response for both burned and nonburned rats is shown in Figure 1. In both groups, PFC production reached a maximum 4 days following immunization. The response of the burned animals was

TABLE 1. Body Weight and Tissue Zinc Concentration Data (Mean ± SE)

Treatment Group	n	Body Weight (g) Start	End	Change	Zinc Plasma (µg/dl)	Zinc Liver (µg/g)
BZD	18	342 ± 10	340 ± 11	− 2 ± 2[a]	72 ± 11[a]	86 ± 3[a]
BZS	18	341 ± 10	344 ± 10	+ 3 ± 2[a]	137 ± 8[b]	121 ± 9[b]
CZD	18	342 ± 11	352 ± 9	+10 ± 2[b]	75 ± 10[a]	76 ± 5[a]
CZS	18	341 ± 10	354 ± 9	+13 ± 2[b]	149 ± 9[b]	94 ± 5[a]
CPC	5	−	−	−	153 ± 7[b]	72 ± 5[a]

Those sharing common letters are not significantly different ($P < 0.05$). BZD indicates 30% total body surface area burn and saline injections; BZS, 30% total body surface area burn and zinc injections; CZS, zinc injections only; CZD, saline injections only; and CPC, Purina chow only.

higher than control animals on both days 4 and 5 following immunization.

PFC data expressed as both total plaques per 10^5 lymphocytes and as percent of control values are shown in Table 2. Mean responses (PFC/10^5 cells) are summarized in Figure 2. While no significant difference was found due to repetition ($P = 0.525$), burn injury alone caused a highly significant difference ($P < 0.001$) and zinc treatment resulted in a small, but statistically significant effect ($P = 0.039$). Based on the Duncan's multiple range test of mean differences, no significant difference was found between the BZD, CZD, and CZS groups while the mean PFC response for the BZS group was significantly higher ($P < 0.05$) than the other three treatment groups.

DISCUSSION

Zinc restriction after burn injury caused a reduction in the humoral response to a T-dependent antigen when compared to animals maintained on adequate zinc intake. While these results suggest a possible relationship between zinc nutriture, burn injury, and the ability of rats to generate a humoral response, the mechanisms by which zinc affects immunocompetence in adult animals is unclear. One possibility is a reduction in activity of a zinc-dependent thymic hormone which influences both intrathymic (10) and extrathymic (11) T-cell differentiation.

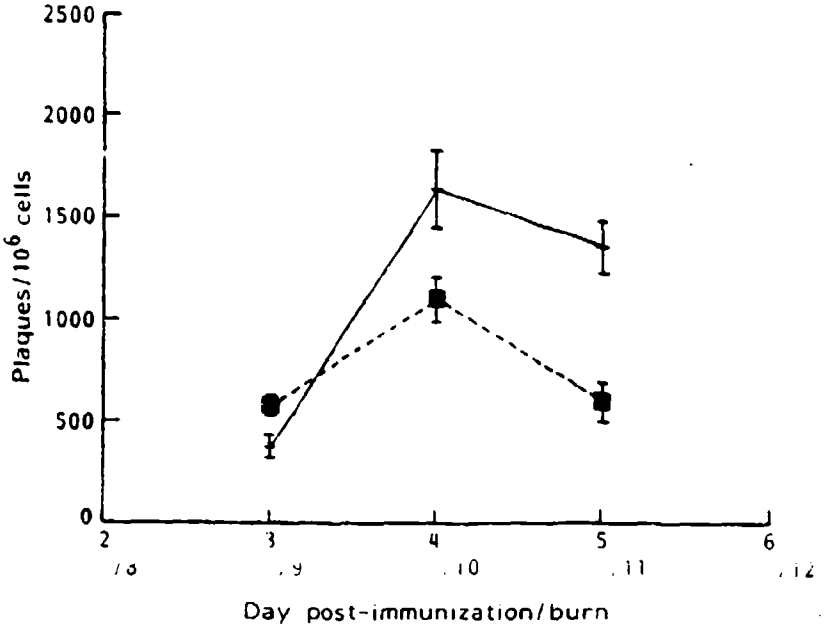

FIGURE 1. Kinetic response of control (broken line, n=18) and burned rats (continuous line, n= 21) on days 3, 4, and 5 postinjection of 0.2 ml of 20% suspension of SRBCs (mean ± SEM).

James et al (12) have suggested that short-term dietary intervention may not directly affect peripheral T-cell functional capacity. They have shown that macrophages from mice fed a complete diet could restore the in vitro proliferation capacity of T cells from mice fed a zinc-deficient diet. Thus, zinc might affect humoral responsiveness in burned animals by depressing helper T-cell and macrophage activities.

It has been well established that the essential fatty acids, linoleic acid and arachidonic acid, can be immunosuppressive presumably because they serve as precursors for prostaglandins and thromboxane synthesis (13,14). This is of particular importance because burn injury leads to release of dienoic prostaglandins and thromboxanes (15,16).

Alexander et al (17) have used a flame burn guinea pig model to show significant improvements in weight gain, metabolic expenditure, cell-mediated immune responses, and higher transferrin and lower C3 serum concentrations when the animals were given diets containing fats high in eicosapentaenoic acid in place of linoleic acid (safflower oil). Guinea pigs given indomethacin, an inhibitor of prostaglandin synthesis, and maintained on diets containing safflower oil had significantly lower metabolic rates than control animals.

TABLE 2. Primary PFC Response (Mean ± SEM)

	Repetition 1 (n=24)				Repetition 2 (n=24)				Repetition 3 (n=24)			
Group *	BZD	BZS	CZD	CZS	BZD	BZS	CZD	CZS	BZD	BZS	CZD	CZS
Total PFCs/10^5	463 ± 130	623 ± 147	248 ± 49	280 ± 71	603 ± 213	996 ± 304	390 ± 53	428 ± 112	303 ± 39	531 ± 154	172 ± 32	266 ± 56
% of Control Values	165 ± 46	223 ± 53	89 ± 18	116 ± 25	141 ± 50	233 ± 71	91 ± 12	100 ± 26	114 ± 15	200 ± 58	65 ± 12	100 ± 21

BZD indicates 30% total body surface area burn and saline injections; BZS, 30% total body surface area burn and zinc injections; CZS, zinc injections only; and CZD, saline injections only.

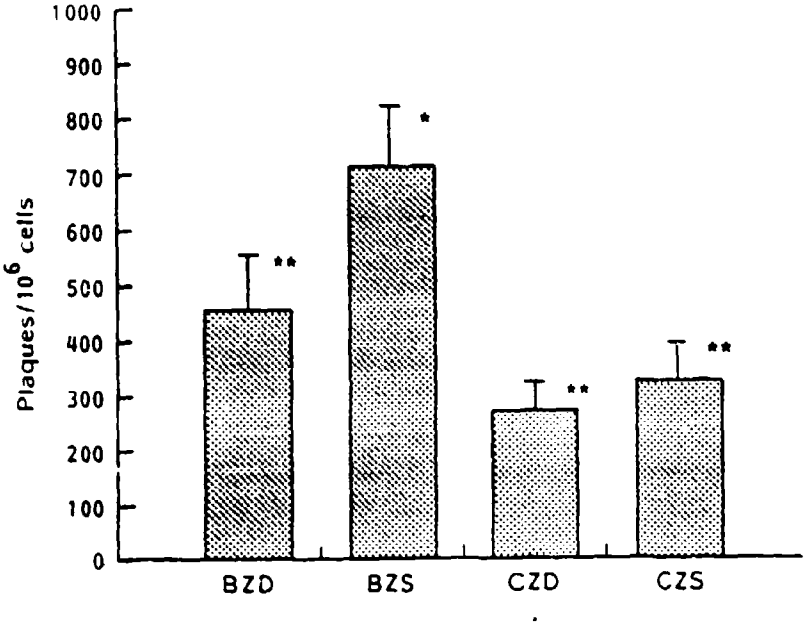

FIGURE 2. Primary PFC response expressed as PFC/10^5 cells of the CZS rats (mean ± SEM). BZD indicates 30% total body surface area burn and saline injections; BZS, 30% total body surface area burn and zinc injections; CZS, zinc injections only; and CZD, saline injections only. Treatments sharing common symbols are not significantly different from each other at $P < 0.05$.

The relationship between changes in fatty acid metabolism and thermal injury is relevant to the present study since zinc deficiency has been shown to effect arachidonic acid metabolism (18,19). Both the chick and rat models have shown that severe skin lesions due to zinc deficiency are associated with a high proportion of arachidonate in the skin fatty acids (18). The pathology of the double deficiency, low essential fatty acids and low zinc intake, with the rat model is greater than the sum of the single deficiencies.

An hypothesis could be made that the effect of zinc deficiency on the immune response in our study may be associated with altered fatty acid metabolism and subsequently prostaglandin synthesis. Future work will be designed to investigate this possible interaction.

There is precedence in the literature for the increased humoral response that we have seen in zinc-sufficient, burn-injured rats. Soderberg et al (20) have reported that the secondary PFC response was increased when mice were immunized with SRBCs following burn injury.

Recent studies have demonstrated that cultures of peripheral blood lymphocytes in vitro from burn patients spontaneously produce large quantities of IgM and IgG antibodi s (21). In order to insure that the increased response we observed was not due to spontaneous polyclonal expansion caused by burn injury, studies were performed using splenic lymphocytes from burned rats immunized with SRBCs in a plaque assay that utilized either SRBCs or human erythrocytes (HRBC) as target cells. While the usual numbers of PFCs were produced when SRBCs were used as target cells, no plaques were produced when HRBC were employed as target cells. Furthermore, no PFCs were observed when spleen cells from either nonimmunized, burned or nonburned rats were tested.

The results of the present study support the hypothesis that an interrelationship between zinc nutriture and burn injury exists. We were careful in our rat model to insure that all the rats had sufficient zinc body stores prior to administering the burn injury and starting the zinc restriction regimen. When considering studies reporting immunocompetence in burn patients, it is likely that the burn patient population used in a particular study is heterogenous with respect to preburn nutritional status. We feel that our studies involving zinc nutriture indicate that some of the variability, both within and between studies, may be due to the nutritional status of the patients.

PRESENTATIONS/PUBLICATIONS

Koppenheffer T: Serum antibody titer response to sheep red blood cells in a burn rat model. Presented at the 2nd International Congress on Immune Consequences of Trauma, Shock, and Sepsis, Munich, Germany, 6 March 1991.

Shippee RL: Effect of burn injury and zinc nutriture on IL2-receptor expression during the anamestic responses to sheep red blood cells in a burn rat model. Presented at the 2nd International Congress on Immune Consequences of Trauma, Shock, and Sepsis, Munich, Germany, 6 March 1991.

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ABSTRACT

PROJECT NUMBER: 3M161102BS14, Research

PROJECT TITLE: Preliminary Studies on Zinc Homeostatic Control and Immunocompetence in a Burned Animal Model: ELISPOT

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

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The ELISPOT method has been utilized to enumerate the number of plasmacytes in response to antigen-hapten complexes. The objective of this phase of our study was to adapt the method to our research. Preliminary studies have indicated that the ELISPOT technique may be a sensitive method for delineating the effect of nutritional status and burn injury on the humoral response. Additional experience with the rat model will be needed to further define the primary and secondary response to antigen-hapten complexes.

PRELIMINARY STUDIES ON ZINC HOMEOSTATIC CONTROL AND IMMUNOCOMPETENCE IN A BURNED ANIMAL MODEL: ELISPOT

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.

During our investigations concerning the interactions among zinc nutriture, immunocompetence, and burn injury, we have consistently observed a heightened humoral response to a single injection of sheep RBCs in the rat model. Both the Jerne plaque assay and serum hemagglutination titers have supported the finding of an increased humoral response in burn-injured rats when the injection was given 24 h or 4 days postburn. This is contrast to the highly referenced reports by Alexander and Moncrief (3-4).

The use of RBCs as an immunizing antigen presents a number of problems. It is difficult to extrapolate the results from these studies to the response in burn patients from infectious agents. Further, the RBCs contain multiple antigenic proteins which do not allow the type of studies that could be performed if a single antigen-hapten was used.

Czerkinsky et al (5) have described a method, ELISPOT, to enumerate the number of plasmacytes responding to antigen-hapten complexes. The objective of this phase of our study was to adapt the method to our research.

MATERIALS AND METHODS

Study Design. Lewis 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. At either 24 h or 5 days after burn injury, the animals were sacrificed and the small intestine was removed.

Description of Procedures. Male Lewis rats weighing ± 250 g were anesthetized, 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. At either 24 h or 5 days after burn injury, the animals were anesthetized and sacrificed by exsanguination.

ELISPOT. The ELISPOT method employs a combination of two immunoenzyme visualization systems yielding distinct color products. The method permits the simultaneous enumeration of mononuclear cells secreting IgG or IgM antibodies. The original method was modified to allow detection of IgG- or IgM-secreting spleen lymphocytes from rats injected with dinitrophenol (DNP). DNP-KLH (DNP-hemocyanin conjugate, keyhole-limpet) was prepared as indicated in Table 1. The rats were injected intraperitoneally with 0.5 ml (2 mg DNP-KLH) of the DNP-KLH suspension.

After the animals were sacrificed, the spleens were excised, teased apart in HBSS, and transferred to 15-ml conical plastic tubes. After washing twice in HBSS, the final pellet was suspended in 0.1 X HBSS for 20 sec followed by addition of 2 X HBSS. Finally, 4 ml of 1 X HBSS was added followed by underlaying of 1 ml heat-inactivated FCS. Tubes were centrifuged and the final pellet suspended in RPMI culture media. This isotonic lysing procedure consistently produces lymphocytes that are > 90% viable with a greatly reduced amount of RBC contamination.

Cell concentrations were adjusted to 2 X 10^6 cells/ml and then serially diluted three times. Cells were plated in 96-well nitrocellulose bottom microtiter plates that had been previously coated with DNP-bovine albumin conjugate (DNP-BSA) as described in Table 2.

After incubation for 4 h, free cells were washed off the nitrocellulose and the staining antibodies were added. Alkaline phosphatase conjugated to goat anti-rat Fc fragment and peroxidase conjugated to goat anti-rat Mu chain were used to identify IgG and IgM-secreting cells, respectively. After incubation overnight at 4°C, the antibodies were washed off with subsequent staining with substrates as described in detail in Table 2. As the enzymes act on their respective substrates, the products precipitate into the nitrocellulose, producing blue or red spots. Performed as described above, the method allows enumeration of IgG or IgM-secreting cells by counting the number of blue and red spots, respectively.

TABLE 1. IgG and IgM Response to Dinitrophenol-Keyhole-Limpet Hemocyanin (DNP-KLH), Mean ± SEM

Day Postburn	2	3	4	5	6	7	8
Day Post-DNP-KLH	1	2	3	4	5	6	7
Burn Group							
IgG	0 ± 0	0 ± 0	0 ± 0	2 ± 2	46 ± 24	353 ± 142	214 ± 2
IgM	62 ± 7	192 ± 27	135 ± 40	227 ± 34	363 ± 35	313 ± 69	172 ± 25
n=	3	3	5	8	9	4	2
Control Group							
IgG	0 ± 0	0 ± 0	0 ± 0	19 ± 13	99 ± 34	363 ± 111	413 ± 71
IgM	73 ± 7	220 ± 9	198 ± 47	424 ± 85	272 ± 48	174 ± 24	117 ± 14
n=	3	3	6	7	9	5	2

TABLE 2. ELISPOT Procedure

MATERIALS AND REAGENTS
1. Millipore 96-well nitrocellulose bottom microtiter plates.
2. PBS tablets (#P4417, Sigma Chemical Company, St. Louis, MO).
3. Tween 20.
4. Dimethyl formamide.
5. 3-amino-9-ethyl-carbazole (AEC, #A6926, Sigma Chemical Company).
6. 5-bromo-4-chloro-e-indolyl phosphate (BCIP, #B0274, Sigma Chemical Company).
7. Nitro blue tetrazolium (NBT, #N6876, Sigma Chemical Company).
8. PO_4 citrate-urea tablets.
9. Tris, pH 8.1.
10. NaCl.
11. $NaHCO_3$.
12. $MgCO_3$.
13. RPMI.
14. Goat anti-rat IgG and IgM (#112-005-044 and #112-035-020, respectively, Jackson Immunology Research Laboratories).

TABLE 2 (Continued)

FORMULATIONS

1. PBS tablets = 1 tablet/200 ml dH_2O.

2. PBS-T = PBS + 50 µl Tween + 1 ml FCS/100 ml.

3. Tris saline = 0.05M Tris + 0.125M NaCl.

   a. 35 g Trizma 8.1.
   b. 43.5 g NaCl.
   c. QS to 500 ml.
   d. Dilute 1:10 for working solution.

4. 1M $NaHCO_3$ with 10 µM $MgCO_3$.

5. AEC/H2O = 20 mg tablet of AEC + 1.6 ml DMF. Dissolve and then add 76 ml pH 5.0 PO_4 citrate-urea. Add 30 µl H_2O_2. Filter and store in dark at 4°C.

6. BCIP/NBT = 25 mg BCID tablet in 1.66 ml DMF/30 mg NBT in 1 ml H2O. Add 1 ml of NBT and 1 ml of BCIP to 100 ml of $NaHCO_3$ solution and filter. Store in dark at 4°C.

7. DNP-KLH = DNP-hemocyanin conjugate, keyhole limpet (#324121, Calbiochem, La Jolla, CA).

   a. Weigh out 40 mg DNP-KLH.
   b. Add, slowly, to 10 ml PBS.
   c. Add 4.5 ml 1M $NaHCO_3$.
   d. Slowly add 10 ml 0.2M aluminum potassium sulfate.
   e. Wash 3X with PBS.
   f. Adjust to final volume of 10 ml (4 ml/ml).
   g. For immunization, dilute 1 part stock DNP-KLH with 4 parts saline and inject 0.5 ml intraperitoneally.

8. DNP-BSA = DNP-albumin conjugate, bovine (#324101, Calbiochem).

TABLE 2 (Continued)

COATING PLATES
1. Add 0.1 ml DNP-BSA at 5 µg/ml (stock = 5 mg/ml dilute with PBS). The amount is for a particular assay is calculated and then a third of this volume is made up for the BSA control wells. With 4 cell concentrations per animal, triplicates within cell concentration 1.2 ml of DNP-BSA is needed per animal and 0.4 ml of BSA control (7.2 ml DNP-BSA and 2.4 BSA ml per plate).
2. Let sit at least 24 h at 4°C.

TABLE 2 (Continued)

CELL INCUBATION
NOTE: During wash cycles, the plates were prepared by washing 3X with PBS and then immersing in PBS for 5 min. They were blotted dry and 0.1 ml of RPMI with 10% FCS was added to each well and incubated for at least 30 min.
1. Tease spleens in HBSS.
2. Wash once, suspend pellet in 5 ml HBSS and wash again.
3. Remove supernatant and tap pellet into suspension. Remove 100 µl into clean tube.
4. Add 1 ml 0.1 X HBSS and aspirate for 20 sec. Add 1 ml 2 X HBSS (cold) followed by 4 ml of 1 X HBSS.
5. Underlay with 1 ml FCS and centrifuge 1000 rpm for 10 min.
6. Remove supernatant and suspend in 5 ml RPMI-FCS and count.
7. Adjust to 1 X 10^6 and then make serial dilutions.
8. Remove plate from incubator and add 0.1 ml of cell suspensions and incubate for 4 h at 37°C.

TABLE 2 (Continued)

ADDITION OF CONJUGATED Ab

1. Rinse wells 3X with PBS and 3X with PBS-T.

2. Immerse in PBS-T for 5 min, blot dry, and add bottom to plate.

3. Add 0.1 ml Ab conjugates: AP goat anti-rat IgG and HRP goat anti-rat u-chain (concentration must be determined for each lot, diluent is PBS-T containing 1% FCS. Calculations were as follows:

   a. Need 0.25 µg Ab/0.1 ml.

   b. If stock is Ab-goat anti-rat, Fc = 600 µl/ml
   If stock is Ab-goat anti-rat, u = 1000 µl/ml

   c. If 6 ml needed for assay:

   2.5 µg/ml X 6 ml = 15 µg of each Ab
   15 µg X ml/600 = 0.025 ml
   15 µg X ml/1000 = 0.015 ml
   0.060 ml FCS (1%)
   5.900 ml PBS-T

For 6 animals (1 plate), use 42 µl of anti-Fc and 25 µl of anti-u to 9.93 ml of PBS-T.

TABLE 2 (Continued)

STAINING
1. Wash 3X with PBS and blot.
2. Place in tray with Tris and float for 5 min.
3. Remove and blot dry.
4. Replace bottom.
5. Add 0.1 ml BCIP stain and monitor development.
6. Rinse in PBS.
7. Blot dry.
8. Add 0.01 ml AEC and monitor development.
9. Rinse in tap water.

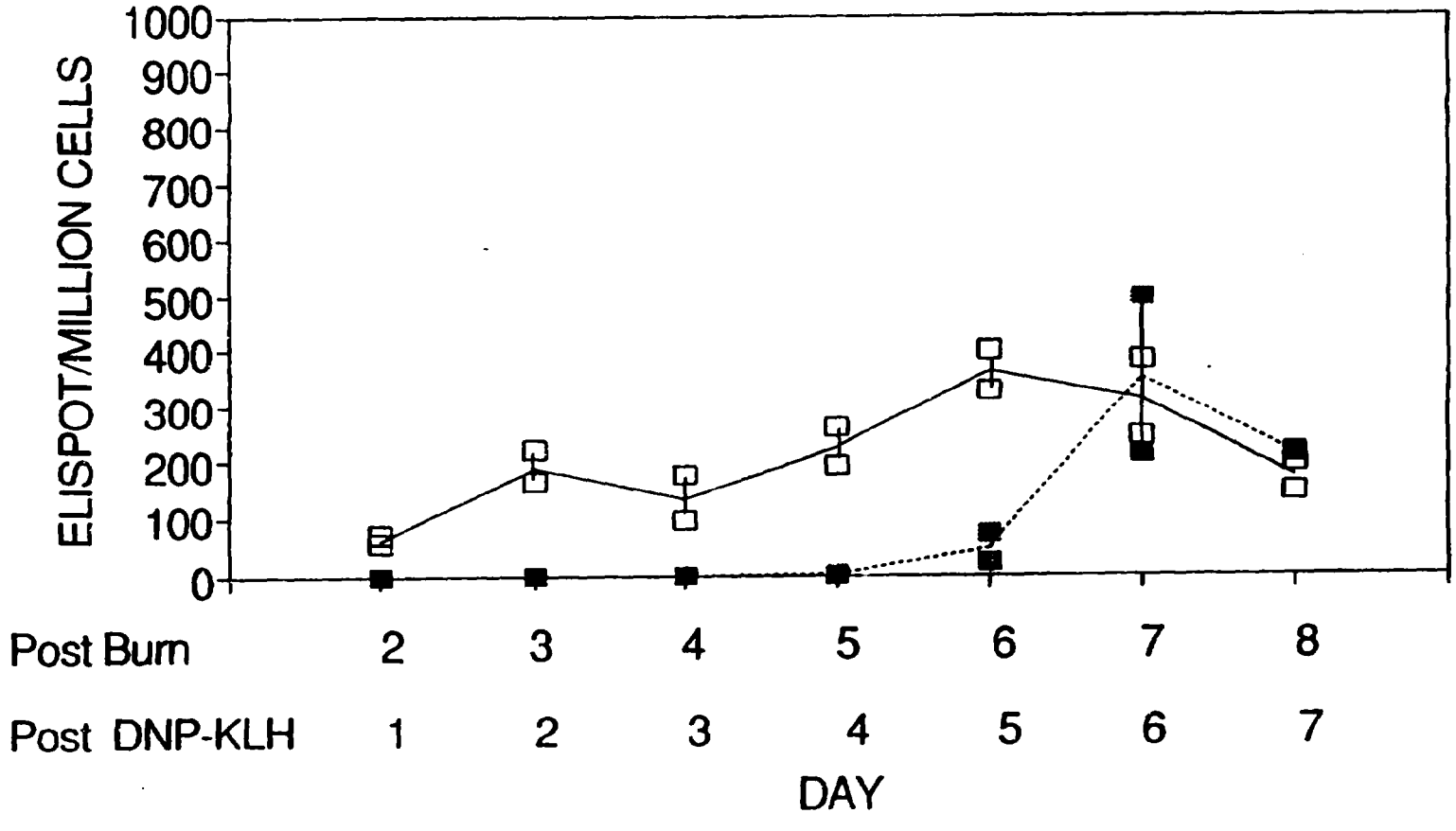

FIGURE 1. IgG (broken line) and IgM (solid line) response for the burn group (mean ± SEM).

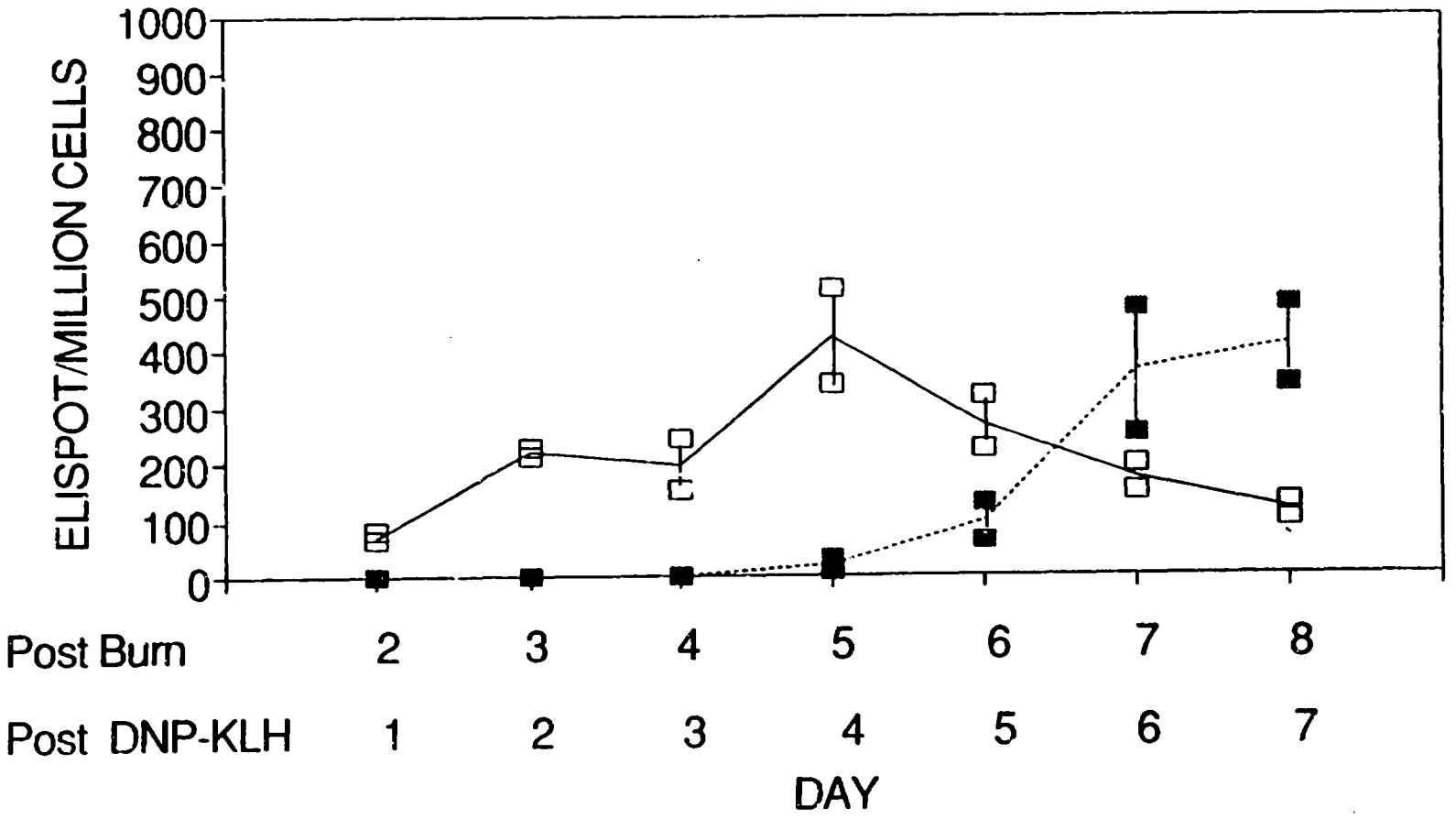

FIGURE 2. IgG (broken line) and IgM (solid line) response for the control group (mean ± SEM).

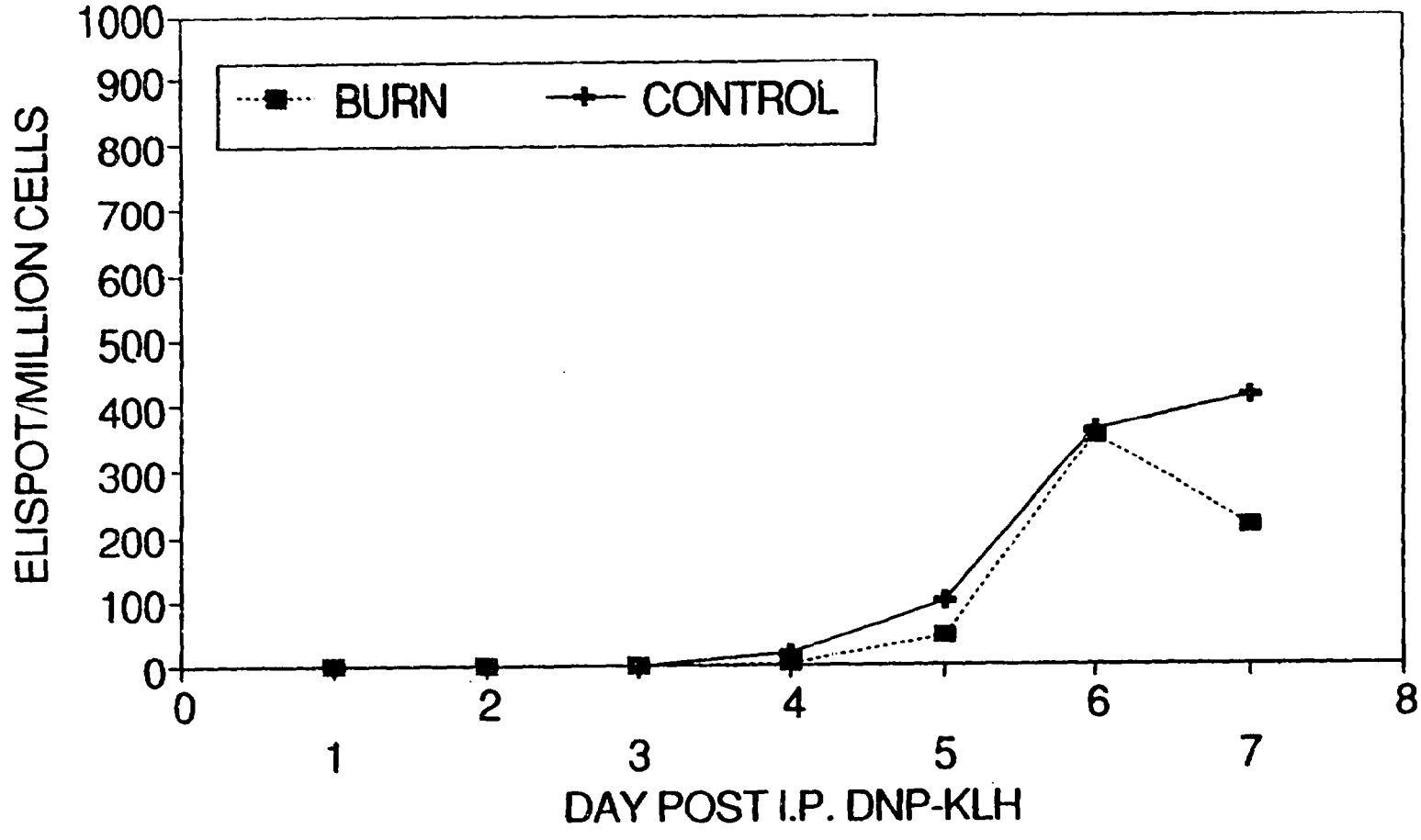

FIGURE 3. IgG response for burn and control groups.

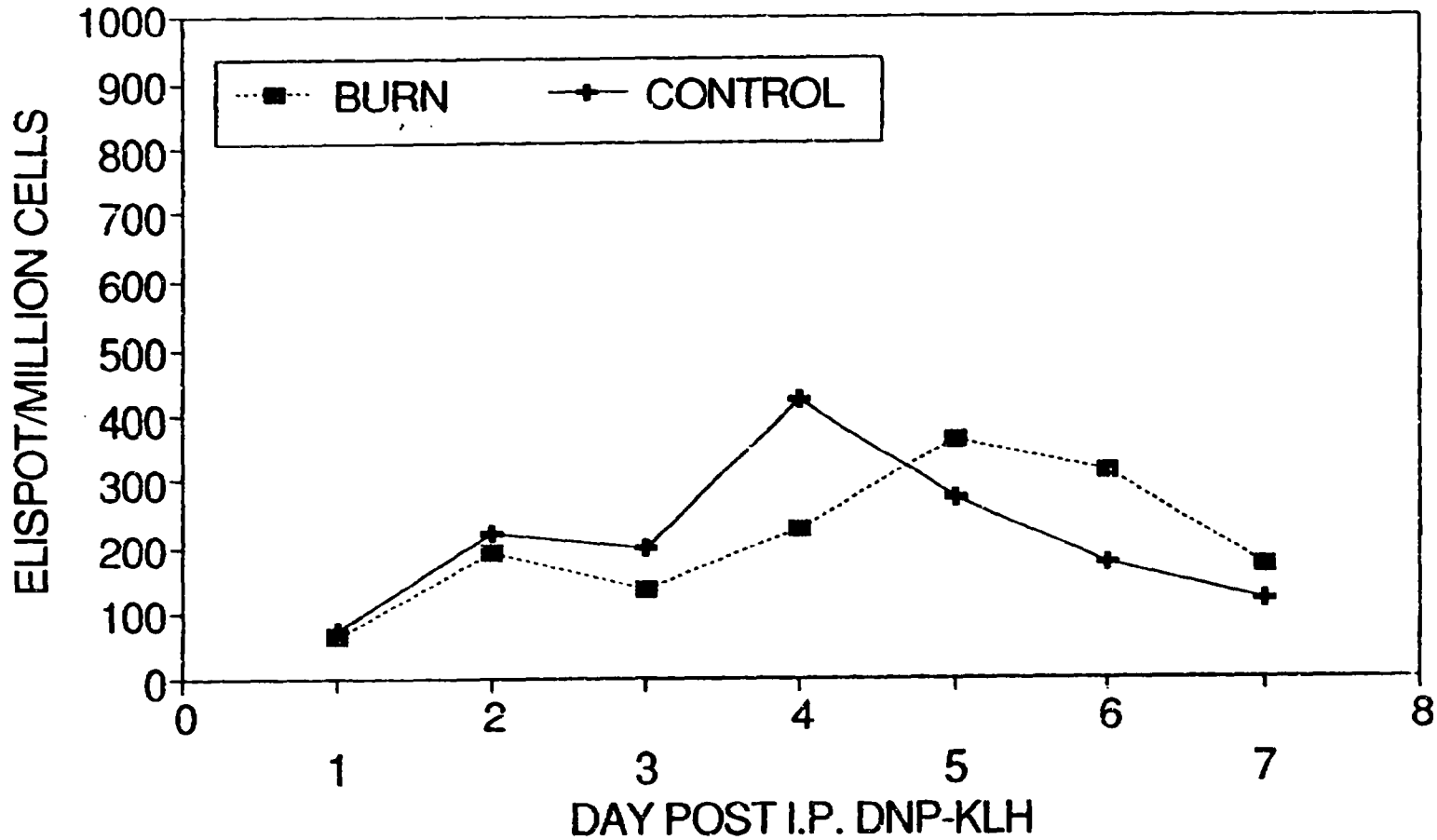

FIGURE 4. IgM response for burn and control groups.

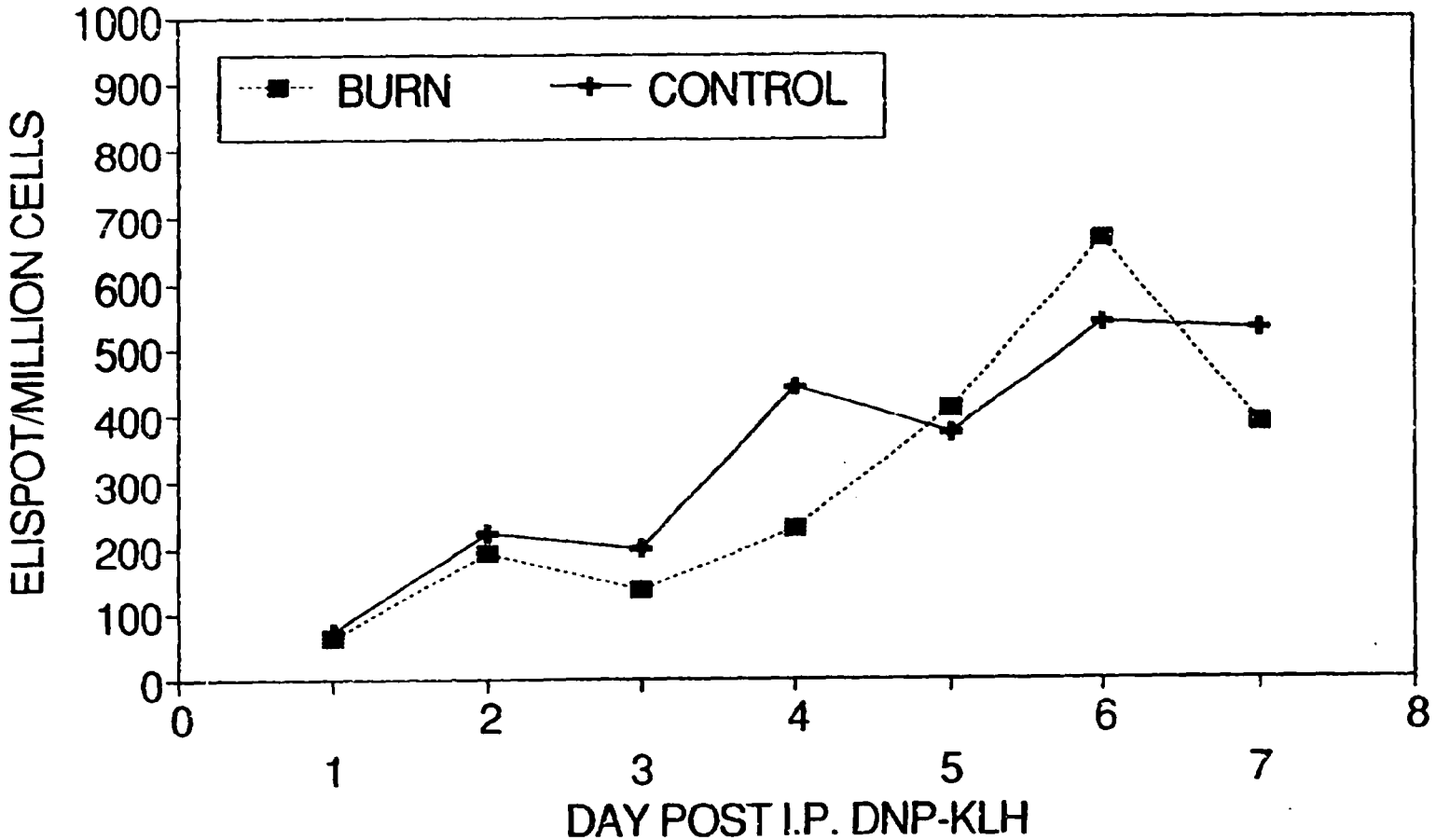

FIGURE 5. Total response for burn and control groups.

RESULTS

The results of preliminary studies to determine the effect of burn injury on the kinetics of the response to DNP-KLH are shown in Table 1 and graphed in Figures 1-5. The kinetics of the primary response to DNP-KLH in the control animals (fig 1) followed a predictable pattern of an increased appearance of IgM-producing plasmacytes followed by a later appearance of IgG-producing plasmacytes. The peak day for the IgM response appeared on day 4 postimmunization. Although the pattern of the response in the burn-injured animals was similar to animals in the control group, the peak IgM response was a day later and appeared to be slightly weaker.

DISCUSSION

The ELISPOT technique may be a sensitive method for delineating the effect of nutritional status and burn injury on the humoral response. Additional experience with the rat model will be needed to further define the primary and secondary response to antigen-hapten complexes. Probably the methods strongest appeal is it allows a transition to the clinical situation.

PRESENTATIONS/PUBLICATIONS

None.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION DA318368	2. DATE OF SUMMARY 01 Oct 91	REPORT CONTROL SYMBOL DD-DR&E(AR) 636
3. DATE PREV SUM'RY 01 Oct 90	4. KIND OF SUMMARY D. Chg	5. SUMMARY SCTY U	6. WORK SECURITY U	7. REGRADING	8. DISP'N INSTR'N CX	9. LEVEL OF SUM A WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS14	FA	307
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code) (U) Interleukin 1 (IL1) Activity in the Serum of Burned Rats and Thermally Injured Patients

12. SUBJECT AREAS
0601 Biochemistry 0605 Medicine and Medical Research

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
8905	9209	DA	C. In-House

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

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

RESOURCES ESTIMATE		
a. FISCAL YEARS	a. PROFESSIONAL WORK YEARS	b. FUNDS (In thousands)
91	1.5	65
92	1.5	70

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME: US Army Institute of Surgical Research	a. NAME: US Army Institute of Surgical Research
b. ADDRESS (Include zip code): Fort Sam Houston, San Antonio, Texas 78234-5012	b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-5012
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR: BURLESON, D G
d. TELEPHONE NUMBER (Include area code): 512-221-2720	d. TELEPHONE NUMBER (Include area code): 512-221-4858
21. GENERAL USE: FIC MILITARY/CIVILIAN APPLICATION: M	e. NAME OF ASSOCIATE INVESTIGATOR (If available): DROST, A C g. NAME OF ASSOCIATE INVESTIGATOR (If available): CIOFFI, W G

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

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

23/24. (U) The objective of this work is to delineate the role of IL1, IL6, and TNF in burn patients, with emphasis on establishing a correlation between serum IL1 levels, IL6, and TNF and the degree of burn injury or infection. The first part of this study includes duplication of the methodology used to detect serum IL1, IL6, and TNF activity in a burned rat model. The second part of the study will involve detection of serum IL1, IL6, and TNF activity in burned patients. If serum IL1, IL6, and TNF activity is significantly increased in burn patients above that of healthy controls, serum IL1, IL6, and TNF activity will be correlated with time postburn, burn size, infection, or other burn-associated manifestations.

25. (U) 9010 - 9109. Twenty-five burn patients and 17 control subjects have been enrolled in the study, with 16 burn patients being enrolled during this reporting period. Plasma IL1ß, TNFα and IL6 were analyzed. IL1ß and IL6 were highest during the first week postinjury and declined over time. Overall, IL1ß and IL6 levels were higher in patients who suffered from infections as compared to patients who remained infection-free. There was no apparent relationship between TNFα levels and infection. IL6 and IL1ß positively correlated with core temperature. Furthermore, there was a positive correlation between IL6 and IL1ß and between IL6 and TNFα, but no correlation between TNFα and IL1ß. Surgery and transfusion did not have an effect on plasma cytokine levels.

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

SUPPLEMENTAL WORK UNIT INFORMATION FOR THE PROJECT ENTITLED "INTERLEUKIN 1 (IL1) ACTIVITY IN THE SERUM OF BURNED RATS AND THERMALLY INJURED PATIENTS"

Subrecord/Linking Accession Number: Not applicable.

Search Control Data: W6L37A/W6L37E, 20 October 1989.

Product Identification: For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report* for fiscal years 1989-91.

Unclassified Special Categories: Volunteers: Adults; RA II.

ABSTRACT

PROJECT NUMBER: 3M161102BS14, 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

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The relationship of plasma cytokine levels to infection, core temperature, transfusion, surgery, and their relationships with each other were examined in patients with thermal injury. IL1β, IL6, and TNF_α were serially measured by ELISA in the plasma of 27 patients with thermal injury and in 16 healthy control subjects. The mean levels of all three cytokines were increased in burn patients who suffered from infection as compared to burn patients who remained infection-free. Mean plasma IL6 levels were higher in samples obtained shortly before infection diagnosis compared to samples taken during infection, after infection, and to samples taken from infection-free burn patients and control subjects. There was no difference in mean IL1β levels before, during, or after infection. There was no apparent relationship between TNF_α levels and infection. IL6 and IL1β positively correlated with core temperature. Furthermore, there was a positive correlation between IL6 and IL1β and between IL6 and TNF_α, but no correlation between TNF_α and IL1β. Surgery or transfusion had no discernable effect on plasma cytokine levels.

These results suggest that IL6 may play a role in the early response to infection during thermal injury. IL1β remains elevated during the course of and after infection without detectable changes over time.

INTERLEUKIN 1 (IL1) ACTIVITY IN THE SERUM OF BURNED RATS AND THERMALLY INJURED PATIENTS

Thermal injury causes increased infection susceptibility which can lead to increased morbidity and mortality. Cytokines, such as IL1β, IL6, and TNF_α, are involved in the response to infection challenge and have been studied in a number of inflammatory diseases (1-6). We previously reported that IL1β and IL6 levels were significantly higher in the plasma of patients with thermal injury than in nonburned control subjects (7). IL1β correlated with burn size and time postburn, whereas IL6 strongly correlated with mortality. TNF_α was not significantly increased in burn patients, but seemed to be transiently elevated in a subpopulation of burn patients.

In the present study, we examined the relationship of all three cytokines with infection occurring after thermal injury. Other clinical events, such as transfusions and surgeries, were also examined. The study was a preliminary attempt to find diagnostic tools for the early detection of infection in severely burned patients.

MATERIALS AND METHODS

Study Participant Data. Seventy-seven burned patients, with burn sizes ranging from 17.5% to 89% and an average age of 35.8 yr (range 21-70) were enrolled in this study. The presence of inhalation injury was not exclusionary. All patients were normotensive and hemodynamically stable after uneventful resuscitation. Of the 27 patients studied, 12 had at least one infection during the study period. There were 7 incidences of pneumonia, 2 of vaginitis, 1 of burn wound invasion, 1 of enterocolitis, and 3 of bacteremia. Other infections, such as bronchitis, urinary tract infections, or cellulitis were not considered to be systemic infections. Control subjects had an average age of 37.9 yr (range 21-58).

Study Procedure. Blood was drawn between 0500 and 0600 h into sterile EDTA blood collection tubes three times a week for 6 weeks. The tubes were centrifuged at 750 rpm for 20 min and a 1-ml aliquot of the plasma was placed into a tube and stored at -70°C until assayed. Platelets were removed by further centrifugation at 11,000 g for 1 min immediately before assaying. Plasma IL1β concentrations were measured in 253 samples from 21 patients. Four hundred and nine samples from 27 patients were tested for TNF_α content and IL6 was measured in 419 samples. The number of IL1β specimens was lower because many samples did not have enough plasma to run all three assays. Seventeen plasma samples from the 16 control subjects were used as controls.

ELISA. Cytokines were measured by ELISA. The IL1β and TNF_α ELISA kits were purchased from Cistron Biotechnology (Pine Brook, NJ) and the IL6 ELISA kits from Genzyme Corporation (Cambridge, MA). All three procedures were sandwich ELISAs in which the molecule of interest was first bound by an immobilized primary monoclonal antibody, washed free of plasma components, and subsequently bound by a rabbit polyclonal antibody. The reactions were amplified using goat anti-rabbit IgG conjugated to horseradish peroxidase and visualized by production of color after the addition of peroxidase substrate. The color intensity was proportional to the amount of bound conjugate, and therefore, to the amount of cytokine present. The color development was measured with a MR600 microplate reader (Dynatech Laboratories, Inc., Alexandria, VA) at 490 nm or 450 nm.

Test Standards. Cytokine concentrations were calculated by comparing their absorbance to the regression line of a standard curve made up of pooled plasma from control subjects enriched with increasing amounts of recombinant human cytokine. Pooled control plasma did not contain detectable concentrations of endogenous cytokines when compared to a bovine serum albumin standard curve. Control plasma 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 different from 0 when their mean absorbance was two standard deviations above that of the nonspecific binding of controls. Pooled control plasma without cytokine added was used to determine nonspecific binding in all ELISAs.

Statistical Analysis. Statistical significance was determined by the Mann-Whitney U test, or Spearman rank correlation when appropriate (BMDP Statistical Software, Los Angeles, CA).

RESULTS

Plasma cytokine levels in 27 burned patients were examined for their relationship with infections. The patient population was divided into patients who became infected and patients who remained infection-free. Table 1 shows that the mean IL6 concentration was significantly higher in 14 infected patients than in 13 patients who remained infection-free ($P < 0.0001$). The mean TNF_α concentration of infected patients was not significantly different from that of infection-free patients or controls. Twelve of 21 patients screened for IL1β developed infections. Their mean IL1β concentration was significantly higher than that of 9 uninfected patients ($P < 0.05$).

Cytokine levels in surviving infected patients were compared to those of nonsurviving infected patients. Table 2 shows that the mean IL6 concentration in patients who became infected and died was significantly higher than that in infected patients who survived ($P < 0.0001$). The mean TNF_α concentration of nonsurviving infected

TABLE 1. Cytokine Levels in Burned Patients With (+) and Without (-) Infection (Mean ± SEM)

Group	IL6 (ng/ml)	IL1β (pg/ml)	TNF_α (pg/ml)
Burn patients			
+ infection	1.096 ± 0.263* (197)	1.869 ± 0.214** (114)	0.879 ± 0.216*** (201)
- infection	0.247 ± 0.026 (222)	1.738 ± 0.195 (140)	1.128 ± 0.313 (208)
Control subjects	0.030 ± 0.015 (17)	0.346 ± 0.025 (17)	0.303 ± 0.303 (17)

( ) indicates number of samples. *P < 0.0001, **P < 0.05, ***not significant. Only infected and uninfected groups were compared statistically.

TABLE 2. Cytokine Levels in Surviving and Nonsurviving Burn Patients with Infection (Mean ± SEM)

Group	IL6 (ng/ml)	TNFα (pg/ml)
Survivors	0.595 ± 0.132 (181)	0.779 ± 0.185 (185)
Nonsurvivors	6.766 ± 2.54* (16)	2.049 ± 1.688** (16)

( ) indicates number of samples. *P < 0.0001, **not significant.

patients was also higher than for surviving infected patients but not statistically different. The number of IL1β samples from nonsurviving patients was too small to be included in this analysis. Because of the difference between these two groups, we will identify subsequent data as being from surviving or nonsurviving patients.

We evaluated the time course of cytokine appearance in infected patients. Only samples from surviving infected patients were included, since the number of plasma samples from nonsurviving patients was too small to be analyzed in this manner. We assigned an 8-day infection window to all surviving infected patients, where day 1 was one day before infection diagnosis. The window was

ascribed with the assumption that invading organisms were present at least one day before the patient symptoms allowed a positive infection diagnosis. The prior 7 days were called preinfection and the recovery period of 8 days following the infection window were defined as postinfection. If a patient developed two or more infections which were diagnosed < 8 days apart, the days between infections were excluded from data analysis. 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 concentration in patients who remained infection-free was significantly lower than in patients in the preinfection stage ($P < 0.05$). IL6 levels of infection-free patients did not differ significantly from IL6 levels of infected patients during or after infections. There was no significant difference in mean TNF_α or IL1β concentrations between any of the groups.

Figure 2 is a temporal profile of plasma IL1β and IL6 levels ± SEM during infections in surviving patients. Infection diagnosis occurred on day 0. Figure 2A shows that there were no sustained changes in IL6 levels during the time period displayed. The mean IL6 concentrations were higher than other periods with greater variation immediately before diagnosis and declined slightly after diagnosis and start of treatment. IL1β, in contrast (fig 2B), increased 3 days before diagnosis and peaked 3 days after infection diagnosis. Plasma Il1β levels remained elevated for almost one week.

The increase of IL6 levels in the nonsurviving infected subpopulation was examined. Figure 3 displays temporal plasma IL6 profiles of 3 burned patients who subsequently died as compared to the mean ± SEM of all infected but surviving patients and controls. Patient 5, who died on postburn day 13, had the highest IL6 levels between postburn days 1 and 5. One day after the plasma IL6 concentration peaked, the patient was diagnosed with pneumonia by x-ray (designated in the figure by an arrow). On postburn day 7, Patient 5 was diagnosed with Aspergillus burn wound invasion. Patient 13 expired on postburn day 18. This patient's IL6 profile peaked on postburn day 3, one day before diagnosis of sepsis due to *Staphylococcus aureus* (designated in the figure by an arrow). On postburn day 10, the patient had a second IL6 peak and underwent surgery within 24 h prior to that measurement. Patient 25 was diagnosed with sepsis on postburn day 3 and died on postburn day 5. Plasma IL6 levels in patients 5 and 13 were lower before they died than when the infections were diagnosed.

We also examined the relationship between cytokine levels and type of infection. Infections were divided into gram-positive, gram-negative, and fungal infections. Only samples taken 8 days before to 8 days after infection diagnosis were included in

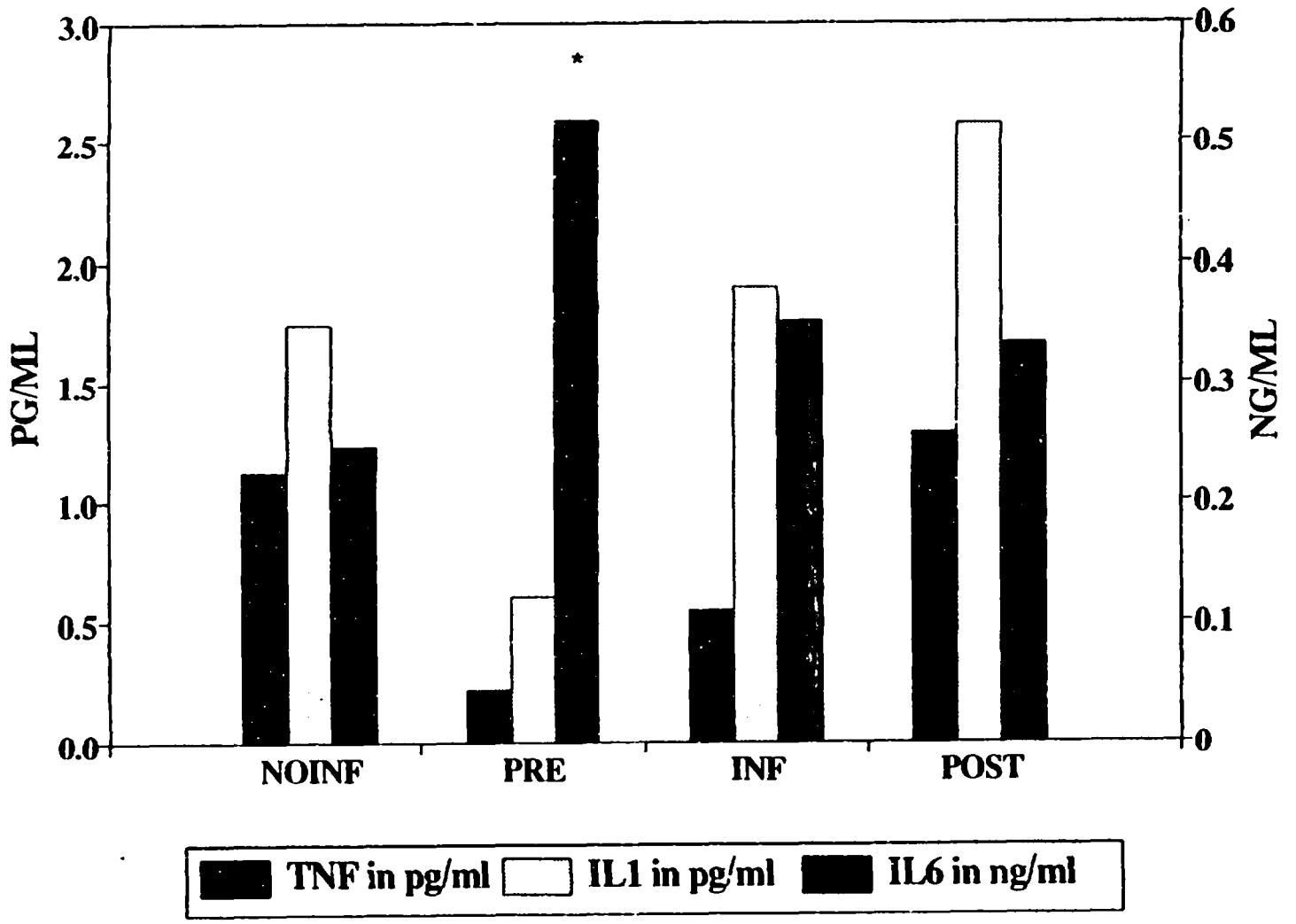

FIGURE 1. Cytokine levels in burned patients with and without (NOINF) infection. NOINF samples were compared to samples from infected patients divided into one of three periods. INF is an 8-day period with day 1 being one day before infection diagnosis, PRE is the 8-day period before INF, and POST reflects an 8-day window after INF. All data are expressed as means ± SEM. **$P < 0.01$.

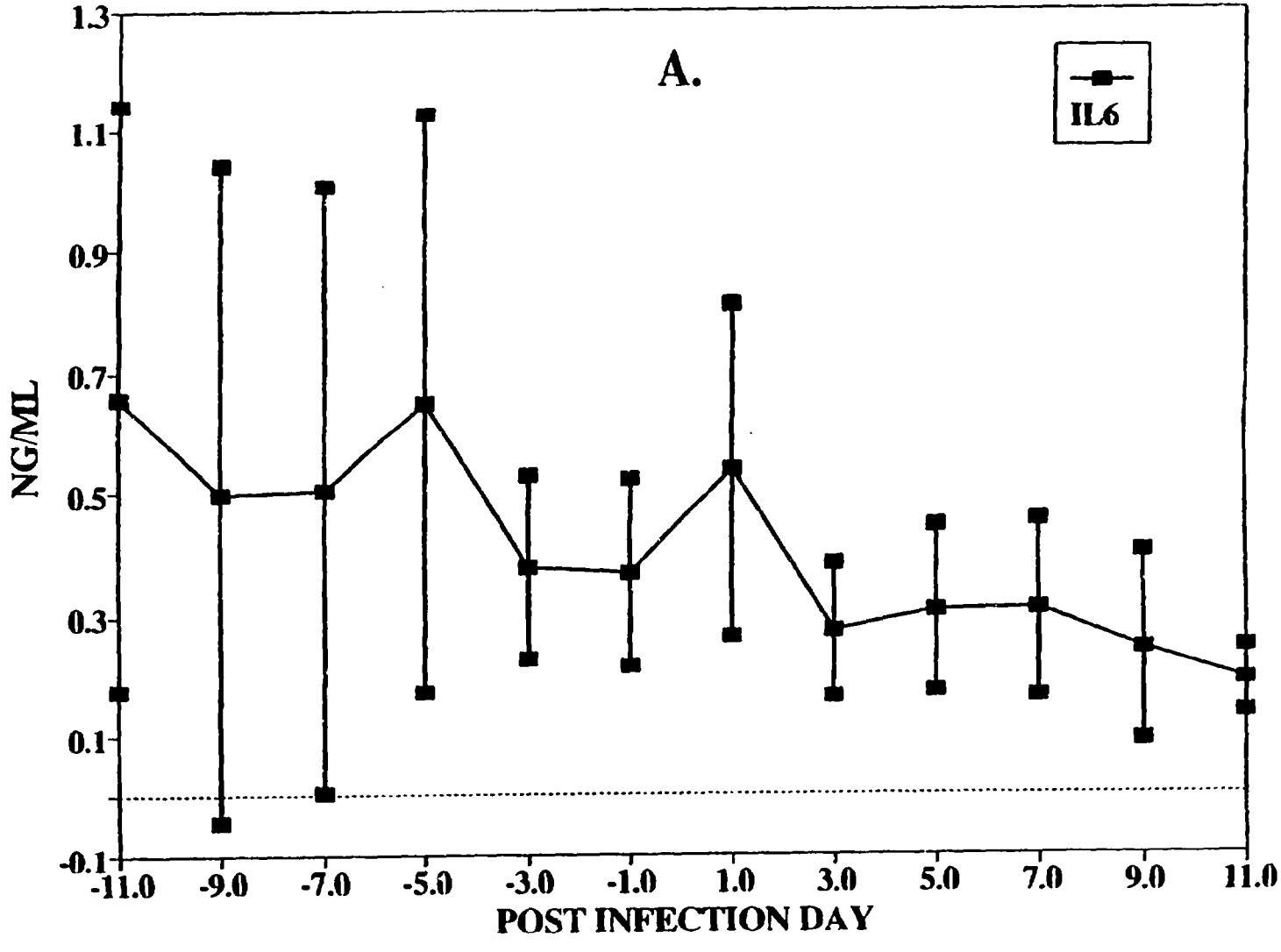

FIGURE 2. Temporal profile of plasma IL6 (A) and IL1β (B) levels during infection. The profiles represent the means ± SEM of samples from different patients on the particular days with respect to infection diagnosis (day 0).

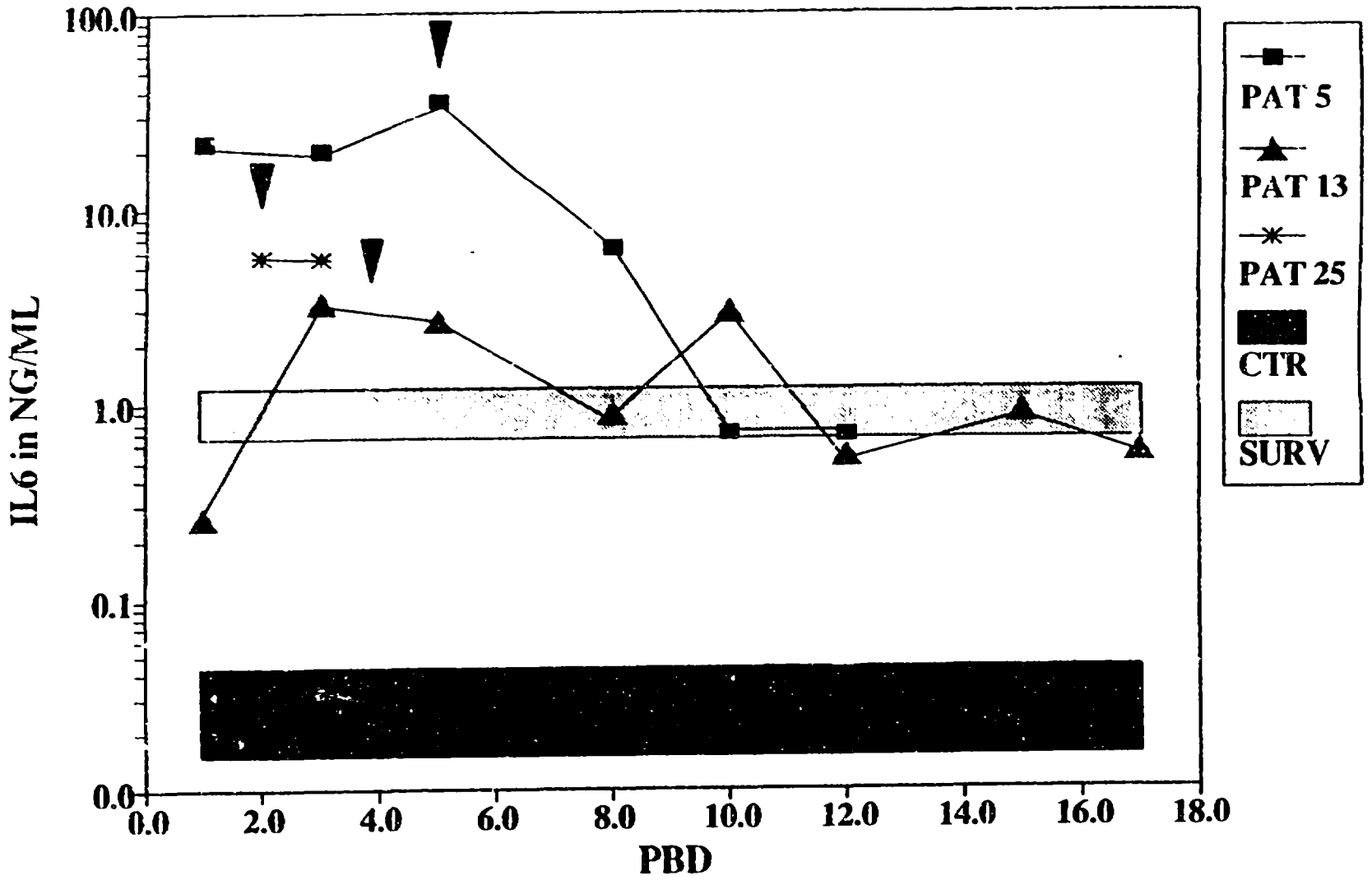

FIGURE 3. Temporal IL6 profiles for 3 nonsurviving burned patients (PAT). Data is expressed on a semilog scale. Arrows indicate the time of infection diagnosis. IL6 concentrations for control subjects (CTR) and surviving burned patients with infection (SURV) are expressed as mean ± SEM.

analyses. Even though there was no statistically significant difference in mean cytokine concentrations bet een gram-positive, gram-negative, or fungal infections (fig 4), mean IL1β and TNF$_\alpha$ levels were numerically greater in bacterial than in fungal infections. The mean IL6 level in patients with gram-negative infections was higher than those in patients with gram-positive or fungal infections.

Relationship of Plasma Cytokines with Each Other. Cytokines levels are interrelated, i.e., IL6 may be induced by IL1β or TNF$_\alpha$. We looked for a possible relationship among plasma cytokines by stepwise linear regression. There was a positive correlation between IL6 and IL1β ($r = 0.4732$, $P < 0.0001$). A weak positive correlation existed between IL6 and TNF$_\alpha$ ($r = 0.2458$, $P < 0.0001$), but TNF$_\alpha$ and IL1β did not correlate significantly.

Effects of Surgery and Transfusions on Plasma Cytokine Levels. Plasma from patients undergoing surgery within 24 h before sample collection were compared to all other plasma samples. Cytokine levels from patients undergoing surgery did not differ significantly from samples taken at all other times.

Another potential trigger for changes in plasma cytokine levels are blood transfusions. There was no significant difference between plasma cytokine levels of samples drawn within 24 h of transfusion and samples collected at other times.

Plasma Cytokines and Fever. The relationship between plasma cytokine levels and core temperature was examined and the results are shown in Figure 5. Rectal temperatures were taken routinely 3-4 h before plasma samples were drawn. Plasma IL6 and IL1β levels positively correlated with core temperature ($r = 0.1656$ and $r = 0.2456$, respectively). There was no significant correlation between plasma TNF$_\alpha$ levels and core temperature. The percentage of positive IL6 and IL1β samples as defined by increases above the 95% confidence level of controls increased with increasing temperature (fig 5). These data affirm previous reports of pyrogenic activities of IL6 and IL1β.

DISCUSSION

The results of this study suggest that plasma IL6 levels are related to systemic bacterial infections. The relationship of IL1β to infection remains less clear. TNF$_\alpha$ does not appear to be altered by postburn infections.

The magnitude of plasma IL6 increases during infections was more than 10-fold higher in patients who subsequently died than in surviving patients. It remains unknown whether these increased IL6 levels in nonsurviving patients were caused by infections or whether they were due to other events occurring soon after burn

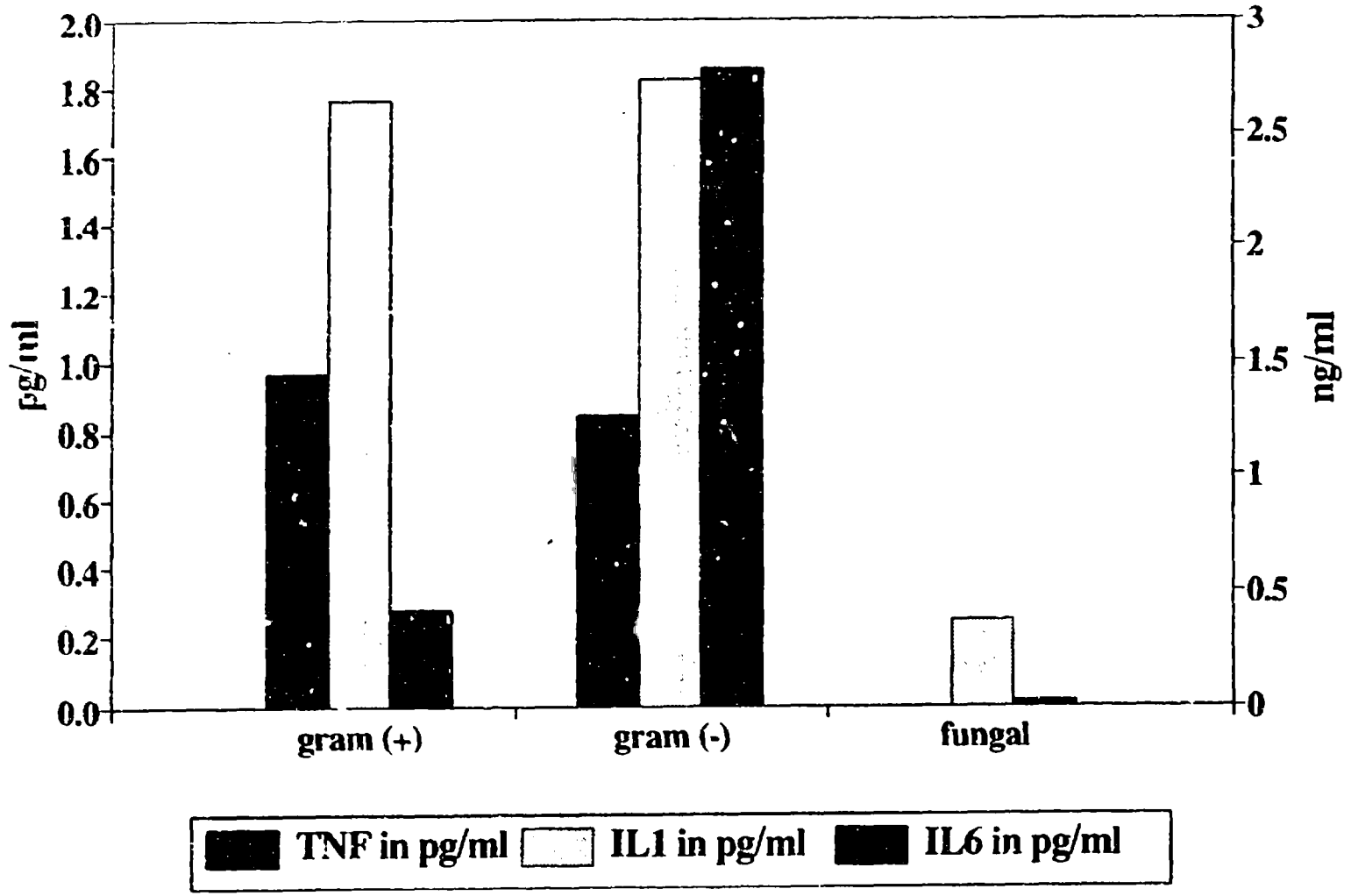

FIGURE 4. Effects of different types of infection on plasma cytokine levels. Plasma samples from burned patients with infection during the infectious period (8 days before to 8 days after infection diagnosis) were grouped by the type of infection and mean cytokine levels were compared.

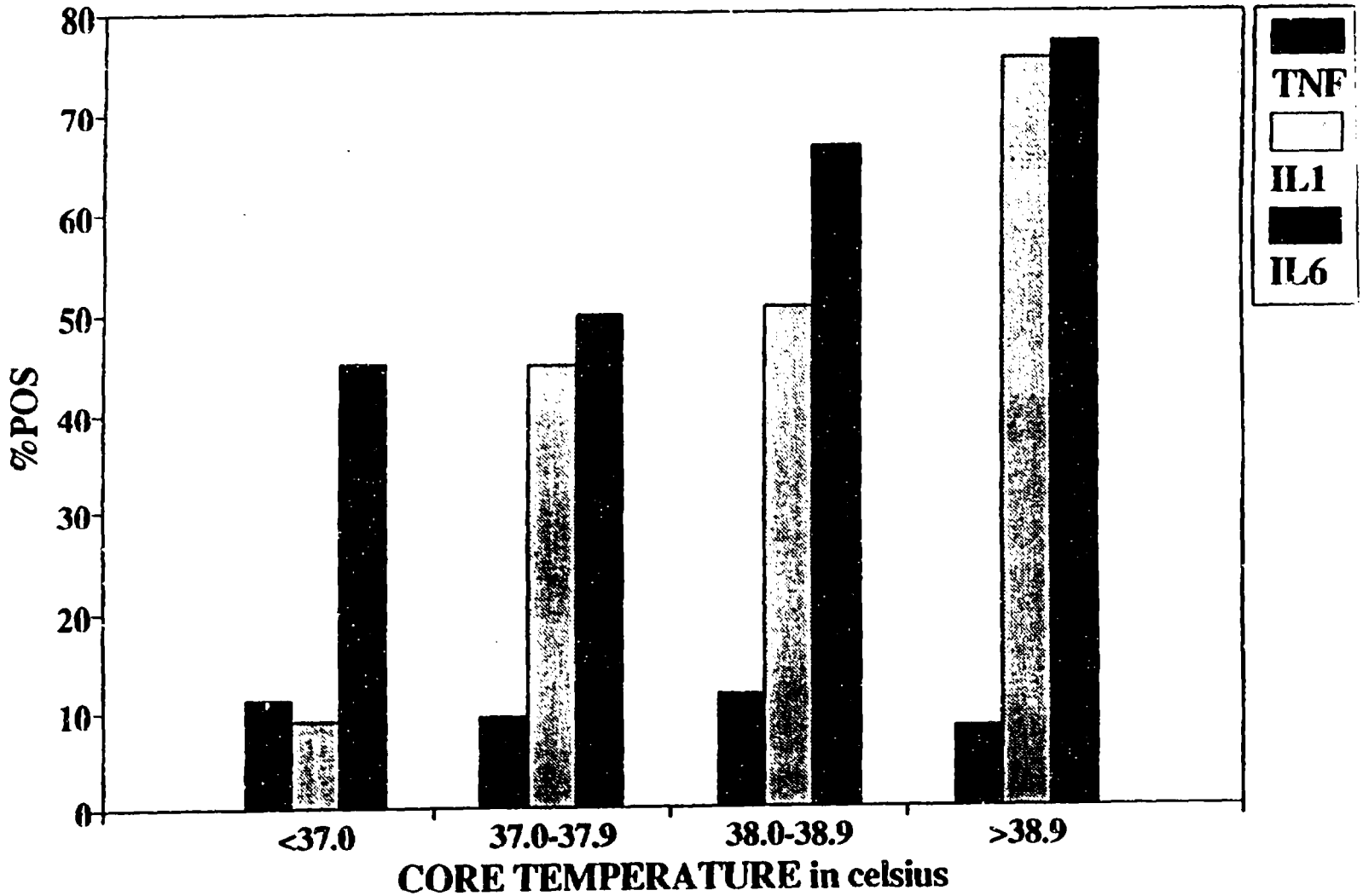

FIGURE 5. Cytokine levels and core temperature in burned patients. Cytokine values were grouped by core temperature as displayed. Bars represent the percentage of samples with cytokine concentrations > 95% confidence level of controls.

injury. We have previously reported that plasma IL6 is elevated soon after burn injury (7).

There is a relationship between IL6 levels and the time before infection diagnosis in surviving patients. IL6 plasma levels were highest shortly before the clinical diagnosis of infection. Considering that some of the infection process precedes the clinical diagnosis of infection, it is not surprising that increased levels of IL6 precede infection diagnosis. Our data would support the findings of Fong et al (8) who injected healthy volunteers with a single intravenous bolus of endotoxin. In their study, plasma IL6 levels peaked 2-4 h after injection, with typical ranges of 4-25 ng/ml. This IL6 concentration range is similar to that reported in this study.

Furthermore, our data indicate that plasma IL6 levels may have some utility in the diagnosis of systemic bacterial infection. Since the increases in plasma IL6 were not always present before infection diagnosis, these routine IL6 plasma measurements would have to be used in conjunction with other early infection indications.

Fong et al (9) have also reported the measurement of circulating IL1β, IL6, and TNF_α levels following intra-aortic infusion of live *Escherichia coli* in 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 indicated that IL1β appears early after infusion followed by IL6. Our findings confirm the increase of both cytokine concentrations during infections, but we observed an increase in IL6 before IL1β. Since we measured cytokine levels only three times a week, we may not have been able to see hourly fluctuations observed by Fong et al (9). Furthermore, the discrepancy may be due to increased variability in a patient study group compared to the controlled baboon study.

A number of investigators have reported increased circulating TNF_α following infectious diseases, sepsis, septic shock, and even thermal injury (2-4,8). Our results are in variance with those findings. Possible explanations for the discrepancy include the use of a different assay, or a different sample source, i.e., serum rather than plasma. Marano et al (10) measured TNF_α in the serum of burned patients and found a positive correlation between TNF_α and sepsis. Our data included only 2 bacteremic patients and 10 patients with other infections, and thus, it is not surprising that the results differ. In fact, Offner et al (4) reported that serum TNF_α levels in patients with septic shock were higher than those in patients who had infections which did not evolve toward sepsis.

We observed that the cytokine concentrations did not differ between different types of infections; but in general, it appeared that bacterial infections led to higher plasma cytokine levels than

fungal infections, e.g, vaginitis. Hack et al (1) examined cytokine levels in patients with sepsis. He reported that there was no difference in plasma IL6 levels whether the infection was gram-negative or gram-positive or whether double infection was present.

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 (11-13). Further studies examining the close time course of cytokine appearance following thermal injury will be necessary to confirm the interaction of these three cytokines.

There was no correlation between plasma cytokine levels and surgery or transfusions. In contrast, Cruickshank et al (14) reported that serum IL6 rose within 2-4 h of incision in patients undergoing elective surgery of varying severity. The response of IL6 correlated with the duration of surgery. One explanation for this discrepancy with our data may be that we did not measure IL6 levels immediately before, during, or after surgery. Our samples were drawn within 24 h of surgery, by which time the IL6 levels may have returned to more normal levels, especially in some patients undergoing minor surgical procedures.

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 our study. This probably accounts for the difference of data. The positive correlation of IL1β and core temperature was expected, as IL1 has been long known as an endogenous pyrogen (16).

PRESENTATIONS/PUBLICATIONS

Burleson DG: The relationship between serum lymphokine levels, burn size, and time postinjury. Presented at the 2nd International Congress on Immune Consequences of Trauma, Shock, and Sepsis, Munich, Germany, 7 March 1991.

Drost AC: Plasma interleukin-1 and interleukin-6 concentrations following thermal injury and their relationship to patient survival. Presented at the 2nd International Congress on Immune Consequences of Trauma, Shock, and Sepsis, Munich, Germany, 8 March 1991.

Drost AC: Interleukin-1-beta (IL-1-beta) measured by ELISA in plasma from patients with thermal injury. Presented at the Joint Meeting of the American Society for Biochemistry and Molecular Biology and the American Association of Immunologists, New Orleans, Louisiana, 6 June 1990.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
				DA313583	01 Oct 91	DD-DR&E(AR) 636
3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISP'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
01 Oct 90	D. Chg	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS14	CK	309
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE *(Precede with Security Classification Code)*
(U) Caloric Requirements of Thermally Injured Children

12. SUBJECT AREAS
0605 Medicine and Medical Research

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
8710	9209	DA	C. In-House

17. CONTRACT/GRANT MILITARY RELEVANCY CERTIFIED / RESOURCES ESTIMATE

a. DATE EFFECTIVE APPROVED BY Donald Dutton	FISCAL YEARS	a. PROFESSIONAL WORK YEARS	b. FUNDS *(In thousands)*
b. CONTRACT/GRANT NUMBER	91	0.5	23
c. TYPE / d. AMOUNT	92	0.5	24
e. KIND OF AWARD / f. CUM/TOTAL			

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME: US Army Institute of Surgical Research	a. NAME: US Army Institute of Surgical Research
b. ADDRESS *(Include zip code)*: Fort Sam Houston, San Antonio, Texas 78234-5012	b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-5012
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR: CIOFFI, W G
d. TELEPHONE NUMBER *(Include area code)*: 512-221-2720	d. TELEPHONE NUMBER *(Include area code)*: 512-221-8440
21. GENERAL USE: FIC MILITARY/CIVILIAN APPLICATION: M	f. NAME OF ASSOCIATE INVESTIGATOR *(If available)*: MILNER, E A g. NAME OF ASSOCIATE INVESTIGATOR *(If available)*: MC MANUS, W F

22. KEYWORDS *(Precede EACH with Security Classification Code)* (U) Burns (Injuries); (U) Nutrition; (U) Metabolism; (U) Therapy

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

23/24. (U) The objective of this work is to define optimal nutritional support for the burned child. On the fifth postburn day, the REE will be calculated as will the RQ. Baseline laboratory data will be collected and liver function and partial thromboplastin time tests will be performed. The patient will then be begun on alimentation that will be adjusted every . days until the caloric need is determined and met for two successive, 3-day cycle measurements as determined by a positive nitrogen balance, a RQ between 0.85 and 1.0, and a caloric intake equal to 1.25 X REE.

25. (U) 9010 - 9109. No suitable pediatric patients were admitted to the Institute during this reporting period.

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SUPPLEMENTAL WORK UNIT INFORMATION FOR THE PROJECT ENTITLED "CALORIC REQUIREMENTS OF THERMALLY INJURED CHILDREN"

Subrecord/Linking Accession Number: Not applicable.

Search Control Data: W6L57B/W6M03C, 20 October 1989.

Product Identification: Not applicable.

Unclassified Special Categories: Volunteers: Children; RA II.

ABSTRACT

PROJECT NUMBER: 3M161102BS14, 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 1990 - 30 September 1991

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 US Army Institute of Surgical Research Human Use Committee on 9 October 1987. No suitable pediatric patients were admitted to the Institute during this reporting period. Patients will be asked to enroll in the study as they become available.

CALORIC REQUIREMENTS OF THERMALLY INJURED CHILDREN

The optimum nutritional support program for the thermally injured child has not been determined. The caloric requirements of a burned child are only marginally estimated by the existing formulas. The Curreri formulas, the Harris-Benedict equations, and the Wilmore nomograms all differ in their estimation of the caloric requirements for children, e.g., a 2-yr-old girl weighing 12 kg (50th percentile) and measuring 86 cm in length (50th percentile) who has sustained a 40% total body surface area burn will have an estimated daily caloric requirement of 2,120 kcal by the original Curreri formula for children or 2,200 kcal by the Curreri "Junior" formula for 1- to 3-yr-olds, 1,839 kcal by the Harris-Benedict equation, and 1,600 kcal by the Wilmore nomograms. Determination of adequate nutritional support is important since inadequate caloric intake may result in protein wasting and malnutrition, whereas excess caloric intake can result in fatty infiltration of the liver, the fat intoxication syndrome, dehydration secondary to hyperglycemia and glucosuria, and excess carbon dioxide production with subsequent ventilator weaning failure. All of these potential problems could be avoided by the administration of the correct number of calories distributed between protein, fat, and carbohydrate.

Nitrogen requirements in thermally injured patients are increased over those in uninjured people. Numerous studies have demonstrated that injured, hypermetabolic patients demonstrate ineffective utilization of administered protein and have an optimum nitrogen to calorie ratio between 1:135 and 1:200 (grams nitrogen to nonprotein kilocalories). An optimum ratio of 1:150 has been recommended by Goodwin (3). Larger amounts of protein create a progressively more positive nitrogen balance but have not been shown to improve survival (7).

The role of fat as a source of nonprotein calories is dependent upon the extent of injury and the other nutrients administered. When diets lacking in protein are used, carbohydrate is more effective in sparing body protein than fat. However, when a "balanced" alimentation regimen containing protein, fat, and carbohydrate is devised, consideration is given to the administration of sufficient calories as fat not only to prevent essential fatty acid deficiency, but also to supply a large number of calories. Fat administration in excess of 3 g/kg/day in normal infants and 4 g/kg/day in normal adults can produce a fat overload syndrome (4). This has been described as consisting of hyperlipidemia, coagulopathy, fever, cholestatic jaundice, and gastrointestinal distress. This syndrome is believed to occur when the rate of infusion exceeds the maximum rate of peripheral clearance.

Studies comparing the utilization of fat and carbohydrate as energy sources have been undertaken in unburned surgical patients. A controlled study by MacFie et al (6) demonstrated that the administration of as little as 17% of calories as fat can reduce the loss of lean body tissue and the accumulation of body fat which is seen when glucose is used as the sole nonprotein energy source. A positive nitrogen balance has been achieved in postoperative patients with regimens supplying 33-38% of nonprotein calories from intravenous fat (9). The fat infusions depressed the 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 will be enrolled in the study. Properly signed and witnessed DA Forms 5303-R, Volunteer Agreement Affidavit, will be obtained for each patient prior to enrollment in the study.

Patient Inclusion Criteria. Patients meeting the following criteria will be eligible for enrollment in the study:

1. Patients admitted to the US Army Institute of Surgical Research with burn injury.

2. Male or female patients < 13 yr old.

3. Patients with burn wounds > 30% of the total body surface area.

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

1. Patients ≥ 13 yr old.

2. Patients with burn wounds < 30% of the total body surface area.

3. Patients with electrical injury.

4. Patients with fractures or major associated injuries.

5. Patients with inhalation injury.

6. Patients who are wards of the state or any other agency, institution, or entity.

Patient Assent. For children from 6–12 yr old, judgment by the primary investigator and the attending surgeon will be made as to whether the child is capable of assent. In determining whether the child is capable of assent, the primary investigator and the attending surgeon will take into account the age, maturity, and psychological state of the child involved. This judgment will be made for each child. If it is deemed that the child is capable of assent, then the research protocol will be explained to that child in terms that he/she will understand. The child will then be enrolled in the study if his/her assent is given and permission is obtained from the child's parent or legal guardian. If it is deemed that the child is not capable of assent or if the child is ≥ 5 yr of age or younger, then permission will be obtained from the child's parent or legal guardian only.

Study Procedures. On the fifth postburn day, each patient will be transported to the Metabolic Room on Ward 14A prior to the morning dressing change. Oxygen consumption and carbon dioxide production will be measured using the Horizon™ metabolic cart. The environment temperature and humidity will be maintained constant throughout each patient's stay in the Metabolic Room. The REE will be calculated as will the RQ. Baseline laboratory data will include serum electrolytes, creatinine, cholesterol, triglycerides, platelet count, prothrombin time, ketone, and insulin values. Liver function and partial thromboplastin time tests will also be performed. These serum laboratory values will be repeated at the time of each subsequent trip to the Metabolic Room for further measurements. All measurements in the Metabolic Room will take place prior to the morning dressing change. The patient's height and baseline weight will be determined upon admission. Weights will be obtained on a daily basis.

The patient will then be begun on alimentation using either parenteral hyperalimentation or enteral feeding. If possible, enteral feedings will be used to supply the patient's nutrition. If the patient's gastrointestinal tract is not capable of tolerating enteral feedings for any reason, intravenous hyperalimentation will be employed. The total calorie requirement will be based upon the lowest estimated caloric need as calculated from the Wilmore nomograms, the Curreri formulas, and the Harris-Benedict equations. Nitrogen administration will be calculated to produce a 1 g nitrogen to 150 nonprotein kilocalorie ratio. Lipids will be administered at a rate of 3 g/kg/day. Electrolyte composition of the fluids will be adjusted to the patient's needs. Each patient will receive standard vitamin and mineral supplements.

Once the patient's intake has reached the projected requirements and has remained stable for 3 days, the patient will be transported to the Metabolic Room where oxygen consumption and carbon dioxide production will again be measured. A 24-h urine collection will be obtained on that day as well. From this data,

the RQ and REE will calculated. The grams of totally metabolized nitrogen, carbohydrate, and fat as well as the nitrogen balance will also be calculated.

Based on the new RQ, REE, and nitrogen balance measurements, the caloric requirements will be recalculated. If the RQ value is < 0.85, the total number of calories will be increased by 10%, maintaining the 1:150 gram of nitrogen to kilocalories ratio and the 3 g/kg/day lipid infusion rate. If the RQ is > 1.0, nitrogen, carbohydrate, and fat will be examined in an effort to determine which component or components (protein, carbohydrate, fat) should be reduced in order to decrease the total number of calories by 10% (2).

After a 3-day stabilization period, these metabolic measurements will be rechecked and again the caloric intake adjusted to bring the RQ to between 0.85 and 1.0 and to keep the nitrogen balance positive. This 3-day cycle will be repeated until the caloric need is determined and met for two successive, 3-day cycle measurements. This will be determined by a positive nitrogen balance, a RQ between 0.85 and 1.0, and a caloric intake equal to 1.25 X REE. Caloric needs shall be redetermined following any operative procedure after a 3-day stabilization period. During these days, alimentation will be maintained at the preoperative level.

RESULTS

This project was approved by the US Army Institute of Surgical Research Human Use Committee on 9 October 1987. No suitable pediatric patients were admitted to the Institute during this reporting period. Patients will be asked to enroll in the study as they become available.

DISCUSSION

When 20 patients have completed the study, the data will be analyzed to determine the optimum nutritional support program for the thermally injured child.

PRESENTATIONS/PUBLICATIONS

None.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
				DA314706	01 Oct 91	DD-DR&E(AR) 636
3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISP'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
01 Oct 90	D. Chg	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS14	AZ	310
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)
(U) Salt and Water Balance in the Thermally Injured Patient

12. SUBJECT AREAS
0605 Medicine and Medical Research

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
8805	9209	DA	C. In-House

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

18. RESOURCES ESTIMATE

a. FISCAL YEARS	a. PROFESSIONAL WORK YEARS	b. FUNDS (In thousands)
91	0.5	23
92	0.5	24

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME: US Army Institute of Surgical Research	a. NAME: US Army Institute of Surgical Research
b. ADDRESS (Include zip code): Fort Sam Houston, San Antonio, Texas 78234-5012	b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-5012
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR: CIOFFI, W G
d. TELEPHONE NUMBER (Include area code): 512-221-2720	d. TELEPHONE NUMBER (Include area code): 512-221-8440
21. GENERAL USE: FIC MILITARY/CIVILIAN APPLICATION: M	e. NAME OF ASSOCIATE INVESTIGATOR (If available): VAUGHAN, G M g. NAME OF ASSOCIATE INVESTIGATOR (If available): HEIRONIMUS, J D

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Burns (Injuries); (U) Hormones; (U) Peptides; (U) Renin; (U) Aldosterone; (U) Angiotensin; (U) Inulin;

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

23/24. (U) The objective of this work is to describe the alterations of plasma levels of ADH, atrial natriuretic peptide, and the renin-angiotensin-aldosterone axis following thermal injury as related to plasma volume, osmolality, and tonicity. Twenty consecutive patients will be entered into this study. On postburn days 2, 5, and 10, intravascular volume measurements will be made utilizing chromium-labeled RBCs to measure red cell volume. Also on postburn day 5, the glomerular filtration rate will be measured utilizing inulin and a radiopharmaceutical. Effective renal plasma flow will be measured using a colorimetric hippurate method. The two methods will then be compared.

25. (U) 9010 - 9109. Fourteen burn patients and 10 control subjects have been enrolled in the study to date, 4 burn patients during this reporting period. Data analysis revealed that despite a consistent hyperdynamic state documented by increased cardiac output and renal plasma flow, the 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.

DD FORM 83 MAR 1498 EDITION OF MAR 88 IS OBSOLETE. * U.S.G.P.O. 1988-691-003/50329

SUPPLEMENTAL WORK UNIT INFORMATION FOR THE PROJECT ENTITLED "SALT AND WATER BALANCE IN THE THERMALLY INJURED PATIENT"

Subrecord/Linking Accession Number: Not applicable.

Search Control Data: W6M09B/W6MJ0A, 20 October 1989.

Product Identification: For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report* for fiscal years 1988-91.

Unclassified Special Categories: Volunteers: Adults; RA II.

ABSTRACT

PROJECT NUMBER: 3M161102BS14, Research

PROJECT TITLE: Salt and Water Balance in the Thermally Injured Patient

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

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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 [$a_1\exp(b_1 \cdot \text{time}) + a_2\exp(b_2 \text{time})$] 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 $TBSA^{1.289}$; 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, †$P < 0.05$, ‡$P < 0.01$, §$P < 0.001$.

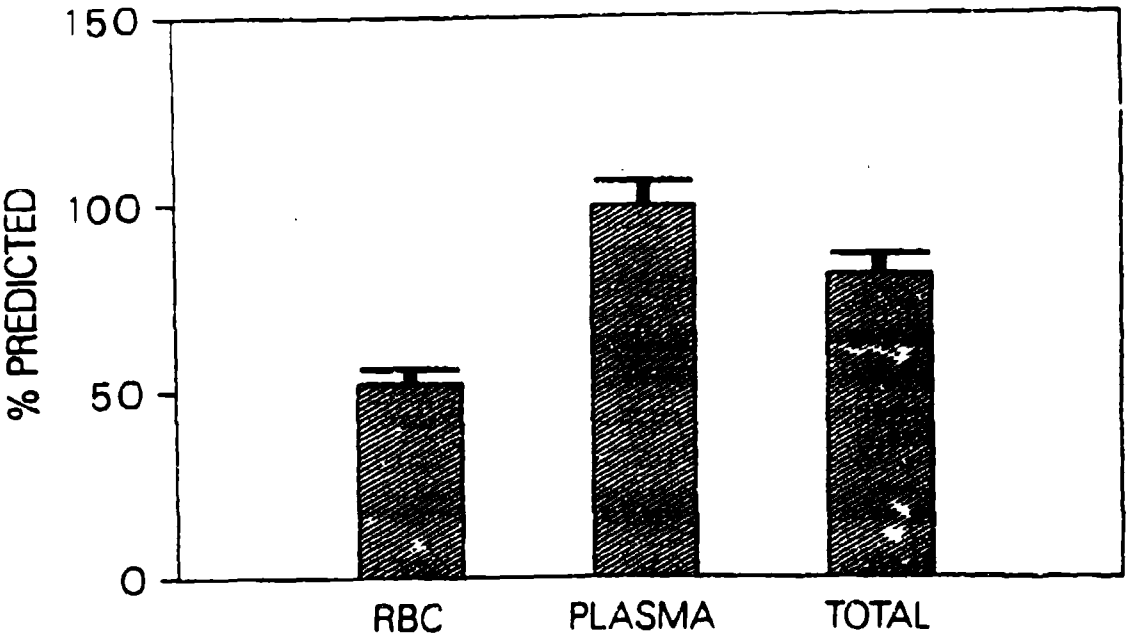

FIGURE 1. The percentage of predicted RBC volume, plasma volume, and total blood volume for all patients is depicted. RBC and total blood volumes differ significantly from predicted values ($P < 0.01$) while calculated plasma volume was normal.

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

Cioffi WG Jr: Dissociation of blood volume and flow in regulation of salt and water balance in burn patients. Presented at the 111th Annual Meeting of the American Surgical Association, Boca Raton, Florida, 11 April 1991.

PUBLICATIONS

Cioffi WG Jr, Vaughan GM, Heironiums JD, Jordan BS, Mason AD Jr, and Pruitt BA Jr: Dissociation of blood volume and flow in regulation of salt and water balance in burn patients. *Ann Surg* 214(3):213-20, September 1991.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION DA315349	2. DATE OF SUMMARY 01 Oct 91	REPORT CONTROL SYMBOL DD-DRA&MAR) 636
3. DATE PREV SUM'RY 01 Oct 90	4. KIND OF SUMMARY D. Chg	5. SUMMARY SCTY U	6. WORK SECURITY U	7. REGRADING	8. DISP'N INSTR'N CX	9. LEVEL OF SUM A. WORK UNIT

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS14	EA	311
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code) (U) Effect of Growth Factors on the Healing of Partial-Thickness Scald Wounds in the Guinea Pig

12. SUBJECT AREAS
0604 Anatomy and Physiology 0605 Medicine and Medical Research

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
8701	9209	DA	C. In-House

17. CONTRACT/GRANT MILITARY RELEVANCY CERTIFIED. RESOURCES ESTIMATE
APPROVED BY

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

(a) FY	a. PROFESSIONAL WORK YEARS	b. FUNDS (In thousands)
91	0.5	32
92	0.5	33

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME: US Army Institute of Surgical Research	a. NAME: US Army Institute of Surgical Research
b. ADDRESS (Include zip code): Fort Sam Houston, San Antonio, Texas 78234-5012	b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-5012
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR: CIOFFI, W G
d. TELEPHONE NUMBER (Include area code): 512-221-2720	d. TELEPHONE NUMBER (Include area code): 512-221-8440
21. GENERAL USE: FIC MILITARY/CIVILIAN APPLICATION: M	e. NAME OF ASSOCIATE INVESTIGATOR (If available) g. NAME OF ASSOCIATE INVESTIGATOR (If available)

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Burns (Injuries); (U) Contraction; (U) Healing; (U) Therapy; (U) Histology

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

23/24. (U) The objective of this work is to determine whether infusion of a recently described copolymer will enhance burn epithelization in a deep partial-thickness burn wound in the guinea pig. After receiving deep partial-thickness burns, male guinea pigs will receive either saline or the copolymer. In the first phase, guinea pigs will be sacrificed at 72 h and the effects of the copolymer on the zone-of-stasis studied histologically. In the second phase, guinea pigs will be sacrificed at 5, 10, and 20 days following injury and the effects of the copolymer on reepithelization and hair follicle survival will be measured. The extent of healing by contraction will be assessed by planimetry and the extent of reepithelization will be assessed histologically.

25. (U) 9010 - 9109. 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.

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

SUPPLEMENTAL WORK UNIT INFORMATION FOR THE PROJECT ENTITLED "EFFECT OF GROWTH FACTORS ON THE HEALING OF PARTIAL-THICKNESS SCALD WOUNDS IN THE GUINEA PIG"

Subrecord/Linking Accession Number: DA312335.

Search Control Data: W6M16F/W6M17E, 20 October 1989.

Product Identification: For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report* for fiscal years 1987-91.

Unclassified Special Categories: Lab Animals: Guinea Pigs; RA II.

ABSTRACT

PROJECT NUMBER: 3M161102BS14, 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 1990 - 30 September 1991

INVESTIGATORS: William G. Cioffi, Jr., MD, Major, MC
Chi-Sing Chu, MD
Carlin V. Okerberg, DVM, PhD, Lieutenant Colonel, MC
Arthur D. Mason, Jr., MD
Basil A. Pruitt, Jr., MD, Colonel, MC

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				1. AGENCY ACCESSION DA315350	2. DATE OF SUMMARY 01 Oct 91	REPORT CONTROL SYMBOL DD-DR&E(AR) 636
3. DATE PREV SUM'RY 01 Oct 90	4. KIND OF SUMMARY D. Chg	5. SUMMARY SCTY U	6. WORK SECURITY U	7. REGRADING	8. DISP'N INSTR'N CX	9. LEVEL OF SUM A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS14	CH	312
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code) (U) Cellular Host Defense Function After Thermal Injury: Assessment by Flow Cytometry of Peripheral Blood Cells

12. SUBJECT AREAS
0601 Biochemistry 0605 Medicine and Medical Research

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
8605	9209	DA	C. In-House

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

a. DATE EFFECTIVE		18. RESOURCES ESTIMATE		
b. CONTRACT/GRANT NUMBER		a. FISCAL YEARS	a. PROFESSIONAL WORK YEARS	b. FUNDS (In thousands)
c. TYPE	d. AMOUNT	91	0.5	42
e. KIND OF AWARD	f. CUM/TOTAL	92	0.5	43

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME: US Army Institute of Surgical Research	a. NAME: US Army Institute of Surgical Research
b. ADDRESS (include zip code): Fort Sam Houston, San Antonio, Texas 78234-5012	b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-5012
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR: BURLESON, D G
d. TELEPHONE NUMBER (Include area code): 512-221-2720	d. TELEPHONE NUMBER (Include area code): 512-221-4858
21. GENERAL USE: FIC MILITARY/CIVILIAN APPLICATION: M	f. NAME OF ASSOCIATE INVESTIGATOR (If available): MASON, A D g. NAME OF ASSOCIATE INVESTIGATOR (If available):

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

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

23/24. (U) The objective of this work is to analyze the complex leukocyte mixtures seen in the blood of burned patients, quantitate the changes that occur, and correlate those changes with changes in cell function as well as clinical outcome. The immune status of burn patients will be assessed in terms of lymphocyte subpopulation composition and function using flow cytometry to differentiate the subpopulations. Data will be correlated with patient morbidity and compared to data from healthy control subjects.

25. (U) 9010 - 9109. Thirty-two patients and 31 control subjects have been enrolled in the study to date. Data analyses have revealed several interesting facts about lymphocyte subpopulations from burned patients. The proportion of CD8+ (suppressor/cytotoxic) cells was negatively correlated with severity of injury during the 2 months the patients were on the study. The proportion of CD4+ (helper/inducer) cells was negatively correlated with severity of injury during the first month following injury, but not the second. The proportion of NK or CD16+ cells was positively correlated with severity of injury during the second month postburn but not earlier. A possible mechanism to explain some of these phenotypic changes in burn patient lymphocyte subpopulations may be the selective loss of the lymph node-homing receptor (LHR). The proportion of circulating lymphocytes expressing LHR after thermal injury was reduced about 20% below healthy control subjects. The proportion of CD4+, LHR+ lymphocytes was decreased by 17%, while the proportion of CD4-, LHR+ lymphocytes was decreased 25%. After stimulation with ConA, about one-half as many LHR+ cells from burned patients

expressed IL2 receptor as did LHR- cells. Burn injury perturbed the expression of this cell adhesion molecule, which may explain some of the changes in phenotype and function seen after thermal injury.

SUPPLEMENTAL WORK UNIT INFORMATION FOR THE PROJECT ENTITLED "CELLULAR HOST DEFENSE FUNCTION AFTER THERMAL INJURY: ASSESSMENT BY FLOW CYTOMETRY OF PERIPHERAL BLOOD CELLS"

Subrecord/Linking Accession Number: DA311488.

Search Control Data: W6M25D/W6M27E, 20 October 1989.

Product Identification: For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report* for fiscal years 1986-91.

Unclassified Special Categories: Volunteers: Adults; RA II.

ABSTRACT

PROJECT NUMBER: 3M161102BS14, 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 1990 - 30 September 1991

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

Coincident with an increase in susceptibility to infection, circulating lymphocyte subpopulation function and phenotype are drastically altered after thermal injury. The mechanisms underlying these changes are unknown. The leukocyte-endothelial cell adhesion molecule (proposed as the lymph node-homing receptor or LHR) may influence the movement of lymphocytes in and out of the circulation. We have measured the expression of LHR on lymphocytes after thermal injury. The proportions of lymphocytes bearing LHR were measured in 26 patients with thermal injury and compared to 28 healthy control subjects. Lymphocyte subpopulations bearing LHR were determined by staining with monoclonal antibodies and anti-LHR (anti-Leu-8). Lymphocytes were purified on Ficoll-Hypaque™ gradients from heparinized whole blood samples obtained from patients twice weekly for up to 8 weeks postburn. Freshly isolated cells and cells cultured for 24 h in the presence and absence of ConA were stained with the appropriate antibody and analyzed by flow cytometry. Expression of IL2 receptor (IL2R) after cell culture was used as an indicator of mitogenic stimulation. The proportion of freshly isolated cells expressing LHR was decreased by an average of 15% after injury compared to the control population. CD4+ lymphocytes showed a decrease of 17% in the proportion of cells expressing LHR; in CD4- cells, expression was not decreased. After 24-h stimulation with ConA, LHR expression decreased 33% in both groups. The proportion of LHR+ cells expressing IL2R after 24-h stimulation was 44% lower for burn patients than for control subjects, while LHR- cells expressing IL2R were decreased by only 32%. We conclude that thermal injury perturbs the normal expression of LHR. Cells expressing the homing receptor appear less able to respond mitogenically to ConA stimulation after thermal injury than other cells. These changes are unevenly distributed across the lymphocyte subpopulations and

may contribute to postburn changes in phenotype and function of circulating cells.

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

The ability of lymphocytes to travel throughout the body is crucial to their ability to fight infection. Lymphocytes must be able to respond to the secretion of cytokine "signals" and travel to the site of inflammation or the place of antigen processing. In response to the proper cytokines or chemotactic signals, they adhere to the capillary venules and extrude themselves across the vessel wall and into the tissue. They also travel to lymph organs such as the lymph nodes, spleen, liver, or even the skin. The mechanism of the lymphocytes ability to adhere to and "home" to specific tissues is just now beginning to be understood. Lymphocytes and other leukocytes have a series of adhesion molecules which they use to adhere to specific receptors on various types of tissue (1). Three types of adhesion molecules have been identified so far. The integrins, the LECAMs, and the ICAMs. Of these, one of the LECAM molecules has been tentatively identified as the lymph node-homing receptor (LHR) (2). It reacts with the monoclonal antibody anti-Leu-8, which marks a large number of circulating lymphocytic cells.

MATERIALS AND METHODS

Study Participant Data. Data are based on 26 patients with thermal injury and 28 control subjects enrolled in this study. Burn patients were enrolled in the study within 5 days of injury. The average total body surface area burn size was 44.8%. The average age of the burn patients was 44.5 yr and 33.9 yr for the control subjects.

Cell Preparation. Heparinized blood samples were obtained from patients twice weekly for up to 8 weeks postburn. Lymphocytes were isolated on Ficoll-Hypaque™ gradients. After washing, one portion of the cells were used for staining with monoclonal antibodies and analyzed by flow cytometry, a second portion was used for the preparation of a slide (cytospin) to determine the extent of nonlymphocyte contamination, and a third portion was used for a 24-h culture with and without the mitogen ConA.

Cell Staining. Cells were stained with monoclonal antibodies (Becton Dickinson, Mountain Home, CA) bound to either phycoerythrin, fluorescein isothiocyanate, or biotin. Allophycocyanin was bound to biotin-labeled primary antibodies by adding streptavidin conjugated to allophycocyanin. Anti-Leu-3 (CD4), anti-Leu-8 (LHR), anti-Leu-18 (CD45RA), and anti-IL2 (CD25) were used to identify subpopulations. IgG1 or IgG2 conjugated with the appropriate dye marker were employed as isotypic controls. The staining procedure followed that specified by the manufacturer of the monoclonal antibody. Cells were fixed immediately after staining in 1% paraformaldehyde.

Mitogen Stimulation. Cells were cultured for 24 h using RPMI-1640 (GIBCO, Grand Island, NY) supplemented with 50 Mm glutamine, 100 U/ml penicillin, 50 mg/L streptomycin sulfate, and 10% fetal bovine serum as culture medium. The cell concentration was maintained at 1 X 10^6 lymphocytes/ml in the presence and absence of ConA (10 µg/ml). Cells were placed in sterile 17 X 75-mm polypropylene culture tubes and maintained in an humidified atmosphere containing 5% CO_2 at 37°C. After culture, the cells were washed twice in RPMI culture medium without ConA and once with HBSS. The cell suspension was counted and the cell concentration adjusted for staining.

Flow Cytometry Analysis. Subpopulations were analyzed by flow cytometry using a FACSTAR+ (Becton Dickinson) flow cytometer. Electronic gates were set on forward angle and side scatter intensity using normal human peripheral blood lymphocytes as guides. Nonlymphoid cell contamination was monitored by observing the level of anti-Leu-M3 positives (anti-Leu-M3 binds monocytes and weakly binds granulocytes). The positive cutoff was set at a point that defined 1% or less of the electronically gated isotypic control sample as positive.

Data Analysis. Data were compared by ANOVA using BMDP Program 7D (BMDP Statistical Software, Los Angeles, CA).

RESULTS

Patient and control cells were analyzed for subpopulation proportions by flow cytometry. Figure 1 depicts the changes in CD4+ and LHR+ lymphocytes after thermal injury. There are significant decreases in CD4+ cells and LHR+ cells in the burn patient compared to the control population. Analysis of the cells stained for both CD4 and LHR show that the decrease in LHR expression occurs only in the CD4+ cells and not CD4- cells. This decrease might reduce the ability of CD4+ cells to home to the lymph nodes.

In addition to CD4 and LHR, the isolated cells were stained simultaneously for CD45RA. CD45RA is expressed by naive lymphocytes which have not responded to antigen. CD45RA- cells includes those cells which have responded to antigen previously. These cells are able to respond much more quickly to the same antigen and are termed "memory" cells. Figure 2 compares the expression of LHR on CD4+ and CD4- memory cells. Memory cells from both the CD4+ and CD4- subpopulations expressed a lower proportion of LHR than did those same subpopulations from control subjects. In contrast, naive cells did not have decreased expression of LHR.

Functional response to mitogenic stimulation was measured by the expression of IL2R 24 h after the addition of ConA. Cells from burn patients and control subjects were purified, placed in culture with the mitogen ConA, harvested after 24 h in culture, and

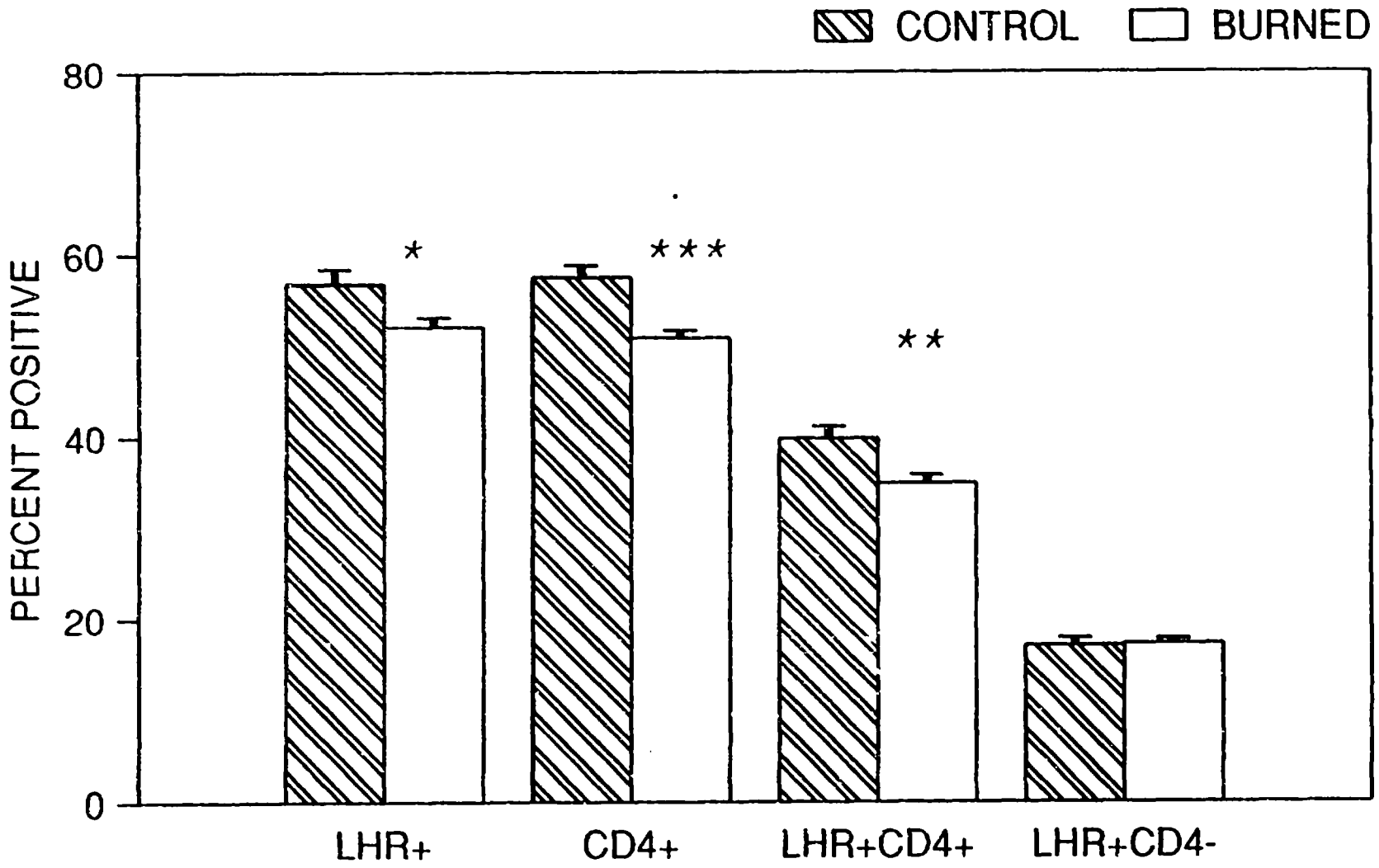

FIGURE 1. The difference in CD4+ and LHR+ lymphocyte subpopulations in peripheral blood from burn patients and control subjects (mean ± SEM). *P < 0.05, **p < 0.01, ***P < 0.001.

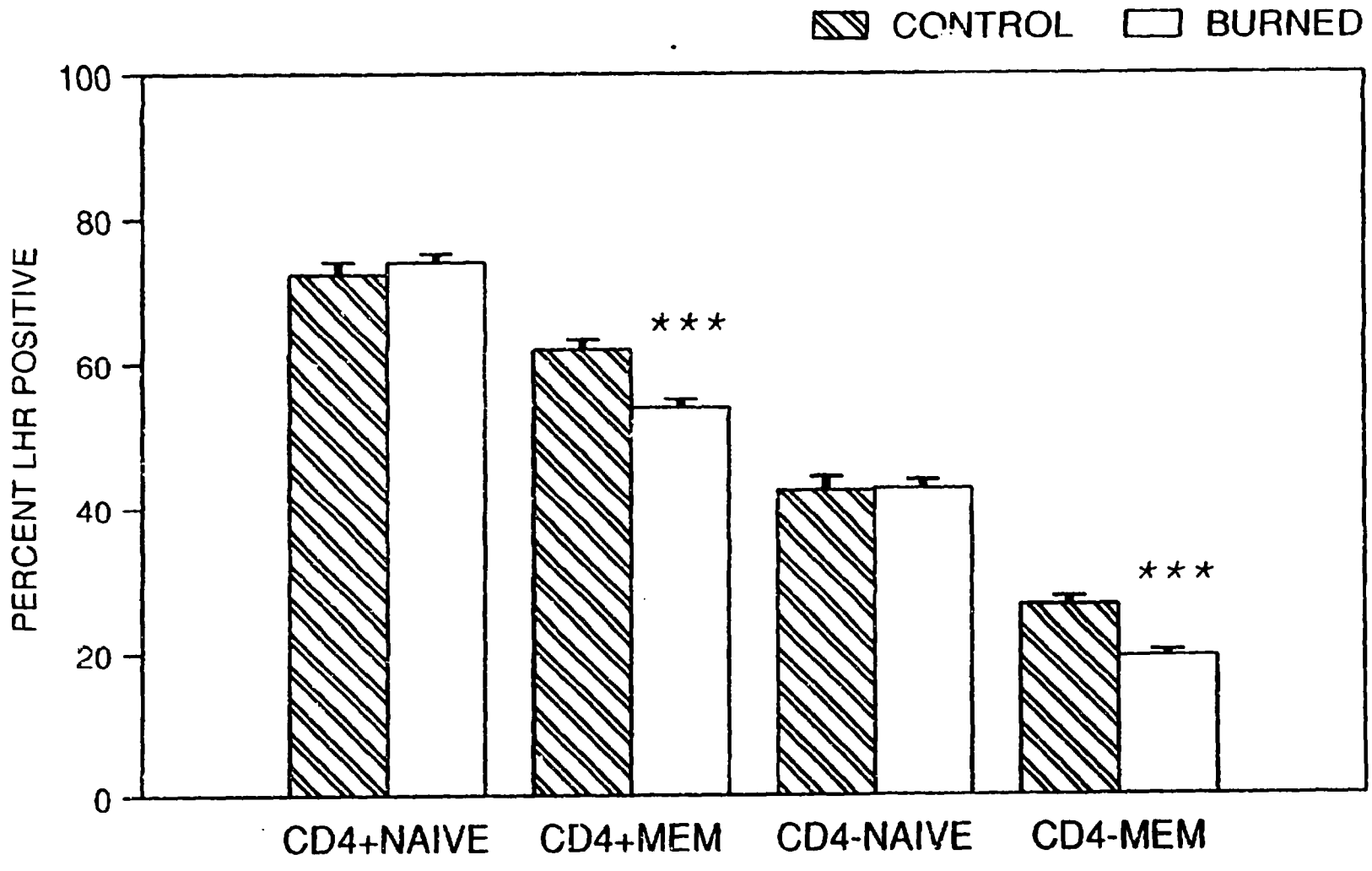

FIGURE 2. The proportion of LHR+ in CD4+ and CD4- memory and naive cells in peripheral blood from burn patients and control subjects. Error bars are ± SEM. ***p < 0.001.

analyzed for IL2 expression by flow cytometry. The cells were stained with anti-CD4 and anti-LHR as well as anti-IL2R. As shown in Figure 3, all four subpopulations of cells (CD4+ LHR+, CD4+ LHR-, CD4- LHR+, CD4- LHR-) from burn patients had a sharply deceased proportion of IL2 expressed than did cells from control subjects. There appeared to be no functional discrimination between cell subpopulations expressing LHR or CD4.

DISCUSSION

There are two possible explanations for the selective decrease in LHR expression in the memory cells from the CD4+ and CD4- subpopulations. One is reduced expression by the circulating cells. The second is a selective removal of these cells from the circulation. The first would indicate a potential defect in the ability of the cells to move into and out of the circulation. The second would be a residual effect of the cells leaving the circulation, probably in response to lymphokine signals from the burn wound. If the second possibility is true, it is interesting that principally memory cells were removed from circulation rather than naive cells. The selective removal of memory cells is supported by Picker et al (3) and Shimizu et al (4), who have shown that memory cells are selectively bound to inflamed endothelium by ELAM-1. ELAM-1 is expressed by endothelium after inflammation or exposure to inflammatory lymphokines. McKay (5) proposes that the differential expression of adhesion molecules on memory and naive cells is responsible for their different migration pathways and removal from circulation. Memory cells tend to migrate to nonlymphoid tissue while naive cells tend to migrate to lymphoid tissue. Activation of cells results in down-regulation of LHR and lowered affinity for lymphoid tissue.

PRESENTATIONS

Burleson DG: The relationship of lymphocyte subpopulations to mortality predictors in thermal injury. Presented at the 27th Annual Meeting of the Society for Leukocyte Biology and the 12th International RES Congress, Heraklion, Crete, Greece, 16 October 1990.

Burleson DG: Cluster analysis of multi-parameter data using VGA color graphics on the IBM-PC. Presented at the 15th Congress of the International Society for Analytical Cytometry, Bergen, Norway, 26 August 1991.

Burleson DG: Selective loss of the lymph node-homing receptor on lymphocytes from burned patients. Presented at the 29th National Meeting of the American Association for the Surgery of Trauma and 9th Annual Meeting of the Trauma Association of Canada, Philadelphia, Pennsylvania, 14 September 1991.

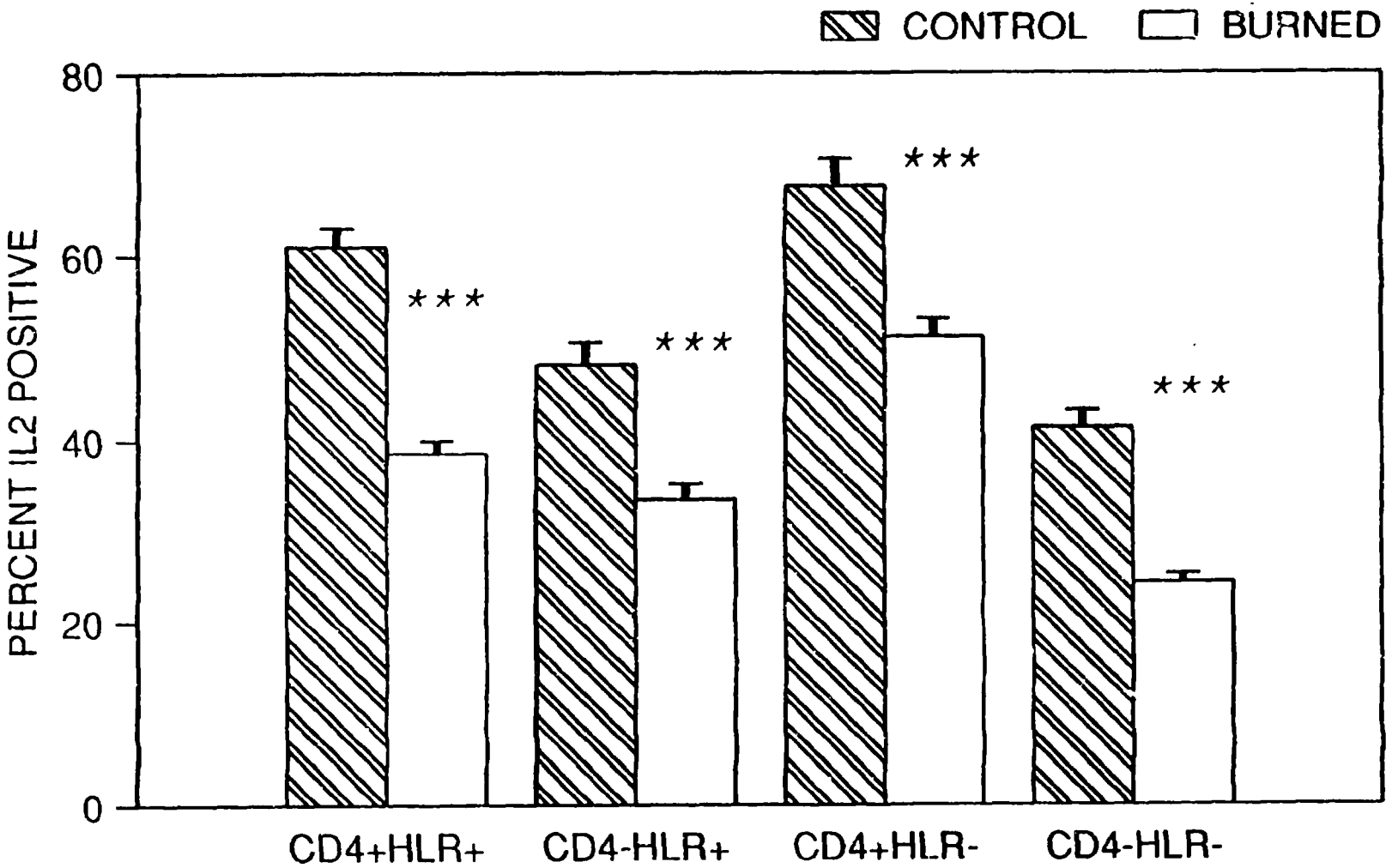

FIGURE 3. Proportion of IL2R+ cells after 24 h of stimulation with the mitogen ConA. The percentage of each of the CD4, LHR subpopulations from control subjects and burn patients that express IL2R are shown as ± SEM. ***P < 0.001.

PUBLICATIONS

Burleson DG, Wolcott KM, Mason AD Jr, and Pruitt BA Jr: The relationship of lymphocyte subpopulations to mortality predictors in thermal injury (abstr). *J Leuk Biol* (Suppl 1):41, October 1990.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION: DA315351	2. DATE OF SUMMARY: 01 Oct 91	REPORT CONTROL SYMBOL: DD-DRAR(AR) 636
3. DATE PREV SUM'RY: 01 Oct 90	4. KIND OF SUMMARY: D. Chg	5. SUMMARY SCTY: U	6. WORK SECURITY: U	7. REGRADING:	8. DISP'N INSTR'N: CX	9. LEVEL OF SUM: A. WORK UNIT

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS14	AZ	313
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) A Study of Biochemical Changes in the Cellular Environment of Tissue of the in vivo Partial-Thickness Rat Burn Wound

12. SUBJECT AREAS: 0601 Biochemistry 0604 Anatomy and Physiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
8609	9209	DA	C. In-House

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

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

18. RESOURCES ESTIMATE

a. FISCAL YEARS	a. PROFESSIONAL WORK YEARS	b. FUNDS (In thousands)
91	1.5	110
92	1.5	113

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME: US Army Institute of Surgical Research	a. NAME: US Army Institute of Surgical Research
b. ADDRESS (Include zip code): Fort Sam Houston, San Antonio, Texas 78234-5012	b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-5012
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR: BROWN, W L
d. TELEPHONE NUMBER (Include area code): 512-221-2720	d. TELEPHONE NUMBER (Include area code): 512-221-4652
21. GENERAL USE: FIC MILITARY/CIVILIAN APPLICATION: M	e. NAME OF ASSOCIATE INVESTIGATOR (If available): MASON, A D g. NAME OF ASSOCIATE INVESTIGATOR (If available):

22. KEYWORDS (Precede EACH with Security Classification Code): (U) Burns (Injuries); (U) Wounds and Injuries; (U) Metabolism; (U) Metabolites

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

23/24. (U) The objective of this work is to determine the biochemical and metabolic changes that occur in 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. Microelectrodes will be used to measure changes in extracellular potassium ion concentration and in pH and/or carbon dioxide partial pressure at various sites in vivo in the burn wound. 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.

25. (U) 9010 - 9109. The time of measurement of changes in metabolite concentrations in the blood and burn wounds of animals subjected to 20% total body surface area sham or partial-thickness scald burns was extended to 7 days postburn. There was no difference in blood ATP and lactate concentrations between burned and sham-burned animals either 3 or 7 days postburn. Burn wound ATP concentrations were higher 3 and 7 days postburn than at 48 h postburn but remained lower than those in sham-burn wound controls. Tissue lactate concentrations did not differ at 3 or 7 days postburn. Methods for measurement of intracellular pH in the burn wound are currently being tested.

DD FORM 83 MAR 1498 EDITION OF MAR 88 IS OBSOLETE. * USGPO: 1988-691-003/50329

SUPPLEMENTAL WORK UNIT INFORMATION FOR THE PROJECT ENTITLED "A STUDY OF BIOCHEMICAL CHANGES IN THE CELLULAR ENVIRONMENT OF TISSUE OF THE IN VIVO PARTIAL-THICKNESS RAT BURN WOUND"

Subrecord/Linking Accession Number: DA311489.

Search Control Data: W6M37B/W6N22D, 20 October 1989.

Product Identification: For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report* for fiscal years 1986-91.

Unclassified Special Categories: Lab Animals: Rats; RA II.

ABSTRACT

PROJECT NUMBER: 3M161102BS14, 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

INVESTIGATORS: Wanda L. Brown, MS
Beverly Worley, MS
Carlin V. Okerberg, DVM, PhD, Lieutenant Colonel, VC
Arthur D. Mason, Jr., MD
Basil A. Pruitt, Jr., MD, Colonel, MC

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

The time of measurement of biochemical changes in the blood and wounds of rats subjected to 20% total body surface area sham or partial-thickness scald burns has been extended. The water content of the burn wound was still 0.7 ml greater at 7 days postburn (PB) and 0.5 ml greater at 14 days PB than that of sham wounds. Lactate and ATP content of blood and lactate content of wounds of sham and burned rats were not different at 3 and 7 days PB. ATP content of burn wounds increased from its lowest value of 19% of sham content at 24 h PB to 39% and 57% of sham at 3 and 7 days PB, respectively.

Routine histological examination of burn wound tissue showed that blood vessels in the upper and lower dermis contained clumped erythrocytes during the early postburn period but those in the lower dermis began to clear after 24 h PB. Some new vessels began to appear in the subcutaneous tissue by 48 h PB. At 24 h PB, the epidermis was completely necrotic but there was surviving epithelium in some of the hair follicles. At 7 days PB, the necrotic epithelium was raised above the surface of the wound and there was a viable layer composed of squamous epithelial cells underneath.

A different method for isolation of cells from wound tissue in which the diced tissue was first incubated in a solution of collagenase and hyaluronidase, washed, and then incubated in a solution of protease XXV produced a greater yield of viable cells. These cells are being used to develop methods for measurement of intracellular pH and calcium using fluorescent probes.

A STUDY OF BIOCHEMICAL CHANGES IN THE CELLULAR ENVIRONMENT OF TISSUE OF THE IN VIVO PARTIAL-THICKNESS RAT BURN WOUND

The progressive increase in the depth of irreversibly injured tissue of the burn wound during the first 24 to 48 h postburn (PB) has been attributed to ischemia (1), but the sequence of metabolic and biochemical changes which occur in vivo in the burn wound has not been well characterized. However, the changes that occur in other ischemic organs have been well documented (2-4). These include a rapid depletion of glycogen and high-energy phosphate compounds, an accumulation of lactate, carbon dioxide, reduced nicotinamide adenine dinucleotide, and protons from other metabolic processes, and, ultimately, a cessation of all energy dependent functions. Since the changes that occur are similar for heart, brain, liver, kidney, and muscle, it is possible that they may also occur in ischemic skin, although they may differ in time and extent because of differences in the relative contributions of different pathways for energy production in a particular organ. The objective of this study is to determine the in vivo biochemical and metabolic changes in partial-thickness rat burn wounds during the early postburn period and to identify those changes which either foster or impede recovery of cellular function in such wounds.

In previous reports (5,6), we described the changes in water content of wound and of ATP and lactate content in blood and wound of rats with 20% total body surface area sham and burn wounds during the first 48 h PB. In this report, we describe the changes that occur in the later postburn period.

MATERIALS AND METHODS

Male Sprague-Dawley rats weighing 180-200 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 fine-mesh gauze beneath a layer of 1/4-in foam padding held in place by tubular elastic netting to protect the wounds from mechanical trauma. The wound covers were left in place for 3-7 days PB.

The procedures for collection and analysis of samples for water content, ATP, and lactate have previously been described in detail (5,6). Tissue samples for histological examination were taken from the center of the wound, the margin of the wound, and from the belly of the burned animals and from equivalent areas of sham-burned animals at 1, 3, 6, 12, 24, 48, and 72 h and 7 days PB. These were processed for routine light microscopy, thin plastic sections, and for electron microscopy to provide a baseline for

evaluation of effects of potential treatments which might be applied to the wound.

A different method for isolation of cells from the wound was used in this part of the study in an effort to obtain a greater yield of viable cells (7). The entire wound area was excised and as much of the muscle tissue as possible was removed from the underside. The tissue was cut into 4- to 6-mm squares and weighed. Approximately half of each wound was placed in each flask. The tissue in each flask was incubated for 15 min in 15 ml MCDB-151 culture medium (Sigma Chemical Company, St. Louis, MO) at 37°C in an orbital shaking water bath at 125 rpm. The solution was decanted and tissue was rinsed three times with a portion of cold culture medium. The tissue was then incubated for 45 min in a solution containing 1.25 mg/ml collagenase CLS4 (Worthington Biochemical Corporation, Freehold, NJ), 1 mg/ml hyaluronidase (Wyeth Laboratories, Inc., Philadelphia, PA), 4 mg/ml bovine serum albumin, and 1 mg/ml glucose in Ca++ and Mg++-free HBSS. Afterwards, the solution was decanted, the tissue was rinsed twice, and the washings were combined with the supernate from the incubation. Tissue fragments were then incubated in a solution containing 12.5 mg protease XXV from *Streptomyces griseus* (Sigma Chemical Company, St. Louis, MO) and 1 mg/ml glucose in HBSS containing Mg++ but no Ca++ for 1 h. Then two volumes of fetal bovine serum was added to each flask and the solution was rapidly chilled before being filtered through nylon filter cloth. The residue on the filter was washed three times with cold HBSS. The supernates and washes from the collagenase and protease incubations for each individual sample were combined and centrifuged at 800 g for 2 min. The supernate was aspirated and discarded. The cells were resuspended in MCDB-151 culture medium containing 1 µg/ml deoxyribonuclease (DNase), allowed to stand 10 min in an ice bath, vortexed, and centrifuged as before. This was repeated twice before a final wash without DNase in the solution. A portion of the cell suspension was mixed with four parts of trypan blue dye and the numbers of viable and nonviable (trypan blue-permeable) cells were counted on a hemocytometer. Slides from the cell suspensions were prepared and stained with H&E or Giemsa stain. If analysis of the cells was delayed, 0.1 vol fetal bovine serum was added to the culture medium in which they were stored.

Methods for measuring intracellular pH using fluorescent probes are currently being tested. BCECF (Molecular Probes, Inc., Eugene, OR) was loaded into cells as the membrane permeant acetoxymethyl ester (BCECF-AM). Intracellular esterases hydrolyze the acetoxymethyl esters to release BCECF-free acid which is retained by the cells. The extracellular probe was removed by washing and 2 ml of the cell suspension (20-30 X 10^6 cells/ml) was placed in a 10 mm^2 cuvette for analysis using a Model LS-50 luminescence spectrometer (Perkin Elmer, Norwalk, CT). A change in pH results in a shift in the maximum excitation wavelength and the ratio of the fluorescent intensities (440/490 nm) was used to calculate the

intracellular pH by relating the ratio to ratios obtained using standard solutions.

Intracellular Ca++ can be determined by loading cells with the fluorescent probe Fura-2AM and measuring the excitation ratio (340/380) of the fluorescence intensities of the sample. A calibration run in which the ratio is determined when the probe was fully saturated with metabolite (Rmax value) and the ratio in which the probe was present in unbound form only (Rmin value) was used to calculate the Ca++ content of the test sample using the method described by Grynkiewicz et al (6).

RESULTS

We previously reported that the water content of the 20% total body surface area partial-thickness rat burn wound increased to a maximum of 7.8 ml more than the content of the equivalent sham wound at 18 h PB, then began to decrease slowly. We have now measured the water content of burn wound in the later PB period and found that the wound still contained 0.7 ml excess water at 7 days PB and 0.5 ml excess at 14 days PB.

The ATP content of blood and the lactate content of blood and wound of sham and burned rats were not significantly different at 3 and 7 days PB. The ATP content of burn wound increased from its lowest value of 19% of sham at 24 h PB to 37% and 59% of sham at 3 and 7 days PB, respectively.

At 1 h PB, the vessels in the upper dermis were slightly dilated and filled with erythrocytes. The vessels in the lower dermis were dilated but the erythrocytes were not clumped. At 6 h PB, the vessels in the upper dermis were dilated and contained clumped erythrocytes while the vessels in the lower dermis were open. By 6 h PB, there was separation at the dermal epidermal junction in multifocal areas and there was visible edema. At 12 h PB, the vessels in the upper and lower dermis were dilated and contained erythrocytes that appeared to be clumped. At 24 h PB, the epidermis was completely necrotic and the hair follicles had necrosis of the epithelium in some places, but surviving epithelium in other places. The vessels in the upper dermis still were dilated and contained what appeared to be clumped erythrocytes but those of the lower dermis, although also dilated, contained erythrocytes that did not appear to be clumped. Some new blood vessel formation was evident in the subcutaneous tissues at 48 h PB. Sebaceous glands showed multifocal loss of structure and disruption of architecture. Hair follicles had some areas with viable epithelium, particularly adjacent to the hair bulb. There were occasional mitotic figures. At 7 days PB, the necrotic epithelium was raised above the surface of the wound and a viable epidermal layer composed of squamous epithelial cells was present underneath. The epithelium connected with viable hair follicles. In the lower dermis, there were multifocal mineralized hair

follicles. We have other samples taken as late as 28 days PB in which the surface appears to have been completely covered with new epidermis.

Tissues for thin plastic sections and for electron microscopy have not yet been processed.

DISCUSSION

The fact that a viable epidermal layer is present at 7 days PB may account for the increase in the ATP content of the burn wound since the rate of ATP synthesis is greatest in the epidermis.

We are currently trying to develop methods that will be suitable for measuring intracellular Ca++ and pH in cells from sham and burn wounds. The method for isolation of the cells reported here is an improvement over the previous method. There are apparently unique conditions that must be met for each cell type to obtain valid measurements. We are testing several buffers and substrates to find the ones most suitable for analyzing cells isolated from wound tissue.

PRESENTATIONS/PUBLICATIONS

None.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION DA314353	2. DATE OF SUMMARY 13 May 91	REPORT CONTROL SYMBOL DD-DRA(AR) 636
3. DATE PREV SUM'RY 01 Oct 90	4. KIND OF SUMMARY K. Comp	5. SUMMARY SCTY U	6. WORK SECURITY U	7. REGRADING	8. DISP'N INSTR'N CX	9. LEVEL OF SUM A. WORK UNIT

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS14	CH	314
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code) (U) The Effect of Interleukin 2 Administration on Mortality to Rats with Pseudomonas Burn Wound Sepsis

12. SUBJECT AREAS
0604 Anatomy and Physiology 0605 Medicine and Medical Research

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
8710	9105	DA	C. In-House

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

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

18. RESOURCES ESTIMATE

FISCAL YEARS	a. PROFESSIONAL WORK YEARS	b. FUNDS (In thousands)
90	0.1	63
91	0.1	66

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME: US Army Institute of Surgical Research	a. NAME: US Army Institute of Surgical Research
b. ADDRESS (Include zip code): Fort Sam Houston, San Antonio, Texas 78234-5012	b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-5012
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR: CIOFFI, W G
d. TELEPHONE NUMBER (Include area code): 512-221-2720	d. TELEPHONE NUMBER (Include area code): 512-221-8440
21. GENERAL USE: FINA MILITARY/CIVILIAN APPLICATION: M	e. NAME OF ASSOCIATE INVESTIGATOR (If available) f. NAME OF ASSOCIATE INVESTIGATOR (If available)

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Burns (Injuries); (U) Septicemia; (U) Prostaglandin; (U) Cells; (U) Pseudomonas

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

23/24. (U) The objective of this work is to study the effects of exogenous IL 2 administration following burn wound infection in a rodent model. Due to the failure of IL2 to improve survival in this infection model, the study was extended to evaluate the effects of indomethacin, gamma interferon, and other cytokines on IL2 receptor expression.

25. (U) 8710 - 9105. The optimal dose which could be tolerated by the septic animal was identified. In LD_{100} and LD_{50} models, IL2 administration concomitant with burning and seeding of the burn wound with Pseudomonas failed to improve survival. To increase IL2 receptor expression, both high (5 mg/kg) and low (0.5 mg/kg) doses of indomethacin were administered with the IL2. This failed to improve survival in the LD_{100} model. An addendum for measuring IL2 receptor expression in rodent splenocytes and peripheral blood mononuclear cells was developed and approved. Indomethacin, PGE_2, and gamma interferon failed to improve IL2 receptor expression.

DD FORM 83 MAR 1498 EDITION OF MAR 88 IS OBSOLETE. * USGPO: 1988-491-003/50539

SUPPLEMENTAL WORK UNIT INFORMATION FOR THE PROJECT ENTITLED "THE EFFECT OF INTERLEUKIN 2 ADMINISTRATION ON MORTALITY TO RATS WITH PSEUDOMONAS BURN WOUND SEPSIS"

Subrecord/Linking Accession Number: DA313322.

Search Control Data: W6N47A/W6N48A, 20 October 1989.

Product Identification: For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report* for fiscal years 1987-91.

Unclassified Special Categories: Lab Animals: Rats; RA II.

ABSTRACT

PROJECT NUMBER: 3M161102BS14, Research

PROJECT TITLE: The Effect of Interleukin 2 (IL2) Administration on Mortality to Rats with Pseudomonas Burn Wound Sepsis

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

PERIOD COVERED IN THIS REPORT: 1 October 1990 - 13 May 1991

INVESTIGATORS: William G. Cioffi, Jr., MD, Major, MC
Bryan S. Jordan, RN, MSN
David G. Burleson, PhD, Colonel, MS
Basil A. Pruitt, Jr., MD, Colonel, MC

Decreases in IL2 production, activity, and receptor (IL2R) expression have been described following injury. Previous investigators have reported increased survival following pretreatment with recombinant IL2 in secondary infection, thermal injury models. Attempts to improve survival in a rat invasive burn wound infection model have been unsuccessful. Not only is IL2 production decreased following injury, but the presentation of IL2R is also altered. PGE_2 production from macrophages is increased following thermal injury and these elevated levels have been shown to decrease IL2 production and possibly decrease IL2R expression on T cells. The current studies were designed to investigate the role of PGE_2 on T-cell subpopulations and IL2R expression following thermal injury. Preliminary data indicated that exogenous administration of a long-acting form of PGE had no significant effect on splenic T-cell subpopulation numbers as measured by flow cytometry. The administration of indomethacin in an attempt to decrease PGE_2 production following thermal injury also had no significant effect on splenic T-cell subpopulations. Additionally, indomethacin administration had no effect on peripheral blood T-cell subpopulations.

THE EFFECT OF INTERLEUKIN 2 (IL2) ADMINISTRATION ON MORTALITY TO RATS WITH PSEUDOMONAS BURN WOUND SEPSIS

Following a severe thermal injury, alterations in the host immune system develop, which lead to depression of the immune response (1-3). Defects in cellular and humoral systems have been reported. These defects are manifested by increased susceptibility to sepsis, impaired delayed hypersensitivity reaction, and prolongation of allograft rejection.

As the mechanisms for T-cell interactions become clearer, it is apparent that IL2 (T cell growth factor) (4) is produced by T helper cells. The stimulus for this IL2 production comes from circulating IL-1, which is itself released from macrophages when they are exposed to foreign antigens (5). The IL2 then binds to specific IL2 receptors (IL2R) to promote the proliferation of T lymphocytes, regardless of their antigenic specificity (6). The number of IL2R, the amount of IL2 circulating, and the length of contact between the two all seem to be important in the magnitude of the T-cell response.

Levels of interleukin 1 produced in man in response to thermal injury have been measured and found to be increased immediately postburn and then subsequently return to normal. IL2 production has been shown to be significantly reduced postburn and returns to normal only in those patients who eventually survive (5). IL2R are also decreased on T cells following thermal injury (7). This has been related to the ability of IL2 to modulate the expression of its own receptors.

Previous investigators have reported increased survival following pretreatment with recombinant IL2 in secondary infection, thermal injury models. Gough et al (8) treated mice with recombinant IL2 for 7 days following thermal injury. On postburn day 10, cecal ligation and puncture were performed. Animals receiving only saline showed 100% mortality by postburn day 14, while those receiving IL2 had a 55% mortality. The splenocytes harvested from IL2-treated mice showed improved responses to T-cell mitogens in vitro when compared to saline controls. In contrast to these findings, we have previously failed to show any benefit of exogenous IL2 administration in a rat model of invasive burn wound sepsis (9). The failure of exogenous IL2 to increase survival in this model may result from the fact that exogenous IL2 administration may only increase low affinity interleukin receptors which do not result in an increased T cell response to antigenic stimulation (10). Thus, a functional defect is maintained in the milieu of increased IL2 levels. Hancock et al (11) have shown that macrophages fail to express IL2R in response to gamma interferon when PGE_2 is added in vitro. LaLa et al (12) have suggested that PGE can down-regulate IL2R expression on activated T cells.

The suggestion that PGE may modulate IL2R expression in vitro and the fact that PGE_2 levels are elevated following injury led us to investigate the role of PGE on splenic and peripheral blood T-cell subpopulations and IL2R expression following thermal injury.

MATERIALS AND METHODS

Male Sprague-Dawley rats weighing approximately 200 g were used throughout the study. All rats received a 20% total body surface full-thickness scald or sham burn (13). Appropriate groups were administered either indomethacin (5 mg/kg IP) or a long-acting PGE analogue (100 µg/kg IP), 16,16-dimethyl-prostaglandin E (dPGE), for 7 days following injury. At the end of the 7 days, the animals were anesthetized with sodium pentobarbital (60 mg/kg IP) and sacrificed by exsanguination. Peripheral blood mononuclear cells and splenocytes were then harvested and analyzed for T cell numbers and subpopulations as well as IL2R density following culture and stimulation as previously described (14).

RESULTS

Phase I. Four groups of animals were used for this phase of the study. Two groups received scald burns and two groups received sham burns. One group from the scald burn group and one group from the sham burn group received dPGE and the remaining animals received saline. On postburn day 7, splenocytes were harvested and analyzed by flow cytometry for T-cell subpopulations (see Table 1).

TABLE 1. Percentage of Splenic Suppressor, Pan, and Helper T Cells in Burned Rats Treated with dPGE or Saline (Mean ± SD)

Group	n=	Suppressor	Pan	Helper
Sham/Saline	7	19.6 ± 1.1	63.1 ± 3.8	39.5 ± 3.9
Burn/Saline	5	19.9 ± 4.0	56.1 ± 4.2	38.5 ± 6.3
Sham/dPGE	7	20.6 ± 3.0	55.7 ± 4.2	35.2 ± 3.3
Burn/dPGE	6	21.6 ± 2.9	61.6 ± 8.4	37.5 ± 4.9

Phase II. For this phase, two groups of animals received scald burns. Following burn injury, one group was administered indomethacin for 7 days and the other group was administered saline. On postburn day 7, the animals were sacrificed, the spleens were removed, and splenocytes were harvested and counted for T-cell subpopulations by flow cytometry (Table 2). Because a

TABLE 2. Percentage of Splenic Suppressor, Pan, and Helper T Cells in Burned Rats Treated with Indomethacin or Saline (Mean ± SD)

Group	n=	Suppressor	Pan	Helper
Burn/saline	8	24.7 ± 2.4	55.0 ± 3.4	38.3 ± 2.6
Burn/indomethacin	6	26.7 ± 2.9	64.4 ± 3.7	43.0 ± 3.0

slight increase in helper cell numbers and total T cells were seen following indomethacin administration, the study was repeated (see Table 3).

TABLE 3. Percentage of Splenic Suppressor, Pan, and Helper T Cells in Burned Rats Treated with Indomethacin or Saline (Mean ± SD)

Group	n=	Suppressor	Pan	Helper
Burn/saline	8	21.6 ± 3.2	66.1 ± 3.1	42.5 ± 4.5
Burn/indomethacin	8	21.1 ± 2.2	58.3 ± 3.7	40.9 ± 1.5

Phase III. For this phase, two groups of animals received scald burns. Following burn injury, one group received indomethacin and the other group received saline. On postburn day 7, the animals were sacrificed, peripheral blood mononuclear cells were harvested, T-cell subpopulations were analyzed by flow cytometry. The study was repeated twice. No effect of indomethacin on T-cell subpopulations in peripheral blood could be identified (Tables 4 and 5).

Phase IV. Failing to show any significant effect of indomethacin or PGE on splenic T-cell subpopulations following thermal injury, we next sought to identify whether these drugs would alter IL2R expression on either splenic T cells or peripheral blood T cells following 24 h of culture and stimulation. Preliminary studies were carried out to determine the optimal antibody and ConA concentration for maximal IL2R expression on splenic T cells from normal rats (Table 6).

DISCUSSION

The optimal dose which could be tolerated by the septic animal was identified. In LD_{100} and LD_{50} models, IL 2 administration

TABLE 4. Percentage of Peripheral Blood Suppressor, Pan, and Helper T Cells in Burned Rats Treated with Indomethacin or Saline (Mean ± SD)

Group	n=	Suppressor	Pan	Helper
Burn/Saline	5	22.4 ± 1.8	87.6 ± 3.4	60.6 ± 5.1
Burn/Indomethacin	5	27.4 ± 4.8	83.3 ± 4.7	55.8 ± 3.5

TABLE 5. Percentage of Peripheral Blood Suppressor, Pan, and Helper T Cells in Burned Rats Treated with Indomethacin or Saline (Mean ± SD)

Group	n=	Suppressor	Pan	Helper
Burn/Saline	5	22.4 ± 1.8	87.6 ± 3.4	60.6 ± 5.1
Burn/Indomethacin	5	27.4 ± 4.8	83.3 ± 4.7	55.8 ± 3.5

TABLE 6. IL2R Antibody Concentration (µl/ml) vs ConA Concentration (µg/ml) for Maximal IL2R Expression in Normal Rats

	IL2R Antibody				
ConA	5.0	2.5	1.25	0.625	0.313
0	1.1	9.9	7.9	7.0	6.7
2.5	13.4	31.0	30.3	28.7	22.5
5	13.1	43.1	39.0	33.9	33.7
10	14.5	53.5	47.0	48.1	44.8

concomitant with burning and seeding of the burn wound with Pseudomonas failed to improve survival. To increase IL2R expression, both high doses (5 mg/kg) and low doses (0.5 mg/kg) of indomethacin were administered with the IL2. This failed to improve survival in the LD_{100} model. An addendum for measuring IL2 receptor expression in rodent splenocytes and peripheral blood mononuclear cells was developed and approved. Indomethacin, PGE2, and gamma interferon failed to improve IL2 receptor expression.

PRESENTATIONS/PUBLICATIONS

None.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION DA315355	2. DATE OF SUMMARY 01 Oct 91	REPORT CONTROL SYMBOL DD-DR&E(AR) 636
3. DATE PREV SUM'RY 01 Oct 90	4. KIND OF SUMMARY D. Chg	5. SUMMARY SCTY U	6. WORK SECURITY U	7. REGRADING	8. DISS'N INSTR'N CX	9. LEVEL OF SUM A. WORK UNIT

10. NO./CODES.	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS14	CA	316
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE *(Precede with Security Classification Code)* (U) The Effect of High Frequency Ventilation on V_A/Q in Sheep with Inhalation Injury

12. SUBJECT AREAS
0604 Anatomy and Physiology 0605 Medicine and Medical Research

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
8701	9209	DA	C. In-House

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

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

18. RESOURCES ESTIMATE

a. FISCAL YEARS	b. PROFESSIONAL WORKYEARS	b. FUNDS *(In thousands)*
91	0.5	39
92	0.5	40

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME: US Army Institute of Surgical Research	a. NAME: US Army Institute of Surgical Research
b. ADDRESS *(Include zip code)*: Fort Sam Houston, San Antonio, Texas 78234-5012	b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-5012
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR: CIOFFI, W G
d. TELEPHONE NUMBER *(Include area code)*: 512-221-2720	d. TELEPHONE NUMBER *(Include area code)*: 512-221-8440
21. GENERAL USE: FIC MILITARY/CIVILIAN APPLICATION: M	e. NAME OF ASSOCIATE INVESTIGATOR *(If available)* g. NAME OF ASSOCIATE INVESTIGATOR *(If available)*

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

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

23/24. (U) The objective of this work is to compare the effects of volumetric diffusive ventilation and conventional ventilation on pulmonary and hemodynamic indices which are altered in an ovine inhalation injury model. Inhalation injury will be induced using the standard ovine smoke inhalation model developed at this Institute. Animals will be randomized to treatment with 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.

25. (U) 9010 - 9109. Data indicates that high-frequency oscillatory ventilation employing a percussive ventilator is inadequate for support of sheep with inhalation injury 24 h following injury.

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

SUPPLEMENTAL WORK UNIT INFORMATION FOR THE PROJECT ENTITLED "THE EFFECT OF HIGH-FREQUENCY VENTILATION ON V_A/Q IN SHEEP WITH INHALATION INJURY"

Subrecord/Linking Accession Number: DA312336.

Search Control Data: W6003E/W6006A, 20 October 1989.

Product Identification: For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report* for fiscal years 1987-91.

Unclassified Special Categories: Lab Animals: Sheep; RA II.

ABSTRACT

PROJECT NUMBER: 3M161102BS14, 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 1990 - 30 September 1991

INVESTIGATORS: William G. Cioffi, Jr., MD, Major, MC
Bryan S. Jordan, RN, MSN
Avery A. Johnson, BS
Basil A. Pruitt, Jr., MD, Colonel, MC
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 utilizing 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 indicate that high-frequency oscillatory ventilation employing a percussive ventilator was inadequate for support of sheep with inhalation injury 24 h following injury. Prior to continuing this project, benchwork utilizing the high-frequency oscillatory ventilator in an attempt to increase volume output will be necessary.

THE EFFECT OF HIGH-FREQUENCY VENTILATION 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 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 utilizing 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 utilized. 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 following 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 following 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. $\dot{V}_A/Q$ distributions were then measured utilizing 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 following 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 utilizing 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 utilizing the student's t test.

RESULTS

Data indicate that high-frequency oscillatory ventilation employing a percussive ventilator was inadequate for support of sheep with inhalation injury 24 h following injury.

DISCUSSION

Prior tc continuing this project, benchwork utilizing 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				1. AGENCY ACCESSION: DA315356	2. DATE OF SUMMARY: 01 Oct 91	REPORT CONTROL SYMBOL: DD-DR&E(AR) 636
3. DATE PREV SUM'RY: 01 Oct 90	4. KIND OF SUMMARY: D. Chg	5. SUMMARY SCTY: U	6. WORK SECURITY: U	7. REGRADING:	8. DISP'N INSTR'N: CX	9. LEVEL OF SUM A. WORK UNIT:

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS14	AZ	317
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code) (U) Effects of Replacement Therapy on Hemodynamic Parameters in an Ovine Model of Controlled Pure Plasma Loss

12. SUBJECT AREAS
0604 Anatomy and Physiology 0605 Medicine and Medical Research

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
8701	9209	DA	C. In-House

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

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

18. RESOURCES ESTIMATE

a. FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
91	0.5	30
92	0.5	31

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME: US Army Institute of Surgical Research	a. NAME: US Army Institute of Surgical Research
b. ADDRESS (Include zip code): Fort Sam Houston, San Antonio, Texas 78234-5012	b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-5012
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR: SHIRANI, K Z
d. TELEPHONE NUMBER (Include area code): 512-221-2720	d. TELEPHONE NUMBER (Include area code): 512-221-4652
21. GENERAL USE: FIC MILITARY/CIVILIAN APPLICATION: M	e. NAME OF ASSOCIATE INVESTIGATOR (If available): MASON, A D g. NAME OF ASSOCIATE INVESTIGATOR (If available)

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Burns (Injuries); (U) Albumins; (U) Shock (Pathology); (U) Resuscitation

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

23/24. (U) The objective of this work is to determine the hemodynamic consequences of controlled pure plasma loss in sheep using a method to simulate the acute burn. A plasmapheresis filter will be used to produce intravascular plasma loss similar to that caused by burn injury. This device selectively removes plasma while leaving the formed elements of blood in the vascular system.

25. (U) 9010 - 9109. This project was transferred to a new principal investigator. 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.

DD FORM 83 MAR 1493 — EDITION OF MAR 88 IS OBSOLETE.

SUPPLEMENTAL WORK UNIT INFORMATION FOR THE PROJECT ENTITLED "EFFECTS OF REPLACEMENT THERAPY ON HEMODYNAMIC PARAMETERS IN AN OVINE MODEL OF CONTROLLED PURE PLASMA LOSS"

Subrecord/Linking Accession Number: DA312334.

Search Control Data: W6021F/W6023D, 20 October 1989.

Product Identification: For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report* for fiscal years 1987-91.

Unclassified Special Categories: Lab Animals: Sheep; RA II.

ABSTRACT

PROJECT NUMBER: 3M161102BS14, 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 1990 - 30 September 1991

INVESTIGATORS: William G. Cioffi, Jr., MD, Major, 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.

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).

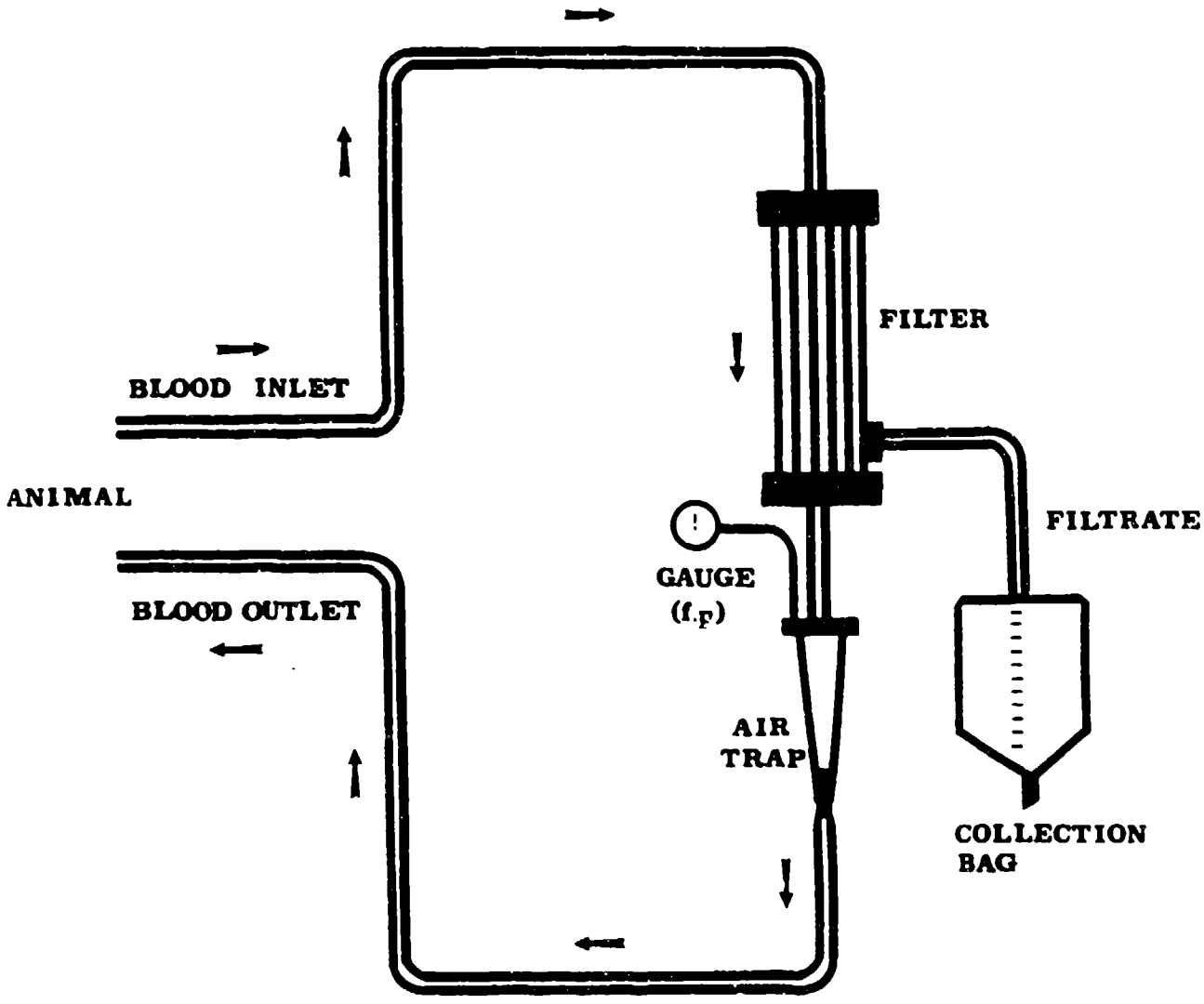

FIGURE 1. Graphic representation of the filtration circuit.

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.

PRESENTATIONS/PUBLICATIONS

None.

REFERENCES

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3. Pruitt BA Jr, Mason AD Jr, Moncrief JA: Hemodynamic changes in the early postburn patient: the influence of fluid administration and of a vasodilator (hydralazine). *J Trauma* 11:36-46, 1971.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION: DA315357	2. DATE OF SUMMARY: 01 Oct 91	REPORT CONTROL SYMBOL: DD-DR&E(AR) 636

3. DATE PREV SUMMARY	4. KIND OF SUMMARY	5 SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISP'N INSTR'N	9 LEVEL OF SUM A WORK UNIT
01 Oct 90	D. Chg	U	U		CX	

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS14	EA	318
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE *(Precede with Security Classification Code)*: (U) Antibacterial and Wound Healing Effects of Silver-Nylon Electrodes with Weak Direct Current

12. SUBJECT AREAS
0601 Biochemistry 0605 Medicine and Medical Research

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
8808	9209	DA	C. In-House

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

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

18. RESOURCES ESTIMATE

FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
91	1.6	134
92	1.6	139

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME: US Army Institute of Surgical Research	a. NAME: US Army Institute of Surgical Research
b. ADDRESS *(Include zip code)*: Fort Sam Houston, San Antonio, Texas 78234-5012	b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-5012
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR: CHU, C S
d. TELEPHONE NUMBER *(Include area code)*: 512-221-2720	d. TELEPHONE NUMBER *(Include area code)*: 512-221-3411
21. GENERAL USE: FIC MILITARY/CIVILIAN APPLICATION: M	e. NAME OF ASSOCIATE INVESTIGATOR *(If available)*: MC MANUS, A T g. NAME OF ASSOCIATE INVESTIGATOR *(If available)*: OKERBERG, C V

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

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

23/24. (U) The objective of this work is to determine the antimicrobial and wound healing effects of weak direct current and to measure silver concentrations of burn eschar and underlying tissue as a function of time and direct current. The guinea pig will be used to study healing in deep second degree burns, third degree burns, donor site wounds, and skin grafts with and without stimulation with weak direct current.

25. (U) 9010 - 9109. Direct current treatment using silver-nylon dressings enhanced the speed and quality of healing of partial-thickness scald burns and significantly reduced edema in full-thickness injuries in guinea pigs and rats. The earlier the time of application, the more pronounced the effect. This effect was noted at both anodal and cathodal polarity.

DD FORM 83 MAR 1498 — EDITION OF MAR 68 IS OBSOLETE. — • U.S.G.P.O. 1988-491-003/20329

SUPPLEMENTAL WORK UNIT INFORMATION FOR THE PROJECT ENTITLED "ANTIBACTERIAL AND WOUND HEALING EFFECTS OF SILVER-NYLON ELECTRODES WITH WEAK DIRECT CURRENT"

Subrecord/Linking Accession Number: Not applicable.

Search Control Data: W6O35C/W6O36B, 20 October 1989.

Product Identification: For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report* for fiscal years 1988-91.

Unclassified Special Categories: Lab Animals: Guinea Pigs; Rats; RA II.

ABSTRACT

PROJECT NUMBER: 3M161102BS14, 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 1990 - 30 September 1991

INVESTIGATORS: Chi-Sing Chu, MD
Albert T. McManus, PhD
Arthur D. Mason, Jr., MD
Carlin V. Okerberg, DVM, PhD, Lieutenant Colonel, VC
Basil A. Pruitt, Jr., MD, Colonel, MC

Preliminary results suggest that silver-nylon dressings with direct current may have utility in reducing wound edema-related alterations in microcirculation during the early postburn period.

ANTIBACTERIAL AND WOUND HEALING EFFECTS OF SILVER-NYLON ELECTRODES WITH WEAK DIRECT CURRENT

The loss of physical and immunological barriers of the skin following thermal injury is an obvious example of trauma-related immunosuppression. The correction of these defects by autografting skin from the patient's unburned areas was a major advance in burn care. With major burns, however, the burned area is commonly greater than the unburned surface and closing of the wounds with autograft becomes a staged series of operations, where graft donor sites after healing are repeatedly harvested. This process may take several months and the susceptibility of the open wounds to infection is a major liability. Numerous temporary wound coverings have been proposed. These include allograft skin, cutaneous xenografts, and several varieties of synthetic wound dressings. As an adjunct in burn wound care, the value of topical application of antimicrobial agents such as mafenide acetate, 0.5% silver nitrate solution, and silver sulfadiazine to wounds has been clinically well established. The value of the 0.5% silver nitrate solution and silver sulfadiazine in the treatment of infected wounds is very limited. The reason for such failure appears to be the limited tissue penetration of silver, the principal antimicrobial activity of these agents.

We have developed a silver-nylon dressing that can be used as a topical antimicrobial barrier against wound contamination (1). In addition, the silver-nylon dressing may be used as a source of silver ions when anodal direct current (DC) is applied. The silver ions when generated from the anodal dressing ionophorese into the wound in a current-dependent manner. Such electric dressings have been found to be experimentally effective, even for deeply infected burn wounds (2). As an adjunctive observation in such studies, we observed that animals that survived wound infections because of the antimicrobial action of the silver ions also had distinctly accelerated wound healing and reduced scarring.

Investigations into the mechanisms of DC-mediated wound healing have shown that DC treatment significantly shortens the postwounding time to the return of microcirculation. This finding has been verified in grafts, burns, and donor site wounds (3). During the past year, we have examined the possible effects of DC treatment on edema in full-thickness burns.

MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats were observed for a minimum of one week prior to entry into the study to exclude the possibility of any preexisting diseases. All animals were individually housed in plastic cages inside the animal intensive care unit. Temperature was maintained between 78°F and 82°F and

humidity was approximately 50%. All animals were fed food and water ad libitum throughout the study period.

Burn Wound Model. Animals were anesthetized with sodium pentobarbital (35 mg/kg IP). The dorsal hair was clipped and a depilatory cream (Nair™) was applied for 15 min. Full-thickness scald wounds were inflicted by a 10-sec exposure of the depilated areas to 100°C water using a Walker-Mason burn template with a window for a 20% total body surface area. Following injury, animals had dorsal and ventral silver-nylon dressings and electrodes applied as previously described (1). DC was applied either as immediately after burning and maintained until time of sacrifice or after a postburn delay and then constantly until time of sacrifice (see RESULTS). Control groups included unburned animals and animals burned and covered with silver-nylon without current.

Edema was measured as the difference in dry-to-wet weights of excised burn wounds after drying for 4 days at 70°C. Excised tissue included the panniculus muscle.

RESULTS

As shown in Figure 1, application immediately postburn of 40 μA DC significantly reduced edema at all time points measured. As shown in Figure 2, delay in application of < 8 h appears to have reduced the edema content of animals sacrificed at 48 h postburn. These preliminary results suggest that silver-nylon dressings with DC may have utility in reducing wound edema--related alterations in microcirculation during the early postburn period.

DISCUSSION

Future study will examine the effects of silver-nylon and DC on serum protein content of wound edema.

PRESENTATIONS

Chu C-S: Iontophoretic treatment of burn wound sepsis using silver-nylon dressings. Presented at the 8th International Congress on Burn Injuries, New Delhi, India, 15 November 1990.

Chu C-S: Improved healing and expansion with animal growth of deep partial thickness scalds treated with weak direct current silver-nylon dressings. Presented at the 23rd Annual Meeting of the American Burn Association, Baltimore, Maryland, 3 April 1991.

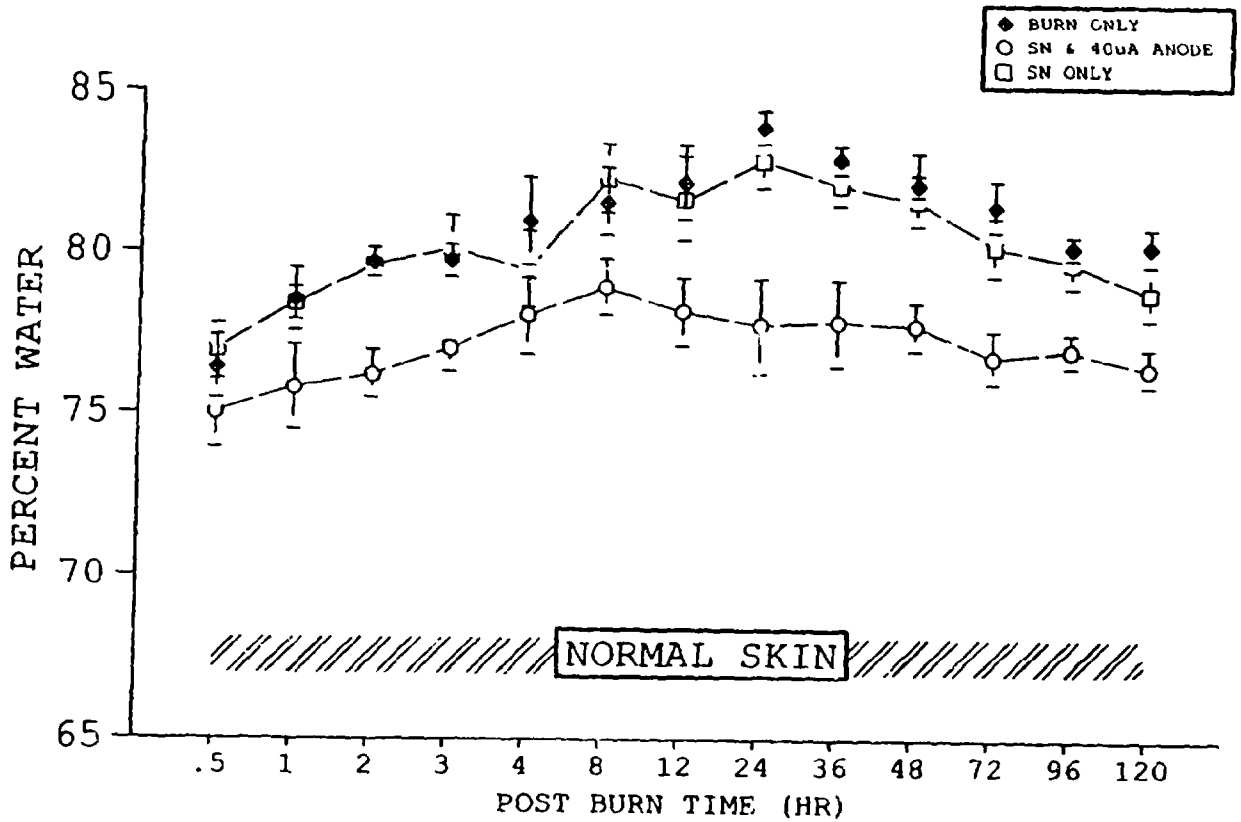

FIGURE 1. Effect of DC on accumulation of edema after 20% total body surface area full-thickness scald burns. Silver-nylon with 40 μA anodal current was applied immediately postinjury. Animals were sacrificed at indicated times postinjury and water content was measured. Control groups, burn only and silver-nylon without DC, were sacrificed at the same postinjury periods. Shaded area represents the 95% confidence limits for unburned dorsal rat skin.

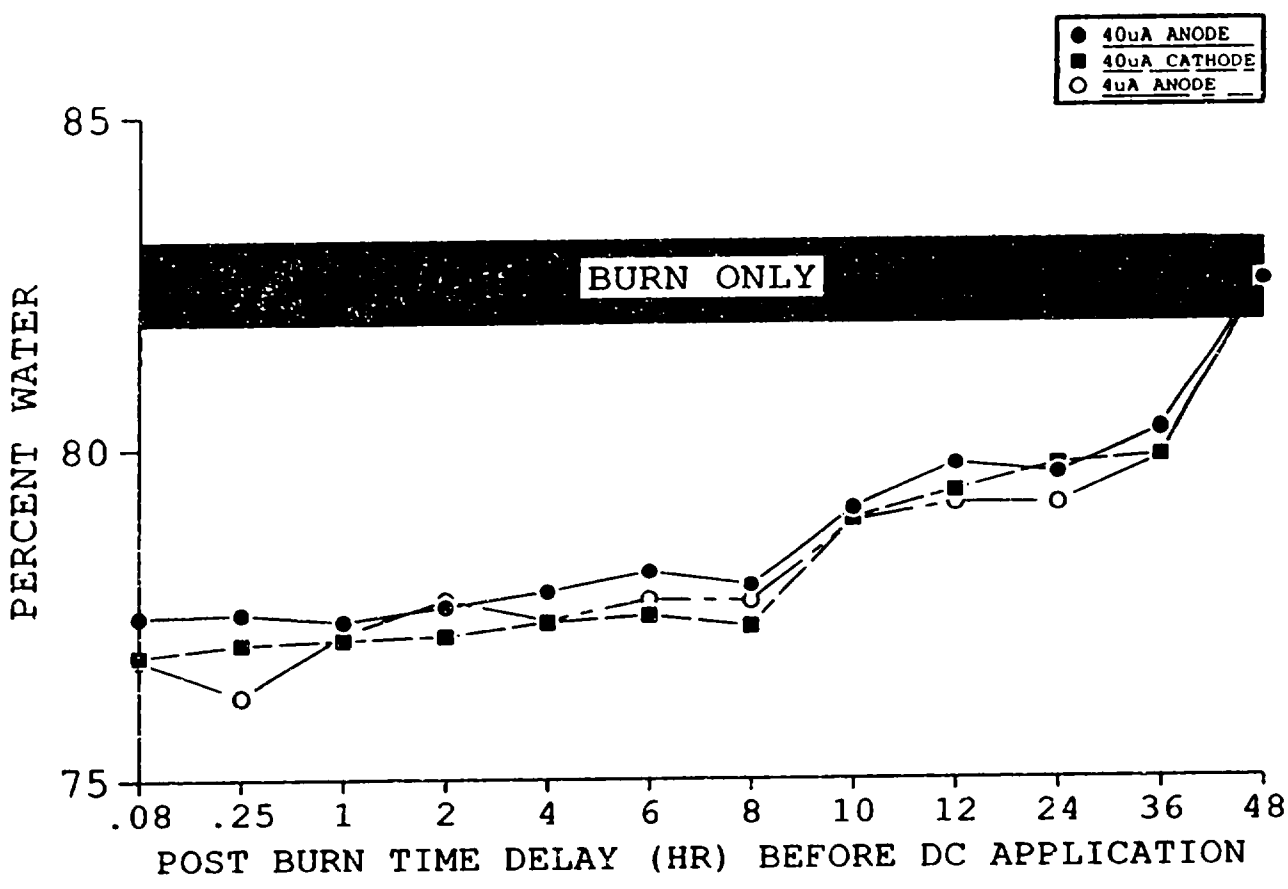

FIGURE 2. Effect of postburn time of DC application on burn wound edema at 48 h after 20% total body surface area full-thickness scald burns. DC was applied after the indicated delay period and then continued until animals were sacrificed at 48 h postinjury. Shaded area represents the 95% confidence limits for burned dorsal rat skin at 48 h postburn.

PUBLICATIONS

Chu C-S, McManus AT, Okerberg CV, Mason AD Jr, and Pruitt BA Jr: Weak direct current accelerates split-thickness graft healing of tangentially excised second-degree burns. *J Burn Care Rehabil* 12(4):285-93, July-August 1991.

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2. Walker HC, Mason AD Jr: A standard animal burn. *J Trauma* 8:1049-51, 1968.

3. Chu C-S, McManus AT, Mason AD, et al: Multiple graft harvestings from deep partial-thickness scald wounds healed under the influence of weak direct current. *J Trauma* 30:1044-50, 1990.

4. Chu C-S, McManus AT, Okerberg CV, et al: Weak direct current accelerates split-thickness graft healing on tangentially excised second-degree burns. *J Burn Care Rehabil* 12(4):285-93, 1991.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION: DA318371	2. DATE OF SUMMARY: 01 Oct 91	REPORT CONTROL SYMBOL: DD-DR&E(AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISP'N INSTR'N	9. LEVEL OF SUM / WORK UNIT
01 Oct 90	D. Chg	U	U		CX	

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS14	AZ	321
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code) (U) Effect of Resuscitation Fluid on Hepatic Blood Flow and High Energy Phosphate Production in a Swine Hemorrhagic Shock Model

12. SUBJECT AREAS
0605 Medicine and Medical Research

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
8905	9209	DA	C. In-House

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

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

18. RESOURCES ESTIMATE

FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
91	0.5	36
92	0.5	37

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME: US Army Institute of Surgical Research	a. NAME: US Army Institute of Surgical Research
b. ADDRESS (Include zip code): Fort Sam Houston, San Antonio, Texas 78234-5012	b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-5012
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR: BECKER, W K
d. TELEPHONE NUMBER (Include area code): 512-221-2720	d. TELEPHONE NUMBER (Include area code): 512-221-3349
21. GENERAL USE: FIC MILITARY/CIVILIAN APPLICATION: M	e. NAME OF ASSOCIATE INVESTIGATOR (If available): MASON, A D g. NAME OF ASSOCIATE INVESTIGATOR (If available):

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Burns (Injuries); (U) Hemorrhage; (U) Hemorrhagic Shock; (U) Trauma; (U) Resuscitation; (U) Liver; (U) Blood; (U) Colloids

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

23/24. (U) The objective of this work is to evaluate the effects of various resuscitation formulae on hepatic blood flow and hepatic high-energy phosphate production following hemorrhagic shock. The effective use of resuscitation fluids is vital to the management of battle casualties. Liver high-energy phosphate levels, hepatic blood flow, and oxygen delivery will be measured in swine at baseline, after 25% and 50% hemorrhage, and after administration of various resuscitative fluids.

25. (U) 9010 - 9109. Studies have been completed in 62 animals. Results indicated that hypertonic saline-dextran administration following 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 following dehydration. Results from the amended portion of the project indicate that hypertonic saline is effective for early resuscitation following hemorrhage in animals with 5% isotonic dehydration.

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

SUPPLEMENTAL WORK UNIT INFORMATION FOR THE PROJECT ENTITLED "EFFECT OF RESUSCITATION FLUID ON HEPATIC BLOOD FLOW AND HIGH-ENERGY PHOSPHATE PRODUCTION IN A SWINE HEMORRHAGIC SHOCK MODEL"

Subrecord/Linking Accession Number: Not applicable.

Search Control Data: W6055C/W6056F, 20 October 1989.

Product Identification: For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report* for fiscal years 1989-91.

Unclassified Special Categories: Lab Animals: Swine; RA II.

ABSTRACT

PROJECT NUMBER: 3M161102BS14, 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 1990 - 30 September 1991

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. We conclude that hypertonic saline-dextran may provide a period of organ support when other resuscitation practices are impractical.

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

To further evaluate hypertonic saline-dextran, an unanesthetized swine model will be studied. The model will include a preliminary procedure under general anesthesia. A splenectomy will be performed and flow probes will be placed on the portal vein and hepatic artery. An arterial catheter will be placed in the aorta through the sacral artery and catheters will be placed in the portal and hepatic veins. A Swan-Ganz catheter will be placed in the pulmonary artery. Catheters will be tunneled subcutaneously, protected by Velcro™ patches, and flushed daily with a heparin-saline solution. Approximately 5 days after these procedures, the animals will be placed into a modified Pavlon™ sling for the shock portion of the study.

PRESENTATIONS

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, 3 June 1991.

PUBLICATIONS

Becker W, Cioffi W, Mason A, and Pruitt B: Hypertonic saline-dextran and the recovery of hepatic blood flow and high energy phosphate content following hemorrhage. *Circ Shock* 34(1):35, May 1991.

REFERENCES

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2. Martins MA, Younes RN, Lin CA, et al: Hypovolemic shock resuscitation with hyperosmotic 7.5% NaCl: effects on respiratory system mechanics. *Circ Shock* 26:147-55, 1988.

3. Wade CE, Hannon JP: Confounding factors in the hemorrhage of conscious swine: a retrospective study of physical restraint, splenectomy, and hyperthermia. *Circ Shock* 24:175-82, 1988.

4. Doucet JP: Comparison of electrophysiologic effects of small volume resuscitation with 7.5% NaCl and 6% Dextran 70 with standard resuscitation following hemorrhage. (Personal communication).

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
				DA346176	01 Oct 91	DD-DR&E(AR) 636
3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISP'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
01 Oct 90	D. Chg	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS14	CK	323
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code) (U) Correlation of Plasma Amino Acid and Pyridoxal-5'-Phosphate (PLP) Levels in Thermally Injured Patients

12. SUBJECT AREAS
0601 Biochemistry 0605 Medicine and Medical Research

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
8906	9309	DA	C. In-House

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

18. RESOURCES ESTIMATE

a. FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
91	0.5	51
92	0.5	53

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME: US Army Institute of Surgical Research	a. NAME: US Army Institute of Surgical Research
b. ADDRESS (Include zip code): Fort Sam Houston, San Antonio, Texas 78234-5012	b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-5012
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR: BECKER, W K
d. TELEPHONE NUMBER (Include area code): 512-221-2720	d. TELEPHONE NUMBER (Include area code): 512-221-3349
21. GENERAL USE: FIC MILITARY/CIVILIAN APPLICATION: M	e. NAME OF ASSOCIATE INVESTIGATOR (If available) f. NAME OF ASSOCIATE INVESTIGATOR (If available)

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Burns (Injuries); (U) Trauma; (U) Septicemia; (U) Morbidity; (U) Amino Acids; (U) Enzymes; (U) Metabolism

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

23/24. (U) The objectives of this work are to measure PLP levels in thermally injured patients and correlate those with abnormalities in amino acid metabolism. One hundred burn patients will have plasma PLP and amino acid profiles drawn on admission, weekly, and when indicated by a change in clinical status. Burn size, presence of inhalation injury, morbidity, mortality, liver function test results, nitrogen balance, calories predicted and received, usage of aminoglycosides, theophylline, and/or digoxin, and the amount of vitamin B_6 supplementation received in tube feedings or hyperalimentation will be recorded. Multiple regression will be used to detect relationships between the various independent variables which are measured and the dependent variables.

25. (U) 9010 - 9109. Twenty-four patients have been enrolled in the study to date, 15 patients during this reporting period. Data 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. A question arises as to whether the depression in PLP levels observed in thermally injured patients represents a true deficiency of the vitamin or alternatively represents dilution and/or redistribution of the vitamin. Therefore, the study was amended to determine whether this depression represents a true vitamin deficiency and further work will be initiated shortly.

DD FORM 83 MAR 1498 EDITION OF MAR 88 IS OBSOLETE. * U.S.G.P.O. 1988-491-003/50329

SUPPLEMENTAL WORK UNIT INFORMATION FOR THE PROJECT ENTITLED "CORRELATION OF PLASMA AMINO ACID AND PYRIDOXAL-5'-PHOSPHATE (PLP) LEVELS IN THERMALLY INJURED PATIENTS"

Subrecord/Linking Accession Number: Not applicable.

Search Control Data: W6R20M/W6R22N, 29 May 1990.

Product Identification: For technical repor refer to the *US Army Institute of Surgical Research Annual Research Progress Report* for fiscal years 1989-92.

Unclassified Special Categories: Volunteers: Adults; RA II.

ABSTRACT

PROJECT NUMBER: 3M161102BS14, 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 1990 - 30 September 1991

INVESTIGATORS: William K. Becker, MD, Lieutenant Colonel, MC[1]
William G. Cioffi, Jr., MD, Major, MC[1]
Bryan S. Jordan, RN, MSN[1]
J. Enriquez, Sr.[2]
Basil A. Pruitt, Jr., MD, Colonel, MC[1]

Results from the first 24 patients enrolled in this study 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.

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, this study was expanded in an attempt to answer this question.

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, prior to beginning the study.

1. Male or female patients ≥ 18 yr old.

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 old.

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 from the first 24 patients enrolled in this study 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.

An addendum to this study was developed and approved during this reporting period. In addition to measuring PLP levels, peripheral blood mononuclear cells will be isolated from the blood sample obtained and the level of the enzyme, ornithine decarboxylase, will be measured before and after mitogen stimulation in the cell culture. Ornithine decarboxylase is a vitamin B_6-dependent enzyme and activity of this enzyme is required for peripheral blood mononuclear cells to proliferate. In addition to the measurement of this enzyme activity, erythrocyte PLP levels will also be measured. Erythrocyte PLP levels may give a more accurate indication of body stores of vitamin B_6 when compared to plasma levels. Ornithine decarboxylase will be measured using a technique that involves the liberation of $^{14}CO_2$ from C^{14} ornithine. The measurement of RBC PLP will be the same as that for plasma PLP. Correlation between levels of PLP, ornithine carboxylase, and erythrocyte PLP activity will be performed.

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. Therefore, this study was expanded in an attempt to answer this question.

Upon enrollment of the next 26 patients in the amended study, all data will be analyzed as indicated.

PRESENTATIONS/PUBLICATIONS

None.

REFERENCES

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
				DA346164	01 Oct 91	DD-DR&E(AR) 636
3. DATE PSV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISPOSITION	9. LEVEL OF SUM A. WORK UNIT
01 Oct 90	D. Chg	U	U		CX	

10. NO/CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS14	CK	324
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code) (U) Effect of Arginine Deprivation on the Response to Thermal Injury in Burn Wound Infection in the Rat - A Pilot Study

12. SUBJECT AREAS
0605 Medicine and Medical Research

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
9002	9209	DA	C. In-House

17. CONTRACT/GRANT — MILITARY RELEVANCY CERTIFIED — APPROVED BY

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

18. RESOURCES ESTIMATE

a. FISCAL YEARS	b. PROFESSIONAL WORKYEARS	c. FUNDS (In thousands)
91	1.0	65
92	1.0	69

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME: US Army Institute of Surgical Research	a. NAME: US Army Institute of Surgical Research
b. ADDRESS (Include zip code): Fort Sam Houston, San Antonio, Texas 78234-5012	b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-5012
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR: BECKER, W K
d. TELEPHONE NUMBER (Include area code): 512-221-2720	d. TELEPHONE NUMBER (Include area code): 512-221-3349
21. GENERAL USE: FIC MILITARY/CIVILIAN APPLICATION: M	f. NAME OF ASSOCIATE INVESTIGATOR (If available): CIOFFI, W G g. NAME OF ASSOCIATE INVESTIGATOR (If available): MC MANUS, A T

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Burns (Injuries); (U) Nutrition; (U) Metabolism; (U) Therapy

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

23/24. (U) The objective of this work is to determine the effect of a diet deficient in arginine on the response to thermal injury and burn wound infection. Sprague-Dawley rats will be fed regular, arginine-deficient, or arginine-sufficient diets for 5 wk. Weekly weight gain and urinary orotic acid and polyamine excretion will be measured. At the end of 5 wk, the animals will be administered 30% scald or sham wounds. One-half of these animals will be inoculated with *Pseudomonas*. Blood and urine samples will be obtained from any animal not surviving to 15 days and surviving animals sacrificed at 15 days for plasma amino acid analysis, BUN determination, and urine orotic acid and polyamine secretion. Mortality will be compared by life-table analysis. Differences in biochemical parameters will be studied by ANOVA, and where warranted, intergroup significance determined by the Scheffe' technique.

25. (U) 9010 - 9109. Studies during the last reporting period 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 did demonstrate differences in plasma amino acid levels and also in growth curves, both prior to and following thermal injury. Further work will be performed to elucidate the mechanisms of these effects and to determine factors that control ureagenesis and nitrogen balance following injury.

DD FORM 93 MAR 1498 EDITION OF MAR 88 IS OBSOLETE.

SUPPLEMENTAL WORK UNIT INFORMATION FOR THE PROJECT ENTITLED "EFFECT OF ARGININE DEPRIVATION ON THE RESPONSE TO THERMAL INJURY IN BURN WOUND INFECTION IN THE RAT - A PILOT STUDY"

Subrecord/Linking Accession Number: Not applicable.

Search Control Data: W6M22E/W6M25A, 8 January 1990

Product Identification: For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report* for fiscal years 1990-1.

Unclassified Special Categories: Lab Animals: Rats; RA II.

ABSTRACT

PROJECT NUMBER: 3M161102BS14, 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 1990 - 30 September 1991

INVESTIGATORS: William K. Becker, MD, Lieutenant Colonel, MC
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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.

This study demonstrated that the modification of dietary urea-cycle enzyme levels can markedly alter weight gain, plasma amino acid levels, and the excretion of orotic acid. Our results also suggest that thermal injury with the resulting large increase in urea synthesis did not stress the urea cycle or result in a delay in flow of metabolic intermediates resulting in orotic acid excretion. It is also interesting to note that the activity of the five urea-cycle enzymes did not change, suggesting that none of the enzymes were rate-limiting and that posttranslational control mechanisms which were not measured in this study were likely responsible for the ability to produce the increased urea output.

Because these amino acids play such a diverse role in protein synthesis and intermediary metabolism, the physiologic significance of dietary manipulation following trauma will require further investigation.

EFFECT OF ARGININE DEPRIVATION ON THE RESPONSE TO THERMAL INJURY AND BURN WOUND INFECTION IN THE RAT - A PILOT STUDY

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. Arginine, which has generally been considered to be a nonessential amino acid in the adult (1), has been reported to have a number of metabolic properties that warrant further investigation to determine the exact role of this amino acid in the response to injury.

In the rat, consumption of an arginine-deficient diet has been associated with a decrease in water intake and urine output, but growth and weight gain were normal (2). Arginine plays a central role in both amino acid and nitrogen metabolism. It is incorporated into proteins and is also a key intermediate in the urea cycle which is the principal pathway of nitrogen elimination in mammalian species (3). Intake of an arginine-deficient diet has been associated with a marked increase in orotic acid excretion in the urine (4). Carbamoyl phosphate, a urea cycle intermediate and a precursor to orotate, is presumably unable to enter the urea cycle in sufficient quantity during periods of arginine deprivation and is directed to the cytosol and converted to orotic acid, a pyrimidine precursor. Excess orotic acid then leaves the cell and is excreted in the urine. Several studies suggest that arginine may become an essential amino acid during times of stress, injury, or illness (5,6). A relative deficiency of arginine may lead to abnormalities of both the urea cycle and protein synthesis, with concomitant abnormalities in wound healing and immunologic function. Other metabolites of arginine, such as polyamines, are thought to regulate macrophage response to cytokines such as interleukin-1 and tumor necrosis factor and may also be affected by arginine deficiency (7).

The following studies were performed 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.

MATERIALS AND METHODS

Study Design.

Phase I. The effects of modification of dietary urea-cycle amino acids on weight gain and plasma aminograms were studied in the rat model. Adult Lewis rats were placed on defined amino acid diets that varied only in the content of the urea-cycle amino

acids. The first diet, the amino acid control diet (Arg+), contained 12.1 g/kg L-arginine. The arginine-deficient diet (Arg-) was identical except that L-arginine was omitted and the sucrose content was increased to 502.98 g/kg. The ornithine diet (Orn) was identical to the amino acid control diet except that L-arginine was omitted, 15.19 g/kg L-ornithine was added, and sucrose was changed to 487.79 g/kg. The citrulline diet (Cit) was identical to the amino acid control diet except that L-arginine was omitted, 13.42 g/kg L-citrulline was added, and sucrose was changed to 489.56 g/kg. These diets were complete and contained supplemental minerals and vitamins. Animals were placed in individual metabolic cages and weight gain, food intake, and urinary output were measured on a daily basis for a period of 5 weeks. At the end of the study period, the animals were sacrificed with sodium pentobarbital (35 mg/kg IP) and exsanguinated. Blood samples and, in some cases, hepatic tissues were obtained for further analysis. Plasma amino acid levels were performed and the activity of the urea cycle, the five urea cycle amino acids, carbamoyl-phosphate synthetase, ornithine transcarbamylase, argininosuccinate synthetase, argininosuccinase, and arginase were performed on fresh hepatic homogenates.

Phase II. Adult Lewis rats were adapted for a period of 5 days to the amino acid control diet. On day 0, baseline weight, food intake, and nitrogen balance studies were performed. The animals were divided into two groups, burn and sham-burn. Animals were anesthetized with sodium pentobarbital (35 mg/kg IP). The dorsal area was shaved and the animal placed in an insulated template which exposed 30% of the total body surface area. Animals assigned to the burn group were then immersed in 100°C water for 10 sec to produce a 30% total body surface area full-thickness scald burn. Animals assigned to the sham-burn group were immersed in water equal to room temperature for 10 sec. Animals were then randomized to one the diet groups described under Phase I and studied for either 3 or 8 days. Daily nitrogen balance studies were performed and urinary orotic acid excretion and polyamines were measured. On postburn day 3 or 8, animals were sacrificed with sodium pentobarbital (60 mg/kg IP) and exsanguinated. Blood samples were obtained and analyzed for amino acids. Hepatic tissues were analyzed for hepatocellular urea-cycle amino acid levels.

Determination of Number of Animals Required. Since this was a pilot study, the number of animals requested was based upon an estimate of the number of animals per group required to give a large response within each group. No previous data were available to estimate the variance expected to result.

Data Analysis Plan. Differences in biochemical parameters were studied by ANOVA and, where warranted, intergroup significance determined by Scheffe' technique.

RESULTS

Phase I. Weight gains for each group are listed in Table 1. The greatest weight gain was in the Cit group. Animals in the Arg- group had markedly decreased weight gain. Animals in the Orn group had intermediate weight gain. After 5 days on a similar diet, the Arg- group had significantly decreased T-cell helper suppressor ratios (Arg+, 3.04 ± 0.51; Arg-, 2.24 ± 0.17 ($P < 0.05$)) and at 14 days, orotic acid excretion was markedly increased (Arg+, 0.08 ± 0.02 μg/mg creatinine; Arg-, 10.9 ± 5.1 ($P < 0.01$)). Plasma amino acid levels are listed in Table 2. The Arg- group had markedly elevated levels of glutamine at 900 ± 136 μM/l. The Cit group had significantly elevated levels of citrulline and arginine in the plasma. Arginine levels were depressed in the Arg- and Orn groups compared to the Arg+ group.

TABLE 1. Weight Gain (Mean ± SD)

Group	n	Weight (g)
Amino acid control (Arg+)	10	79.4 ± 13.9
Arginine-deficient (Arg-)	10	14.8 ± 7.4*
Ornithine (Orn)	10	40.1 ± 5.9*
Citrulline (Cit)	10	98.8 ± 8.9

*P < 0.05 vs Arg+, ANOVA.

TABLE 2. Plasma Amino Acid Levels (Mean ± SD)

Group	Arginine (μm/l)	Ornithine (μm/l)	Citrulline (μm/l)	Glutamine (μM/l)
Amino acid control (Arg+)	151 ± 14	82 ± 24	66 ± 11	554 ± 154
Arginine-deficient (Arg-)	82 ± 51*	68 ± 32	69 ± 13	900 ± 136*
Ornithine (Orn)	76 ± 43*	74 ± 51	67 ± 9	595 ± 45
Citrulline (Cit)	295 ± 44*	104 ± 13	235 ± 59*	496 ± 20

*P < 0.05 vs Arg+, ANOVA.

Phase II. Results are listed in Table 3. Thermal injury resulted in a significant increase in UUN excretion and negative

TABLE 3. Nitrogen Balance and Enzyme Levels

Group =	Postburn Day 3		Postburn Day 8	
	Sham-Burn	Burn	Sham-Burn	Burn
Nitrogen balance (mg/day)	90 ± 18	-59 ± 33*	79 ± 11	80 ± 17
Nitrogen intake (mg/day)	340 ± 10	330 ± 22	328 ± 11	377 ± 21*
Nitrogen output (mg/day)	253 ± 15	389 ± 33*	250 ± 9	297 ± 26
Orotic acid (μg/day)	106 ± 11	65 ± 7*	114 ± 5	129 ± 6
Ornithine transcarbamylase (U)	123 ± 12	119 ± 14	151 ± 17	144 ± 21
Arginine (U)	260 ± 27	228 ± 23	260 ± 20	245 ± 18

*$P < 0.05$ vs sham-burn group.

nitrogen balance, despite a nitrogen intake equal to sham-burned animals. There was no increase in the activity of any of the levels of the urea-cycle enzymes and orotic acid excretion was significantly lower for animals assigned to the burn group, suggesting adequacy of urea-cycle enzyme function.

DISCUSSION

These studies demonstrated that the modification of dietary urea-cycle enzyme levels can markedly alter weight gain, plasma amino acid levels, and the excretion of orotic acid. The factors that control urea synthesis in normal animals and, in particular, following trauma with resulting large increases in urea output are not well understood. Urea-cycle enzymes have a long half-life and minute-to-minute or hour-to-hour control appears to be largely at the posttranslation level. The excretion of orotic acid is elevated in subjects that have genetic deficiencies in one of the urea-cycle enzymes. It is interesting that this also occurs in rats, particularly when arginine is deleted from the diet. The finding of markedly increased levels of plasma, citrulline, and arginine for the Cit group is interesting. Recent speculation suggests that there is a citrulline-arginine cycle involving the liver, small intestine, and kidney. In the liver, all of the enzymes necessary for arginine synthesis are present, but because of the high levels of the enzyme, arginase, it is likely that almost all arginine present is immediately broken down to ornithine and urea. In contrast, the small intestine and kidney contain enzymes that result in synthesis of arginine from citrulline but arginase is either present in extremely low levels or entirely absent from these tissues. Thus, these two tissues may be net producers of arginine. It is possible that in the Cit group,

arginine synthesis and export from the small intestine or the kidneys was markedly enhanced.

In our observations of burn and sham-burned animals in the ARG+ group, nitrogen balance was markedly negative on the third day postinjury but orotic acid excretion was, in fact, decreased compared to the sham-burned animals. This suggests that thermal injury with the resulting large increase in urea synthesis did not stress the urea cycle or result in a delay in flow of metabolic intermediates resulting in orotic acid excretion. It is also interesting to note that the activity of the five urea-cycle enzymes did not change, suggesting that none of the enzymes was rate-limiting and that posttranslational control mechanisms which were not measured in this study were likely responsible for the ability to produce the increased urea output. The urea-cycle amino acids, arginine, ornithine, and citrulline, are involved in diverse metabolic processes other than the synthesis of urea. These include the synthesis of polyamines, the synthesis of nitric oxide, arginine is also required for protein synthesis and arginine in particular has been described as being involved in a number of immunologic control mechanisms. Because these amino acids play such a diverse role in protein synthesis and intermediary metabolism, the physiologic significance of dietary manipulation following trauma will require further investigation.

It is planned to follow up the current study with a series of studies to determine the mechanisms that control urea synthesis postinjury, to ascertain whether dietary manipulation of urea-cycle amino acids results in alterations in polyamine and nitric oxide syntheses, and to elucidate the pathophysiologic significance of any alteration in the previously described biologic processes.

PRESENTATIONS/PUBLICATIONS

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 CICI, Stockholm, Sweden, 25 August 1991.

REFERENCES

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of other urea cycle intermediates in mouse liver. *J Nutr* 119:1188-95, 1989.

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7. Flescher E, Bowlin TL, Talal N: Polyamine oxidation down-regulates IL-2 production by human peripheral blood mononuclear cells. *J Immunol* 142:907-12, 1989.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1 AGENCY ACCESSION	2 DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA146163	30 Sep 91	DD-R&AR(AR)636

3 DATE FOR SUMMARY	4 KIND OF SUMMARY	5 SUMMARY DATA	6 MARK SECURITY	7 REGRADING	8 DISTRIBUTION	9 LEVEL OF SUM & WORK UNIT
1 Oct 90	M, Term	U	U		CX	

10 NO. (FUNDING)	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	611102A	3M161102BH15	FB	125
b. CONTRIBUTING				
c. CONTRIBUTING				

11 TITLE (Include with Security Classification Code): (U) Development of an Aspergillus Rat Burn Wound Infection Model - A Pilot Study

12 SCIENCE AREAS
0604 Anatomy and Physiology, 0603 Medicine and Medical Research

13 START DATE	14 ESTIMATED COMPLETION DATE	15 FUNDING ORGANIZATION	16 PERFORMANCE METHOD
9002	9109	DA	C. In-House

17 CONTRACT/GRANT: MILITARY RELEVANCY CANCELED
APPROVED BY [illegible]
a. DATE EFFECTIVE
b. EXPIRATION
c. CONTRACT/GRANT NUMBER
d. TYPE
e. AMOUNT
f. DUNS NO.

18 RESOURCES ESTIMATE	FISCAL YEARS	a. PROFESSIONAL MANYEARS	b. FUNDS (In thousands)
	91	0.5	32
	92	0.0	0

19 PERFORMING ORGANIZATION
a. NAME: US Army Institute of Surgical Research
b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-5012
c. NAME OF PRINCIPAL INVESTIGATOR: BECKER, W K
d. TELEPHONE NUMBER (Include Area Code): 512-221-3349
e. NAME OF ASSOCIATE INVESTIGATOR (If Available): MC MANUS, A T
f. NAME OF ASSOCIATE INVESTIGATOR (If Available): FIM, E H

20 RESPONSIBLE INDIVIDUAL
a. NAME: FRUITT, B A
b. TELEPHONE NUMBER (Include Area Code): 512-221-4720
c. SIGNATURE: PIC
d. MILITARY/DIVISION APPLICATION: II

21 KEYWORDS (Precede Each with Security Classification Code): (U) Burns (Injuries); (U) Fungi; (U) Histopathology

22 TECHNICAL OBJECTIVE OR APPROACH 23 PROGRESS (Precede Text with Security Classification Code)

22/23. (U) The objective of this work is to develop an animal model of Aspergillus burn wound infection. Sprague-Dawley rats will be administered 30% scald wounds and inoculated with *Aspergillus flavus* at various times postburn. Group 1 will serve as the control group, Group 2 will receive streptomycin 10 days prior to injury, Group 3 will receive cortisone acetate 24 h prior to injury, and Group 4 will receive cyclosporine 24 h prior to injury. Wounds will be examined daily and the appearance recorded. Any animal not surviving to 15 days and surviving animals sacrificed at 15 days will undergo necropsy and histopathologic examination. Results of wound appearance, mortality, wound culture, and histopathology and any evidence of disseminated infection will be recorded.

24. (U) 9002 - 9109. Despite modifications in species, no significant fungal burn wound infection was produced. It is likely that rodents are resistant to fungal burn wound infection and are not a good species choice to study this problem. Therefore, this study has been terminated.

DD FORM 1498 (ADAR) — EDITION OF MAR 88 IS OBSOLETE — ADAPTED (MM-491-003/MM)

SUPPLEMENTAL WORK UNIT INFORMATION FOR THE PROJECT ENTITLED "DEVELOPMENT OF AN ASPERGILLUS RAT BURN WOUND INFECTION MODEL - A PILOT STUDY"

Subrecord/Linking Accession Number: Not applicable.

Search Control Data: W6L16M/W6L18M, 9 January 1990.

Product Identification: For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report* for fiscal years 1990-1.

Unclassified Special Categories: Lab Animals: Rats; Mice; RA II.

ABSTRACT

PROJECT NUMBER: 3M161102BS14, Research

PROJECT TITLE: Development of an Aspergillus Rat Burn Wound Infection Model - A Pilot Study

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

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

INVESTIGATORS: William K. Becker, Lieutenant Colonel, MC
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Fungal burn wound infection is currently the most common type of burn wound infection at this Institute. Filamentous fungi, such as Aspergillus, are the most common organisms associated with invasive burn wound infection. Because of the success of rodent models of bacterial burn wound infection in laboratory investigations, it would be desirable to have a similar model of fungal burn wound infection. Previous studies of fungal burn wound infections in rodents have been induced or modified by various types of immunosuppression. It was the purpose of this study to investigate various methods of immunologic manipulation to develop a reliable model of fungal burn wound infection in small rodents.

Studies were conducted in rats and Beige mice (these rodents having a T-cell defect). Burn injury and postburn wound inoculation with fungus failed to yield a satisfactory and reliable burn wound infection in any of the study groups despite multiple methods of altering immune function, including methods successfully used by other investigators to induce other types of fungal infections in these species. It was determined that small rodents are resistant to fungal burn wound infection and that further model development should be conducted using other species.

DEVELOPMENT OF AN ASPERGILLUS RAT BURN WOUND INFECTION MODEL - A PILOT STUDY

Fungal burn wound infection has replaced bacterial burn wound infection as the most common type of infectious burn complication seen in patients admitted to this Institute (1). Aspergillus species are the most common organisms involved in fungal burn wound infection. Treatment of fungal burn wound infection involves surgical debridement of involved areas. Topical and systemic antifungal chemotherapies are also used, but there is little objective data to support the efficacy of these treatments.

The development of animal models of bacterial burn wound infection has been quite useful in the development of effective treatment strategies in the management of this wound complication. It would be desirable to develop a small animal model of fungal burn wound infection so that various treatments could be tested in a laboratory setting. Previous work at this Institute demonstrated inconsistent response of rats to experimental fungal burn wound infection, which has limited the usefulness of this model (2,3). In other types of experimental fungal infection induced in small rodents, immunosuppression by various methods has been extremely useful in providing a more reliable and consistent model (4,5). It was the purpose of this study to attempt to use these methods in an attempt to develop a more reliable model of fungal burn wound infection in small rodents.

MATERIALS AND METHODS

Study Design.

Phase I. Sprague-Dawley rats were randomized to one of four groups. Group 1 served as the control group. These animals received 20% total body surface area full-thickness scald burns only. Animals in Group 2 were administered streptozocin 10 days prior to burn injury to induce a diabetic state. Animals in Group 3 received cortisone acetate 24 h prior to burn injury. Animals in Group 4 received cyclosporine 24 h prior to burn injury and continuing for 5 days. Animals in each group were further randomized into four subgroups. Animals in Groups 1A, 2A, 3A, and 4A received burn injury only. Animals in Groups 1B, 2B, 3B, and 4B received burn injury following by immediate inoculation with 10^6 *Aspergillus flavus*. Animals in Groups 1C, 2C, 3C, and 4C received burn injury followed by inoculation at 24 h. Finally, animals in Groups 1D, 2D, 3D, and 4D were administered burn injury following by inoculation at 48 h. Animals were followed for 15 days.

Phase II. Sprague-Dawley rats were randomized to one of six groups. All animals were administered a 20% total body surface area full-thickness scald burn followed by burn wound excision. Split-thickness skin grafts were taken from the abdomen of donor

animals and transplanted to the open wound of recipient animals. Animals in Group 1 received burn injury and excision only. Animals in Group 2 were inoculated with 10^2 fungus just prior to placing the skin graft, Group 3 with 10^3 fungus, Group 4 with 10^4 fungus, Group 5 with 10^5 fungus, and Group 6 with 10^6 fungus. Animals were followed for 15 days.

Phase III. Sprague-Dawley rats were randomized to one of six groups as indicated for Phase II. Animals were further randomized to one of 5 subgroups. Animals in Subgroup A were treated with dry dressings. Animals in Subgroup B had saline applied to the dressing twice daily for 15 days, C received 5% mafenide acetate solution, D received 1% amphotericin B, and E received Dakin's solution. Animals were followed for 15 days.

Phase IV. Beige mice received 20% total body surface area full-thickness scald burns. The burn wounds were inoculated with 10^6 cfu *Aspergillus flavus*. These animals were followed for 3 weeks.

Description of Procedures. Two hundred and eighty male Sprague-Dawley rats weighing ± 350 g and 30 Beige (C56Bl/6/bgJ/bgJ) mice were anesthetized with sodium pentobarbital (35 mg/kg IP) administered through a 25-ga needle. The dorsal area was shaved and the animals placed in an asbestos-coated template which exposed 30% (rats) or 20% (mice) of the total body surface area. The template was immersed in 100°C water for 10 sec to produce a 30% total body surface area full-thickness scald burn (rats) or in 80°C water for 10 sec to produce a 20% total body surface area full-thickness scald burn (mice).

Phase I. In each of four groups, 10 animals served as controls and 10 animals each were inoculated by painting the wound with 10^6 *Aspergillus flavus* immediately, at 24 h, or 48 h, respectively. Burn injury and inoculation for all groups were identical. Group 1 (n=40) served as the control group. Group 2 animals (n=40) received an injection of streptozocin (50 mg/kg IP) 10 days prior to burn injury. Hyperglycemia was confirmed on the day of burn injury. Group 3 animals (n=40) received cortisone (5 mg SC) 24 h prior to burn injury and Group 4 animals (n=40) received cyclosporine (10 mg/kg IP) daily for 5 days beginning 24 h prior to burn injury. Upon completion of the burn injury, the animals were placed back in their cages and allowed to recover from anesthesia without resuscitation. They were allowed food and water ad libitum. Animals were followed for 15 days, at which time any survivors were sacrificed with sodium pentobarbital (60 mg/kg IP) administered through a 25-ga needle. All animals underwent necropsy and histopathologic examination and culture of wound specimens, either at the time of death or sacrifice. Animals were also selectively biopsied for culture and microscopic examination based on wound appearance. Results of wound appearance, mortality,

wound culture, and histopathology and any evidence of disseminated infection were recorded.

Phase II. For each of the six groups, a uniform wound was excised to the level of the fascia after burn injury. Split-thickness skin grafts were taken at a depth of 89/1000 of an inch from the abdomen of donor animals (n=30) and meshed in a ratio of 1.5:1. The mesh grafts were then transplanted to the open wounds of recipient animals (n=30) and secured with sutures and surgical staples. Animals in Group 1 (n=5) received burn injury and excision only. Animals in Group 2 (n=5) were inoculated with 10^2 fungus just prior to placing the skin graft, Group 3 (n=5) with 10^3 fungus, Group 4 (n=5) with 10^4 fungus, Group 5 (n=5) with 10^5 fungus, and Group 6 (n=5) with 10^6 fungus. An occlusive surgical dressing was then fashioned over the graft. Animals were returned to their cages and offered food and water ad libitum. On postoperative day 7, the surgical dressings were removed and the appearance of the grafted tissue was recorded. Animals were observed through postoperative day 15, at which time all surviving animals were sacrificed with sodium pentobarbital (60 mg/kg IP) administered through a 25-ga needle. Samples were obtained from the wounds for microbiologic and microscopic examination.

Phase III. The procedure of skin grafting was identical to that for Phase II. Following inoculation with fungus and placement of the surgical dressing, animals were randomized to one of five subgroups. Animals in Subgroup A had dry dressings applied to the wound throughout the study. The surgical dressings for animals in Subgroups B, C, D, and E were soaked twice daily with saline, 5% mafenide acetate, 1% amphotericin B, or Dakin's solution, respectively. Follow-up and examination of the wounds was identical to that for Phase II. A scoring system to judge the extent of graft infection, graft loss, and graft healing was maintained for each animal. At the end of 15 days, all surviving animals were sacrificed with sodium pentobarbital (60 mg/kg IP) administered through a 25-ga needle. Histologic and microbiologic examination of the wound was performed.

Phase IV. Immediately following burn injury, the animals (n=30) were inoculated with 10^6 cfu *Aspergillus flavus*. Animals were then returned to their cages and offered food and water ad libitum. These animals were followed for 3 weeks. Any surviving animals were sacrificed with sodium pentobarbital (60 mg/kg IP) administered through a 25-ga needle. Both survivors and nonsurvivors underwent necropsy and histologic examination of the burn.

Determination of Number of Animals Required. Two hundred and eighty Sprague-Dawley rats and 60 Beige mice were necessary for this study. Since this was a pilot study, the number of animals requested was based upon an estimate of the number of animals per

group required to detect a large response within each treatment group.

Safety Considerations for Biohazards. All animals were kept in filter-top cages to prevent dissemination of the wound inoculation. All personnel handling either the animals or the inoculum were required to wear caps, masks, gloves, and protective eye gear.

RESULTS

Burn infection with fungi was observed in < 5% of all animals and immunologic manipulations did not appear to be effective in promoting the development of fungal burn wound infection. Surface colonization of the wound was common and the inoculated organism could be recovered from the wound by culture, indicating that the organism was present on the wound but was not able to invade normal tissue below or adjacent to the burn injury. Biochemical studies demonstrated the interventions utilized were effective, i.e., elevated blood glucose was noted in the streptozocin groups and plasma cyclosporine levels were measured in that group and observed to be in the range predicted for the dose. Also, renal function was adversely affected by dose of cyclosporine utilized. Similar findings were noted in studies using the Beige mouse, with this species being equally resistant to fungal burn wound infection.

DISCUSSION

It would appear, in contrast to bacterial burn wound infection, that small rodents are extremely resistant to the development of fungal burn wound infection. Even the use of fairly severe techniques to immunosuppress the animals were unable to significantly modify the response to the fungal inoculum. The development of fungal burn wound infection was so rare in this study that further attempts to develop this model in these species seems unwarranted and model development will need to proceed using another species. It is difficult to explain this resistance; however, the pathogenesis of fungal burn infection in patients does appear to differ greatly from that associated with bacterial burn wound infection, which responds well to the use of topical chemotherapeutic agents and isolation techniques.

PRESENTATIONS

Becker WK: Fungal colonization of the burn wound. Presented at the 9th International Congress on Burn Injuries, New Delhi, India, 14 November 1990.

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* 126:44-8, January 1991.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION DA346177	2. DATE OF SUMMARY 01 Oct 91	REPORT CONTROL SYMBOL DD-DR&E(AR) 636
3. DATE PREV SUMRY 01 Oct 90	4. KIND OF SUMMARY D. Chg	5. SUMMARY SCTY U	6. WORK SECURITY U	7. REGRADING	8. DISP INSTRN CX	9. LEVEL OF SUM A. WORK UNIT

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS14	CA	326
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code) (U) Effect of Surfactant Replacement on V_A/Q in Sheep with Inhalation Injury

12. SUBJECT AREAS
0605 Medicine and Medical Research

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
9002	9309	DA	C. In-House

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

18. RESOURCES ESTIMATE

a. FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
91	0.5	47
92	0.5	50

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME: US Army Institute of Surgical Research	a. NAME: US Army Institute of Surgical Research
b. ADDRESS (Include zip code): Fort Sam Houston, San Antonio, Texas 78234-5012	b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-5012
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR: CIOFFI, W G
d. TELEPHONE NUMBER (Include area code): 512-221-2720	d. TELEPHONE NUMBER (Include area code): 512-221-8440
21. GENERAL USE: FIC MILITARY/CIVILIAN APPLICATION: M	e. NAME OF ASSOCIATE INVESTIGATOR (If available): JORDAN, B S g. NAME OF ASSOCIATE INVESTIGATOR (If available): MASON, A D

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

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

23/24. (U) The objective of this work is to examine the effect of exogenously administered surfactant on V_A/Q changes seen following inhalation injury in an ovine model. Forty-two sheep will be randomized to one of three groups. Group I will be exposed to a moderate smoke injury and administered surfactant 24 h following injury. Groups II and III will be exposed to a severe smoke injury. Group II will be administered surfactant or saline immediately following injury and every 12 h for 24 h. Group III will be administered surfactant or saline continuously during the first 24 h after injury. Group I data obtained following surfactant replacement will be indexed to pretreatment data and compared by the student's t test. Group II and III data will be compiled and, after analysis of mean and standard deviations, compared utilizing the student's t test and ANOVA.

25. (U) 9010 - 9109. The first phase of this study was completed during this reporting period and data are currently being analyzed.

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SUPPLEMENTAL WORK UNIT INFORMATION FOR THE PROJECT ENTITLED "EFFECT OF SURFACTANT REPLACEMENT ON V_A/Q IN SHEEP WITH INHALATION INJURY"

Subrecord/Linking Accession Number: Not applicable.

Search Control Data: W6O15A/W6O15D, 30 May 1990.

Product Identification: For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report* for fiscal years 1990-1.

Unclassified Special Categories: Lab Animals: Sheep; RA II.

ABSTRACT

PROJECT NUMBER: 3M161102BS14, 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 1990 - 30 September 1991

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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.

MATERIALS AND METHODS

Study Design. 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 (EXOSURF®, Burroughs Wellcome Company,

Research Triangle Park, NC) or surfactant vehicle while remaining intubated and on mechanical ventilatory support. Animals were followed for 48 h and standard physiologic measurements were made.

Description of Procedures. Sixteen neutered male sheep weighing 25-45 g were utilized for this study. Each sheep was housed in a conventional outdoor run and had access to food and water ad libitum. Sheep were dewormed with injectable ivermectin (IvomecR, Merck and Company, Rahway, NJ) 2 weeks before use. Inhalation injury was induced using the standard ovine smoke inhalation model developed at this Institute. Sixteen animals were exposed to a moderate smoke injury, with a carboxyhemoglobin of approximately 70% at completion of the smoke exposure.

On the day of the study, the animals were anesthetized with sodium pentobarbital (35 mg/kg IP). A peripheral venous catheter, a central venous pressure catheter, a balloon-directed thermodilution pulmonary artery catheter (7F, American Edwards Company, Irvine, CA), and a femoral artery catheter were placed. A tracheostomy was placed via a Moline neck incision. Animals were paralyzed with pancuronium bromide (0.03-0.04 mg, 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 (Servo 900C). Tidal volumes were set at 15 ml/kg and respiratory rate was altered to maintain a PCO_2 of 25 torr. The animals were ventilated with an FIO_2 of 0.21 and 5 cm of PEEP. Lactated Ringer's was constantly infused at a rate of 1.5 ml/kg/h. Central venous and pulmonary artery pressures 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, MS).

Animals were allowed to awaken from anesthesia and after an 1-h equilibration period, heart rate, blood pressure, central venous pressure, pulmonary artery pressure, cardiac output, arterial blood gases, mixed-venous blood gases, and tidal volume were measured. Blood samples were analyzed immediately by GC-MS (Model 5985, Hewlett-Packard). After two consecutive measurements spaced 30 min apart that were similar, the animals were paralyzed with succinylcholine and exposed to 7 units of smoke in an attempt to yield a moderate injury. Immediately after smoke exposure, arterial blood gases were obtained and carboxyhemoglobin measurements made. The animals were allowed to awaken from paralysis and were continued on conventional mechanical ventilatory support as previously described.

The animals were maintained in the awake tracheostomized state on conventional ventilatory support which maintained total ventilatory control by keeping the arterial PCO_2 at 25 torr. Arterial PCO_2 was manipulated by varying the respiratory rate while keeping the tidal volume at 15 ml/kg. One hour after smoke

exposure, the animals were randomized to receive aerosolized exogenous synthetic surfactant or 0.914N sodium chloride on a continuous basis. Every 4 h, repeat cardiopulmonary parameters were measured over a 48-h period. At the end of 48 h, the animals were sacrificed with sodium pentobarbital (60 mg/kg) and necropsied. Representative sections of the lung and trachea were obtained for histologic study.

RESULTS

One animal aspirated during initial intubation and was immediately sacrificed. In another animal, the Swan-Ganz catheter could not be placed through either jugular vein, thus making the animal unsuitable for study. In yet another animal, malignant hyperthermia occurred within 30 min of smoke injury, rendering the animal unsuitable for study. Thirteen animals were exposed to a 7-unit smoke injury and randomized into the treatment protocol. Seven animals received surfactant replacement and 6 animals received the sodium chloride vehicle only. The carboxyhemoglobin levels of each group immediately after smoke exposure were 61.8% ± 6.8% for the surfactant-treated group and 76.3 ± 7.9% for the saline-treated group. Baseline cardiac index, pulmonary artery systolic pressure, mean systemic blood pressure, pulmonary capillary wedge pressure, and arterial PO_2 were not different between the two groups. All animals in both groups demonstrated a significant and consistent rise in pulmonary artery pressure over the 48-h study period. There was no difference between the two groups in pulmonary artery pressures at any time point. Likewise, there was no significant difference in mean systemic blood pressure, cardiac index, or pulmonary capillary wedge pressure at any time point. Arterial oxygenation was similar before smoke exposure. However, at 1 h postinjury, the saline group had significantly lower arterial PO_2 than the surfactant group (78.5 ± 3.7 vs 92.4 ± 4.3 torr). By 4 h, this trend had reversed, with mean arterial oxygenation of 85.0 ± 2.4 torr for the saline group and 86.0 ± 7.0 torr for the surfactant group. By 36 h postinjury, the saline group tended to have lower arterial PO_2 values than the surfactant group, a trend which continued to the end of the study (48 h) (Table 1). Two animals in the surfactant group and one animal in the saline group died before completion of the 48-h study period. Of the animals which did not survive, one in each group had an initial carboxyhemoglobin level > 90%. In addition, one animal in the saline group, which had an initial carboxyhemoglobin level < 90%, showed a progressive deteriorating course which was markedly different from all other animals. Because previous work has shown that the severity of inhalation injury can be indexed in a gross manner to the carboxyhemoglobin level, the data from the 3 animals with carboxyhemoglobin levels > 90% were removed and the data reanalyzed. This was done in an attempt to compare animals with more similar injuries.

TABLE 1. Arterial PO_2 Values (Mean ± SEM)

Time Postsmoke (h)	Group		P Value
	Surfactant	No Surfactant	
36	59.6 ± 2.9	48.5 ± 5.7	0.12
40	62.0 ± 8.6	41.8 ± 2.4	0.06
44	59.2 ± 8.3	43.8 ± 2.9	0.1
48	62.2 ± 9.1	42.6 ± 3.7	0.09

Exclusion of these 3 animals left 10 animals for analysis. The mean carboxyhemoglobin level immediately after smoke exposure in the smaller cohort was 66.3% ± 7.6% for the saline-treated group and 56.2% ± 4.6% for the surfactant-treated group. Baseline pulmonary and hemodynamic measurements were not different between the two groups, with the exception of venous admixture which was slightly but significantly greater for the saline-treated group compared to the surfactant-treated group 4 h before injury and 1 h after injury (fig 1). By 4 h postinjury, however, the calculated shunt was similar between the two groups.

Throughout the study period, arterial PCO_2 tended to be lower in the surfactant-treated group than the saline-treated group despite similar respiratory rates (figs 2 and 3). The differences in arterial PCO_2 were significant at 24 h postinjury ($P < 0.05$), a time point when minute ventilation was identical between the two groups. However, this difference in CO_2 clearance did not hold for the remainder of the study period. Hemodynamic variables, to include mean systemic blood pressure, pulmonary capillary wedge pressure, and pulmonary artery systolic pressure, were not different between the two groups at any point during the study period (figs 4-6). Cardiac index was similar before smoke exposure. However, at 1 and 8 h postinjury, the saline-treated group tended to have slightly higher cardiac indices than the surfactant-treated group. This difference did not hold for the remainder of the study. The marked hyperdynamic state of the saline-treated animals returned to more normal values that were similar to the surfactant-treated group (fig 7). Pulmonary artery systolic pressure rose in a similar fashion throughout the study period in both groups so that a marked increase was present during the latter 16 h of study compared to preinjury levels.

Arterial oxygenation declined significantly in both groups throughout the study period (fig 8). Only at 40, 44, and 48 h was there a suggestion of difference between the two groups, with the

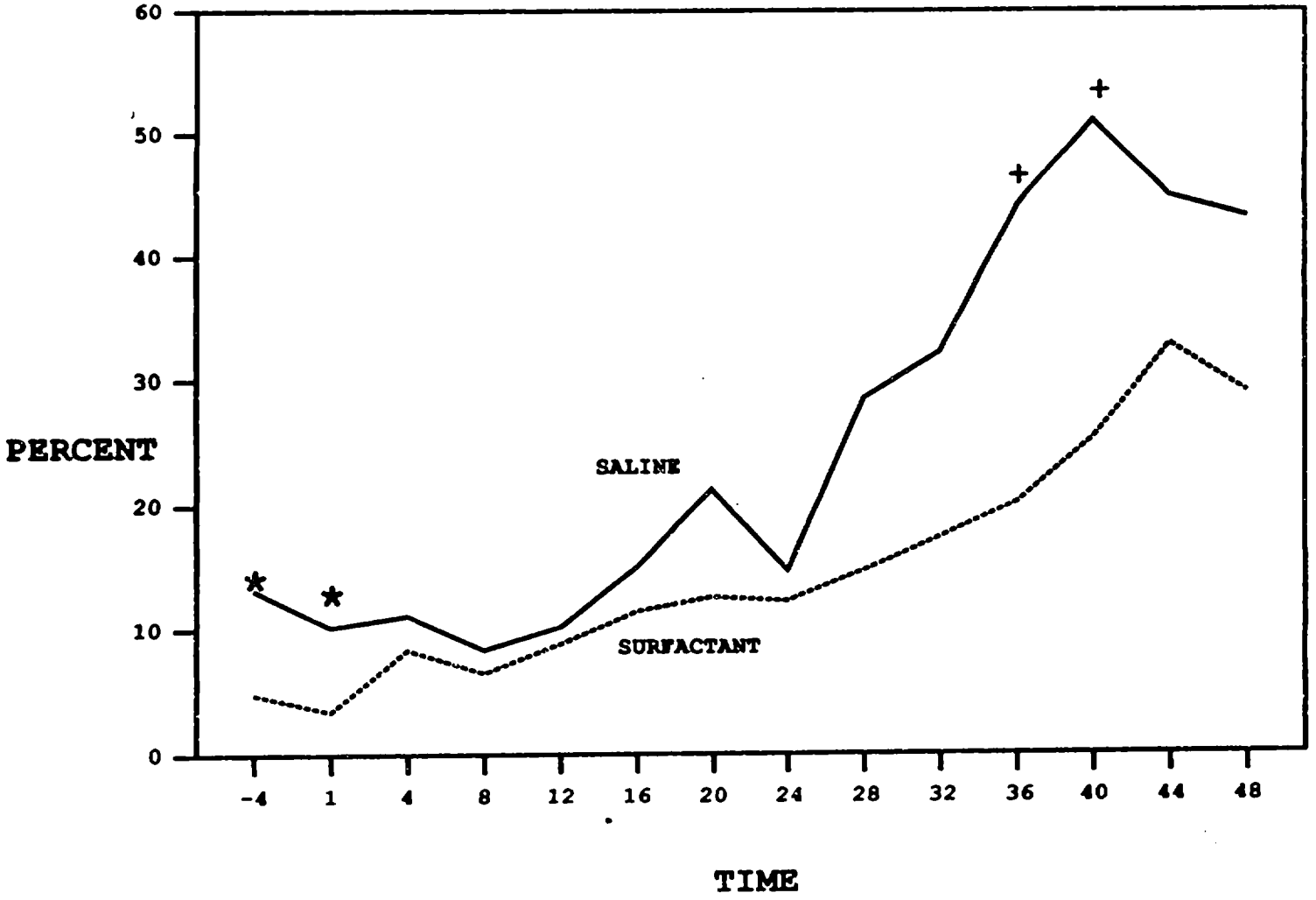

FIGURE 1. Calculated venous admixture (%). The early difference in venous admixture at 4 h before injury and 1 h after injury disappeared during the ensuing 32 h. At 36 and 40 h after injury, the saline group tended to have higher venous admixtures than the surfactant group, although the P value was approximately 0.1. *$P < 0.05$, +$P < 0.1$.

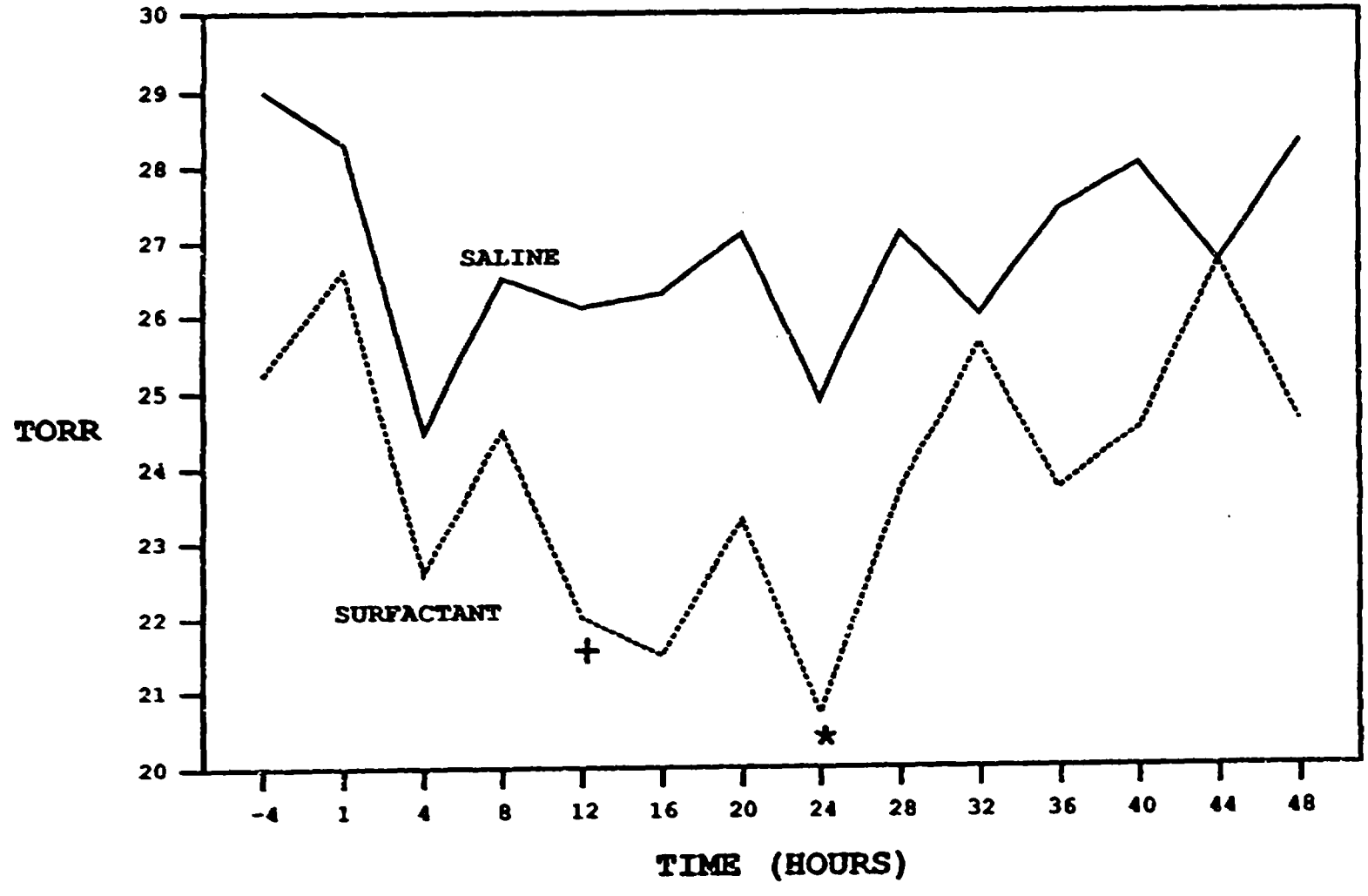

FIGURE 2. Arterial PCO_2 values (torr). At 12 and 24 h after injury, arterial PCO_2 levels were less in the surfactant group than in the saline group, despite similar minute ventilations. *$P < 0.05$, +$P < 0.1$.

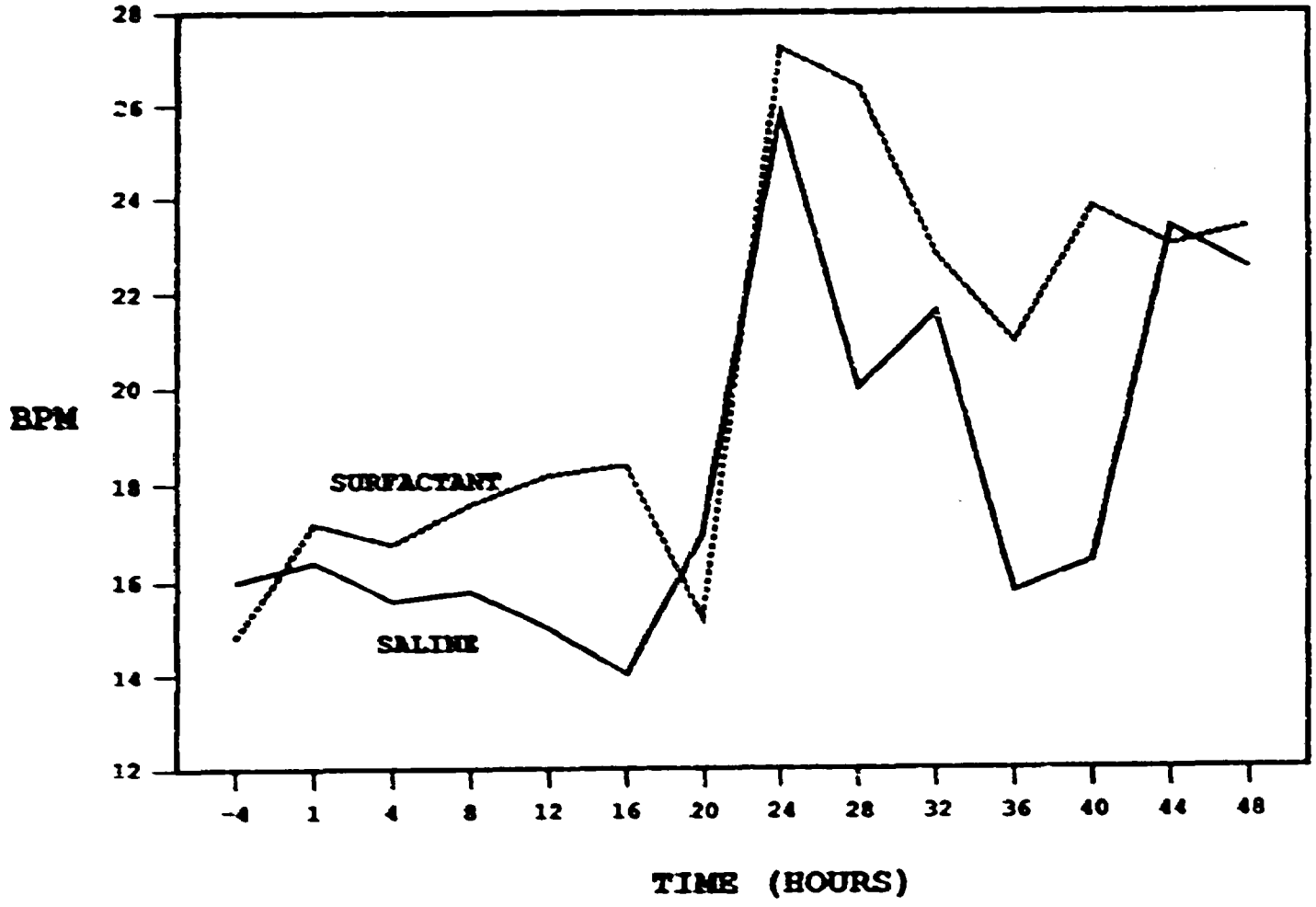

FIGURE 3. Respiratory rates (breaths/min). There were no statistical differences in the respiratory rate, and thus minute ventilation, between the two groups for the duration of the study.

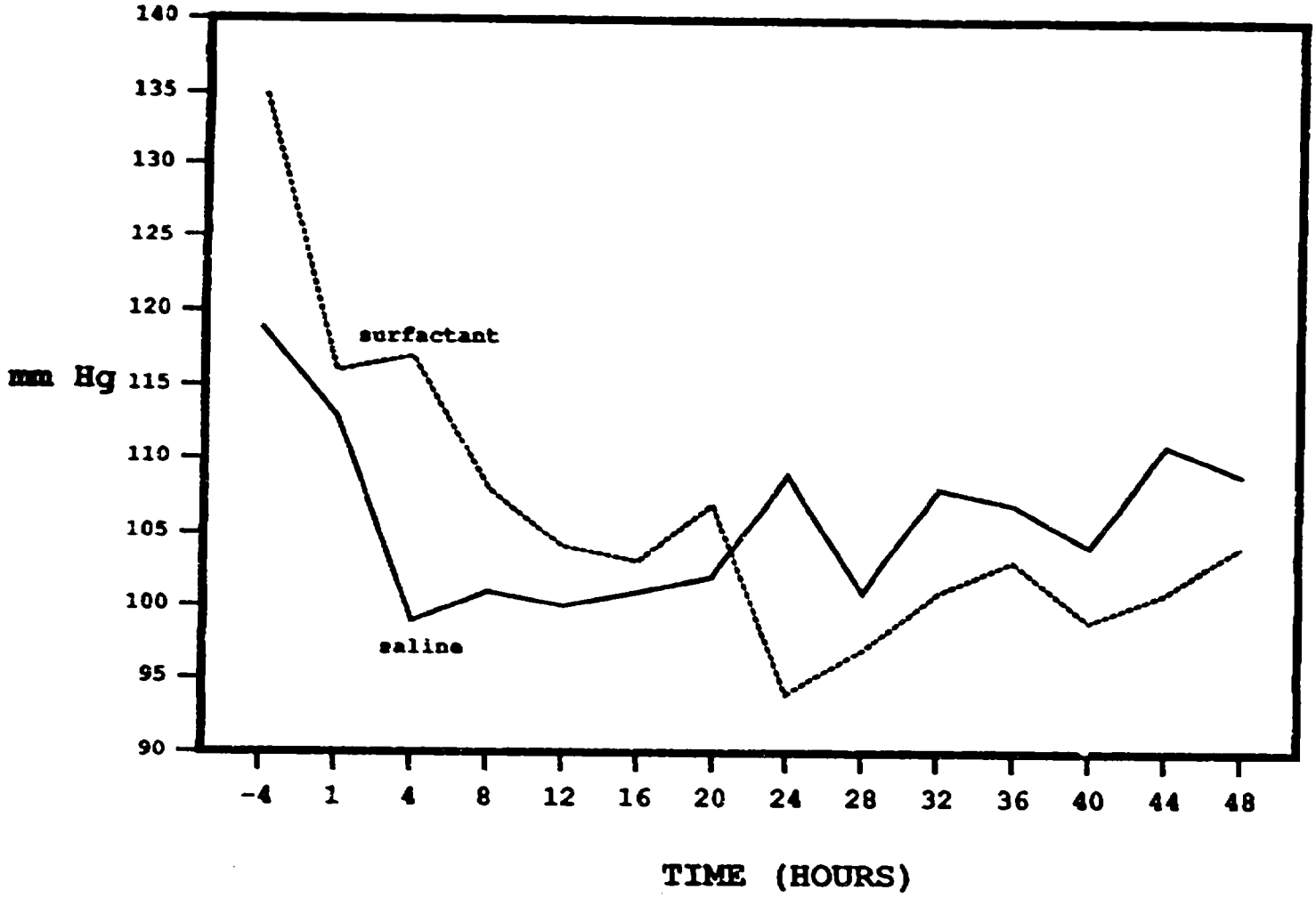

FIGURE 4. Systemic blood pressure (mmHg). Mean systemic blood pressure was not different between the groups at any time point.

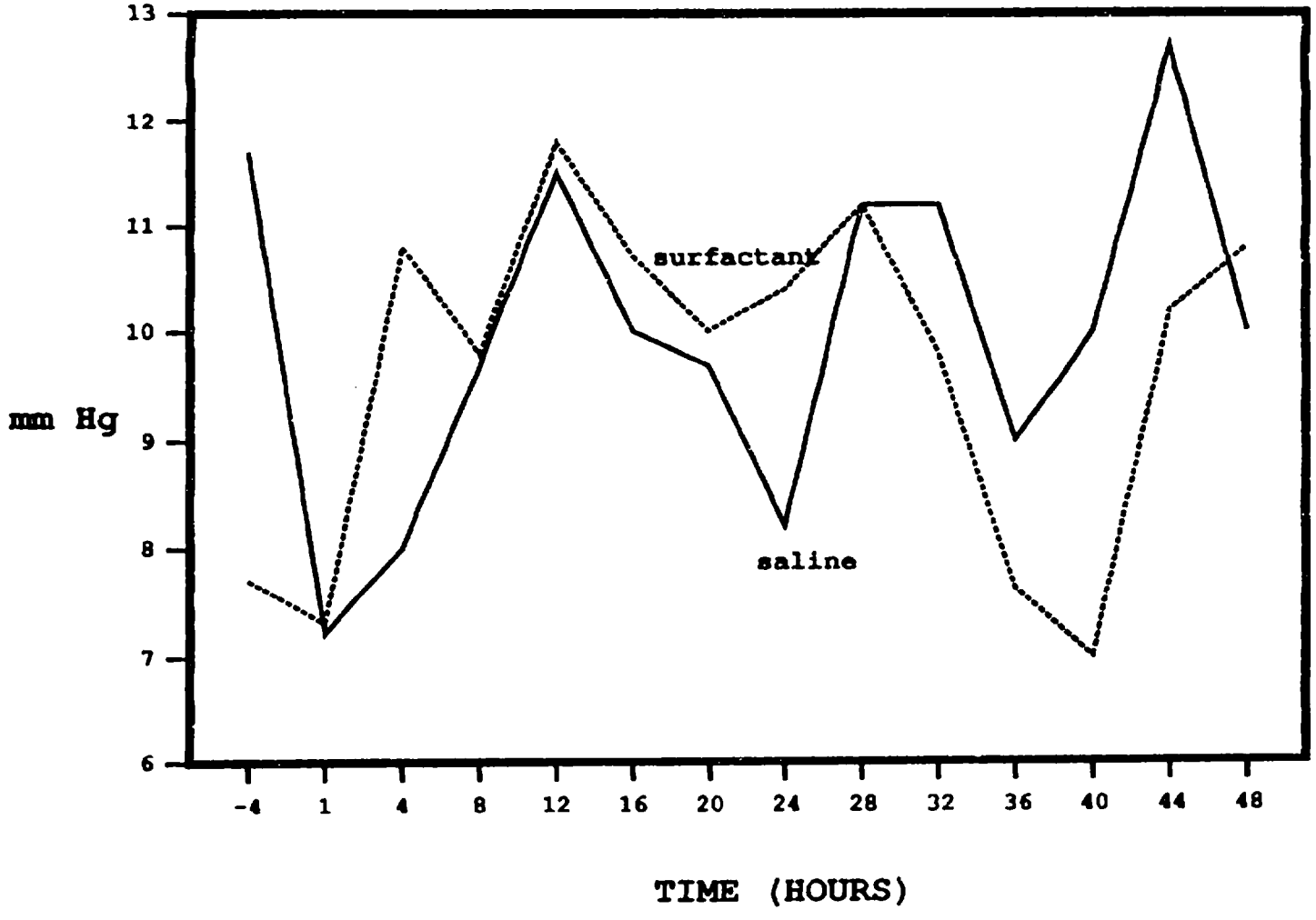

FIGURE 5. Pulmonary artery occlusion pressure (mmHg). There were no differences in pulmonary artery occlusion pressure between the two groups throughout the study period.

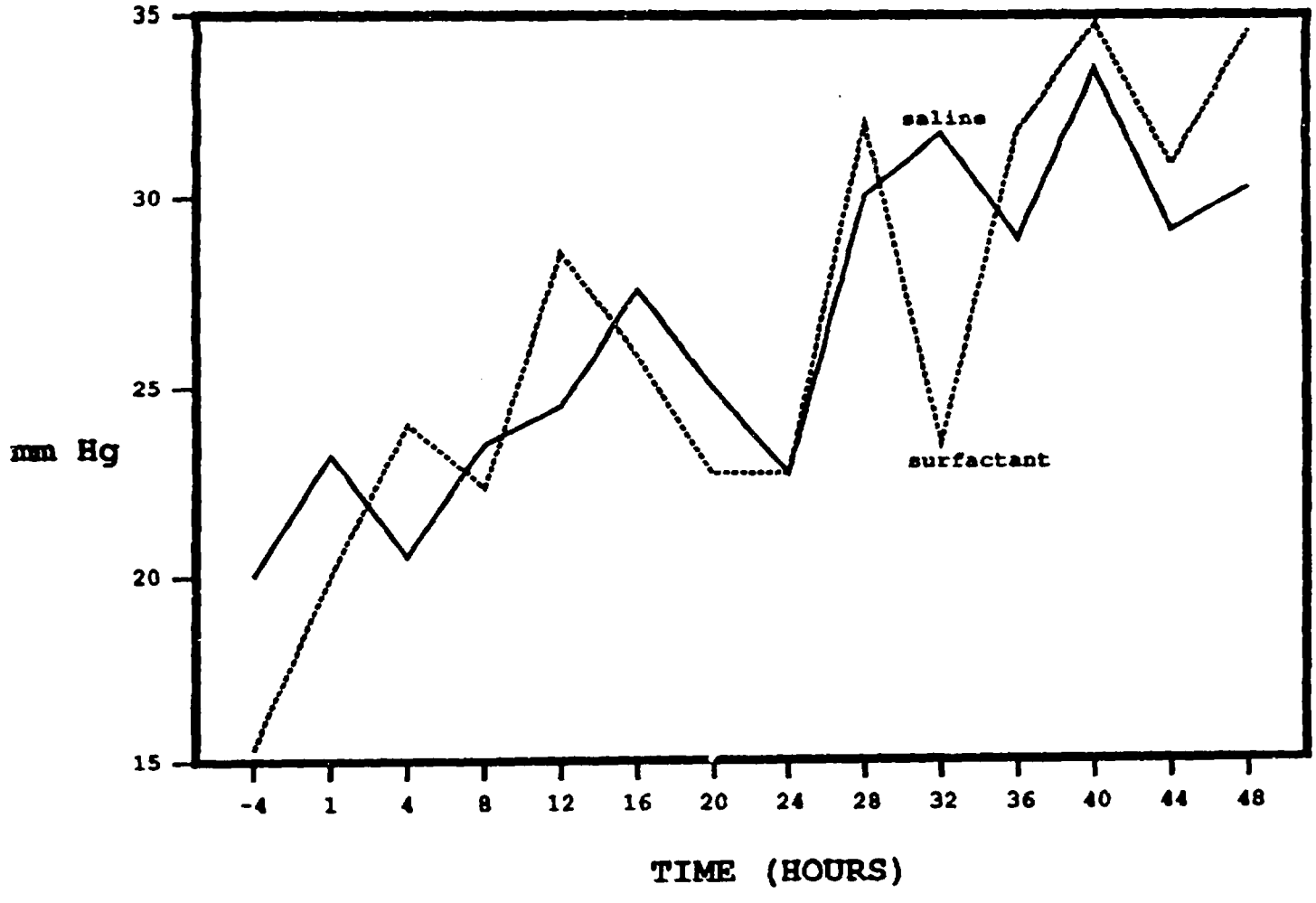

FIGURE 6. Mean systemic blood pressure (mmHg). There were no statistical differences between the two groups in pulmonary artery systolic pressure throughout the study period. Both groups showed a significant rise in pulmonary artery pressure over the course of the study.

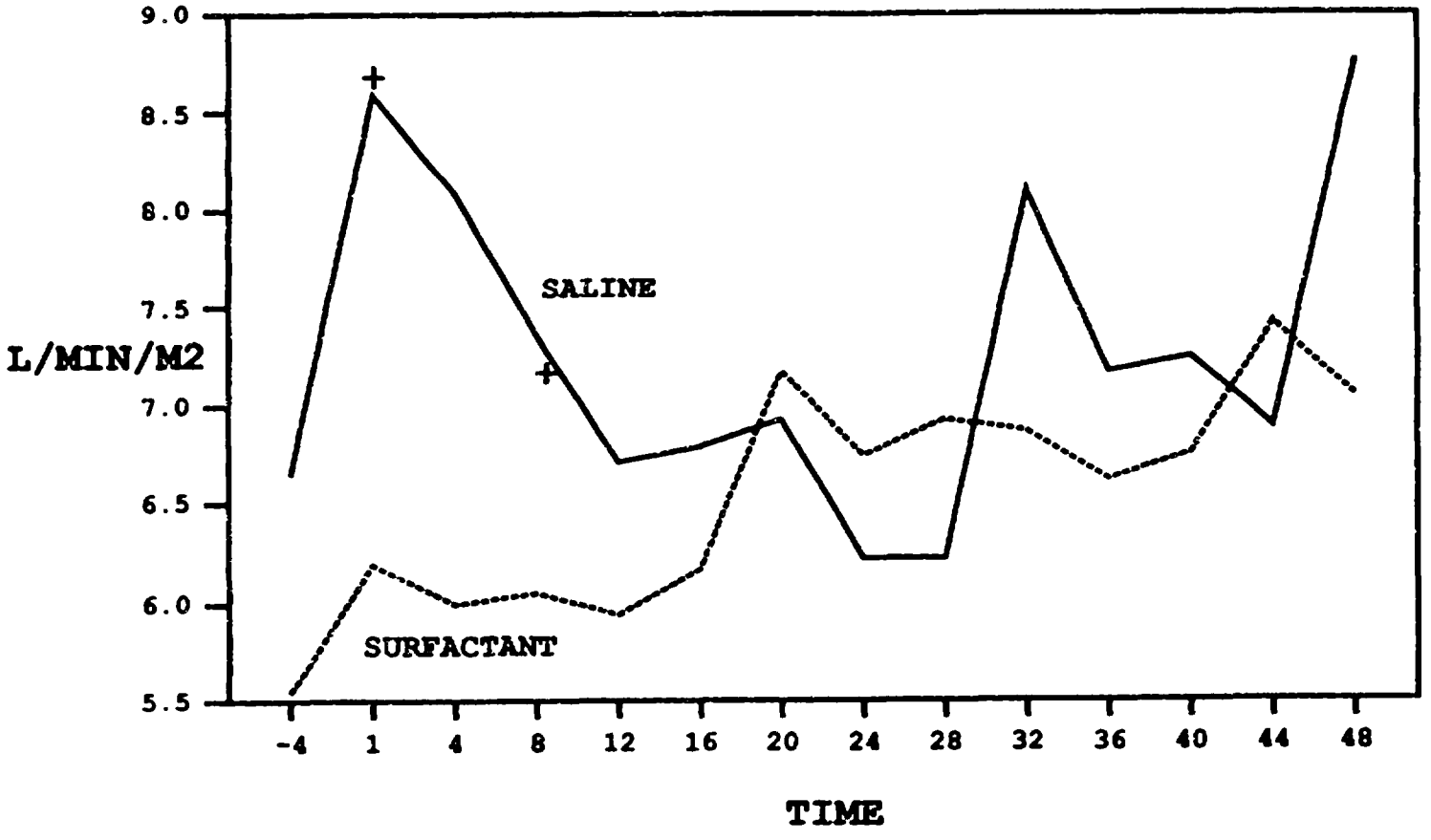

FIGURE 7. Cardiac index (l/min/m^2). The saline group tended to have a higher cardiac index at 1 and 8 h after injury compared to the surfactant group. However, from 12-48 h, there were no differences. +P < 0.1.

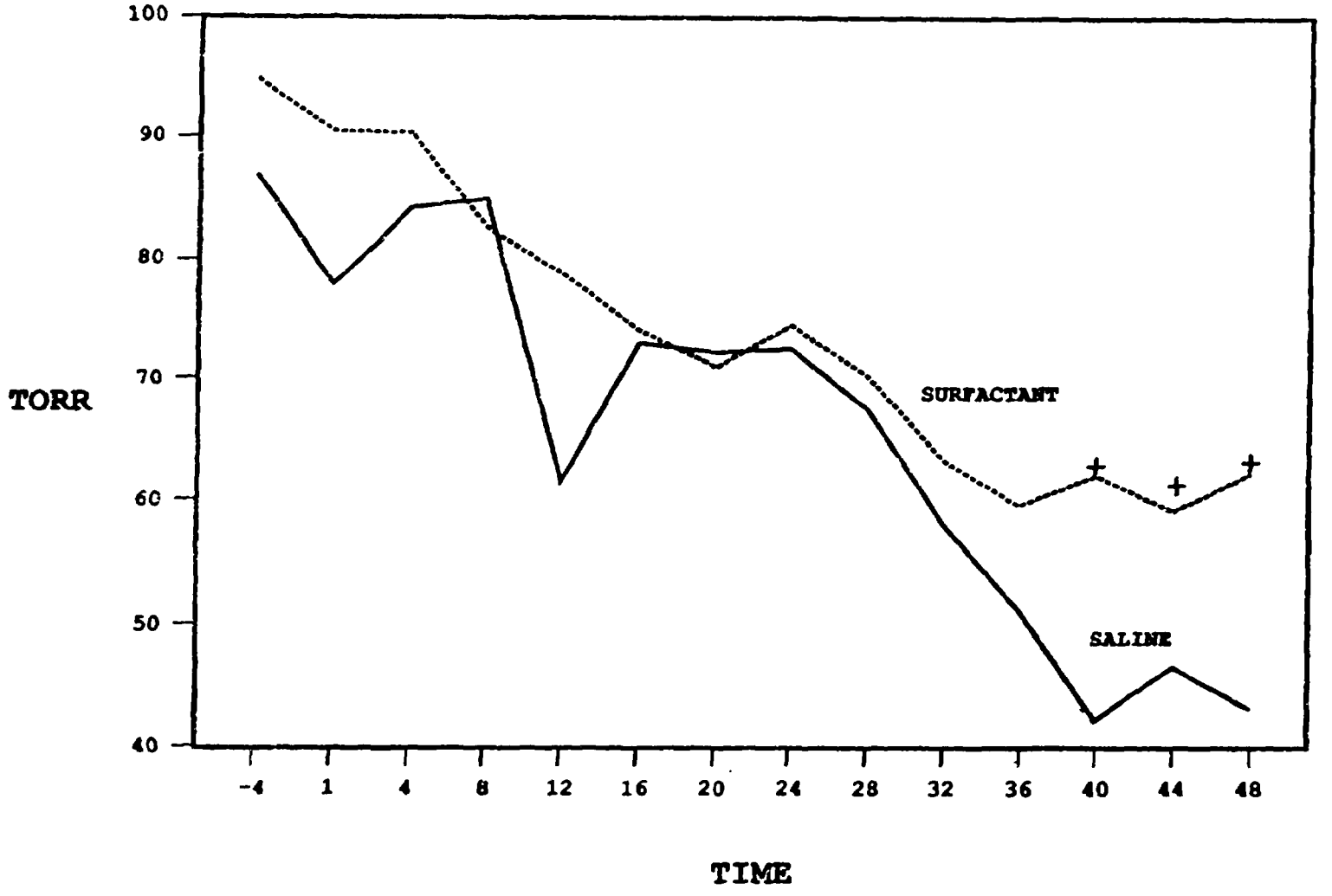

FIGURE 8. Arterial PO_2 (torr). Both groups demonstrated a significant decline in arterial oxygenation throughout the study period. At 40, 44, and 48 h, the surfactant groups tended to have mean PO_2 values which were greater than the saline group ($0.05 > P < 0.1$).

surfactant-treated group having higher mean arterial PO_2 than the saline-treated group ($P = 0.1$). Calculated shunt, although slightly different between the two groups at the beginning of the study period, was identical between 4 and 32 h. At 36 and 40 h, the saline-treated group tended to have higher calculated venous admixtures than the surfactant-treated group ($0.05 < P < 0.1$). By 44 and 48 h, this difference was less apparent.

DISCUSSION

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. These data also suggest, but do 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 limit the validity of these findings. First, although all 13 animals were exposed to 7 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 > 90% were excluded from final analysis, the great variability in initial carboxyhemoglobin levels indicates 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 is another parameter indicating marked heterogeneity in the groups. 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.

After refinement of the smoke exposure protocol, an addendum to this protocol will be written in an attempt to further define the efficacy of surfactant replacement after smoke exposure.

PRESENTATIONS/PUBLICATIONS

None.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION: DA346160	2. DATE OF SUMMARY: 01 Oct 91	REPORT CONTROL SYMBOL: DD-DR&E(AR) 636
3. DATE PREV SUM'RY: 01 Oct 90	4. KIND OF SUMMARY: D. Chg	5. SUMMARY SCTY: U	6. WORK SECURITY: U	7. REGRADING:	8. DISB'N INSTR'N: CX	9. LEVEL OF SUM A. WORK UNIT:

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS14	CG	327
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE *(Precede with Security Classification Code)*
(U) Intestinal Permeability Following Thermal Injury

12. SUBJECT AREAS
0605 Medicine and Medical Research

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

17. CONTRACT/GRANT — MILITARY RELEVANCY CERTIFIED — APPROVED BY

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

18. RESOURCES ESTIMATE

FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS *(In thousands)*
91	0.5	33
92	0.5	34

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME: US Army Institute of Surgical Research	a. NAME: US Army Institute of Surgical Research
b. ADDRESS *(Include zip code)*: Fort Sam Houston San Antonio, Texas 78234-5012	b. ADDRESS: Fort Sam Houston San Antonio, Texas 78234-5012
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR: CIOFFI, W G
d. TELEPHONE NUMBER *(Include area code)*: 512-221-2720	d. TELEPHONE NUMBER *(Include area code)*: 512-221-8440
21. GENERAL USE: FIC MILITARY/CIVILIAN APPLICATION: M	f. NAME OF ASSOCIATE INVESTIGATOR *(If available)*: MC MANUS, W F g. NAME OF ASSOCIATE INVESTIGATOR *(If available)*:

22. KEYWORDS *(Precede EACH with Security Classification Code)* (U) Burns (Injuries); (U) Septicemia; (U) Intestines; (U) Bacteria; (U) Mannitol

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

23/24. (U) The objective of this work is to assess alterations in intestinal permeability in the acute phase of thermal injury. Twenty consecutive patients will have intestinal permeability measured on postburn days 2, 4, 6, 8, 10, and 12 and 10 healthy volunteers will be individually studied for 2 consecutive days. Intestinal permeability will be measured by administering lactulose and mannitol, two different molecular weight sugars which are absorbed via different mechanisms in the gastrointestinal tract. Lactulose/mannitol ratios will be subjected to repeated measures ANOVA and multiple regression analysis to determine variation of lactulose excretion with burn size and postburn day.

25. (U) 9010 - 9109. Eight patients and 10 control subjects were enrolled in the study during this reporting period. Patients who developed infection had a significant increase in intestinal permeability compared to other patients and normal controls which occurred well before the episode of infection.

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SUPPLEMENTAL WORK UNIT INFORMATION FOR THE PROJECT ENTITLED "INTESTINAL PERMEABILITY FOLLOWING THERMAL INJURY"

Subrecord/Linking Accession Number: Not applicable.

Search Control Data: W6L38M/W6L39L, 9 January 1990.

Product Identification: For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report* for fiscal years 1990-1.

Unclassified Special Categories: Volunteers: Adults; RA II.

ABSTRACT

PROJECT NUMBER: 3M161102BS14, 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

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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.

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), utilizing 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.

In an attempt to define the temporal pattern of intestinal permeability changes in thermally injured patients with more precision, we measured the lactulose/mannitol clearance in 15 patients with thermal every 48 h for the first 2 weeks postinjury.

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

Demographics for burn patients and control subjects are presented in Table 1. Patients and control subjects excreted 9.2% and 10.3% of the mannitol administered, respectively. Overall, the patients excreted a mean of 0.96%, or 96.3 mg, of the lactulose administered. Control subjects excreted a mean of 0.14%, or 13.8 mg, of lactulose. The mean L/M ratios for the patients and control subjects were 0.159 ± 0.017 and 0.017 ± 0.003, respectively ($P < 0.001$). There was no statistical difference in mannitol excretion between the two groups.

TABLE 1. Demographic Data for Study Population (Mean ± SEM)

	Burn Patients	Control Subjects
n=	15	10
Mean age (yr)	32.7 ± 3.6	25.6 ± 1.9
Sex (M/F)	13/2	8/2
Inhalation injury (number)	10	-
Burn size (% of total body surface area)	53.5 ± 5.1	-
Nonsurvivors (number)	2	-

There was no correlation between the magnitude of increased intestinal permeability and burn size for the entire patient group ($r < 0.1$). Additionally, there was no correlation between postburn day and increased intestinal permeability for the patient group as a whole, or for patients individually ($r < 0.1$). On each postburn day of study, the L/M ratio was significantly higher for patients than for control subjects. There was no difference in the L/M ratio for patients with inhalation injury compared to those without such injury.

During the 2-week study period, there were 13 diagnosed infections in 9 patients, consisting of 6 cases of pneumonia, 2 of bacteremia, 2 of wound cellulitides, 2 of tracheobronchitides, and

1 of urinary tract infection. The mean burn size for uninfected and infected patients was 52.7% and 53.7% of the total body surface area, respectively. The postburn days on which infections were diagnosed are depicted in Figure 1. No infection was clinically evident before postburn day 4. The L/M ratio for the entire study period for control subjects, noninfected patients, and infected patients were 0.017 ± 0.003, 0.082 ± 0.02, and 0.208 ± 0.02, respectively. The L/M ratio of the infected burn patients was significantly greater than the noninfected patients and normal control subjects ($P < 0.01$). There was no statistically significant difference between the noninfected patients and the control subject ratios. In an attempt to define whether or not the increased permeability occurred before or after the infection, the L/M ratio was examined on postburn day 2, which was before the infection episode in any patient in whom an infection was diagnosed. The mean L/M ratios for control subjects, noninfected patients, and infected patients were 0.017 ± 0.003, 0.044 ± 0.01, and 0.153 ± 0.04, respectively. Once again, the patients who ultimately developed infections had a mean L/M ratio which was significantly greater than the noninfected patients and the control subjects ($P < 0.01$, ANOVA). The L/M ratio for patients who ultimately did not become infected during the first 2 weeks postburn and that of the control subjects did not differ significantly.

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 utilized 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

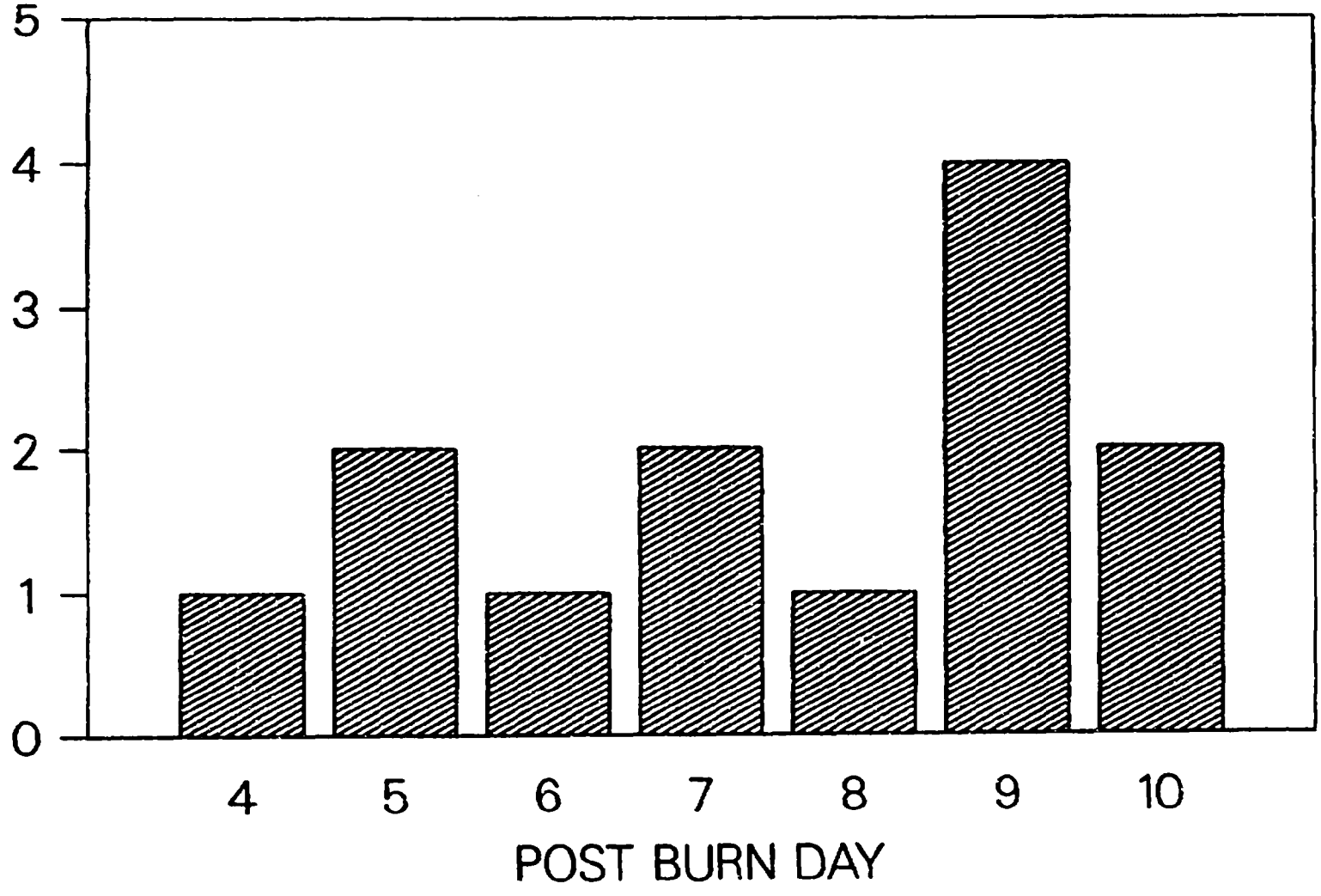

FIGURE 1. Postburn day that each infection was clinically diagnosed is presented. No infection was clinically obvious before postburn day 4 with 69% of infections diagnosed during the second week postburn.

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 utilizing 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 utilized 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 utilizing 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 have 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 is not possible, from this 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.

PRESENTATIONS/PUBLICATIONS

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

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION: DA346161	2. DATE OF SUMMARY: 01 Oct 91	REPORT CONTROL SYMBOL: DD-DRA&E(AR) 636
3. DATE PREV SUM RY: 01 Oct 90	4. KIND OF SUMMARY: D. Chg	5. SUMMARY SCTY: U	6. WORK SECURITY: U	7. REGRADING:	8. DISP'N INSTR'N: CX	9. LEVEL OF SUM A. WORK UNIT:

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS14	CK	328
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code) (U) Mineral Absorption and Metabolism in a Burned Rat Model Using the Everted Gut Sacs Technique

12. SUBJECT AREAS
0601 Biochemistry 0605 Medicine and Medical Research

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
9004	9209	DA	C. In-House

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

18. RESOURCES ESTIMATE

FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
91	0.5	36
92	0.5	38

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME: US Army Institute of Surgical Research	a. NAME: US Army Institute of Surgical Research
b. ADDRESS (Include zip code): Fort Sam Houston, San Antonio, Texas 78234-5012	b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-5012
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR: SHIPPEE, R L
d. TELEPHONE NUMBER (Include area code): 512-221-2720	d. TELEPHONE NUMBER (Include area code): 512-221-4058
21. GENERAL USE: FIC	e. NAME OF ASSOCIATE INVESTIGATOR (If available): YOUNG, E
MILITARY/CIVILIAN APPLICATION: M	f. NAME OF ASSOCIATE INVESTIGATOR (If available): OKERBERG, C V

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Burns (Injuries); (U) Metabolism; (U) Nutrition; (U) Isotopes

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

23/24. (U) The objective of this work is to use the everted gut sac technique to investigate the effect of burn injury on mineral absorption in a burned rat model. Lewis rats will be administered either a 30% full-thickness scald or sham burn. At either 24 h or 5 days after burn injury, the rats will be sacrificed and the small intestine will be removed. The intestinal absorption of four radioactive-labeled nutrients will then be assessed.

25. (U) 9010 - 9109. Our previous studies have shown that, despite changes in redistribution of tissue zinc after burn injury, no effects of burn injury on zinc balance were seen in a burned rat model. Although zinc bound to the zinc storage protein, metallothionein, increased, no changes were seen in intestinal metallothionein. This would suggest uninhibited zinc gut absorption. To investigate this phenomenon further, the everted gut sac technique was used to study zinc absorption following burn injury in the rat. Burn injury caused lower gut zinc absorption on days 2 and 4 postburn; however, by day 8 postburn, absorption was equivalent to control levels.

DD FORM 83 MAR 1490 — EDITION OF MAR 83 IS OBSOLETE

SUPPLEMENTAL WORK UNIT INFORMATION FOR THE PROJECT ENTITLED "MINERAL ABSORPTION AND METABOLISM IN A BURNED RAT MODEL USING THE EVERTED GUT SACS TECHNIQUE"

Subrecord/Linking Accession Number: Not applicable.

Search Control Data: W6R28L/W6R301, 9 January 1990.

Product Identification: For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report* for fiscal years 1990-1.

Unclassified Special Categories: Lab Animals; Rats; RA 11.

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

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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.

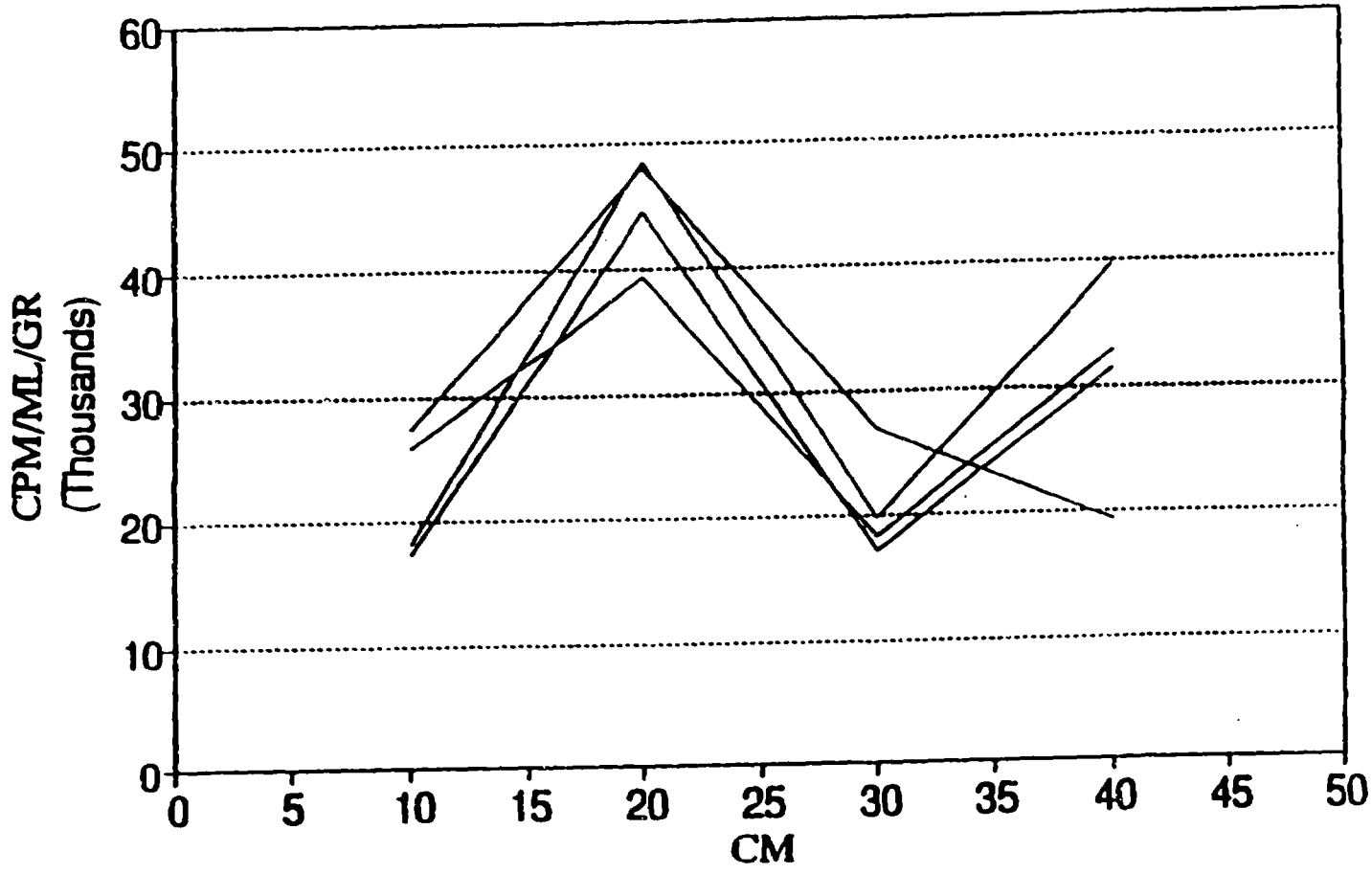

FIGURE 1. ^{65}Zinc absorption of 10-cm segments of small intestine from 4 sham-burn rats.

TABLE 1. Zinc absorption (dpm/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				1. AGENCY ACCESSION: DA335424	2. DATE OF SUMMARY: 01 Oct 91	REPORT CONTROL SYMBOL: DD-DRA&E(AR) 636
3. DATE PREV SUM'RY: 01 Oct 90	4. KIND OF SUMMARY: D. Chg	5. SUMMARY SCTY: U	6. WORK SECURITY: U	7. REGRADING	8. DISP'N INSTR'N: CX	9. LEVEL OF SUM A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS14	CH	330
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code) (U) Donor-Specific Bone Marrow and Antithymocyte Preparations for Establishment of Selective Tolerance to Allografted Skin

12. SUBJECT AREAS
0604 Anatomy and Physiology 0605 Medicine and Medical Research

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
9007	9209	DA	C. In-House

17. CONTRACT/GRANT — MILITARY RELEVANCY CERTIFIED / APPROVED BY [signature] — RESOURCES ESTIMATE

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

a. FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
91	0.5	42
92	0.5	47

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME: US Army Institute of Surgical Research	a. NAME: US Army Institute of Surgical Research
b. ADDRESS (Include zip code): Fort Sam Houston, San Antonio, Texas 78234-5012	b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-5012
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR: RUE, L W
d. TELEPHONE NUMBER (Include area code): 512-221-2720	d. TELEPHONE NUMBER (Include area code): 512-221-8440
21. GENERAL USE: FIC MILITARY/CIVILIAN APPLICATION: M	e. NAME OF ASSOCIATE INVESTIGATOR (If available): CIOFFI, W G g. NAME OF ASSOCIATE INVESTIGATOR (If available): BECKER, W K

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Burns (Injuries); (U) Bone Marrow; (U) Skin Grafts; (U) Immunosuppression; (U) Lymphocytes

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

23/24. (U) The objectives of this work are to demonstrate selective unresponsiveness to skin allografts using antithymocyte preparations and donor-specific bone marrow, to demonstrate the time course of restoration of the MLR to third-party cells following induction of tolerance, and to investigate the need for antithymocyte preparations in induction tolerance in a rat burn model. Groups I and II will be subjected to 30% total body surface area burns. Group I will undergo skin grafting from Brown-Norway rat donors on the second postburn day. Group II will be administered antithymocyte serum on the first postburn day, undergo skin grafting from a Brown-Norway rat donor on the second postburn day, be administered antithymocyte serum on the second postoperative day, and be administered the bone marrow preparation from the Brown-Norway rat donor on the seventh postoperative day. Each of these two groups will undergo MLR surveillance at the time of burn injury, 1 week postinjury, and monthly thereafter.

25. (U) 9010 - 9109. The first phase of this project has been undertaken. Data for the control group (n=40) 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. Further work will be undertaken to refine this model.

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

SUPPLEMENTAL WORK UNIT INFORMATION FOR THE PROJECT ENTITLED "USE OF DONOR-SPECIFIC BONE MARROW AND ANTITHYMOCYTE PREPARATIONS FOR THE ESTABLISHMENT OF SELECTIVE TOLERANCE TO ALLOGRAFTED SKIN IN A RAT BURN MODEL"

Subrecord/Linking Accession Number: Not applicable.

Search Control Data: W6Q13K/W6Q11L, 5 January 1990.

Product Identification: Not applicable.

Unclassified Special Categories: Volunteers: Adults; RA II.

ABSTRACT

PROJECT NUMBER: 3M161102BS14, 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 1990 - 30 September 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 > 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 are 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.

Phase I of this study was completed during this reporting period. Though only in the early phase of investigation, it is encouraging that a statistically significant difference in graft survival time was observed in the animals receiving the combination of antithymocyte serum and donor-specific bone marrow. It is hoped that short-term administration of cyclosporine A will even further augment graft survival time in the animal groups.

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 are 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 involves 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 will be subjected to a 30% total body surface area burn, administered antithymocyte serum on the first postburn day, undergo 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 utilizing 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 will be anesthetized with sodium pentobarbital (35 mg/kg IP), the dorsal surface will be shaved, and a 30% total body surface area scald burn (90°C for 5 sec) will be administered utilizing the Walker-Mason method. They will then be administered antithymocyte serum (2 cc IP) on the first postburn day. On the second postburn day, the wounds will be excised to fascia and skin grafts from Brown Norway rat donors will be applied as described for Group I. Two days following application of the graft, another dose of antithymocyte serum (2 cc IP) will be administered and a standard bone marrow preparation of 5×10^7 viable nucleated cells from the Brown Norway rat donor will be administered intravenously with a 25-ga needle through the dorsal penile vein on postoperative day 6. Wounds will be examined on postoperative day 7 and then daily by tactile and visual inspection for rejection of the graft. When < 10% of the graft remains, it will be considered total rejection.

Each of these two groups will undergo MLR surveillance at the time of burn injury, at 1 week following burn injury, and monthly thereafter to determine the time course of restoration for the MLR.

Determination of Number of Animals Required. This study has not previously been undertaken but it is felt that the number of

animals to be used will allow one to detect a 25% difference with a type I error 0.05 and a type II error of 0.10.

Data Analysis Plan. Descriptive statistics and life-table analyses of survival data will be performed.

RESULTS

Phase I of this 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.

Phase I revealed the mean graft survival time in animals receiving only the allogeneic skin graft was 10.3 ± 0.41 days. Group 2 animals receiving antithymocyte preparations and donor-specific bone marrow had a mean graft survival time of 14.2 ± 0.37 days. The P value < 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.

Currently, Phase II studies are being undertaken and preliminary data is not available for interpretation.

DISCUSSION

Though only in the early phase of investigation, it is encouraging that a statistically significant difference in graft survival time was observed in the animals receiving the combination of antithymocyte serum and donor-specific bone marrow. It is hoped that short-term administration of cyclosporine A will further augment graft survival time in the animal groups.

PRESENTATIONS/PUBLICATIONS

None.

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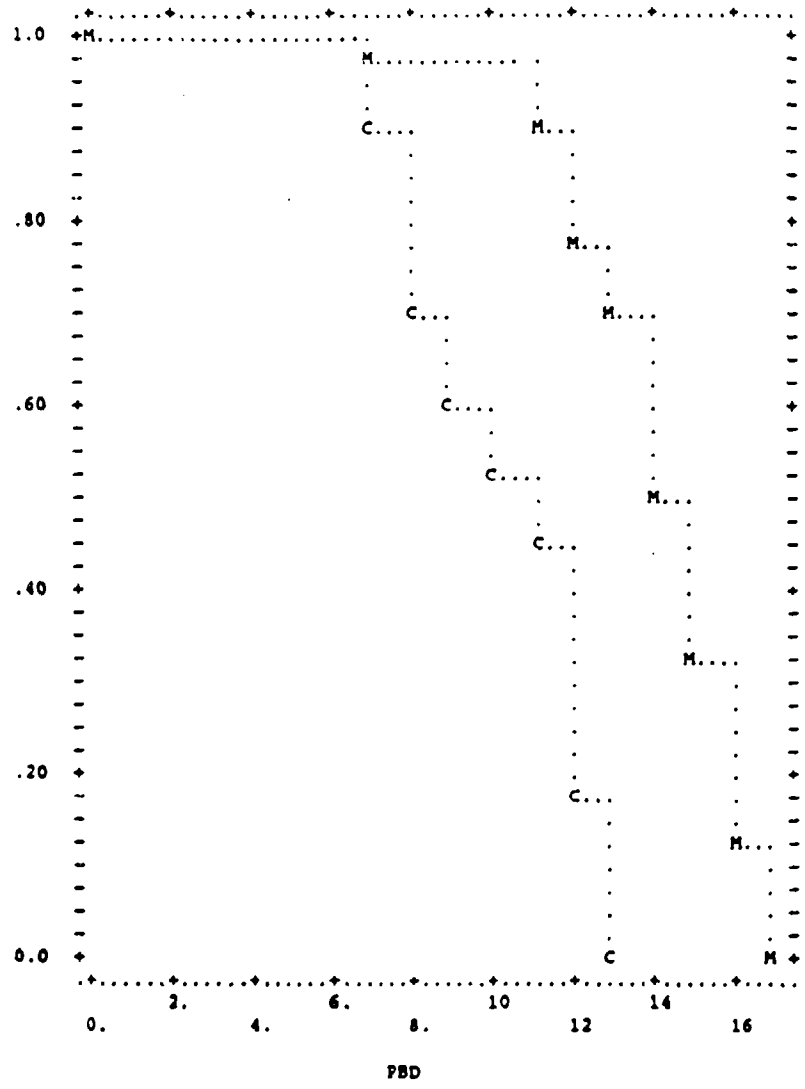

FIGURE 1. Cumulative proportion surviving. C indicates group receiving allogeneic skin grafts only; M, group receiving antithymocyte preparations and donor-specific bone marrow.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION: DA335467	2. DATE OF SUMMARY: 01 Oct 91	REPORT CONTROL SYMBOL: DD-R&E(AR) 636
3. DATE PREV SUM'Y: 01 Oct 90	4. KIND OF SUMMARY: D. Chg	5. SUMMARY SCTY: U	6. WORK SECURITY: U	7. REGRADING	8. DISP'N INSTR'N: CX	9. LEVEL OF SUM: A. WORK UNIT

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS14	CA	331
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code) (U) Protein Turnover, Pulmonary Amino Acid Flux, and Nitrogen Balance in Thermally Injured Patients

12. SUBJECT AREAS
0601 Biochemistry 0603 Medicine and Medical Research

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
9008	9309	DA	C. In-House

17. CONTRACT/GRANT [illegible]

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

18. RESOURCES ESTIMATE

FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
91	1.0	78
92	1.0	82

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME: UU Army Institute of Surgical Research	a. NAME: US Army Institute of Surgical Research
b. ADDRESS (Include Zip Code): Fort Sam Houston San Antonio, Texas 78234-5012	b. ADDRESS: Fort Sam Houston San Antonio, Texas 78234-5012
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A	d. NAME OF PRINCIPAL INVESTIGATOR: BECKER, W K
e. TELEPHONE NUMBER (Include Area Code): 512-221-2720	e. TELEPHONE NUMBER (Include Area Code): 512-221-3349
21. GENERAL USE: PIC	f. NAME OF ASSOCIATE INVESTIGATOR (If available): CIOFFI, W G
MILITARY/CIVILIAN APPLICATION: U	g. NAME OF ASSOCIATE INVESTIGATOR (If available): [illegible], I. W

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Burns (Injuries); (U) Lung; (U) Protein Metabolism; (U) Hypermetabolism; (U) Amino Acids

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

23/24. (U) The objective of this work is to measure protein turnover, amino acid flux, and nitrogen balance across the lungs in thermally injured patients. Improvement in the understanding of the metabolic response to thermal injury may improve the outcome of thermally injured soldiers. This is a pilot study to establish techniques and to determine if amino acid flux in the lung can be demonstrated. Ten patients will undergo studies of protein turnover, pulmonary amino acid flux, and nitrogen balance on postburn day 7. Rates of protein turnover, amino acid flux, and nitrogen balance will be correlated with each other and with patient demographics, such as burn size and inhalation injury, using linear regression.

25. (U) 9010 - 9109. Initial results from the first 8 patients enrolled in this study indicate that following thermal injury, there is net flux of amino acids from the lung. This occurs during a period of increased protein turnover. In order to more clearly define this phenomenon, an addendum was submitted and approved for the infusion of the stable isotope, C^{13} phenylalanine, concomitantly with the N^{15} glycine to measure amino acid flux and protein turnover in 10 additional patients.

DD FORM 83 MAR 1498 EDITION OF MAR 88 IS OBSOLETE. * USGPO: 1988-491-003/50139

SUPPLEMENTAL WORK UNIT INFORMATION FOR THE PROJECT ENTITLED "PROTEIN TURNOVER, PULMONARY AMINO ACID FLUX, AND NITROGEN BALANCE IN THERMALLY INJURED PATIENTS"

Subrecord/Linking Accession Number: Not applicable.

Search Control Data: W6Q13K/W6Q11L, 5 January 1990.

Product Identification: Not applicable.

Unclassified Special Categories: Volunteers: Adults; RA II.

ABSTRACT

PROJECT NUMBER: 3M161102BS14, Research

PROJECT TITLE: Protein Turnover, Pulmonary Amino Acid Flux, and Nitrogen Balance in Thermally Injured Patients

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

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

INVESTIGATORS: William K. Becker, MD, Lieutenant Colonel, MC
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Pulmonary amino acid flux was studied in 9 critically ill patients to better understand the altered protein metabolism in stressed patients. Plasma amino acid profiles were measured in the pre- and postpulmonary circulation via an indwelling pulmonary artery and peripheral artery catheter. Whole body protein turnover was calculated by the N^{15} glycine infusion method. Five burn patients and 4 postoperative general surgery patients were studied. Four preoperative coronary artery bypass patients were used as control subjects.

There was a statistically significant increase in flux of glutamate, glutamine, glycine, and lysine in burn patients compared to control subjects ($P < 0.05$). The trend was for increased release of all amino acids proportional to the degree of hypermetabolism following major surgery and burns. The rate of appearance of glycine, used as an estimate of protein turnover, was not different between postoperative and burned patients.

Lung amino acid flux following thermal injury and surgical stress demonstrates net release of amino acids, probably indicating net pulmonary protein catabolism. This may explain, in part, the pulmonary dysfunction and failure commonly seen in these patients.

PROTEIN TURNOVER, PULMONARY AMINO ACID FLUX, AND NITROGEN BALANCE IN THERMALLY INJURED PATIENTS

Amino acid flux in the setting of burns and other trauma is markedly different from the normal state (1). The hypermetabolic state associated with burns and trauma is classically composed of peripheral amino acid release, primarily from skeletal muscle, with uptake predominantly by visceral organs (gut and liver) and the kidney (2). The role of the lung in amino acid flux in burn patients is unknown. Current opinion would suggest that the lung has a net uptake of amino acids. However, there has recently been a suggestion that the lung may be a major source of amino acid release following trauma (3). If this is true, it may significantly alter our understanding of amino acid flux in critically ill patients. The level of protein turnover is generally increased in the hypermetabolic burn patient. Nitrogen excretion is also increased and may reach levels of 20-30 g/day, predominantly as urea in the urine.

There has been no study of pulmonary amino acid flux (PAAF) correlated with the level of total protein turnover and nitrogen excretion in burn patients. The deterioration of pulmonary function, frequently seen following thermal injury, may be explained in part by factors which cause the net release of amino acids from the lung following injury.

PAAF was studied in critically ill patients to better understand the altered protein metabolism of hypermetabolic illness. This pilot study was designed to compare the pulmonary flux of amino acids in stable preoperative patients, postoperative patients after major abdominal surgery, and severely burned patients to sample the spectrum of catabolic stress. The rate of appearance of glycine was used as a gross estimate of protein metabolism in the latter two groups.

MATERIALS AND METHODS

Study Design: Patients admitted to the US Army Institute of Surgical Research and the Brooke Army Medical Center's SICU were eligible for enrollment in the study. Properly signed and witnessed DA Forms 5303-R, Volunteer Agreement Affidavit, were obtained from each patient prior to initiation of the study. Patients were divided into three groups, i.e., control, postoperative, and burn (see Table 1). Mixed-venous and arterial blood samples were analyzed for plasma amino acids and hematocrit. Cardiac output was measured by the thermodilution technique. Protein turnover was performed by N^{15} prime-dose infusion.

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

TABLE 1. Group and Patient Descriptions

Group	n=	Description	Diagnosis
1	4	Stable preoperative patients awaiting coronary artery bypass with normal left function	Coronary artery disease
2	4	Postoperative patients on day 2 or 3 following intra-abdominal surgery	
		Patient 1	Aortic aneurysm
		Patient 2	Iliac occlusion
		Patient 3	Diverticulitis
		Patient 4	Bowel obstruction
3	5	Patients with burns	Burn injury (mean total body surface area burn size = 66.2%)

1. Male or female patients ≥ 18 yr old.

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.

4. Patients with preexisting pulmonary artery catheter and arterial line for routine clinical care.

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

1. Patients < 18 yr old.

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

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

4. Patients with inhalation injury or pneumonia.

5. Patients with toxic epidermal necrolysis.

6. Patients without preexisting pulmonary artery catheter and/or arterial line for routine clinical care.

7. Patients receiving total parenteral nutrition.

Description of Procedures. Blood was obtained from the distal port of the pulmonary artery catheter (mixed-venous blood) and from an arterial line. These catheters were already in place for the patient's routine clinical care. Spun HCT was performed on fresh blood in microcentrifuge tubes. Blood for amino acid analysis was collected in lithium heparin tubes and frozen at -80°C until analysis. Plasma amino acid analyses were performed by HPLC on an amino acid analyzer (Beckman 6300). Cardiac output was performed by the thermodilution technique. Five values were obtained, the final value being the average of three after excluding the highest and lowest values. Amino acid flux was calculated as follows:

$$\text{Flux} = (Q \times [1 - \text{HCT}/100]) \times \text{AV}$$

where Q = cardiac output, HCT = hematocrit, and AV = arterial-venous amino acid plasma concentration. Calculations were performed for individual and total amino acids.

The rate of appearance of glycine (Ra) was determined by a primed constant infusion of N^{15} glycine (95 atom percent) at a dose of 0.2 μmole/kg/min. Blood samples were collected hourly during the 6-h infusion. The percent enrichment was determined by GC-MS (Hewlett-Packard-5988, chemical ionization). Ra was calculated as follows:

$$\text{Ra} = \text{I}/\text{APE} \times 0.01$$

where I = infusion rate and APE = atom percent excess as described by Wolfe et al (4). Urinary N^{15} glycine measurements are currently being processed.

Data Analysis. ANOVA was used to compare the three patient groups.

RESULTS

The net PAAF is shown for each amino acid in Figures 1 through 3. There were statistically significant increases in PAAF of glutamate, glutamine, glycine, and lysine compared to the control population. The trend was for PAAF to be higher in burn patients compared to postoperative patients who were, in turn, higher than control subjects. The rate of appearance of glycine did not statistically correlate with patient groups with this small number of patients (Table 2).

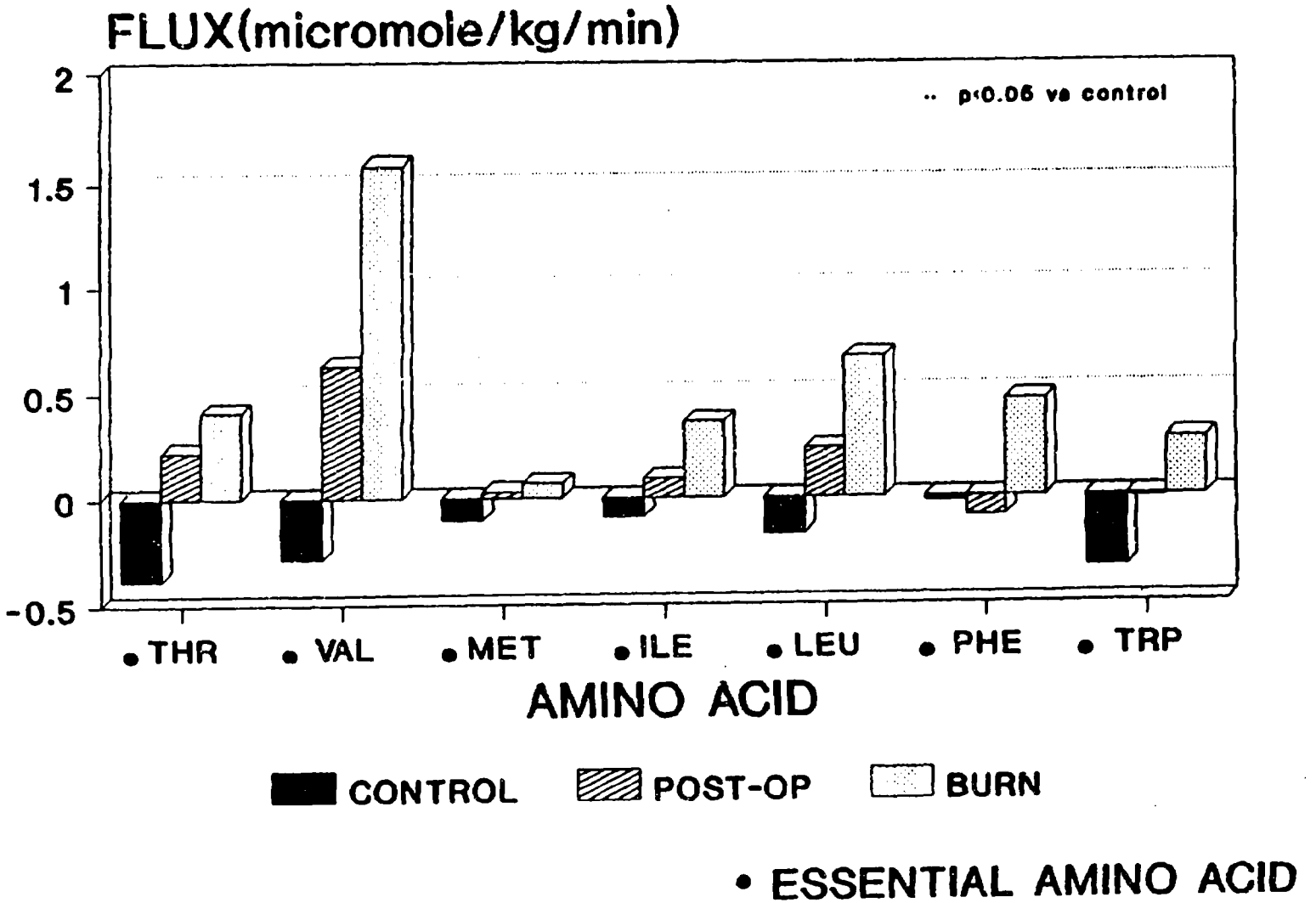

FIGURE 1. Net pulmonary amino acid flux for the amino acids threonine (THR), valine (VAL), methionine (MET), isoleucine (ILE), leucine (LEU), phenylalanine (PHE), and tryptophan (TRP).

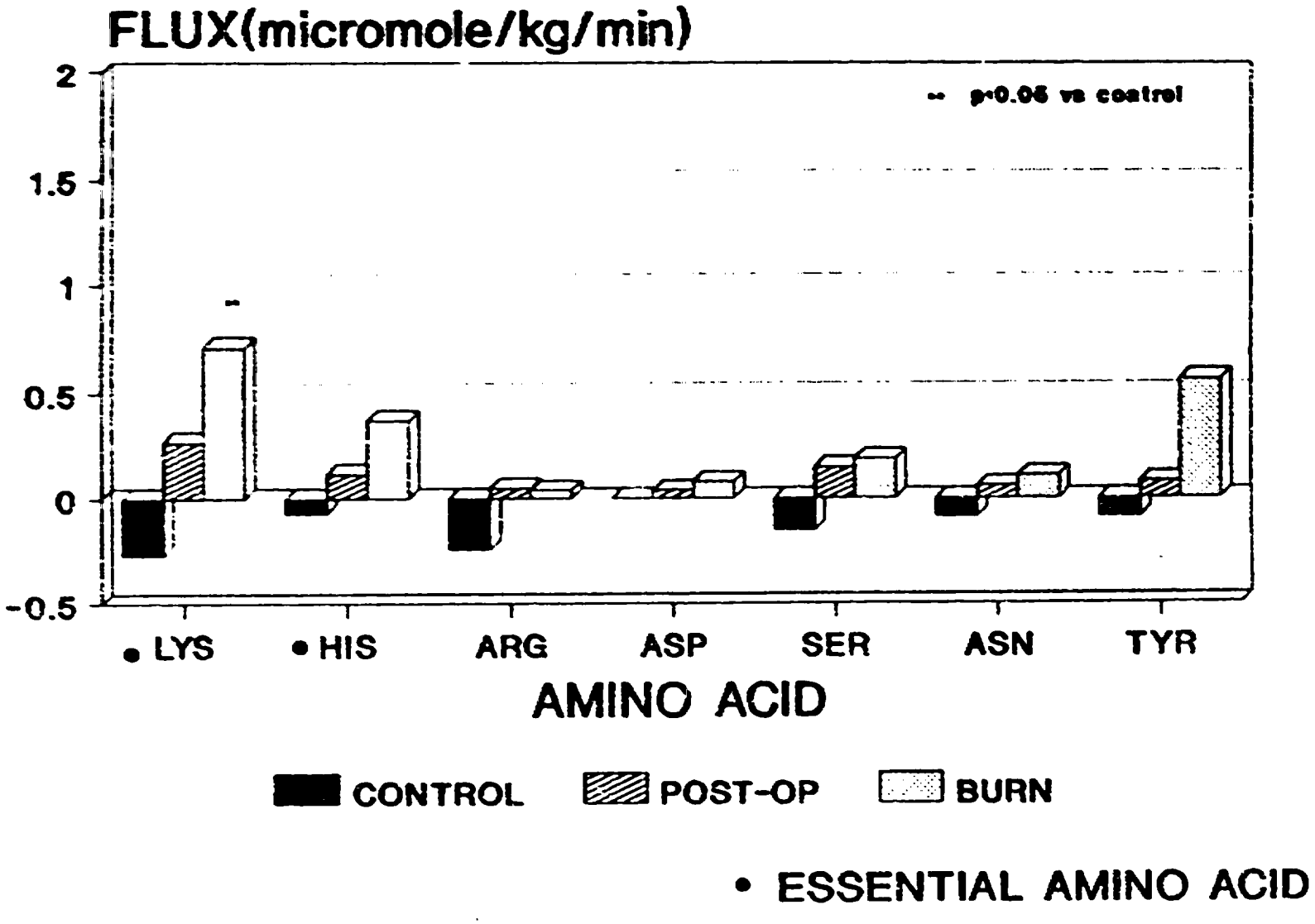

FIGURE 2. Net pulmonary amino acid flux for the amino acids lysine (LYS), histidine (HIS), arginine (ARG), aspartic acid (ASP), serine (SER), asparagine (ASN), and tyrosine (TYR).

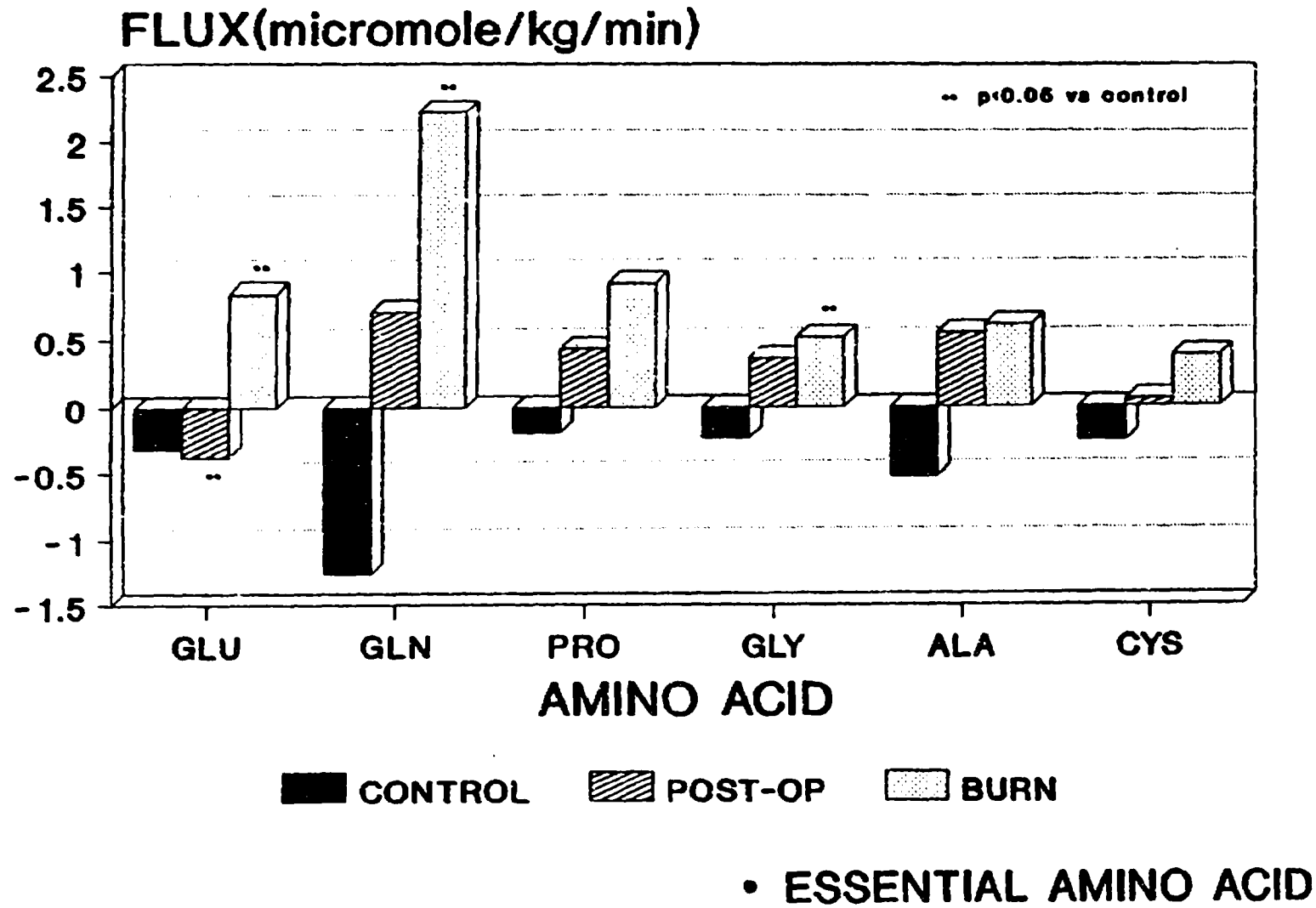

FIGURE 3. Net pulmonary amino acid flux for the amino acids glutamate (GLU), glutamine (GLN), proline (PRO), glycine (GLY), alanine (ALA), and cystine (CYS).

TABLE 2. Rate of Glycine Appearance ± SD

Group	μmole/kg/min
1	Not measured
2	2.56 ± 0.47*
3	3.35 ± 0.55*

*No significant difference by ANOVA.

DISCUSSION

The role of the lung in amino acid homeostasis has not been elucidated. We, and others (3), have demonstrated that there is no net flux of amino acid across the lung in the unstressed state. However, a net release of amino acid similar to that which occurs from skeletal muscle occurs during hypermetabolism. Although only glutamate, glutamine, glycine, and lysine flux were statistically different from the control population, all amino acids showed a similar trend. The lack of significance may reflect a Type II error. This suggests that the release of amino acid may be secondary to protein degradation and not a selective increase in pulmonary amino acid synthesis. Further study will be necessary to define the role of the lung in amino acid metabolism following stress.

CONCLUSIONS

1. The human lung releases glutamate, glutamine, glycine, and lysine in hypermetabolic thermally injured patients compared to metabolically stable controls.

2. Other amino acids were demonstrated to have a positive net flux in the patients, although small sample size may have precluded statistical significance.

3. The lung may serve as a pool for amino acid release similar to skeletal muscle following periods of metabolic stress.

PRESENTATIONS/PUBLICATIONS

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

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4. Wolfe RR (ed): *Traces in Metabolic Research*. New York: Alan R. Liss, Inc., 1983.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION: DA335423	2. DATE OF SUMMARY: 01 Oct 91	REPORT CONTROL SYMBOL: DD-DR&E(AR) 636
3. DATE PREV SUM'RY: 01 Oct 90	4. KIND OF SUMMARY: D. Chg	5 SUMMARY SCTY: U	6. WORK SECURITY: U	7. REGRADING	8. DISP'N INSTR'N: CX	9 LEVEL OF SUM A. WORK UNIT

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	0601101A	3A161101A91C	CA	075
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)
(U) Development of a Rat Model of Inhalation Injury - A Pilot Study

12. SUBJECT AREAS
0605 Medicine and Medical Research

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
9006	9209	DA	C. In-House

17. CONTRACT/GRANT — MILITARY RELEVANCE APPROVED BY [illegible]

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

18. RESPONSIBLE DOD ORGANIZATION

a. NAME: US Army Institute of Surgical Research

b. ADDRESS (Include zip code):
Fort Sam Houston
San Antonio, Texas 78234-5012

c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A

d. TELEPHONE NUMBER (Include area code): 512-221-2720

19. RESOURCES ESTIMATE

FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
91	0.5	48
92	0.5	52

20. PERFORMING ORGANIZATION

a. NAME: US Army Institute of Surgical Research

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

c. NAME OF PRINCIPAL INVESTIGATOR: BECKER, W K

d. TELEPHONE NUMBER (Include area code): 512-221-3349

e. NAME OF ASSOCIATE INVESTIGATOR (If available): CIOFFI, W G

f. NAME OF ASSOCIATE INVESTIGATOR (If available): RUE, L W

21. GENERAL USE: FIC

MILITARY/CIVILIAN APPLICATION: M

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Burns (Injuries); (U) Inhalation; (U) Lung; (U) Carbon Monoxide; (U) Hydrochloric Acid; (U) Mortality; 22. (Continued) (U) Histopathology

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

23/24. (U) The objective of this work is to develop a small animal model of smoke injury and inhalation injury. A smoke delivery system will be developed for the In-Tox™ small animal exposure device, to include a furnace, an air delivery pump, an in-line carbon monoxide monitor, a mixing and cooling chamber, and a temperature monitor. Initial studies will be performed to ensure uniform smoke and hydrochloric acid exposures. Animal studies will include a time-dose mortality curve and measurement of blood carbon monoxide levels. At various times following exposure, histopathological examination of the lungs will be performed.

25. (U) 9010 - 9109. The protocol was amended during the last reporting period to include a second species (guinea pigs) to determine if a species difference in susceptibility to smoke injury was present in this model. No difference in susceptibility to smoke injury was noted in guinea pigs as compared to rats. The smoke-generating apparatus was totally redesigned to decrease the early mortality associated with carbon monoxide poisoning noted with the original exposure device and an addendum for testing this new device is pending.

DD FORM 83 MAR 1498 — EDITION OF MAR 88 IS OBSOLETE — * USGPO: 1988-471-003/10111

SUPPLEMENTAL WORK UNIT INFORMATION FOR THE PROJECT ENTITLED "DEVELOPMENT OF A RAT MODEL OF INHALATION INJURY - A PILOT STUDY"

Subrecord/Linking Accession Number: Not applicable.

Search Control Data: W6R05I/W6R06K, 29 May 1990.

Product Identification: For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report* for fiscal years 1990-1.

Unclassified Special Categories: Lab Animals: Rats; Guinea Pigs; RA II.

ABSTRACT

PROJECT NUMBER: 3A161101A91C, 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 1990 - 30 September 1991

INVESTIGATORS: William K. Becker, MD, Lieutenant Colonel, MC
William G. Cioffi, Jr., MD, Major, MC
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 INJRY - 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. A graphic display of this association is illustrated in Figure 1. 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.

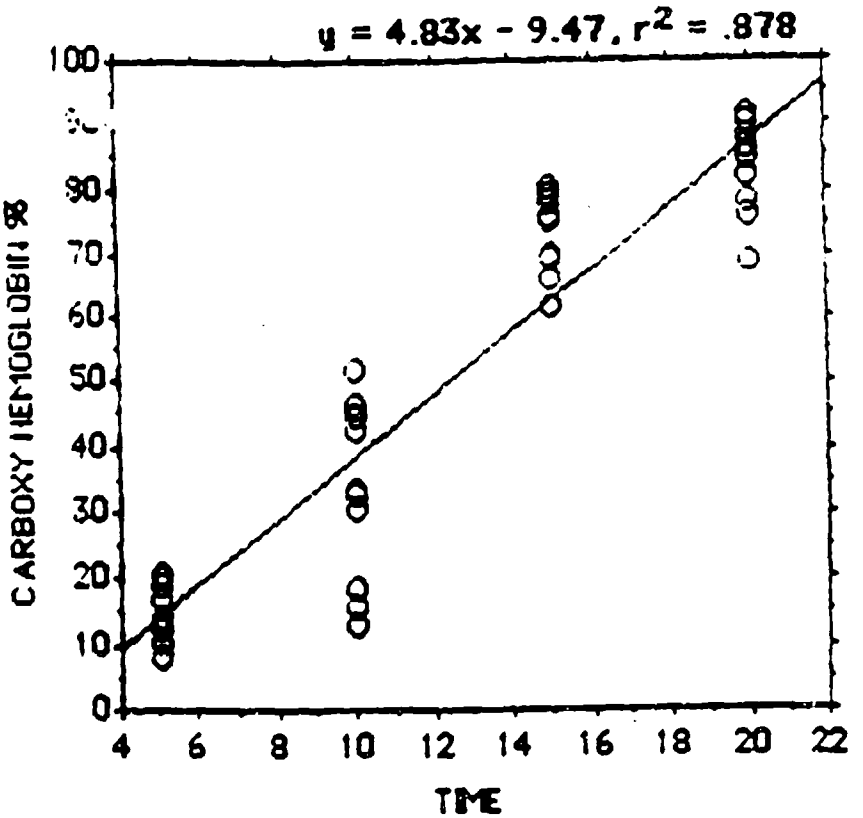

FIGURE 1. The relationship between smoke exposure time and blood carboxyhemoglobin level in rats. O indicates carboxyhemoglobin.

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.

DISCUSSION

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

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.

REFERENCES

1. Shirani KZ, Pruitt BA Jr, Mason AD Jr: The influence of inhalation injury and pneumonia on burn mortality. *Ann Surg* 205:82-7, 1987.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
					21 Nov 90	DD-DR&E(A R) 635
3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISP'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
NONE	A. New	U	U		CX	

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	0601101A	3A161101A91C	CK	076
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code) (U) Effect of Silver Sulfadiazine on Copper Status in Rats with Thermal Injury

12. SUBJECT AREAS
0601 Biochemistry 0605 Medicine and Medical Research

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
9011	9209	DA	C. In-House

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

a. DATE EFFECTIVE		18. RESOURCES ESTIMATE		
b. CONTRACT/GRANT NUMBER		a. FISCAL YEARS	a. PROFESSIONAL WORK YEARS	b. FUNDS (In thousands)
c. TYPE	d. AMOUNT	91	0.1	58
e. KIND OF AWARD	f. CUM/TOTAL	92	0.1	61

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME US Army Institute of Surgical Research	a. NAME US Army Institute of Surgical Research
b. ADDRESS (Include zip code) Fort Sam Houston San Antonio, Texas 78234-5012	b. ADDRESS Fort Sam Houston San Antonio, Texas 78234-5012
c. NAME OF RESPONSIBLE INDIVIDUAL PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR SHIPPEE, R L
d. TELEPHONE NUMBER (Include area code) 512-221-2720	d. TELEPHONE NUMBER (Include area code) 512-221-7138
21. GENERAL USE FINA	f. NAME OF ASSOCIATE INVESTIGATOR (If available) BOOSALIS, M G
MILITARY/CIVILIAN APPLICATION: M	g. NAME OF ASSOCIATE INVESTIGATOR (If available) MC CLAIN, C J

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Burns (Injuries); (U) Sulfadiazine; (U) Copper; (U) Tissue Extracts; (U) Analysis of Variance

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

23/24. (U) The objective of this work is to determine the effect of topical silver sulfadiazine on tissue copper concentration in a rat burn model. Male Sprague-Dawley rats will receive either 30% full-thickness scald or sham burns. Animals randomized to the silver sulfadiazine groups will have silver sulfadiazine cream applied over the dorsal area daily. At 7 days postburn, serum samples will be collected and analyzed for copper, zinc, silver, and ceruloplasmin concentrations. The liver, femur, testes, and kidneys will be excised and processed for determination of copper, zinc, and silver concentrations. Data will be analyzed using a 2 X 2 factorial ANOVA.

25. (U) 8810 - 8909. Not applicable.

(U) 8910 - 9009. Not applicable.

(U) 9010 - 9109. This project 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 1991. Equipment and supplies have been ordered and work will be initiated shortly.

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

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION DA335691	2. DATE OF SUMMARY 01 Oct 91	REPORT CONTROL SYMBOL DD-DR&E(AR) 636
3. DATE PREV SUM'RY 21 Nov 90	4. KIND OF SUMMARY D. Chg	5. SUMMARY SCTY U	6. WORK SECURITY U	7. REGRADING	8. DISP'N INSTR'N CX	9. LEVEL OF SUM A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	0601101A	3A161101A91C	CK	076
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE *(Precede with Security Classification Code)* (U) Effect of Silver Sulfadiazine on Copper Status in Rats with Thermal Injury

12. SUBJECT AREAS
0601 Biochemistry 0605 Medicine and Medical Research

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
9011	9209	DA	C. In-House

17. CONTRACT/GRANT MILITARY RELEVANCY CERTIFIED APPROVED BY Bruce Pruitt 10/91

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

18. RESOURCES ESTIMATE

a. FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS *(In thousands)*
91	0.4	21
92	0.5	24

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME: US Army Institute of Surgical Research	a. NAME: US Army Institute of Surgical Research
b. ADDRESS *(Include zip code)*: Fort Sam Houston, San Antonio, Texas 78234-5012	b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-5012
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR: SHIPPEE, R L
d. TELEPHONE NUMBER *(Include area code)*: 512-221-2720	d. TELEPHONE NUMBER *(Include area code)*: 512-221-4858
21. GENERAL USE: FIC MILITARY/CIVILIAN APPLICATION: M	e. NAME OF ASSOCIATE INVESTIGATOR *(If available)*: BOOSALIS, M G g. NAME OF ASSOCIATE INVESTIGATOR *(If available)*: MC CLAIN, C J

22. KEYWORDS *(Precede EACH with Security Classification Code)* (U) Burns (Injuries); (U) Sulfadiazine; (U) Copper; (U) Tissue Extracts; (U) Analysis of Variance

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

23/24. (U) The objective of this work is to determine the effect of topical silver sulfadiazine on tissue copper concentration in a rat burn model. Male Sprague-Dawley rats will receive either 30% full-thickness scald or sham burns. Animals randomized to the silver sulfadiazine groups will have silver sulfadiazine cream applied over the dorsal area daily. At 7 days postburn, serum samples will be collected and analyzed for copper, zinc, silver, and ceruloplasmin concentrations. The liver, femur, testes, and kidneys will be excised and processed for determination of copper, zinc, and silver concentrations. Data will be analyzed using a 2-by-2 factorial ANOVA.

25. (U) 9011 - 9109. This project 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 1991. 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 considerable 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 advised if copper status is affected by silver sulfadiazine as suggested by this animal model.

SUPPLEMENTAL WORK UNIT INFORMATION FOR THE PROJECT ENTITLED "EFFECT OF SILVER SULFADIAZINE ON COPPER STATUS IN RATS WITH THERMAL INJURY"

Subrecord/Linking Accession Number: Not applicable.

Search Control Data: W6R04J/W6R06J, 9 October 1990.

Product Identification: For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1991.*

Unclassified Special Categories: Lab Animals: Rats; ILIR; RA II.

ABSTRACT

PROJECT NUMBER: 3A161101A91C, 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: 21 November 1990 - 30 September 1991

INVESTIGATORS: Ronald L. Shippee, PhD, Major, MS[1]
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Basil A. Pruitt, Jr., MD, Colonel, MC[1]

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. Bossalis 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

Shippee RL: Effect of silver sulfadiazine on copper status in rats with thermal injury. Presented to the Federation of American Societies for Experimental Biology, Atlanta, Georgia, 24 April 1991.

PUBLICATIONS

Shippee RL, Boosalis M, McClain C, Becker W, and Watiwat S: Effect of topical silver sulfadiazine on plasma copper, zinc, and silver concentrations in a burn rat model (abstr). *FASEB J* 5(5):PA1313, May 1991.

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8. Boosalis M, Solem L, McCall J, McClain CJ: Serum copper, zinc, silver, and selenium concentrations in burn patients (abstr). *JPEN* 8:102, 1984.

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gastrointestinal absorption and extension. *Arch Surg* 114:281-5, 1979.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
					13 Feb 91	DD-DRAB(AR) 636
3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISP'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
NONE	A. New	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	0601101A	3A161101A91C	CA	077
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code) (U) Effects of Pressure-Controlled Inverse Ratio Ventilation (PcIRV) on Smoke Inhalation Injury in an Ovine Model

12. SUBJECT AREAS
0605 Medicine and Medical Research

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
9102	9209	DA	C. In-House

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

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

18. RESOURCES ESTIMATE

FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
91	0.5	55
92	0.5	58

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME: US Army Institute of Surgical Research	a. NAME: US Army Institute of Surgical Research
b. ADDRESS (Include zip code): Fort Sam Houston, San Antonio, Texas 78234-5012	b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-5012
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR: OGURA, H
d. TELEPHONE NUMBER (Include area code): 512-221-2720	d. TELEPHONE NUMBER (Include area code): 512-221-5703
21. GENERAL USE: FINA MILITARY/CIVILIAN APPLICATION: M	e. NAME OF ASSOCIATE INVESTIGATOR (If available): CIOFFI, W G g. NAME OF ASSOCIATE INVESTIGATOR (If available): JOHNSON, A A

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Burns (Injuries); (U) Lungs; (U) Pulmonary Function; (U) Pulmonary Insufficiency

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

23/24. (U) The objective of this work is to determine the effects of PcIRV on smoke inhalation injury in an ovine model as compared with conventional ventilation. Eighteen sheep will be anesthetized, orally intubated, mechanically ventilated, and catheterized. Smoke inhalation injury will be produced by smoke insufflation at a dose that produces a carboxyhemoglobin level of 50-60% (moderate injury). Cardiopulmonary variables and blood gases will be measured every 30 min during mechanical ventilation. Measurement of V_A/Q_C using the multiple inert gas elimination technique will be performed every hour. Blood and expired gas samples will be analyzed by GC. ANOVA for a mixed factorial design and multivariate analysis (regression) will be utilized.

25. (U) 8810 - 8909. Not applicable.

(U) 8910 - 9009. Not applicable.

(U) 9010 - 9109. 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. Equipment and supplies have been ordered and work will be initiated shortly.

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

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
				DA335815	01 Oct 91	DD-DR&E(AR) 636
3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISP'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
13 Feb 91	D. Chg	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	0601101A	3A161101A91C	CA	077
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code) (U) Effects of Pressure-Controlled Inverse Ratio Ventilation (PcIRV) on Smoke Inhalation Injury in an Ovine Model

12. SUBJECT AREAS
0605 Medicine and Medical Research

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
9102	9209	DA	C. In-House

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

18. RESOURCES ESTIMATE

a. FISCAL YEARS	b. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
91	1.0	27
92	1.5	42

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME: US Army Institute of Surgical Research	a. NAME: US Army Institute of Surgical Research
b. ADDRESS (Include zip code): Fort Sam Houston, San Antonio, Texas 78234-5012	b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-5012
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR: OGURA, H
d. TELEPHONE NUMBER (Include area code): 512-221-2720	d. TELEPHONE NUMBER (Include area code): 512-221-3349
21. GENERAL USE: FIC	e. NAME OF ASSOCIATE INVESTIGATOR (If available): CIOFFI, W G
MILITARY/CIVILIAN APPLICATION: M	g. NAME OF ASSOCIATE INVESTIGATOR (If available): JOHNSON, A A

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Burns (Injuries); (U) Lungs; (U) Pulmonary Function; (U) Pulmonary Insufficiency

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

23/24. (U) The objective of this work is to determine the effects of PcIRV on smoke inhalation injury in an ovine model as compared with conventional ventilation. Eighteen sheep will be anesthetized, orally intubated, mechanically ventilated, and catheterized. Smoke inhalation injury will be produced by smoke insufflation at a dose that produces a carboxyhemoglobin level of 50-60% (moderate injury). Cardiopulmonary variables and blood gases will be measured every 30 min during mechanical ventilation. Measurement of V_A/Q_C using the multiple inert gas elimination technique will be performed every hour. Blood and expired gas samples will be analyzed by GC. ANOVA for a mixed factorial design and multivariate analysis (regression) will be utilized.

25. (U) 9102 - 9109. 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. Twelve animals have been studied to date. Data will be analyzed after the study of 6 more animals.

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SUPPLEMENTAL WORK UNIT INFORMATION FOR THE PROJECT ENTITLED "EFFECTS OF PRESSURE-CONTROLLED INVERSE RATIO VENTILATION (PcIRV) ON SMOKE INHALATION INJURY IN AN OVINE MODEL"

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ABSTRACT

PROJECT NUMBER: 3A161101A91C, In-House Laboratory Independent Research

PROJECT TITLE: Effects of Pressure-Controlled Inverse Ratio Ventilation (PcIRV) on Smoke Inhalation Injury in an Ovine Model

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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 74-76°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 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 1.5-2 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				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
					08 May 91	DD-DR&E(AR) 636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
NONE	A. New	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	0601101A	3M161101A91C	CA	078
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE *(Precede with Security Classification Code)* (U) Effects of Pentoxifylline and Protein Kinase C Inhibitor (H-7) on Smoke Inhalation Injury in an Ovine Model

12. SUBJECT AREAS
0605 Medicine and Medical Research

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
9105	9209	DA	C. In-House

17. CONTRACT/GRANT MILITARY RELEVANCY CERTIFIED
APPROVED BY Brad C. Brueckner, M.D.

18. RESOURCES ESTIMATE

a. DATE EFFECTIVE	b. CONTRACT/GRANT NUMBER	c. TYPE	d. AMOUNT	e. FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS *(In thousands)*
				91	0.1	23
				92	0.1	23

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME: US Army Institute of Surgical Research	a. NAME: US Army Institute of Surgical Research
b. ADDRESS *(Include zip code)*: Fort Sam Houston, San Antonio, Texas 78234-5012	b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-5012
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR: OGURA, H
d. TELEPHONE NUMBER *(Include area code)*: 512-221-2720	d. TELEPHONE NUMBER *(Include area code)*: 512-221-5703
21. GENERAL USE: FINA MILITARY/CIVILIAN APPLICATION: M	f. NAME OF ASSOCIATE INVESTIGATOR *(If available)*: CIOFFI, W G g. NAME OF ASSOCIATE INVESTIGATOR *(If available)*: JOHNSON, A A

22. KEYWORDS *(Precede EACH with Security Classification Code)* (U) Burns (Injuries); (U) Lung; (U) Inhalation; (U) Edema; (U) Therapy

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

23/24. (U) The objective of this work is to determine the physiologic effects of pentoxifylline and protein kinase C inhibitor (H-7) on smoke inhalation injury in an ovine model. Twenty-four sheep will be divided into three groups. Group I (n=8) will receive smoke innalation injury without treatment, Group II (n=8) will receive smoke inhalation injury with continuous infusion of pentoxifylline postinjury, and Group III (n=8) will receive smoke inhalation injury with continuous infusion of H-7 postinjury. Cardiopulmonary variables and blood gases will be 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 will be performed at the end of the 24-h study period. After blood sample collections, bronchoalveolar lavage will be 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 will be determined by a gravimetric method upon sacrifice. ANOVA for a mixed factorial design and multivariate analysis (regression) will be utilized.

25. (U) 9105 - 9109. This study was approved by the USAISR Research Council and the 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.

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

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION: DA335806	2. DATE OF SUMMARY: 01 Oct 91	REPORT CONTROL SYMBOL: DD-DR&E(AR) 636
3. DATE PREV SUM'RY: 08 May 91	4. KIND OF SUMMARY: D. Chg	5. SUMMARY SCTY: U	6. WORK SECURITY: U	7. REGRADING	8. DISP'N INSTR'N: CX	9. LEVEL OF SUM A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	0601101A	3A161101A91C	CA	078
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code) (U) Effects of Pentoxifylline and Protein Kinase C Inhibitor (H-7) on Smoke Inhalation Injury in an Ovine Model

12. SUBJECT AREAS
0605 Medicine and Medical Research

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
9105	9209	DA	C. In-House

17. CONTRACT/GRANT MILITARY RELEVANCY CERTIFIED / APPROVED BY: Basil Pruitt — RESOURCES ESTIMATE

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

a. FISCAL YEARS	b. PROFESSIONAL WORKYEARS	c. FUNDS (In thousands)
91	0.1	23
92	0.1	24

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME: US Army Institute of Surgical Research	a. NAME: US Army Institute of Surgical Research
b. ADDRESS (Include zip code): Fort Sam Houston San Antonio, Texas 78234-5012	b. ADDRESS: Fort Sam Houston San Antonio, Texas 78234-5012
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR: OGURA, H
d. TELEPHONE NUMBER (Include area code): 512-221-2720	d. TELEPHONE NUMBER (Include area code): 512-221-3349
21. GENERAL USE: PIC MILITARY/CIVILIAN APPLICATION: M	f. NAME OF ASSOCIATE INVESTIGATOR (If available): CIOFFI, W G g. NAME OF ASSOCIATE INVESTIGATOR (If available): JOHNSON, A A

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Burns (Injuries); (U) Lung; (U) Inhalation; (U) Edema; (U) Therapy

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

23/24. (U) The objective of this work is to determine the physiologic effects of pentoxifylline and protein kinase C inhibitor (H-7) on smoke inhalation injury in an ovine model. Twenty-four sheep will be divided into three groups. Group I (n=8) will receive smoke inhalation injury without treatment, Group II (n=8) will receive smoke inhalation injury with continuous infusion of pentoxifylline postinjury, and Group III (n=8) will receive smoke inhalation injury with continuous infusion of H-7 postinjury. Cardiopulmonary variables and blood gases will be measured presmoke and at 1, 4, 8, 12, 16, 20, and 24 h postsmoke. Measurement of V_A/Q_T using the multiple inert gas elimination technique will be performed at the end of the 24-h study period. After blood sample collections, bronchoalveolar lavage will be 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 will be determined by a gravimetric method upon sacrifice. ANOVA for a mixed factorial design and multivariate analysis (regression) will be utilized.

25. (U) 9105 - 9109. This study was approved by the USAISR Research Council and the 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.

DD FORM 83 MAR 1498 EDITION OF MAR 68 IS OBSOLETE * U.S.G.P.O. 1986-491-003/50329

SUPPLEMENTAL WORK UNIT INFORMATION FOR THE PROJECT ENTITLED "EFFECT OF PENTOXIFYLLINE AND PROTEIN KINASE C INHIBITOR (H-7) ON SMOKE INHALATION INJURY IN AN OVINE MODEL"

Subrecord/Linking Accession Number: Not applicable.

Search Control Data: W6M59E/W6N01F, 30 January 1991.

Product Identification: For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1991.*

Unclassified Special Categories: Lab Animals: Sheep; ILIR; RA II.

ABSTRACT

PROJECT NUMBER: 3A161101A91C, 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: 8 May 1991 - 30 September 1991

INVESTIGATORS: Hiroshi Ogura, MD
William G. Cioffi, Jr., MD, Major, MC
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Paulette Langlinais, MS
Basil A. Pruitt, Jr., MD, Colonel, MC

The physiologic changes after smoke inhalation injury have been observed in a sheep model at this Institute. The influence of medical interventions in this model, however, has not been thoroughly investigated. Pentoxifylline 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 pentoxifylline and protein kinase C inhibitor (H-7) on smoke inhalation injury in an ovine model.

Twenty-four sheep will be randomized to one of three groups. Group I (n=8) will receive smoke inhalation injury without treatment, Group II (n=8) will receive smoke inhalation injury with continuous infusion of pentoxifylline postinjury, and Group III (n=8) will receive smoke inhalation injury with continuous infusion of H-7 postinjury. Cardiopulmonary variables and blood gases will be 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 will be performed at the end of the 24-h study period. After blood sample collections, bronchoalveolar lavage will be 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 will be determined by a gravimetric method upon sacrifice. ANOVA for a mixed factorial design and multivariate analysis (regression) will be utilized.

This study was approved by the USAISR Research Council and the 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.

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 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, pentoxifylline 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, pentoxifylline improved the survival rate, with reduction of TNF formation and platelet aggregation (5,6). In hemorrhagic shock models, the survival rate using pentoxifylline was improved in association with improved tissue oxygenation (7,8). Pentoxifylline 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 pentoxifylline 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 pentoxifylline and protein kinase C inhibitor (H-7) on smoke inhalation injury in an ovine model.

MATERIALS AND METHODS

Study Design. Twenty-four 1- to 2-year-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 wk prior to study. All animals will be fasted for 24 h before smoke exposure and use. The animals will be randomized to one of three groups. Group I (n=8) will receive smoke inhalation injury without treatment, Group II (n=8) will receive smoke inhalation injury with continuous infusion of pentoxifylline postinjury, and Group III (n=8) will receive smoke inhalation injury with continuous infusion of H-7 postinjury.

All animals will be anesthetized, orally intubated, mechanically ventilated, placed in the supine position, and catheterized. Cannulae will be placed in a femoral artery and a femoral vein. A radiopaque sheath will be placed into an external jugular vein and a Swan-Ganz catheter will be inserted through the sheath. Smoke inhalation injury will be produced by smoke insufflation at a dose that produces a carboxyhemoglobin level of 50-60% (moderate injury). After smoke exposure, the animals will be extubated and observed in the awake state for 24 h. Group I will not receive any treatment with pentoxifylline or H-7. Group II will receive pentoxifylline as a bolus intravenous injection just after smoke exposure and a continuous intravenous infusion for 24 h. Group III will receive H-7 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 will be anesthetized with sodium pentobarbital, orally intubated, mechanically ventilated, and placed in the prone position. The animals will then be paralyzed with pancuronium bromide, V_A/Q_C measurements will be obtained, and bronchoalveolar lavage will be performed.

During mechanical ventilation, the tidal volume will be set at 15 ml/kg and the respiratory rate will be 12/min. PEEP will be 5 cmH_2O and FIO_2 will be kept at 0.21 throughout the study period.

Cardiopulmonary variables and blood gases will be measured presmoke and at 1, 4, 8, 12, 16, 20, and 24 h postsmoke. Cardiopulmonary measurements will include systemic blood pressure, heart rate, pulmonary artery pressure, pulmonary capillary wedge pressure, cardiac output, and pulmonary resistance. Arterial and mixed venous blood samples will be analyzed for blood gases. Blood samples for the measurement of 6-keto-PGF_{1a}, thromboxane B_2, and conjugated dienes will be drawn at the same time.

Measurement of V_A/Q_C using the multiple inert gas elimination technique (MIGET) will be performed at the end of the 24-h study period. Lactated Ringer's solution containing six inert gases will be infused. After 30 min when equilibrium of gas exchange occurs, arterial and mixed venous blood samples will be obtained. Mixed-expired gas will be collected from a temperature-controlled copper coil about 1 min after blood sampling. Blood and expired gas samples will be immediately analyzed by GC.

After blood sample collections, bronchoalveolar lavage will be 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), will be determined after sacrifice.

Description of Procedures. Prior to smoke exposure, two Silastic® medical grade cannulae (20 cm) will be inserted into a femoral artery and femoral vein and one radiopaque sheath introducer (8.5F) will be inserted into an external jugular vein

using sterile technique after general anesthesia (sodium pentobarbital, 35 mg/kg IV). 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 (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) will be inserted through the sheath in the jugular vein into the pulmonary artery. Smoke insufflation resulting in a moderate inhalation injury will be produced by the method developed at this Institute (1). Animals in Group I will not receive any treatment with pentoxifylline or H-7. Animals in Group II will be administered pentoxifylline (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. Animals in Group III will be administered H-7 (Seikagaku America, Inc., St. Petersburg, FL) as a bolus injection (1 mg/kg IV) immediately after smoke exposure and a continuous infusion (0.3 mg/kg/h IV) 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 will again be 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 will be measured presmoke and at 1, 4, 8, 12, 16, 20, and 24 postsmoke. Cardiopulmonary measurements will include systemic blood pressure, heart rate, pulmonary artery pressure, pulmonary capillary wedge pressure, cardiac output, and pulmonary 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 1290A 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 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 will be drawn at the same time. After centrifugation, the plasma of these samples will be stored at -70°C for possible future testing. Due to the expense of such measurements, the assays will be 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, will be measured by RIA (17). Conjugated dienes, products of lipid peroxidation, will be determined by techniques described by Ward et al (18). They will be read at an optical density of 233 nm in a spectrophotometer.

Measurement of V_A/Q_C using the MIGET will be 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) 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 (10 cc each) will be drawn anaerobically into preweighed, heparinized syringes (30 ml, matched, glass, Becton, Dickinson, and Company) 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 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 will be infused in the suction port by the use of a syringe on a three-way stopcock. The fluid will be immediately pulled back with suction. This process of lavage and suction will be repeated five times (total fluid = 100 ml).

Extravascular lung water, determined by a gravimetric method (16), will be measured after sacrifice.

Necropsies will be performed on all animals dying spontaneously or sacrificed at the end of the study. A complete set of tissues will be fixed in 10% neutral buffered formalin, processed by standard methods, and stained with hematoxylin-eosin. Histologic evaluation with light microscopy will be 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 will be collected for transmission and scanning electron microscopy, fixed in 2.5% glutaraldehyde, and processed as indicated.

Determination of Number of Animals Required. Eight animals per group for a total of 24 animals should be satisfactory for adequate data collection as a pilot study.

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 the US Army Institute of Surgical Research Animal Care and Use Committee during the third quarter of Fiscal Year 1991.

DISCUSSION

Equipment and supplies have been ordered and work will be initiated shortly.

PRESENTATIONS/PUBLICATIONS

None.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
					21 Aug 91	DD-DR&E(AR) 636
3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISP'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
NONE	A. New	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	0601101A	3A161101A91C	CK	079
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code) (U) Endocrine Responses of the Burned Rat to Infection and Tumor Necrosis Factor (TNF) Challenge

12. SUBJECT AREAS
0601 Biochemistry 0605 Medicine and Medical Research

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16 PERFORMANCE METHOD
9108	9209	DA	C. In-House

17. CONTRACT/GRANT [illegible] MILITARY RELEVANCY VERIFIED APPROVED BY [signature] RESOURCES ESTIMATE

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

a. FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
91	0.0	0
92	0.8	70

18. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME: US Army Institute of Surgical Research	a. NAME: US Army Institute of Surgical Research
b. ADDRESS (Include ZIP code): Fort Sam Houston, San Antonio, Texas 78234-5012	b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-5012
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR: SHIRANI, K Z
d. TELEPHONE NUMBER (Include area code): 512-221-2720	d. TELEPHONE NUMBER (Include area code): 512-221-4652
21. GENERAL USE: FIC	f. NAME OF ASSOCIATE INVESTIGATOR (If available): VAUGHAN, G M
MILITARY/CIVILIAN APPLICATION: M	g. NAME OF ASSOCIATE INVESTIGATOR (If available): MC MANUS, A T

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Burns (Injuries); (U) Metabolism; (U) Hormones; (U) Pseudomonas aeruginosa; (U) Mortality; (U) Analysis of

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

22. (U) Variance

23/24. (U) The objectives of this work are 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-h period (mainly temperature, heart rate, and motor activity, and perhaps metabolic rate), rhythm analysis will be included with standard cosinor models.

25. (U) 9108 - 9109. 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 1991. Equipment and supplies have been ordered and work will be initiated shortly.

DD FORM 83 MAR 1498 EDITION OF MAR 66 IS OBSOLETE. * U.S.G.P.O. 1988-491-003/50329

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
					01 Oct 91	DD-DR&E(AR): 636
3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISP'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
21 Aug 91	D. Chg	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	0601101A	3A161101A91C	CK	079
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code) (U) Endocrine Responses of the Burned Rat to Infection and Tumor Necrosis Factor (TNF) Challenge

12. SUBJECT AREAS
0601 Biochemistry 0605 Medicine and Medical Research

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
9108	9209	DA	C. In-House

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

a. DATE EFFECTIVE		18. RESOURCES ESTIMATE		
b. CONTRACT/GRANT NUMBER		a. FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
c. TYPE	d. AMOUNT	91	0.0	0
e. KIND OF AWARD	f. CUM/TOTAL	92	0.8	70

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME: US Army Institute of Surgical Research	a. NAME: US Army Institute of Surgical Research
b. ADDRESS (Include zip code): Fort Sam Houston, San Antonio, Texas 78234-5012	b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-5012
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR: SHIRANI, K Z
d. TELEPHONE NUMBER (Include area code): 512-221-2720	d. TELEPHONE NUMBER (Include area code): 512-221-4652
21. GENERAL USE: FIC	f. NAME OF ASSOCIATE INVESTIGATOR (If available): VAUGHAN, G M
MILITARY/CIVILIAN APPLICATION: M	g. NAME OF ASSOCIATE INVESTIGATOR (If available): MC MANUS, A T

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Burns (Injuries); (U) Metabolism; (U) Hormones; (U) Pseudomonas aeruginosa; (U) Mortality; (U) Analysis of Variance

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

23/24. (U) The objectives of this work are to determine 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-h period (mainly temperature, heart rate, and motor activity, and perhaps metabolic rate), rhythm analysis will be included with standard cosinor models.

25. (U) 9108 - 9109. 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 1991. Equipment and supplies have been ordered and work will be initiated shortly.

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

SUPPLEMENTAL WORK UNIT INFORMATION FOR THE PROJECT ENTITLED "ENDOCRINE RESPONSES OF THE BURNED RAT TO INFECTION AND TUMOR NECROSIS FACTOR (TNF) CHALLENGE"

Subrecord/Linking Accession Number: Not applicable.

Search Control Data: W6L24K/W6L25M, 5 August 1991.

Product Identification: For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1991.*

Unclassified Special Categories: Lab Animals: Rats; ILIR; RA II.

ABSTRACT

PROJECT NUMBER: 3A161101A91C, 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: 21 August 1991 - 30 September 1991

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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 endotoxin stimulates TNF production and that TNF, in turn, mediates some of the metabolic and hormonal responses to injury and infection by inducing the release and modifying the actions of other cytokines and hormones. What has not been accomplished is observation of the effects of continuous exposure to TNF over a 1-2 week time period, which might simulate the prolonged flow phase of injury, with simultaneous measurement of metabolic rate and the responses in hormonal systems showing major changes after injury. We, therefore, plan to investigate the hormonal, metabolic, and TNF changes and mortality in the rat with untreated or antibiotic-treated experimental infection of the burn wound with *Pseudomonas aeruginosa* (Strain 1244). We will also infuse TNF over a 10-day to 2-week period to assess whether continuous infusion of this hormone reproduces the metabolic disturbances associated with burn wound infection.

MATERIALS AND METHODS

Design. One hundred and sixty male Sprague-Dawley rats weighing 180-200 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.

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

Description of Procedures. One hundred and sixty healthy adult male Sprague-Dawley rats weighing 180-200 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 2 weeks prior to use to exclude the possibility of any preexisting disease.

After the 2-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 5-7 days.

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 6 days. Based on previous experience, we expect infected animals to succumb within 2 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 7 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 1-3 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 25-30°C for sham-burn rats and 30-34°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 7 experimental groups, 2 control groups, and 7 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

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 1991.

DISCUSSION

Equipment and supplies have been ordered and work will be initiated shortly.

PRESENTATIONS/PUBLICATIONS

None.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION DA346162	2. DATE OF SUMMARY 01 Oct 91	REPORT CONTROL SYMBOL DD-DR&E(AR) 636
3. DATE PREV SUM'RY 01 Oct 90	4. KIND OF SUMMARY D. Chg	5. SUMMARY SCTY U	6. WORK SECURITY U	7. REGRADING	8. DISS'N INSTR'N CX	9. LEVEL OF SUM A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	63002A	3M263002D840	CA	081
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code) (U) The Effect of High-Frequency Oscillatory Ventilation on Smoke Inhalation Injury in Baboons

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

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
9005	9209	DA	C. In-House

17. CONTRACT/GRANT MILITARY RELEVANCY CERTIFIED APPROVED BY Paul O Pruitt

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

18. RESOURCES ESTIMATE

FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
91	0.5	88
92	0.5	310

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME: US Army Institute of Surgical Research	a. NAME: US Army Institute of Surgical Research
b. ADDRESS (Include zip code): Fort Sam Houston, San Antonio, Texas 78234-5012	b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-5012
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR: CIOFFI, W G
d. TELEPHONE NUMBER (Include area code): 512-221-2720	d. TELEPHONE NUMBER (Include area code): 512-221-8440
21. GENERAL USE: FIC MILITARY/CIVILIAN APPLICATION: M	e. NAME OF ASSOCIATE INVESTIGATOR (If available): RUE, L W g. NAME OF ASSOCIATE INVESTIGATOR (If available): JORDAN, B S

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Burns (Injuries); (U) Inhalation; (U) Pulmonary Edema; (U) Pulmonary Function; (U) Pulmonary Insufficiency;

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

22. (Continued) (U) Respirations; (U) Morbidity

23/24. (U) The objective of this work was to determine the effects of high-frequency oscillatory ventilation on pulmonary changes following smoke inhalation injury in a baboon model. 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 will be analyzed among groups using repeated measures ANOVA. Data will also be compared at specific time points using ANOVA. Outcome data will be analyzed using Chi square or the Fisher exact test. Nonparametric data will be analyzed using the Kruskall-Wallis test.

25. (U) 8910 - 9109. 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. The remainder of the data, including histopathology and lavage, are currently being analyzed.

DD FORM 83 MAR 1498 EDITION OF MAR 88 IS OBSOLETE. * U.S.G.P.O. 1988-491-003/50329

SUPPLEMENTAL WORK UNIT INFORMATION FOR THE PROJECT ENTITLED "THE EFFECT OF HIGH-FREQUENCY OSCILLATORY VENTILATION ON SMOKE INHALATION INJURY IN BABOONS"

Subrecord/Linking Accession Number: Not applicable.

Search Control Data: W6L59B/W6M00C, 18 April 1990.

Product Identification: For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report* for fiscal years 1990-2.

Unclassified Special Categories: Lab Animals: Baboons; RA II.

ABSTRACT

PROJECT NUMBER: 3M263002D840, 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 1990 - 30 September 1991

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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 strate

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

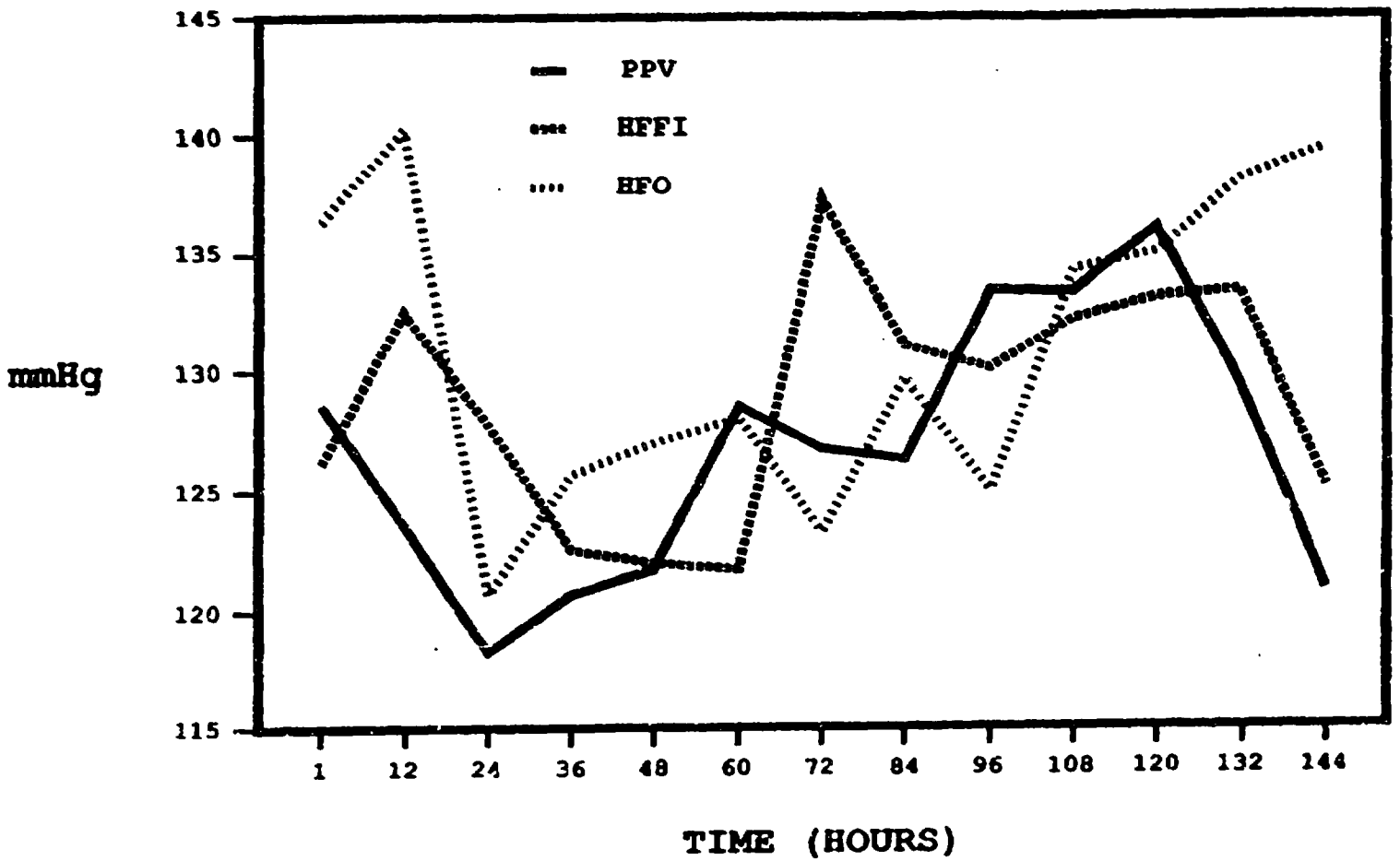

FIGURE 1. Mean blood pressure (mmHg). PPV indicates positive-pressure ventilation; HFFI, high-frequency flow interruption; and HFO, high-frequency oscillatory ventilation.

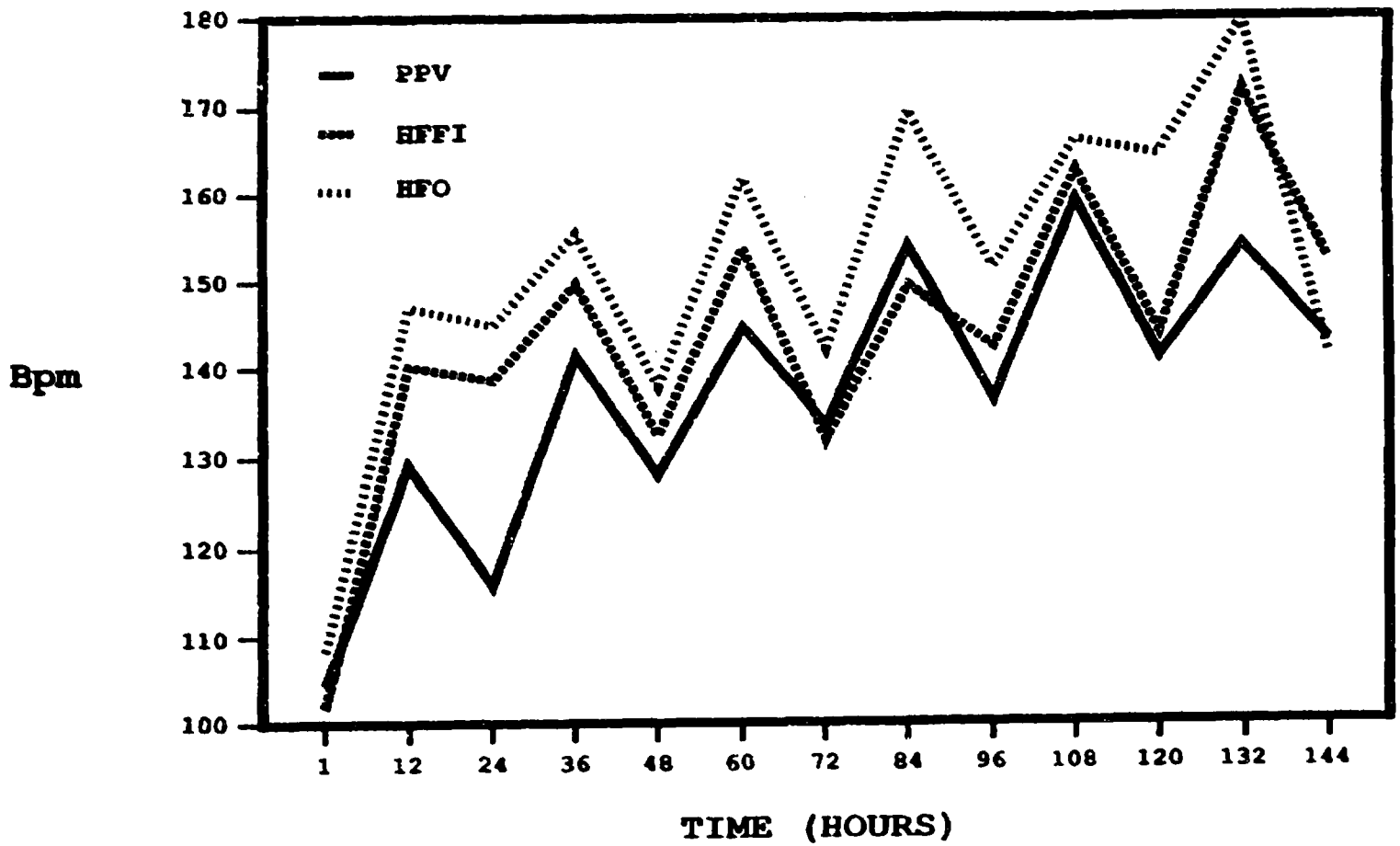

FIGURE 2. Heart rate (beats/min). PPV indicates positive-pressure ventilation; HFFI, high-frequency flow interruption; and HFO, high-frequency oscillatory ventilation.

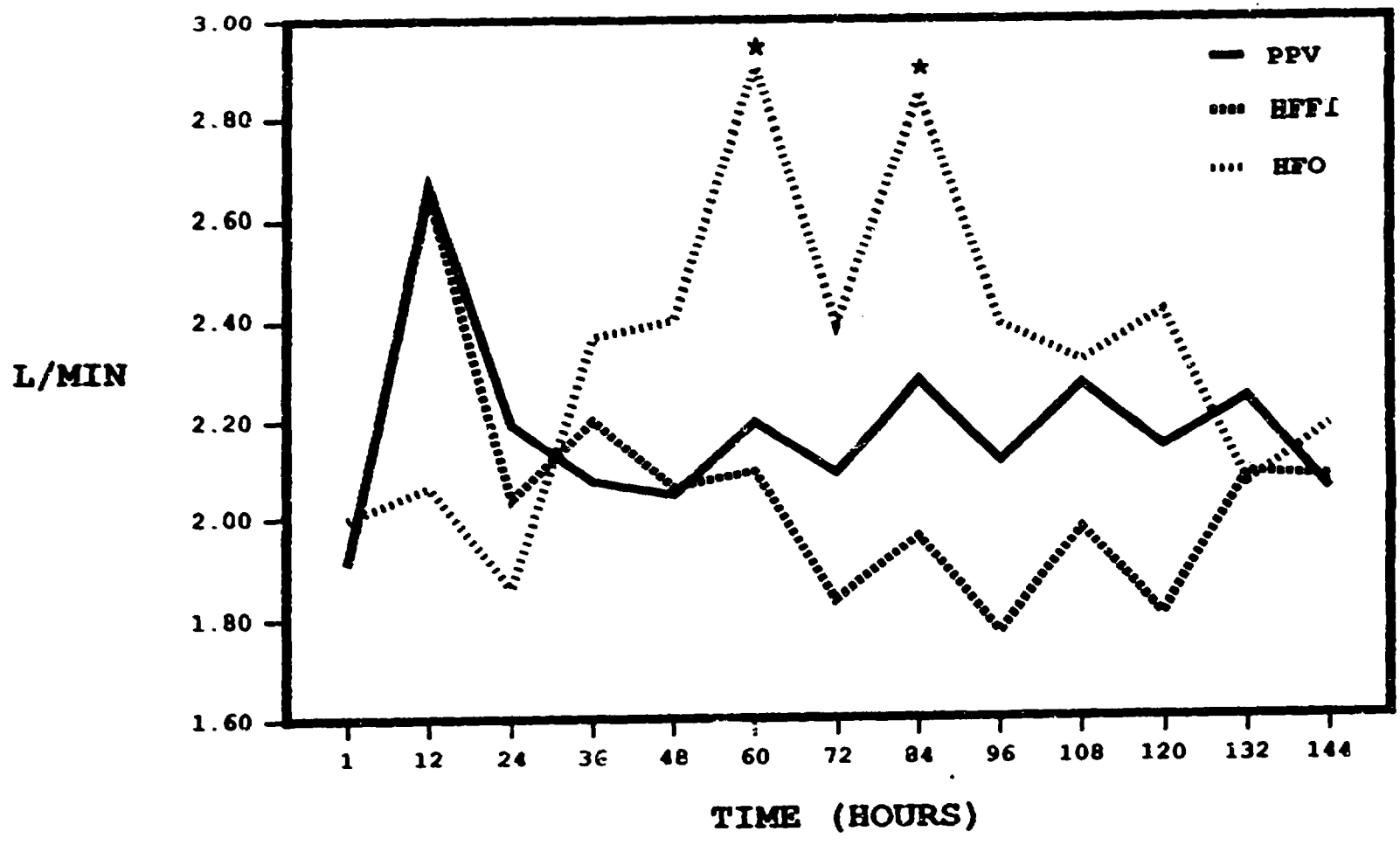

FIGURE 3. Cardiac output (l/min). PPV indicates positive-pressure ventilation; HFFI, high-frequency flow interruption; and HFO, high-frequency oscillatory ventilation. *P < 0.05 vs PPV and HFFI by ANOVA.

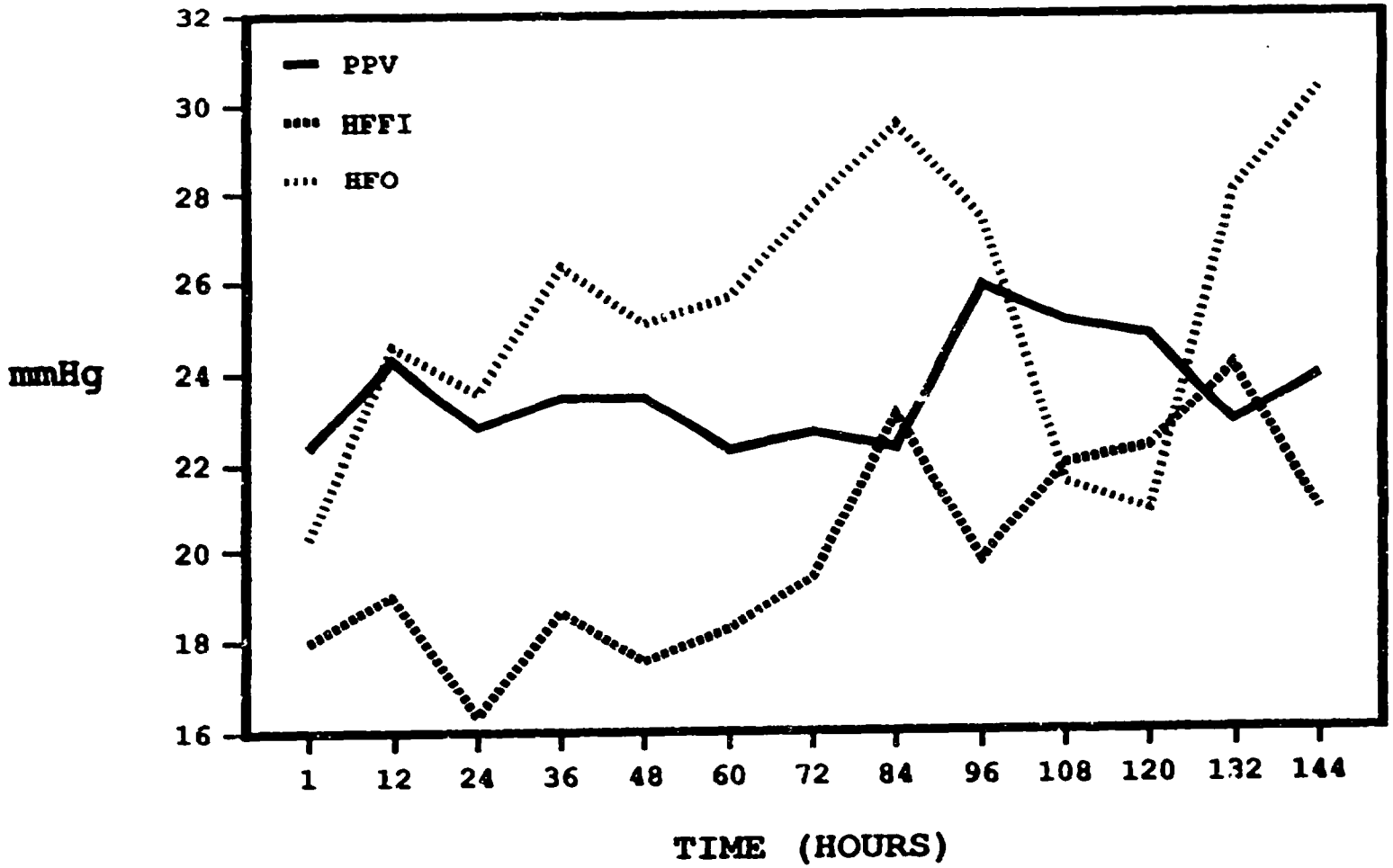

FIGURE 4. Mean pulmonary artery pressure (mmHg). PPV indicates positive-pressure ventilation; HFFI, high-frequency flow interruption; and HFO, high-frequency oscillatory ventilation.

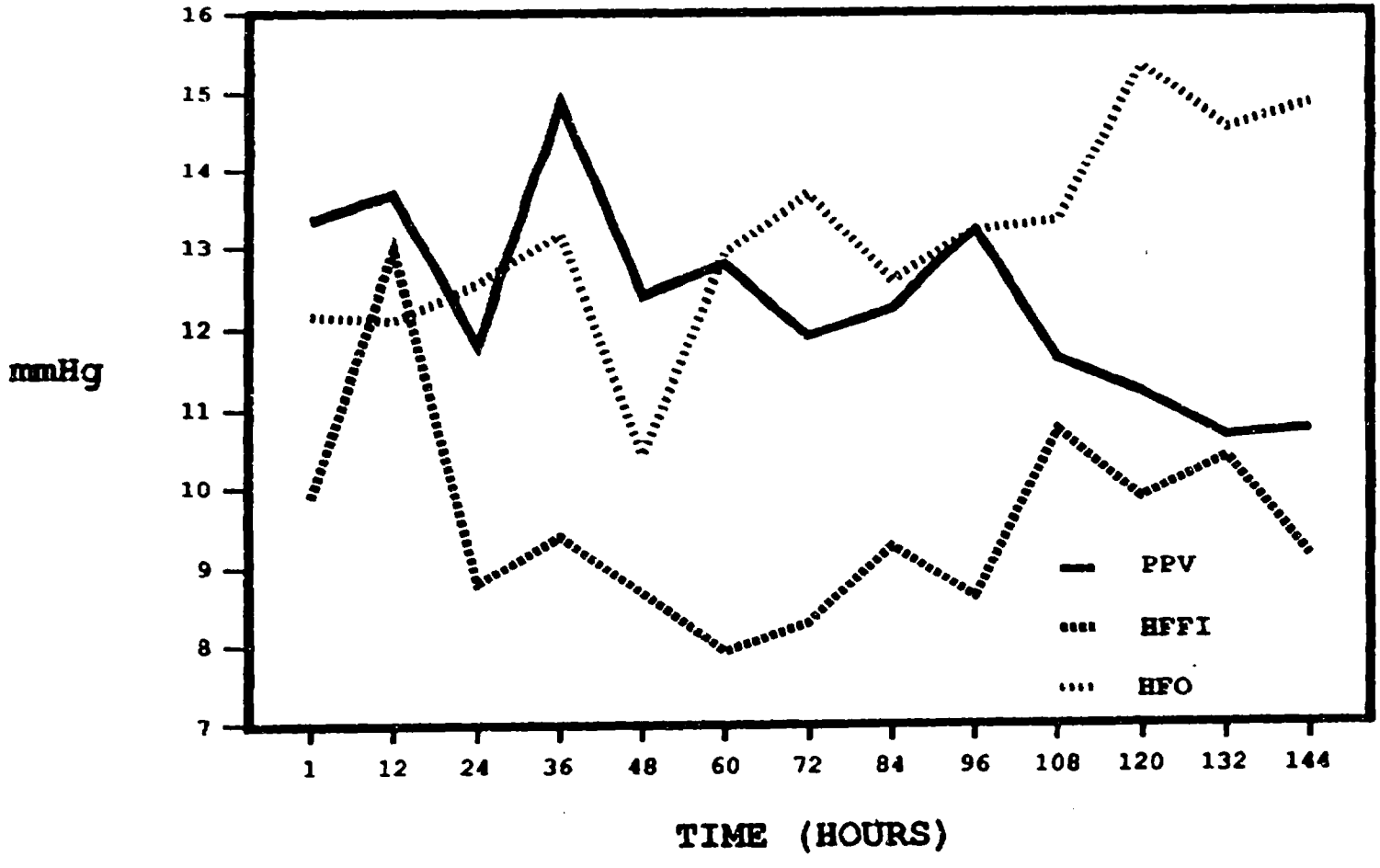

FIGURE 5. Mean pulmonary artery occlusion pressure (mmHg). PPV indicates positive-pressure ventilation; HFFI, high-frequency flow interruption; and HFO, high-frequency oscillatory ventilation.

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).

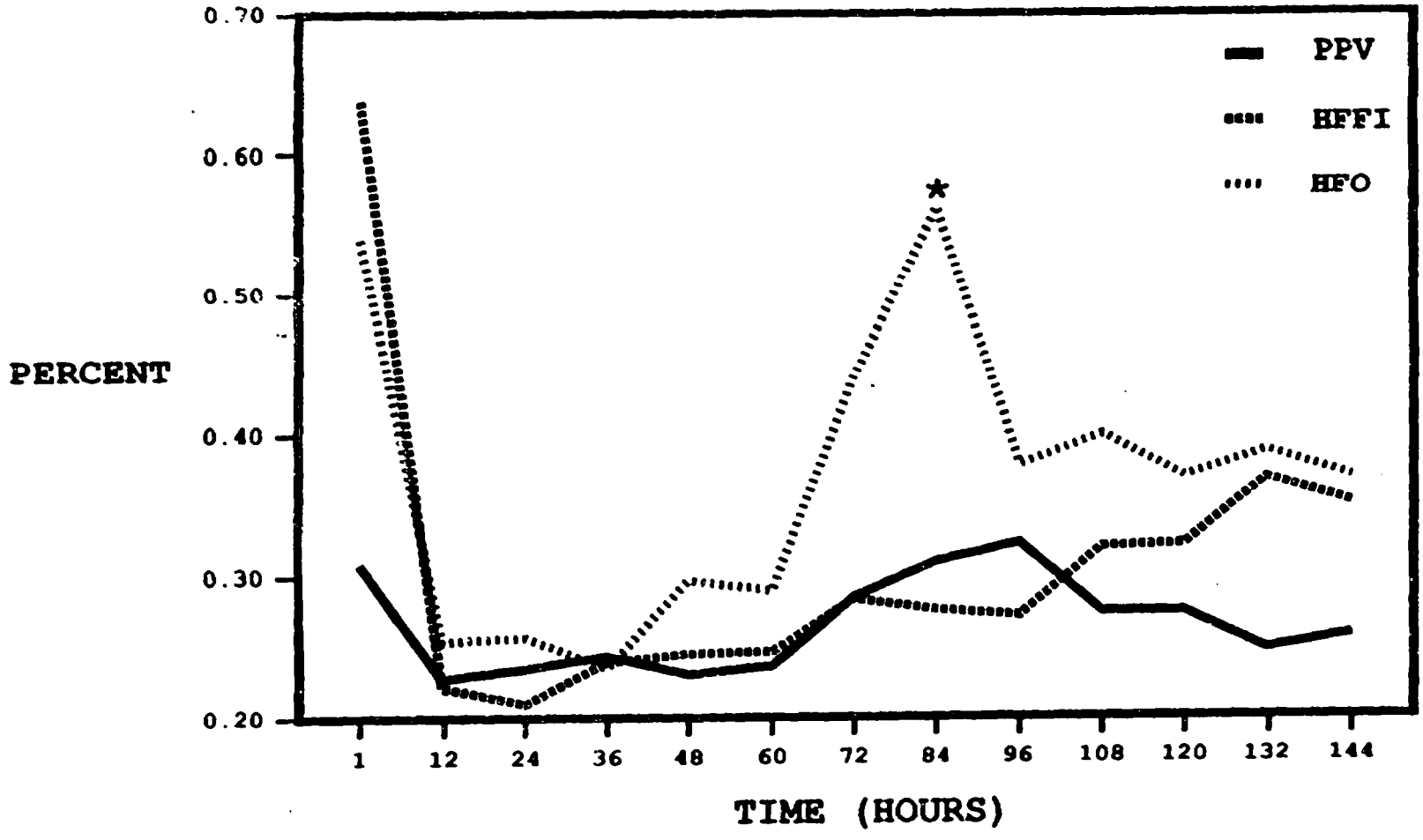

FIGURE 6. FIO_2 (%). PPV indicates positive-pressure ventilation; HFFI, high-frequency flow interruption; and HFO, high-frequency oscillatory ventilation. *$P < 0.05$ vs PPV and HFFI by ANOVA.

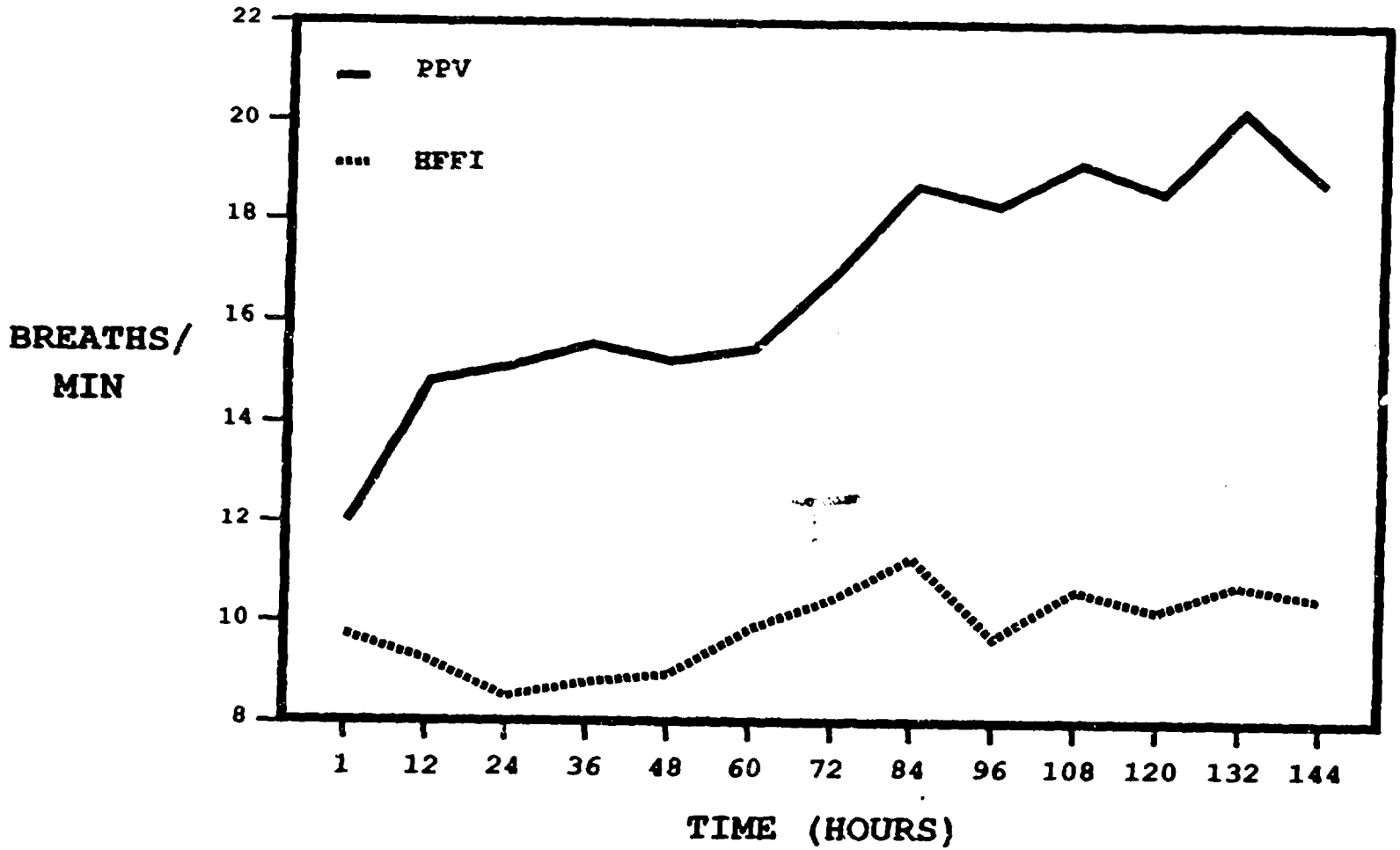

FIGURE 7. Respiratory rate (breaths/min). PPV indicates positive-pressure ventilation, and HFFI, high-frequency flow interruption.

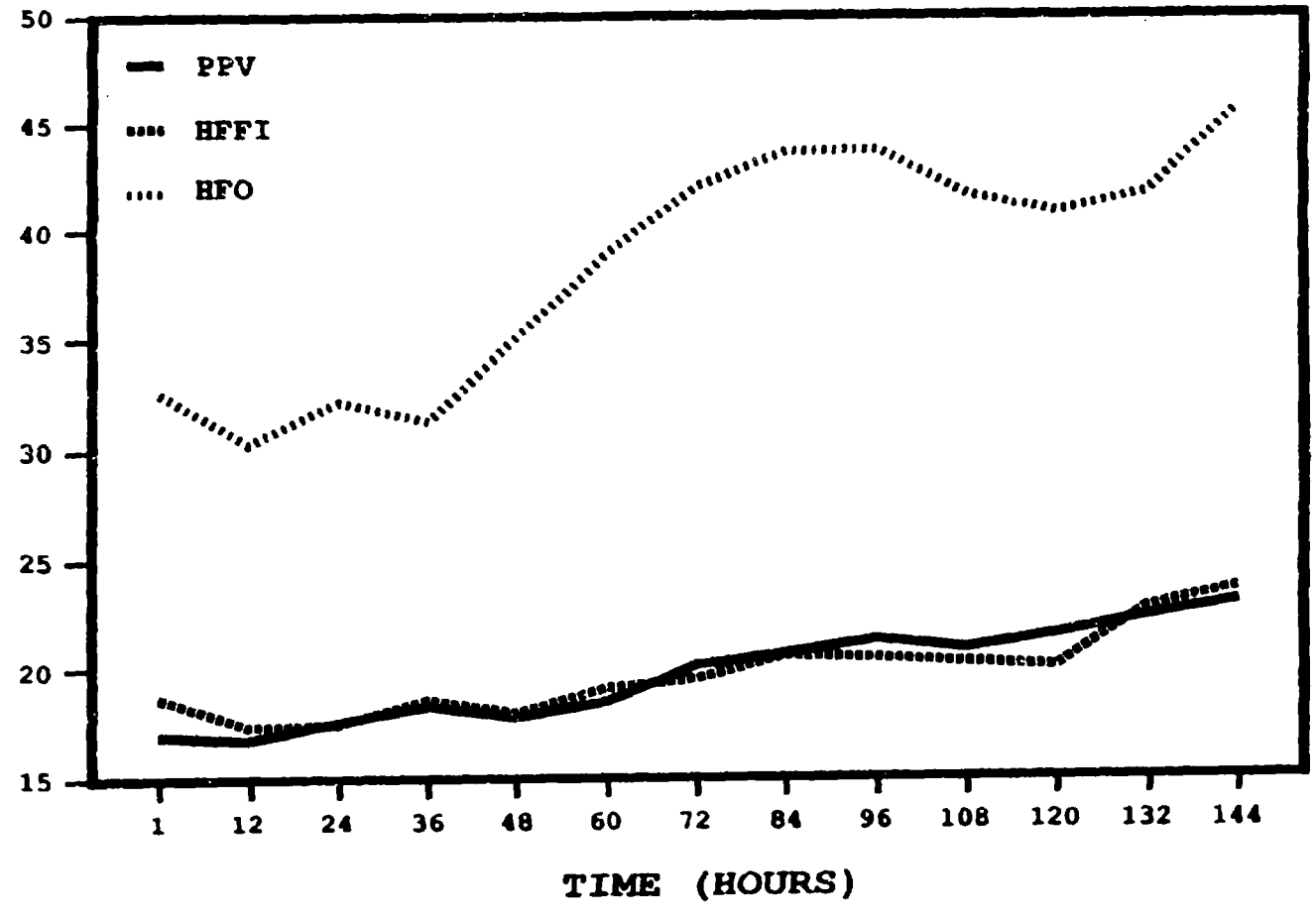

FIGURE 8. Peak airway pressures (cmH_2O). PPV indicates positive-pressure ventilation; HFFI, high-frequency flow interruption; and HFO, high-frequency oscillatory ventilation.

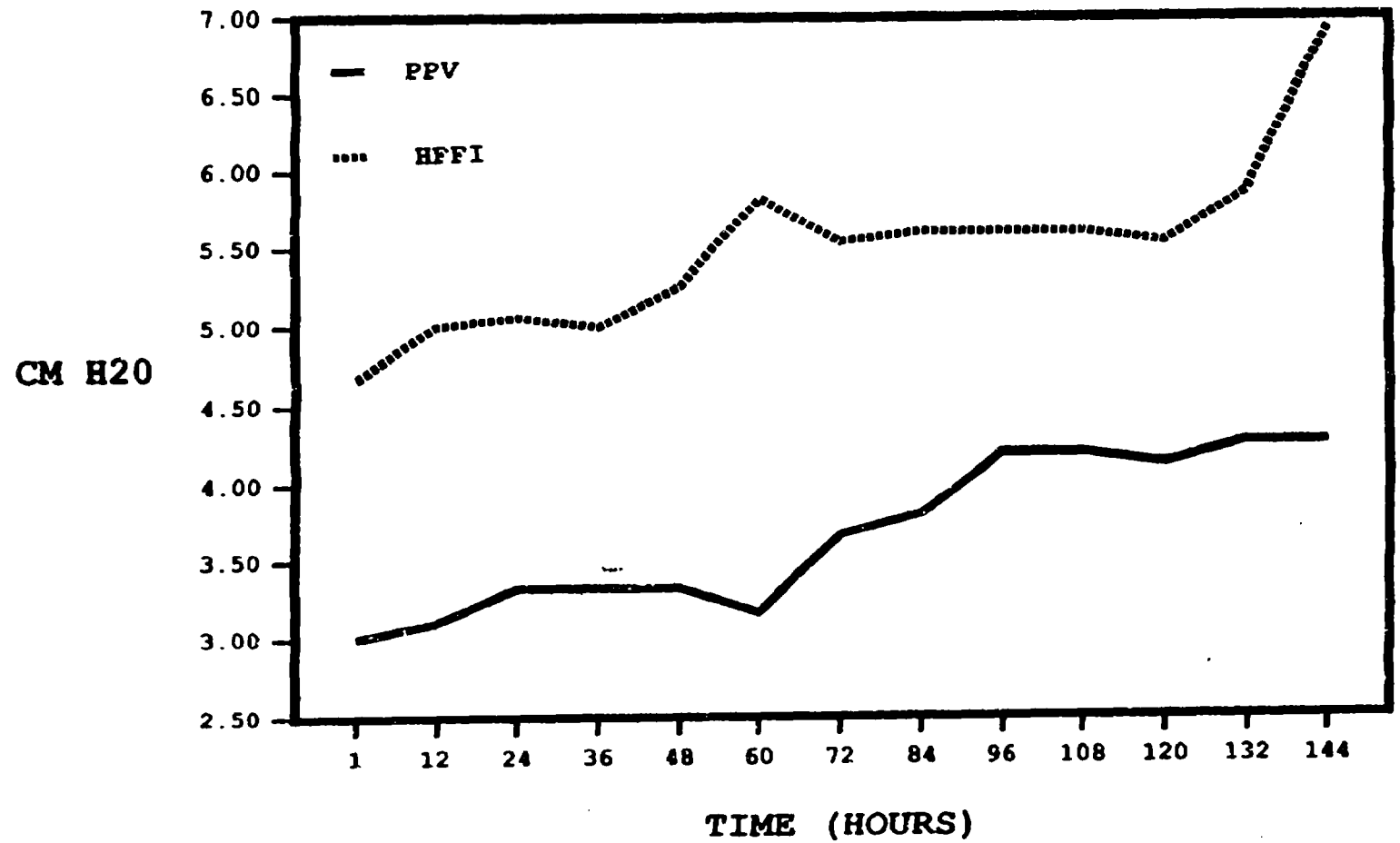

FIGURE 9. Positive-end expiratory pressure (cmH_2O). PPV indicates positive-pressure ventilation, and HFFI, high-frequency flow interruption.

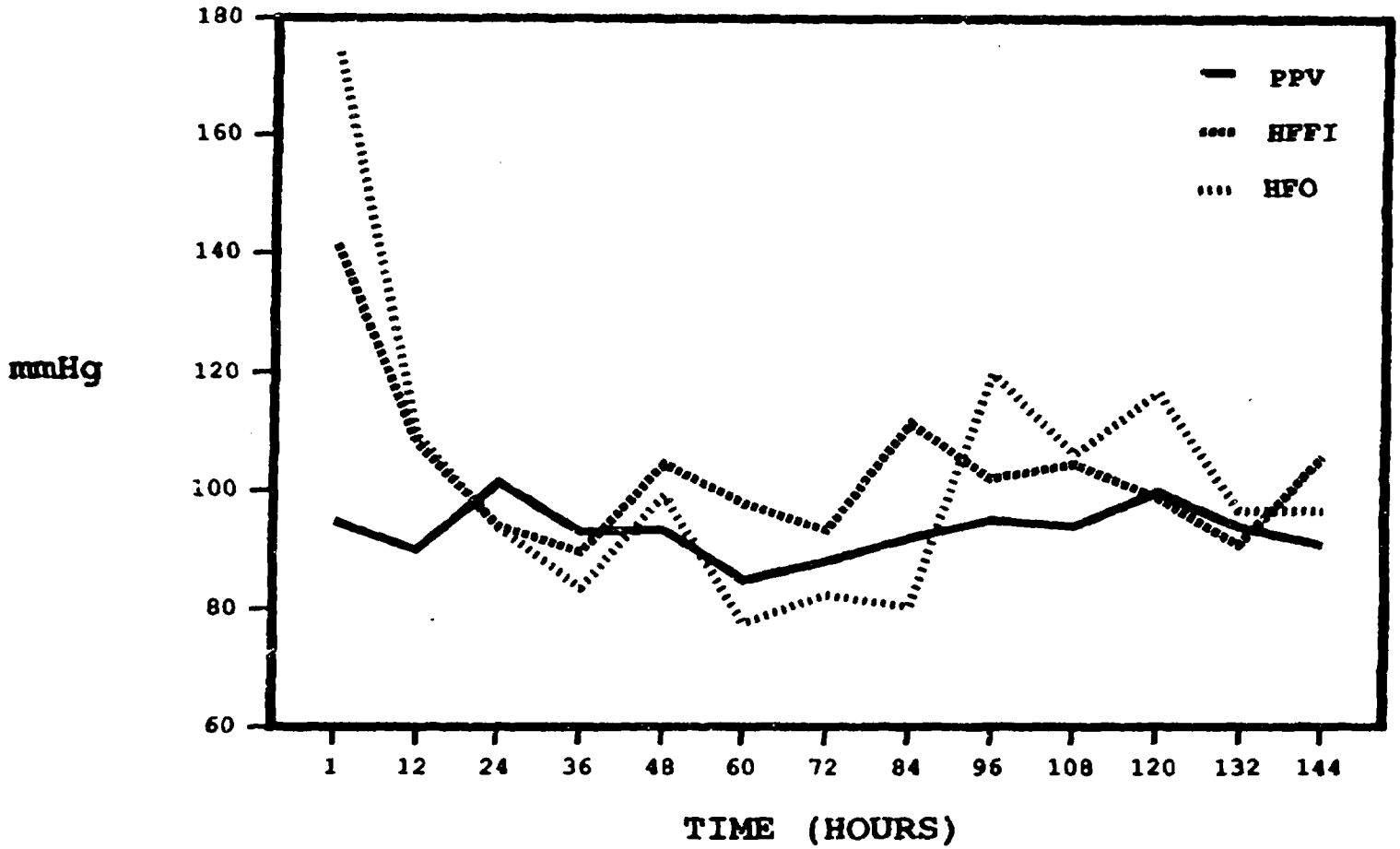

FIGURE 10. PaO_2 (mmHg). PPV indicates positive-pressure ventilation; HFFI, high-frequency flow interruption; and HFO, high-frequency oscillatory ventilation.

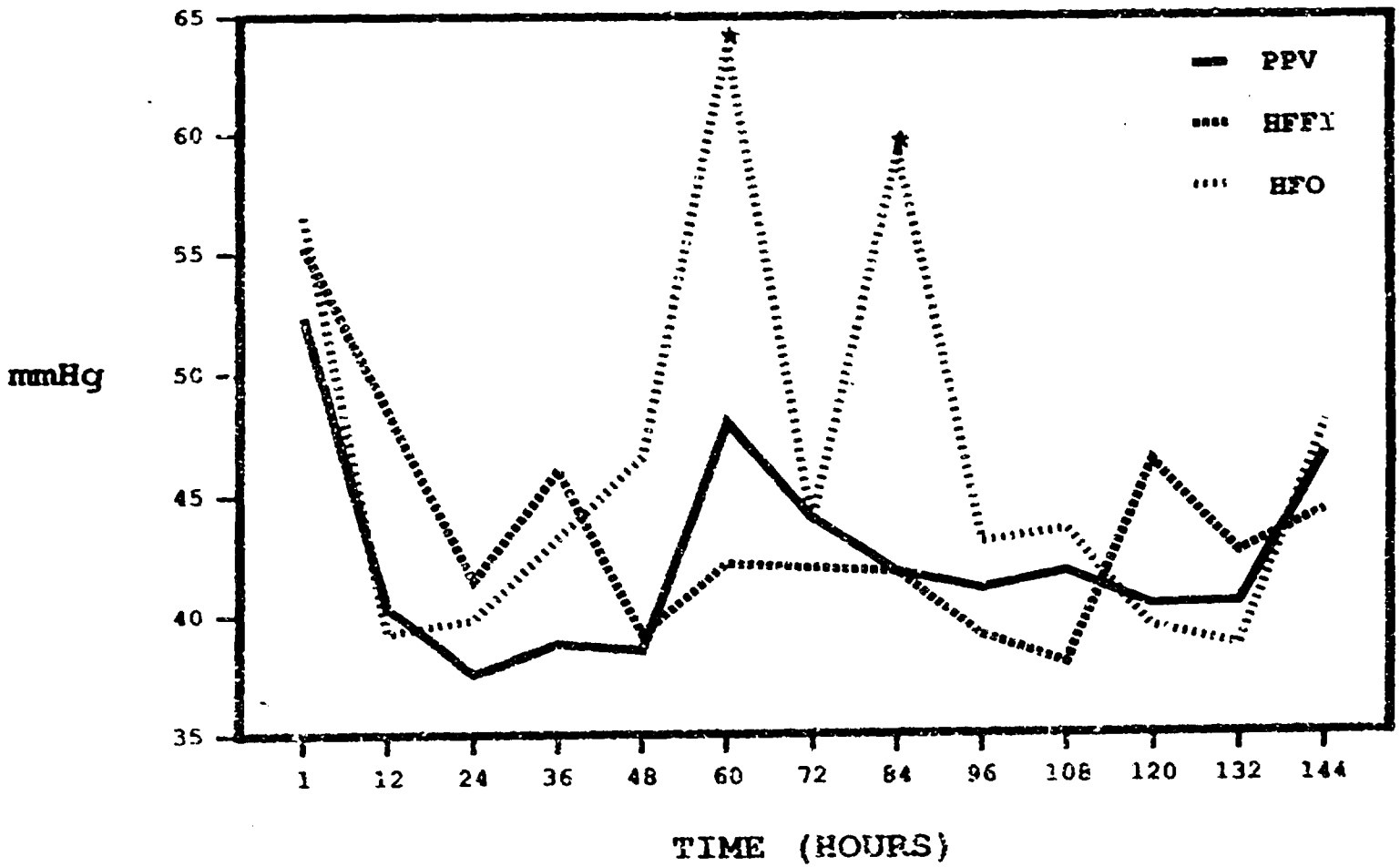

FIGURE 11. $PaCO_2$ (mmHg). PPV indicates positive-pressure ventilation; HFFI, high-frequency flow interruption; and HFO, high-frequency oscillatory ventilation. *P < 0.05 vs PPV and HFFI by ANOVA.

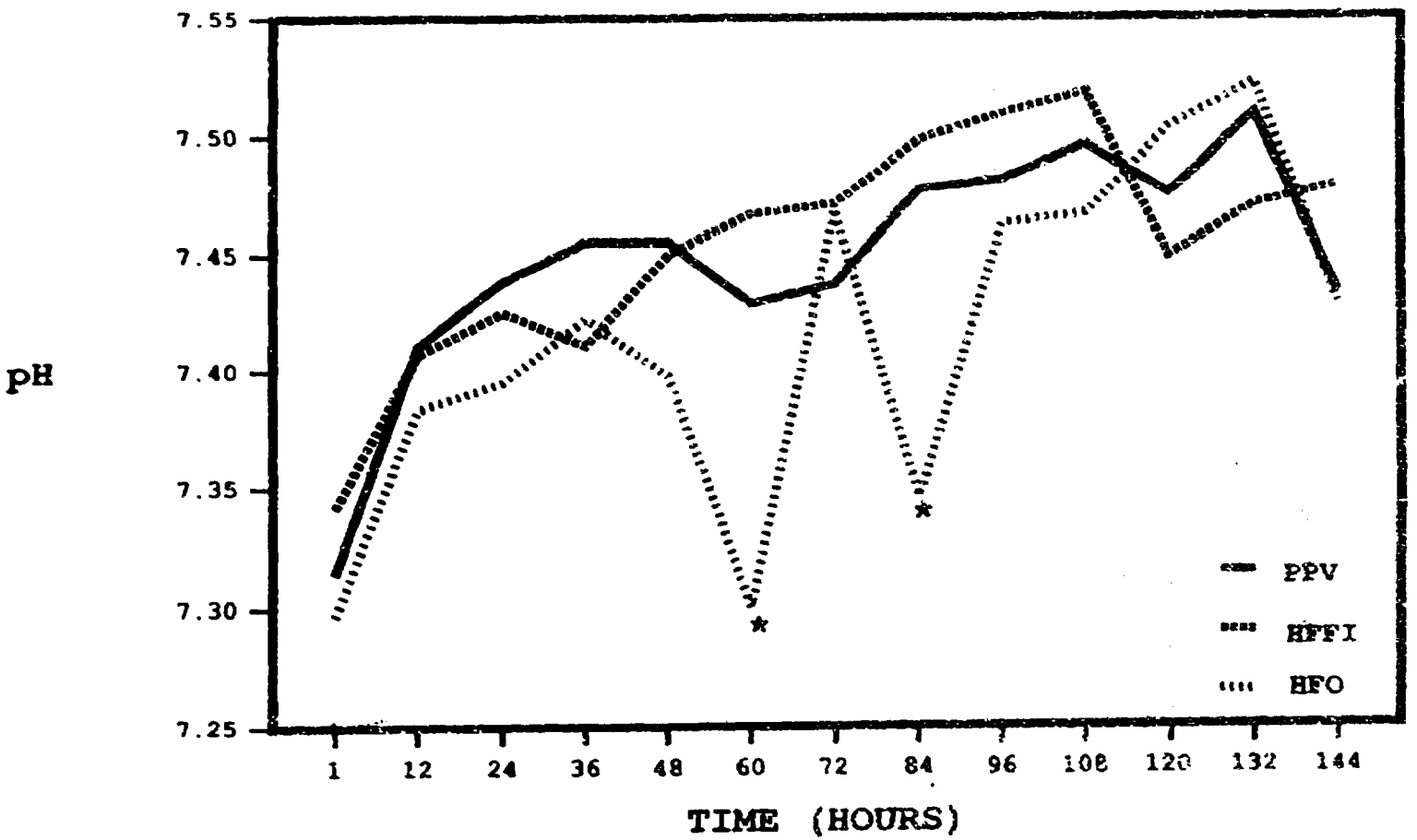

FIGURE 12. pH. PPV indicates positive-pressure ventilation; HFFI, high-frequency flow interruption; and HFO, high-frequency oscillatory ventilation.

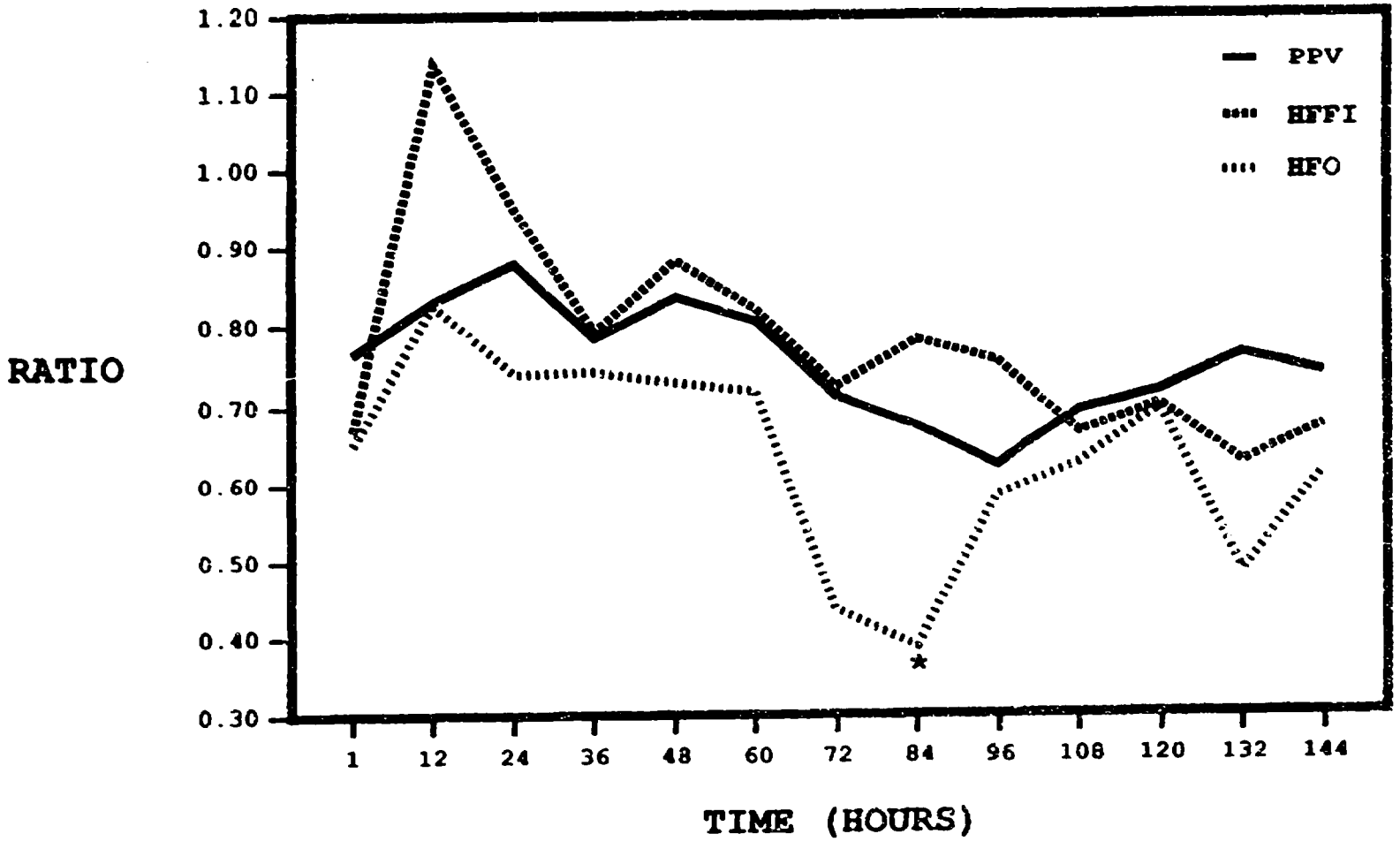

FIGURE 13. Alveolar-arterial O_2 ratio. PPV indicates positive-pressure ventilation; HFFI. high-frequency flow interruption; and HFO, high-frequency oscillatory ventilation. *P < 0.05 vs PPV and HFFI by ANOVA.

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

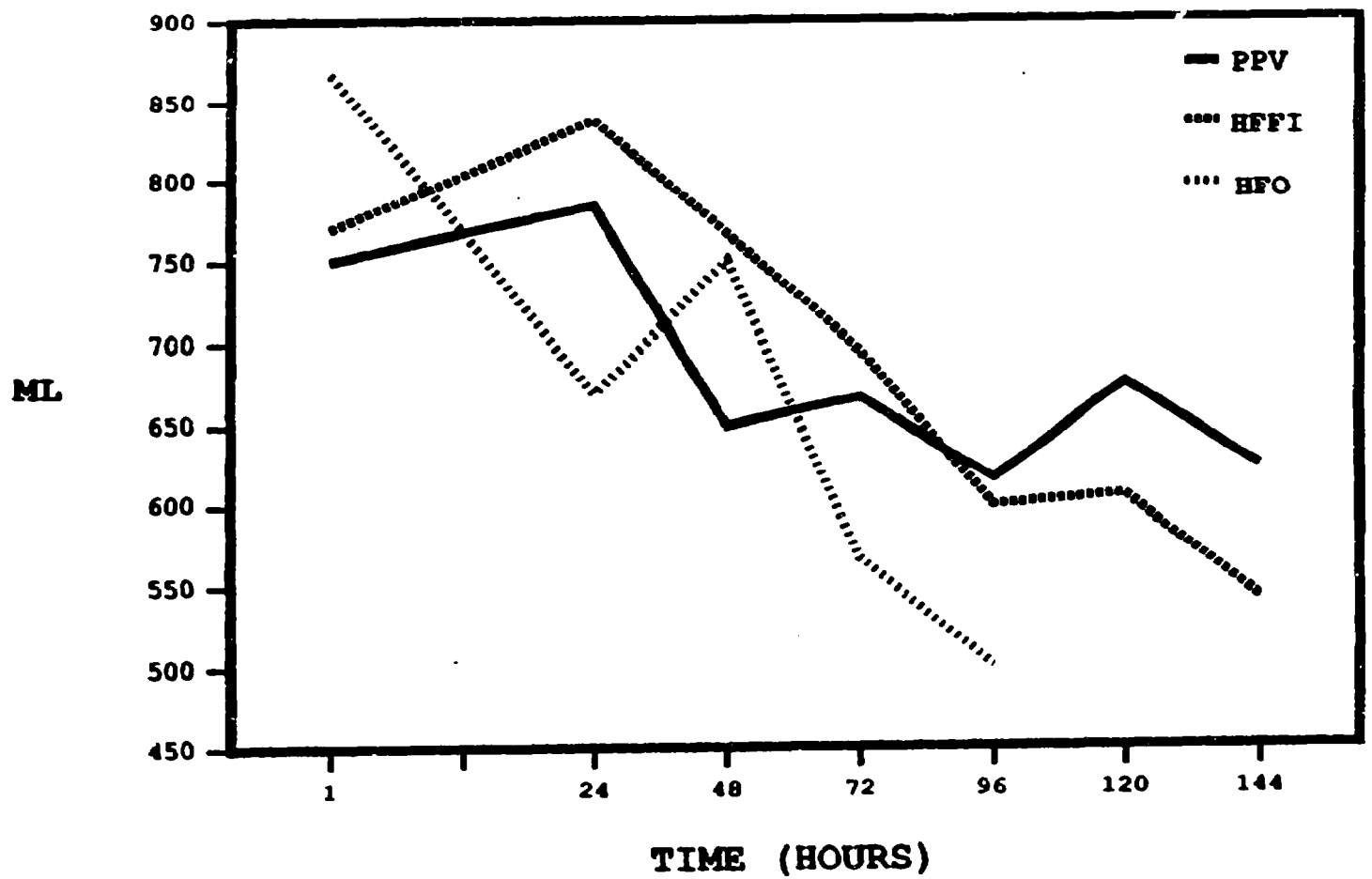

FIGURE 14. Vital capacity (ml). PPV indicates positive-pressure ventilation; HFFI, high-frequency flow interruption; and HFO, high-frequency oscillatory ventilation.

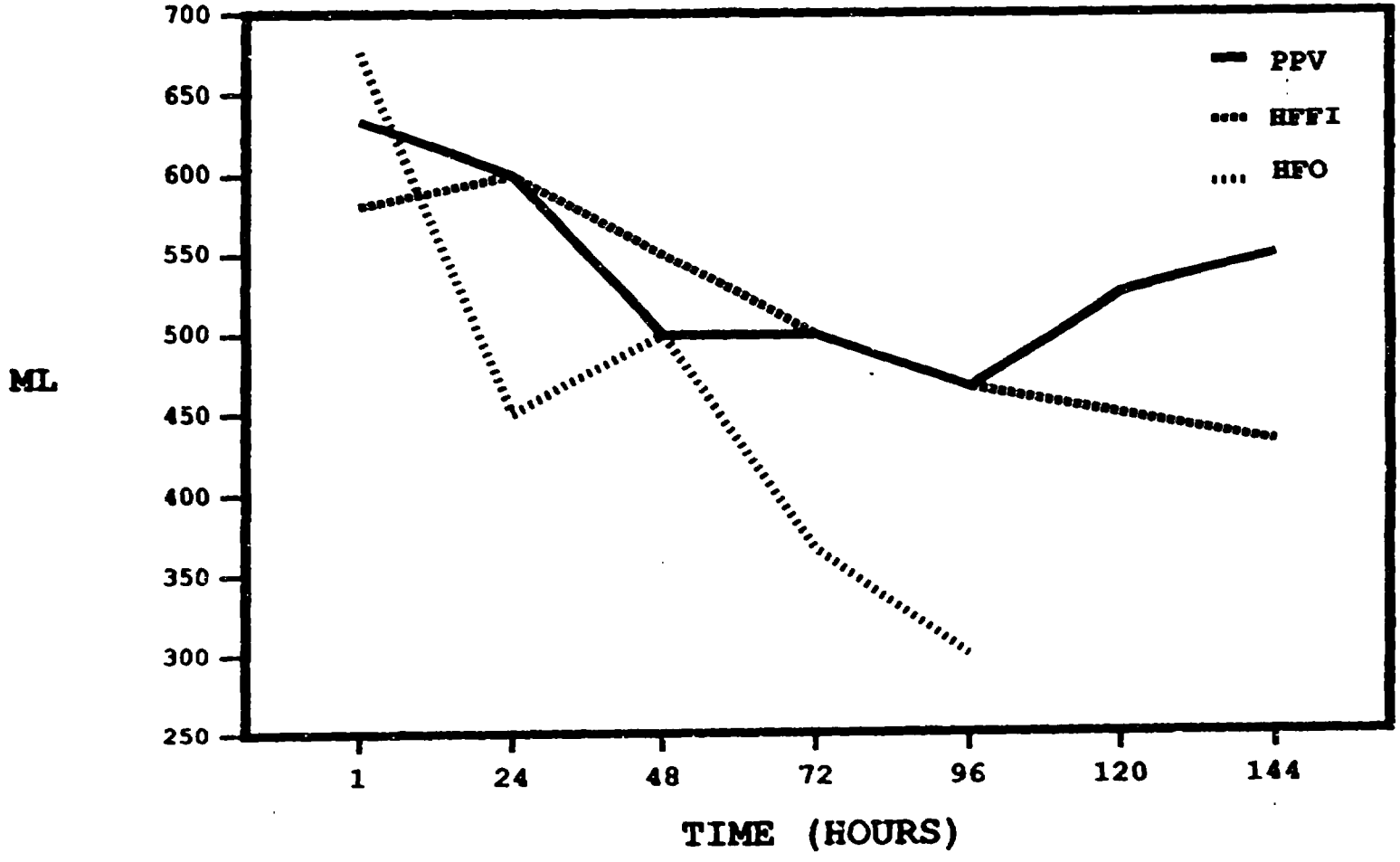

FIGURE 15. Inspiratory capacity (ml). PPV indicates positive-pressure ventilation; HFFI, high-frequency flow interruption; and HFO, high-frequency oscillatory ventilation.

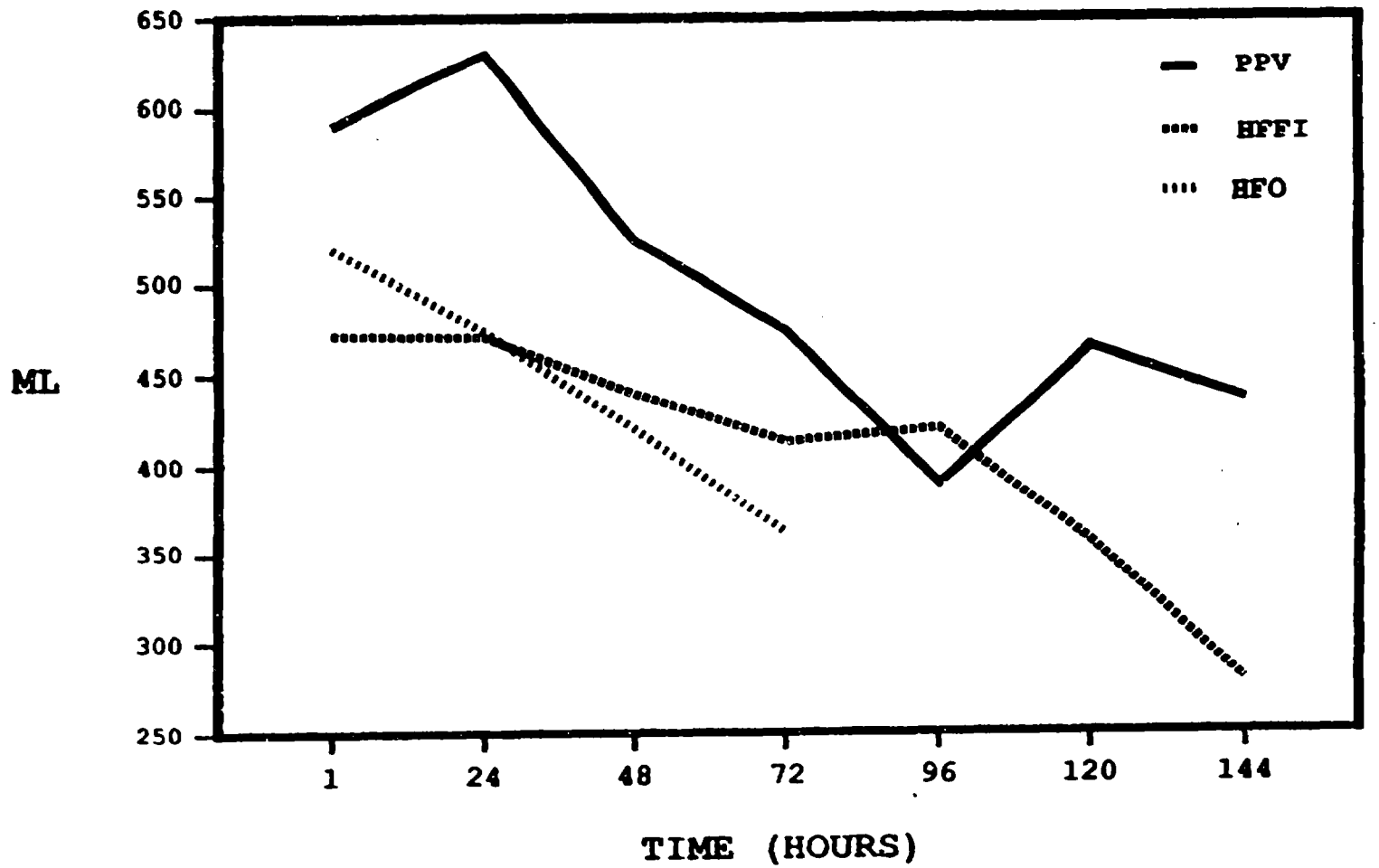

FIGURE 16. Functional residual capacity (ml). PPV indicates positive-pressure ventilation; HFFI, high-frequency flow interruption; and HFO, high-frequency oscillatory ventilation.

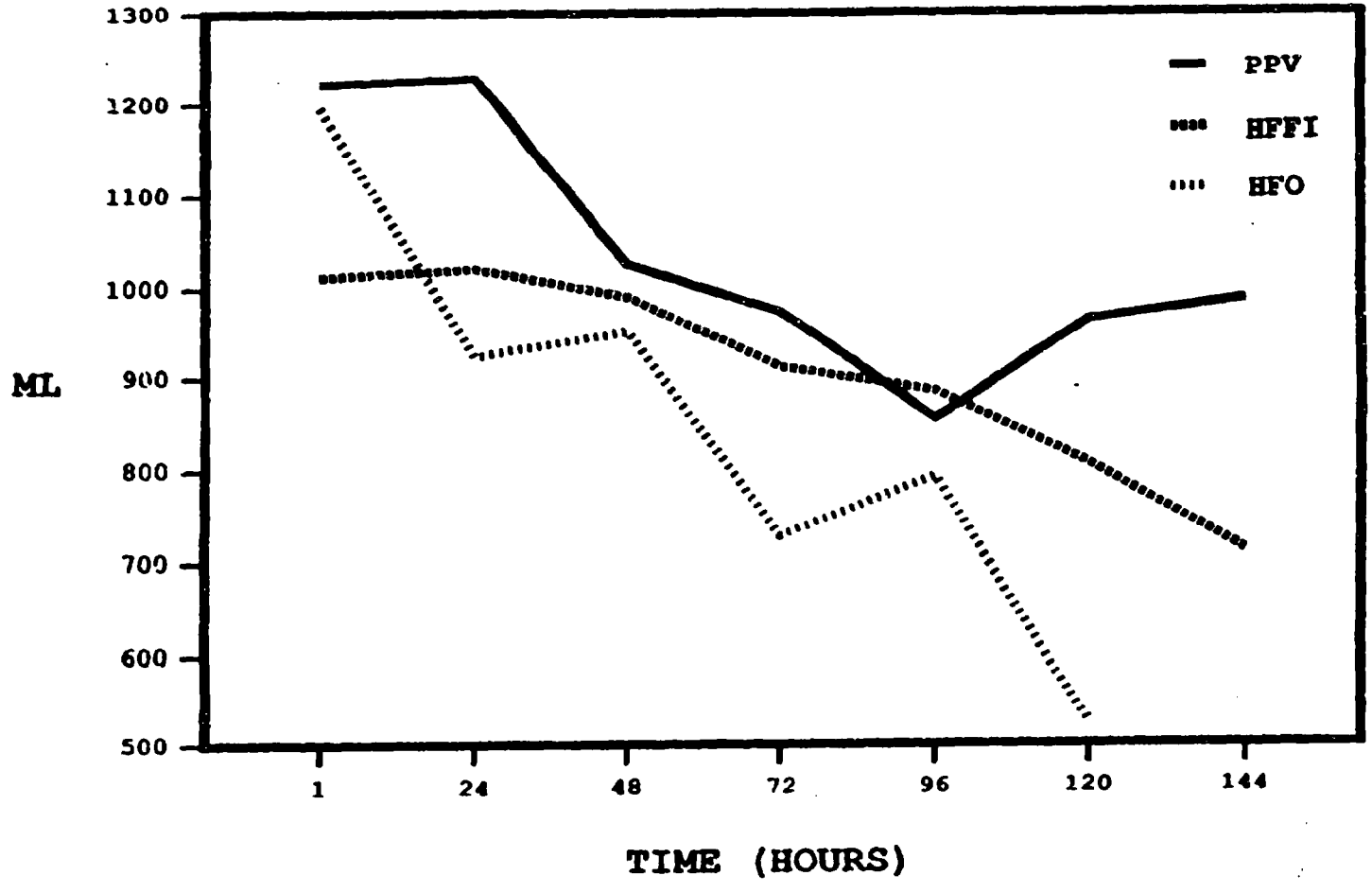

FIGURE 17. Total lung capacity (ml). PPV indicates positive-pressure ventilation; HFFI, high-frequency flow interruption; and HFO, high-frequency oscillatory ventilation.

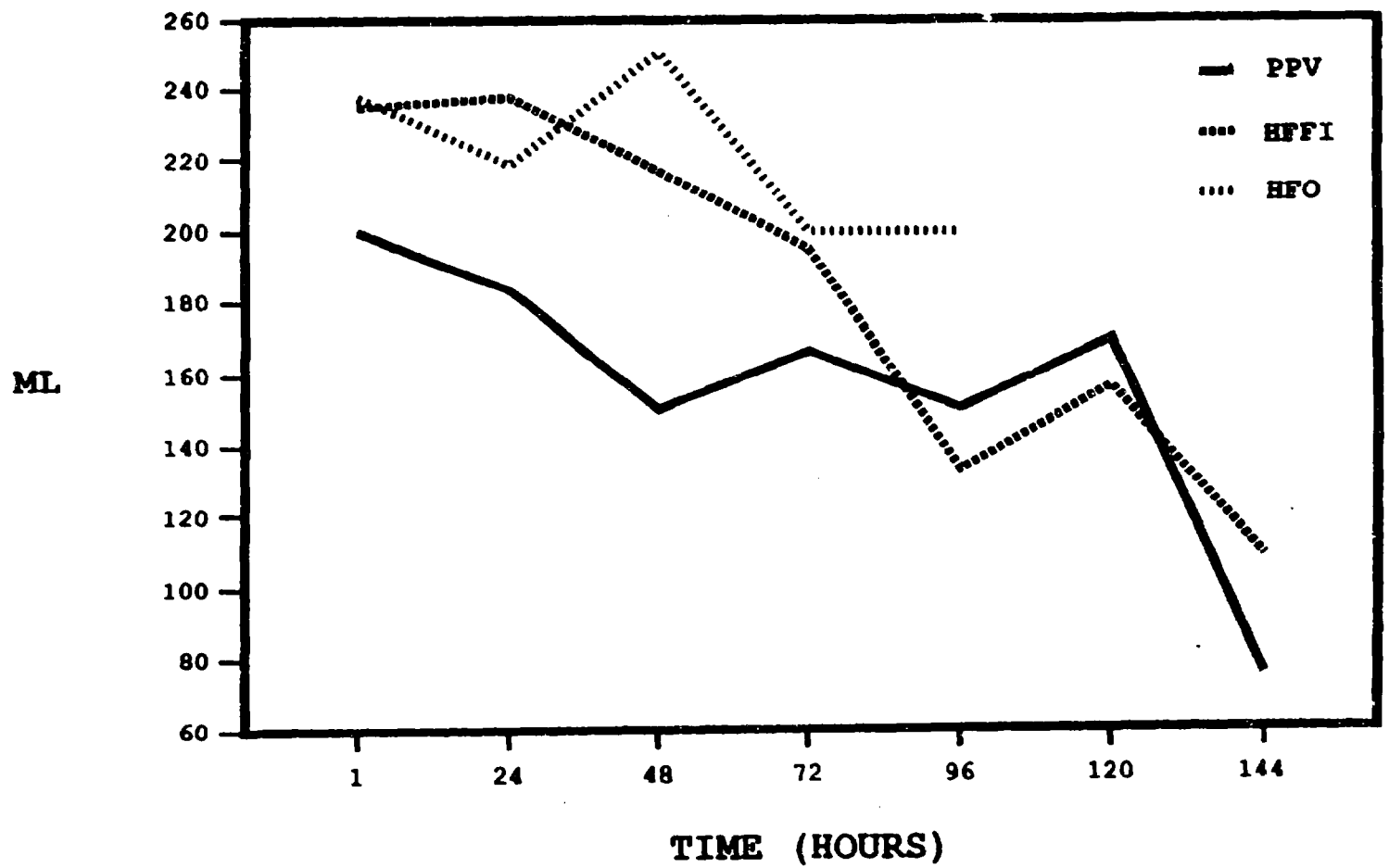

FIGURE 18. Effective residual volume (ml). PPV indicates positive-pressure ventilation; HFFI, high-frequency flow interruption; and HFO, high-frequency oscillatory ventilation.

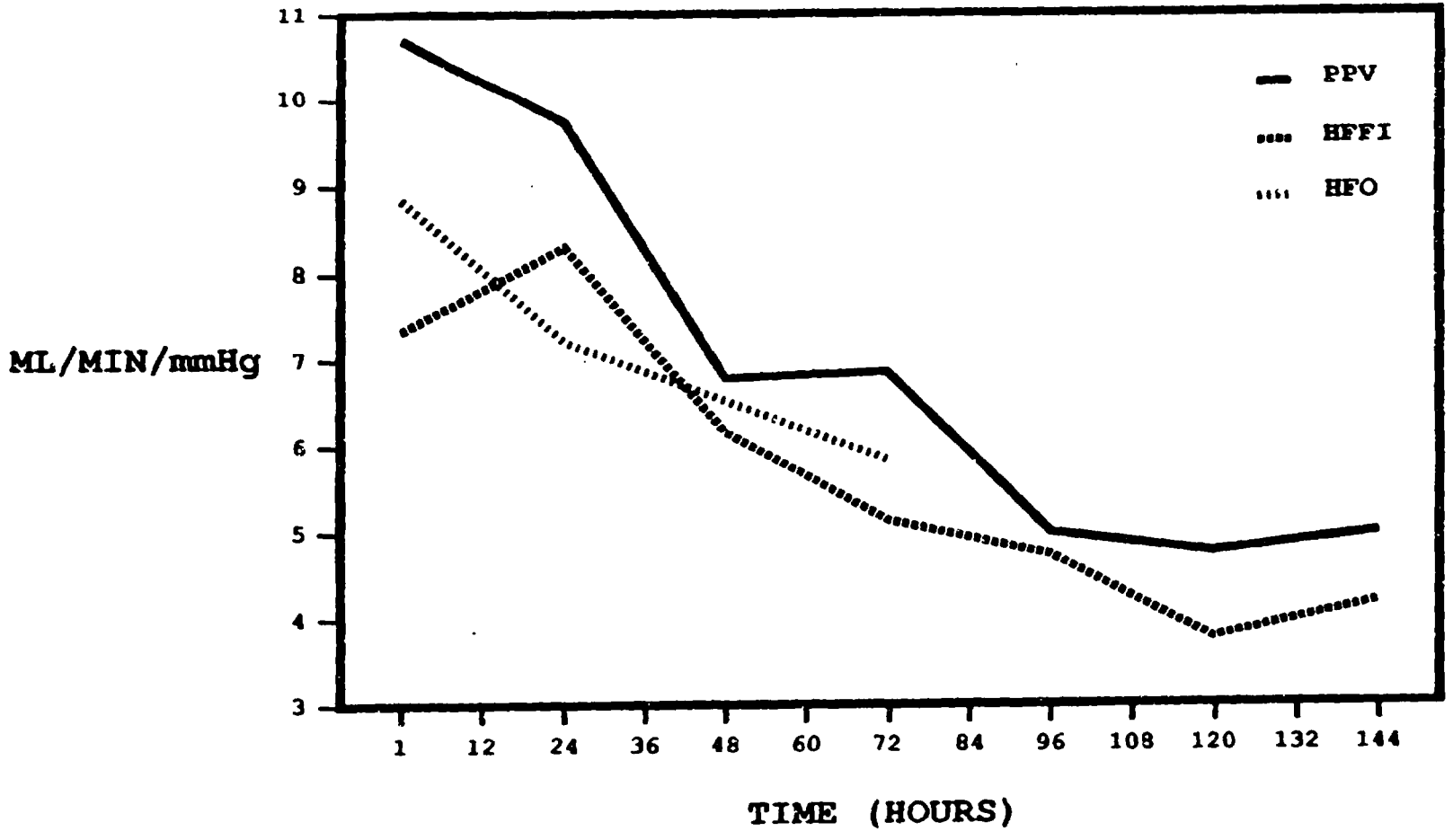

FIGURE 19. Diffusing capacity (ml/min/mmHg). PPV indicates positive-pressure ventilation; HFFI, high-frequency flow interruption; and HFO, high-frequency oscillatory ventilation.

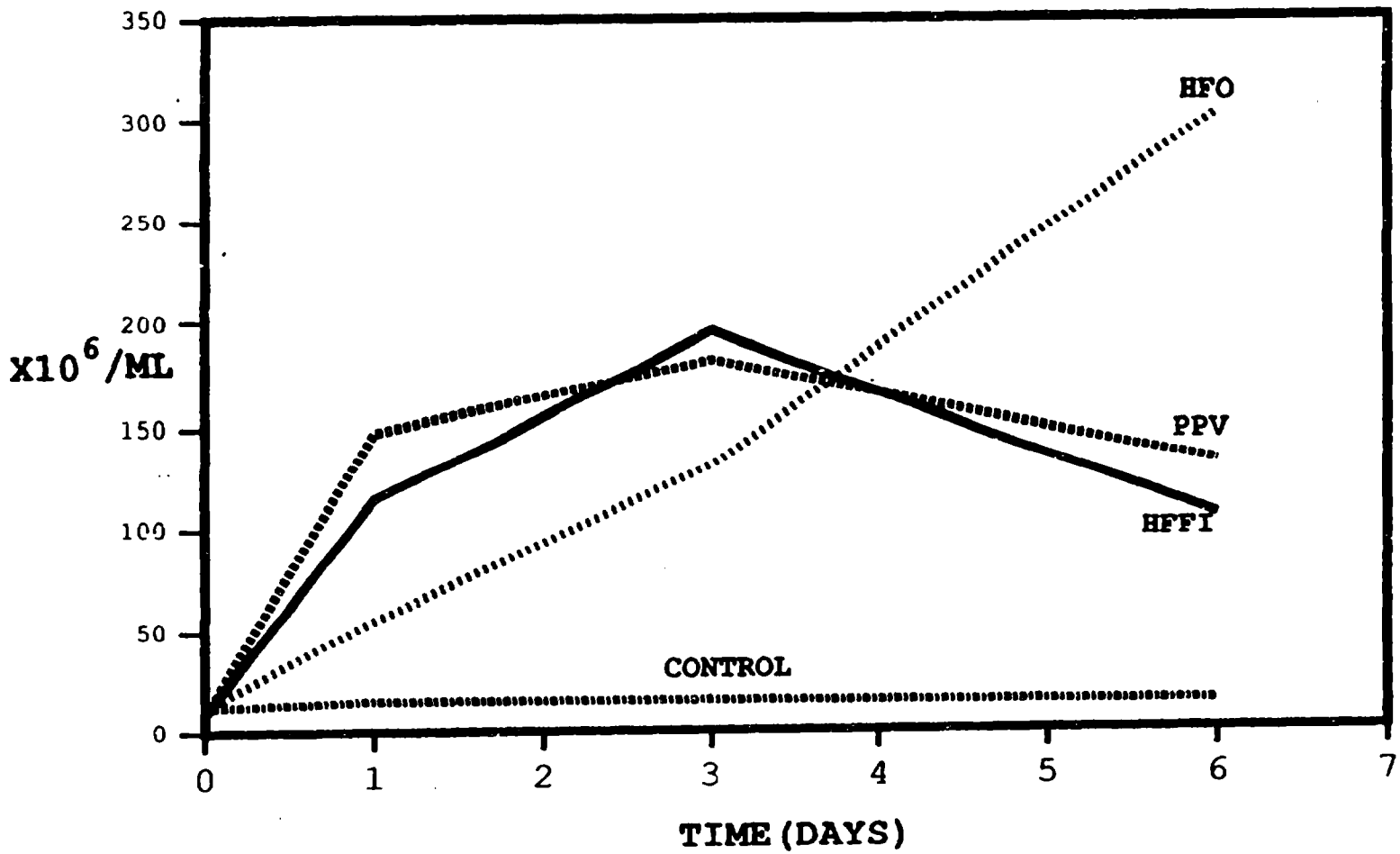

FIGURE 20. Bronchoalveolar lavage WBC count (10^6/ml). PPV indicates positive-pressure ventilation; HFFI, high-frequency flow interruption; and HFO, high-frequency oscillatory ventilation.

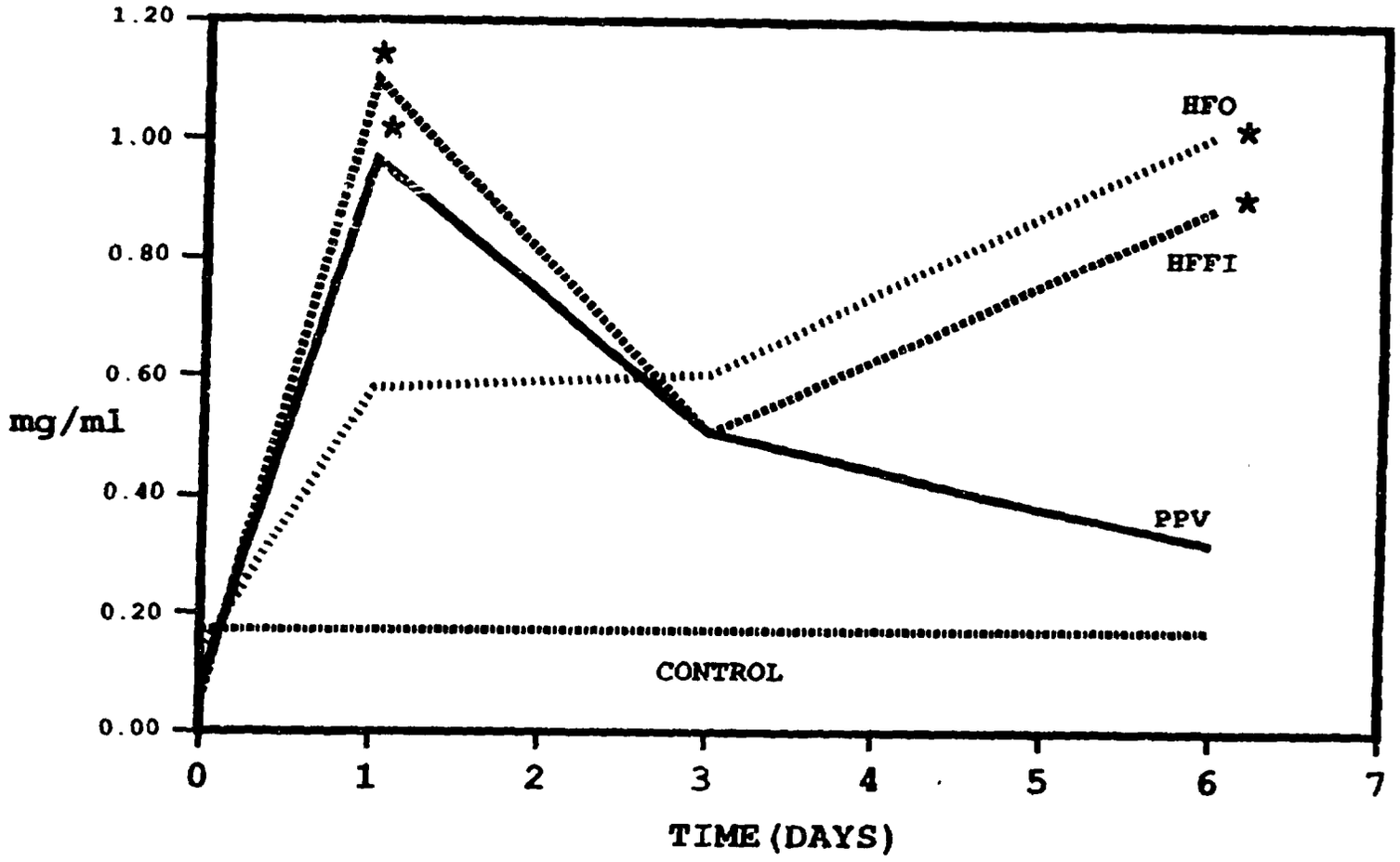

FIGURE 21. Bronchoalveolar lavage protein (mg/ml). PPV indicates positive-pressure ventilation; HFFI, high-frequency flow interruption; and HFO, high-frequency oscillatory ventilation.

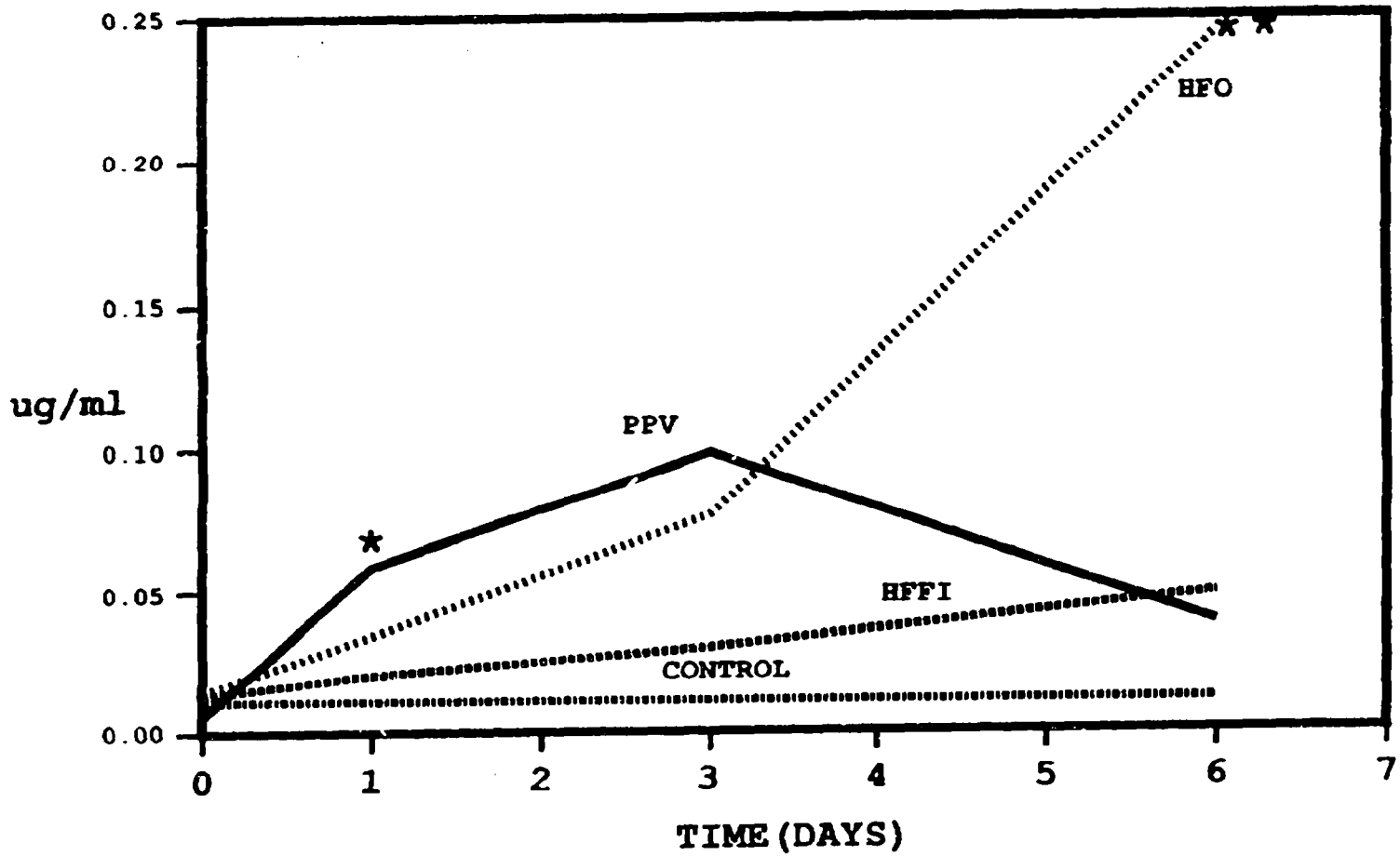

FIGURE 22. Bronchoalveolar lavage elastase (µg/ml). PPV indicates positive-pressure ventilation; HFFI, high-frequency flow interruption; and HFO, high-frequency oscillatory ventilation.

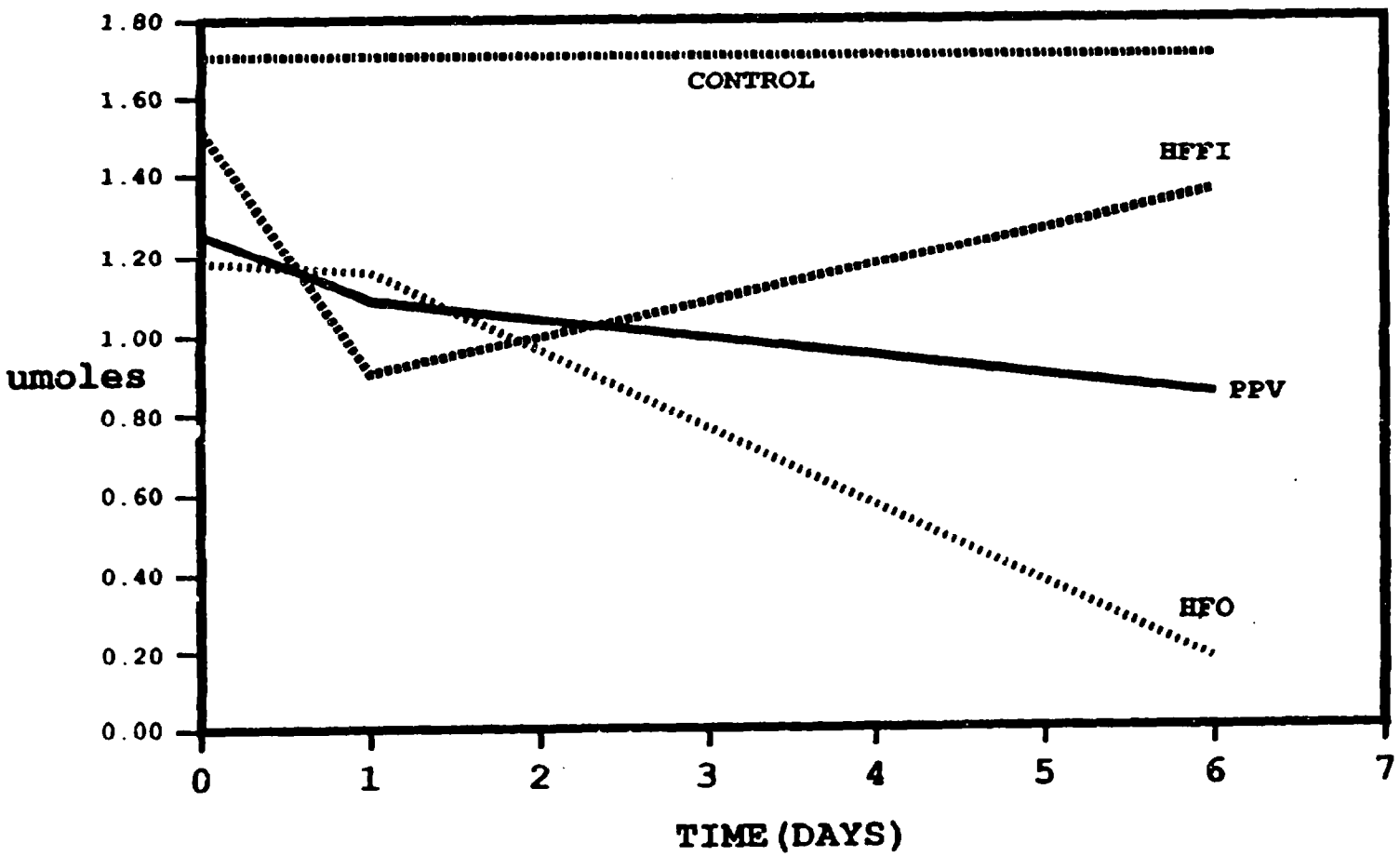

FIGURE 23. Bronchoalveolar lavage surfactant (μmoles). PPV indicates positive-pressure ventilation; HFFI, high-frequency flow interruption; and HFO, high-frequency oscillatory ventilation.

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

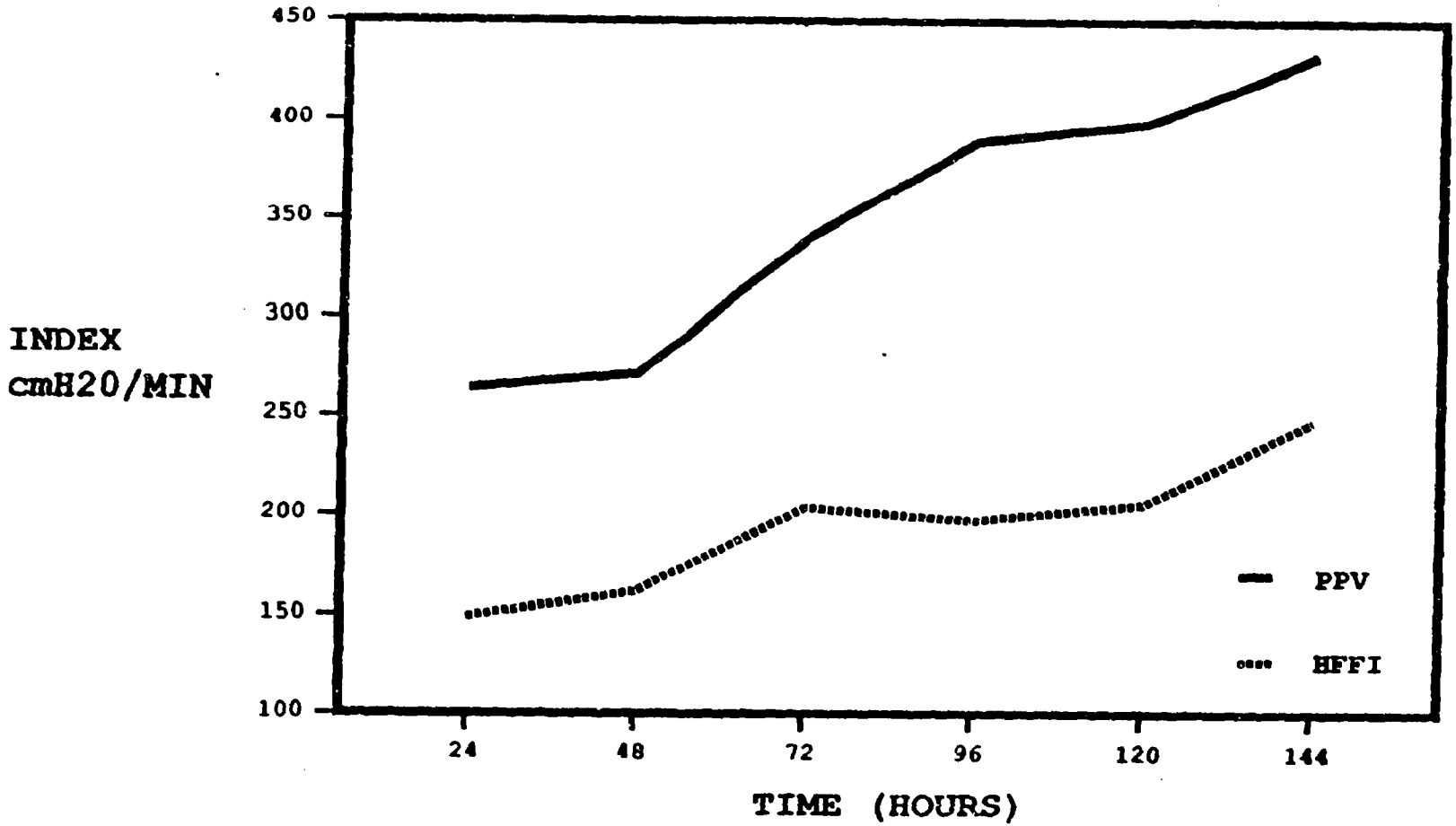

FIGURE 24. Barotrauma index (cmH_2O). PPV indicates positive-pressure ventilation, and HFFI, high-frequency flow interruption.

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.

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None.

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