US ARMY INSTITUTE OF SURGICAL RESEARCH

ANNUAL RESEARCH PROGRESS REPORT

FOR FISCAL YEAR 1989

1 OCTOBER 1988 - 30 SEPTEMBER 1989

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FORT SAM HOUSTON
SAN ANTONIO, TEXAS 78234-5012

PREPARED FOR: US ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
FORT DETRICK
FREDERICK, MARYLAND 21702-5012

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

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

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DEPARTMENT OF THE ARMY
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FORT SAM HOUSTON, TEXAS 78234-5012

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ATTENTION OF

SGRD-USZ (70-9a) 1 October 1989

MEMORANDUM FOR SEE DISTRIBUTION

SUBJECT: US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1989

The *US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1989* is forwarded under the provisions of OTSG Regulation 70-31 dated 2 April 1969.

Basil A. Pruitt Jr.

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

FOREWORD

The effectiveness of this Institute's integration of burn patient care and research is once again confirmed by this annual volume of research reports. In addition to conducting clinical and laboratory research, the Institute responds to emergencies, both civilian and military, both foreign and domestic, to provide on-site care and effect mortality-free aeromedical transfer of severely burned patients. During Fiscal Year 1989, the "real world" benefits of this clinical/laboratory symbiosis have been specifically documented.

On 20 March 1989, a helicopter carrying participants in Operation Team Spirit '89 crashed in South Korea. Nineteen marines were killed outright and 13 sustained burns. Within 24 hours, the Institute dispatched a burn disaster response team consisting of two surgeons, two surgical residents, two nurses, six 91C clinical specialists, and two respiratory therapy technicians. To fulfill the Institute's teaching mission, two military residents were included in the team. Upon arrival in Korea, the team evaluated the patients, effected necessary modifications in therapy, and prepared the patients for aeromedical transfer to San Antonio. The number and type of mission personnel were predicated upon the need to form two burn treatment teams to provide care in each of the two C-141 aircraft needed for the return flight, because 10 of the 13 patients required mechanical ventilation. In-flight monitoring and management ensured the stability and safety of the 13 patients, who were admitted to the burn center on their third postburn day. All 13 patients, having an average burn size of 27% of the total body surface area and, in 10, associated inhalation injury, survived. Seven were returned to active duty.

Less than three months later, early on the morning of 4 June 1989, at a remote rural site west of the Ural Mountains in Bashkiria, USSR, an explosion of leaking natural gas destroyed two passing trains, killing 308 passengers and burning more than 805. The medical facilities at Ufa, Russia, to which many of the burn patients were transferred, were rapidly overwhelmed. Recognition of the disparity between health care needs and clinical resources prompted the Russian government, on 8 June 1989, to accept President Bush's offer to provide medical assistance in the form of a disaster burn care team from this Institute. In less than 24 hours, a 17-member team of surgeons, nurses, respiratory therapists, a microbiologist, and a microbiology technician were dispatched. That team was joined en route by three translators and two administrative officers. Seven tons of equipment and supplies, augmented by ventilators with Russian-compatible power systems provided by the 7th US Army Medical Command, accompanied the team and permitted it to operate without placing additional demands on the already strained local resources. Both clinical and laboratory personnel of the disaster burn care team were readily accepted by

their Russian analogues and quickly integrated into the local medical system's disaster response. In the next four weeks, the team members, in collaboration with Russian health care personnel to whom they taught state-of-the-art techniques of burn care, carried out wound care procedures on 50 patients, performed excision of the burn wounds of 26, and even performed a colon resection for malignant disease.

The rapid responses of the Institute to these two emergencies were made possible by its extensive experience in the aeromedical transfer of burn patients gained in previous military and civilian burn disasters. An earlier national survey of burn disaster health care needs conducted by Institute personnel guided the composition of the two disaster response teams, the clinical components of which were constituted to permit formation of three emergency burn care teams, each comprised of a burn surgeon, a registered burn nurse, two or three 91C clinical specialists, and a respiratory therapist. That staffing was augmented for the Korean mission by the inclusion of two surgical trainees, and for the Russian mission by the addition of an anesthesiologist experienced in burn care, a physical therapist, an occupational therapy technician, and a scrub nurse to address the longer term needs of wound closure and rehabilitation. Clinical expertise gained in caring for large numbers of seriously burned patients each year provided a sound basis for decision about equipment and supplies needed to permit in-flight and on-site operations, respectively, without impediment by local resource limitations. The burn care provided during each mission represented the clinical application of research findings reported in previous editions of this report. Inclusion in the Russian disaster team of microbiology personnel recognized the inseparable amalgam of burn patient care and research and enabled data to be generated that guided antibiotic therapy and identified the epidemiologic characteristics of burn patients treated in a limited resource environment.

The work of both disaster response teams in meeting specific and urgent burn care needs resulted in optimum patient outcome and the Russian experience also identified problems characteristic of a mass casualty, limited resource environment that have already stimulated specific equipment development projects and influenced the planning for equipment, supply, and personnel support. This Institute's response to a military accident in Korea and its Presidentially directed response to the major burn disaster in the USSR confirm the field applicability of the Institute's clinical and research activities and document the importance of maintaining indigenous clinical expertise and the unique military-relevant

global readiness that results from the harmonious union of clinical care and research.

Basil A. Pruitt, Jr.

BASIL A. PRUITT, JR., MD, FACS
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24. (U) Thermally injured patients from the Continental United States and throughout the world are transported to this Institute for intensive, specialized treatment. Carefully controlled evaluations of new treatment technics are conducted by professional staff.

25. (U) 8801 - 8812. Two hundred and twenty seriously burned patients were admitted and treated at this Institute during calendar year 1988. Current clinical research activities include studies of host resistance studies, endocrine changes following injury, development of nutritional support of the burned patient, the use of skin substitutes, and studies on the control of postinjury infection. For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1989.*

ANNUAL RESEARCH PROGRESS REPORT

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

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

1 January 1988 - 31 December 1988

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ABSTRACT

PROJECT NUMBER: 3S162787A874-00, APPLIED RESEARCH

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

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

PERIOD COVERED IN THIS REPORT: 1 Jan 88 through 31 Dec 88

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Two hundred and twenty patients were admitted to this Institute during calendar year 1988. Principal activities included care of severely burned patients, research to improve survival and function of such patients, and education and training of health care professionals and paraprofessionals. Areas of research included an ongoing study of 5% aqueous mafenide acetate soaks for the topical treatment of burn wounds following grafting, studies of neuroendocrine abnormalities in burn injuries, a clinical evaluation of the use of high frequency ventilation in patients with inhalation injury, quantification of dynamic splint forces on metacarpophalangeal function recovery, evaluation of in vitro cultivated keratinocytes as epithelial autografts for the closure of burn wounds, evaluation of imipenem-cilastatin sodium for prophylactic activity against bacterial pneumonias in burned patients with inhalation injury, a clinical study of the safety and efficacy of ceftazidime in the parenteral therapy of infections in hospitalized burn patients, evaluation of serum visceral protein levels as indicators of nitrogen balance, a study of medium-chain

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

CLINICAL OPERATION
CENTER FOR TREATMENT OF BURNED SOLDIERS

During calendar year 1988, 220 patients were admitted to this Institute and there were 220 patient dispositions during the same period. Statistical data are based on the 220 patient dispositions during calendar year 1988. The average total burn size of the entire population was 22.7% of the total body surface area, with an 11.2% average extent of full-thickness injury. The average hospital stay of all patients, excluding convalescent leave for active duty military patients, was 40.6 days.

During calendar year 1988, 924 operative procedures were performed on 161 patients, an average of 5.7 operative procedures per patient. Four hundred and sixty-seven anesthetics were given to 161 patients for an average of 2.9 anesthetics per patient.

ADMISSION DATA

The Clinical Division of this Institute admitted 220 soldiers and other authorized patients with thermal, chemical, or electric injury during calendar year 1988. Aeromedical teams from the Institute conducted 69 missions to transfer 87 (39.5%) of the admitted patients. Sixty-four missions were within the continental United States and 5 were to areas overseas. Twenty-one missions were carried out by rotary-wing aircraft (30.4%) and 48 by fixed-wing aircraft (69.5%). One hundred and three of the 220 patients (46.8%) were admitted within 24 h of injury and 140 (63.6%) were admitted within 48 h of injury. One hundred and seventy-seven patients were male and 43 were female.

DISPOSITION DATA

The following statistics are based on 220 patient dispositions during calendar year 1988. The ages of these patients ranged from 3 months to 91 yr, with an average age of 29.0 yr. Burn sizes averaged 22.7% of the total body surface area, with an average full-thickness component of 11.2%. Forty-four patients were in the pediatric age group (< 15 yr), with an average age of 5.3 yr and an average burn size of 20.4% of the total body surface area. The average hospital stay of all dispositions was 42.9 days when convalescent leave for active duty military patients was included in the calculation and 40.6 days when convalescent leave was excluded. There were 13 patients with high voltage electric injury and 16 patients with chemical injury. The sources of admission are identified in Table 1 and the causes of burn injury are detailed in Table 2.

TABLE 1. Sources of Admission (1988)

AREA	A	AD	AF	AFD	N/MC	ND	VAB	OTHER	TOTAL
First Army	3	1	1	0	0	0	0	0	**5**
Third Army	4	4	3	1	2	1	3	0	**18**
Fifth Army	14	22	10	6	3	1	6	76	**138**
Sixth Army	1	2	2	0	8	0	3	0	**16**
Alaska	0	0	2	0	0	0	0	0	**2**
Cuba	0	0	0	0	4	0	0	0	**4**
Germany	4	0	2	2	3	0	0	1	**12**
Hawaii	1	1	0	0	0	0	0	1	**3**
Honduras	0	0	1	0	0	0	0	1	**2**
Kurt Island	0	0	0	0	0	0	0	1	**1**
Korea	2	0	1	0	0	0	0	1	**5**
Marcus Island	0	0	0	0	1	0	0	0	**1**
Mexico	0	0	0	0	0	0	0	1	**1**
Okinawa	0	0	1	0	0	0	0	0	**1**
Panama	2	3	0	0	1	0	0	1	**7**
Philippines	0	0	1	1	0	0	0	0	**2**
Puerto Rico	1	0	0	0	0	0	0	0	**1**
Thailand	1	0	0	1	0	0	0	0	**1**
TOTAL	**33**	**33**	**24**	**10**	**23**	**2**	**12**	**83**	**220**

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

TABLE 2. Burn Etiology (1988)

Causes	Number of Patients	Disposition (%)	Deaths	Mortality (%)
Hot liquids	46	20.9	-	-
Gasoline, diesel, and kerosene	29	13.2	2	11.1
Open flames	25	11.4	-	-
Motor vehicle accidents	23	10.5	3	16.7
Butane, propane, or natural/sewer gas explosions	20	9.1	1	5.6
Structural fires	17	7.7	7	38.9
Chemical	16	7.3	-	-
Aircraft accidents	15	6.8	4	22.2
Electrical	13	5.9	-	-
Smoking, clothes ignition	9	4.1	1	5.6
Welding	4	1.8	-	-
Bomb, shell, simulator grenade, and gunpowder explosions	3	1.4	-	-
TOTAL	**220**	**100.0**	**18**	**8.2**

Two patients required hemodialysis for acute renal failure. Acute myocardial infarction was seen in 1 patient. Inhalation injury was identified in 45 patients (20.5% of admissions). Seventy-eight patients (35.5%) had some associated injury (includes 45 patients with inhalation injury) which included fractures of dislocations in 14 patients, lacerations in 15 patients, and head injuries in 2 patients.

Morbidity and Mortality. Eighteen of the 220 dispositions (8.2%) died during calendar year 1988. Autopsies were performed in 7 (38.9%) of these hospital deaths. The average burn size of patients who died was 57.9% of the total body surface area and the full-thickness average was 45.2% of the total body surface area. Age ranged from 3 months to 91 yr. Ten of these patients (55.6%) had inhalation injury as a primary or contributing cause of death. Fifteen patients (83.3%) had burn injuries exceeding 30% of the total body surface area, and 5 patients (27.8%) had burn injuries exceeding 80% of the total body surface area. Two of the 18 deaths (11.1%) occurred in pediatric patients. These children had an average total body surface area burn of 70.0% and an average full-thickness burn of 69.5%. The average age of children who died was 3.5 yr. One of these children had an autopsy.

Infection was once again the most common complication following thermal injury, with 40 bacterial pneumonias occurring in 34 patients. The most common organisms isolated in patients with bacterial pneumonia was *Staphylococcus aureus* in 27 patients, Klebsiella species in 10 patients, and *Escherichia coli* in 6 patients. However, only 5 patients demonstrated septicemia and 1 patient had bacterial invasion of the burn wound.

Table 3 lists the effect of age and extent of injury on survival and Table 4 lists mortality rates associated with increments of 10% of the total body surface area for the years 1985-8. Table 5 summarizes the survival of patients with extensive burns from 1963-88. 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 1988.

EDUCATIONAL ACTIVITIES

During calendar year 1988, the professional staff of the Clinical Division continued to provide education to professional an paraprofessioral groups at the local, national, and international levels. A total of 29 resident physicians were attached for periods of 1-2 months, including 8 from Wilford Hall USAF Medical Center, 5 from the University of Texas Health Science Center (San Antonio TX), 4 from Travis Air Force Base Medical Center, 3 from Pensacola Naval Air Station, 2 from Letterman Army Medical Center, and 1 each from William Beaumont Hospital (Royal Oak MI), Providence Hospital (Southfield MI), Butterworth Hospital (Grand Rapids MI), Brooke Army Medical Center, Fitzsimons Army Medical

TABLE 3. Age, Body Surface Involvement, and Mortality (1988)

Age (Yr)	Total Body Surface Area Burn (%)										Cases	Deaths	Mortality (%)
	0-10	11-20	21-30	31-40	41-50	51-60	61-70	71-80	81-90	91-100			
0 - 1	4	-	-	1	-	-	-	-	-	-	5	-	-
1 - 2	4	1	2	-	1	-	-	-	-	-	8	-	-
2 - 3	2	2	2	1	-	-	-	-	-	-	7	-	-
3 - 4	1	3	-	-	1	1	-	-	-	-	6	1	16.7
4 - 5	1	-	-	-	-	-	-	-	1	-	2	1	50.0
5 - 10	3	4	1	-	1	-	-	-	-	-	9	-	-
10 - 15	2	3	1	-	-	-	-	1	-	-	7	-	-
15 - 20	4	4	2	1	2	2	-	1	1	-	17	-	-
20 - 30	22	19	6	8	5	1	3	2	1	2	69	3	4.3
30 - 40	17	5	4	1	5	2	-	-	1	-	35	3	8.6
40 - 50	6	5	1	4	-	2	2	1	-	-	21	3	14.3
50 - 60	5	2	2	2	-	-	1	-	1	-	13	4	30.8
60 - 70	7	4	-	3	1	-	-	1	-	-	16	2	12.5
70 - 80	-	2	-	-	-	-	-	-	-	-	2	-	-
80 - 90	2	-	-	1	1	-	-	-	-	-	2	-	-
90 - 100	-	1	-	-	-	-	-	-	-	-	1	1	100.0
Total Cases	80	55	21	21	16	8	6	6	5	2	220		
Total 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	100.0			8.2

TABLE 4. Total Body Surface Area Burn Involvement (%) and Mortality (1985-8)

	0-10	11-20	21-30	31-40	41-50	51-60	61-70	71-80	81-90	91-100	Total
1988											
Number of Patients	80	55	21	21	16	8	6	6	5	2	220
Number of Deaths	-	2	1	2	2	2	3	1	3	2	18
Mortality (%)	-	3.6	4.8	9.5	12.5	25.0	50.0	16.7	60.0	100.00	8.2
1987											
Number of Patients	67	52	36	20	19	12	10	-	2	3	221
Number of Deaths	-	-	1	2	3	4	6	-	2	3	21
Mortality (%)	-	-	2.8	10.0	15.8	33.3	60.0	-	100.0	100.00	9.5
1986											
Number of Patients	61	40	32	21	19	7	11	7	4	5	207
Number of Deaths	1	2	2	2	5	2	4	2	4	5	29
Mortality (%)	1.6	5.0	6.3	9.5	26.3	28.6	36.4	28.6	100.0	100.00	14.0
1985											
Number of Patients	41	46	28	28	19	11	9	6	5	4	197
Number of Deaths	2	3	5	3	7	3	5	5	5	4	42
Mortality (%)	4.9	6.5	17.9	10.7	36.8	27.3	55.6	83.3	100.0	100.00	21.3

TABLE 5. Survival and Nonsurvival by Year for Patients with Burns > 30% of the Total Body Surface Area (1963–88)

Year	SURVIVORS			NONSURVIVORS		
	Number of Cases	Average Burn (%) Total	3°	Number of Cases	Average Burn (%) Total	3°
1988	56	40.9	20.6	16	58.8	46.4
1987	46	43.7	17.2	21	63.0	44.9
1986	178	21.8	7.3	29	59.8	41.4
1985	48	43.6	21.7	42	54.3	37.1
1984	43	46.4	24.8	32	59.5	38.7
1983	37	43.5	17.5	30	62.8	50.7
1982	53	43.7	24.8	54	53.9	38.3
1981	54	42.7	17.5	43	62.2	39.8
1980	62	42.7	15.1	66	64.3	41.8
1979	61	45.4	13.4	74	65.0	37.0
1978	67	45.7	14.8	69	55.2	33.0
1977	66	42.2	14.4	70	56.9	29.0
1976	69	45.5	15.0	79	64.2	31.1
1975	80	46.1	14.7	94	61.3	32.8
1974	55	43.9	12.2	97	60.8	35.9
1973	47	43.7	19.6	113	60.3	36.2
1972	62	42.0	17.2	103	56.7	35.9
1971	63	41.9	140	68	60.8	38.0
1970	92	39.4	10.7	70	51.9	32.6
1969	113	43.2	11.1	70	58.7	26.4
1968	143	44.2	12.6	38	54.6	24.6
1967	103	42.7	13.3	51	59.9	32.3
1966	68	41.5	14.9	59	59.9	31.3
1965	47	43.8	21.0	33	66.0	33.4
1964	40	41.8	14.8	37	65.0	42.4
1963	28	45.8	19.6	57	69.0	41.0

TABLE 6. Comparison of Burn Mortality Rates (1962-3 and 1964-88)

	TOTAL BODY SURFACE AREA BURN (%)														
	0-30			31-40			41-50			51-60			61-100		
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
1964-87	3,329	123	3.7	879	159	18.1	701	215	30.6	494	232	46.9	903	743	82.3
1988	156	3	1.9	21	3	1.4	16	1	6.3	10	2	20.0	17	9	52.9

TABLE 7. Causes of Death (1988)

Patient	Age	Sex	BURN SIZE (%) Total	3°	Postburn Day	Cause of Death
1	20	M	46	100	1	*100% total body surface area burn with resuscitation failure.
2	23	M	94	94	1	*94% total body surface area burn with inhalation injury.
3	24	M	89	89	5	*89% total body surface area burn with inhalation injury.
4	4	M	87	87	38	*87% total body surface area burn with inhalation injury and pneumonia.
5	52	F	81	67	8	*81% total body surface area burn with inhalation injury and pneumonia.
6	63	M	71	19	17	71% total body surface area burn with inhalation injury and herpes virus pneumonia.
7	59	M	68	59	6	68% total body surface area burn with inhalation injury.
8	59	M	65	62	115	*65% total body surface area burn with acute inhalation injury and pneumonia.
9	35	M	65	65	17	65% total body surface area burn with multiorgan failure secondary to pneumonia and septicemia.

TABLE 7 (Continued)

Patient	Age	Sex	BURN SIZE (%) Total	3°	Postburn Day	Cause of Death
10	40	M	59	15	8	*59% total body surface area burn with inhalation injury.
11	3	M	53	52	21	53% total body surface area burn with acute inhalation injury and bronchopnuemonia.
12	66	F	47	32	73	*47% total body surface area burn with severe arteriosclerosis and massive cerebrovascular accident.
13	29	F	47	15	51	*47% total body surface area burn with bacterial endocarditis and systemic sepsis.
14	53	M	36	28	21	36% total body surface area burn with hepatic failure and liver cirrhosis.
15	41	M	31	16	11	*31% total body surface area burn with acute myocardial infarction.
16	32	M	21	20	35	*21% total body surface area burn with closed head injury.
17	48	M	20	-	29	20% total body surface area burn with inhalation injury and herpes virus pneumonia.
18	91	F	12	4	96	13% total body surface area burn with hypertrophic cardiomyopathy and bronchopneumonia.

*Autopsy not performed.

Center, William Beaumont Army Medical Center, and Mt. Clemons Hospital (Mt. Clemons MI). Five interns from Brooke Army Medical Center (San Antonio TX) rotated through the Institute. A total of 7 medical students rotated through the Institute, including 3 students from the Uniformed Services University of Health Sciences and 1 each from Tufts University, the University of Texas (San Antonio TX), the University of Houston, and the Texas College of Osteopathic Medicine. In addition, 1 critical care fellow from Brooke Army Medical Center rotated through the Institute. A total of 35 physicians visited from foreign countries for periods ranging from 1 day to 3 wk, which included 5 each from Japan and Pakistan, 4 each from Germany, Saudi Arabia, and Korea, 3 from Canada, 2 each from Australia, the Philippines, and Norway, and 1 each from Peru, Sweden, China, and France. The Respiratory Therapy Branch had 213 trainees, the Physical Therapy Branch had 58 trainees, and the Occupational Therapy Branch had 110 trainees. Twenty-three scientific publications appeared in refereed medical journals and approximately 194 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.

PRESENTATIONS

Carlson DE: Nutritional care of the critically ill. Presented to the Brooke Army Medical Center Dietetic Interns, Fort Sam Houston, San Antonio, Texas, 15 January 1988.

Zelenka JP: Occupational therapy in burn care. Presented to the Occupational Therapy Course (91L), Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 20 January 1988.

Waymack JP: The effect of blood transfusions on resistance to infectious complications. Presented at the Symposium on Biologic Effect of Blood Transfusions on Immune Function, Snowbird, Utah, 21 January 1988.

Duncan DJ: Standards of nursing care for the large burn patient in the initial 48 hours. Presented to the OT/PT Management of Burns in the Theater of Operations Course, Fort Sam Houston, San Antonio, Texas, 1-5 February 1988.

Hollan E: The importance of infection control. Presented to the OT/PT Management of Burns in the Theater of Operations Course, Fort Sam Houston, San Antonio, Texas, 1-5 February 1988.

Jordan BS: Management of pain. Presented to the OT/PT Management of Burns in the Theater of Operations Course, Fort Sam Houston, San Antonio, Texas, 1-5 February 1988.

Latona PS: Functioning in an ICU environment. Presented to the OT/PT Management of Burns in the Theater of Operations Course, Fort Sam Houston, San Antonio, Texas, 1-5 February 1988.

Maly DW: Transport of the burn patient. Presented to the OT/PT Management of Burns in the Theater of Operations Course, Fort Sam Houston, San Antonio, Texas, 1-5 February 1988.

Pruitt BA Jr: Burn injury as a military problem: epidemiology and triage. Presented to the OT/PT Management of Burns in the Theater of Operations Course, Fort Sam Houston, San Antonio, Texas, 1-5 February 1988.

Summers TM: Psychosocial complications of burn injuries. Presented to the OT/PT Management of Burns in the Theater of Operations Course, Fort Sam Houston, San Antonio, Texas, 1-5 February 1988.

Pruitt BA Jr: Diagnosis and treatment of opportunistic infections in surgical patients. Presented to the Cincinnati Surgical Society, Cincinnati, Ohio, 2-3 February 1988.

Pruitt BA Jr: Current therapy of burns. Presented to the Officer Advanced Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 5 February 1988.

Molter NC: USAISR nursing service. Presented at the Commander's Conference, US Army Research and Development Command, Fort Sam Houston, San Antonio, Texas, 10 February 1988.

Gutierrez RT: Thermal injuries: Physical therapy management. Presented to the Physical Therapy Specialists Course (91J), Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 10 February 1988.

Summers TM: Families in crisis. Presented to the Critical Care Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 11 February 1988.

Kennan JR: Initial management of burn patients. Presented to the Nursing Service Branch, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 11 February 1988.

Jordan BS: Review of current research at USAISR. Presented to the OT/PT Management of Burns in Theater of Operations Course, Fort Sam Houston, San Antonio, Texas, 12 February 1988.

Luster SH: Rehabilitation procedures in burn care. Presented to the Occupational and Physical Therapy Section, St. Rose Rehabilitation Center, San Antonio, Texas, 16 February 1988.

Pruitt BA Jr: Current management of the burn patient. Presented to the Department of Surgery, University of Tennessee Medical Center, Knoxville, Tennessee, 16-17 February 1988.

Luster SH: Rehabilitation of acute burn patients. Presented to the Occupational and Physical Therapy Section, St. Rose Rehabilitation Center, San Antonio, Texas, 17 February 1988.

Beverly ED: Wear and apparel of the military uniform. Presented at the Noncommissioned Officer Development Program, Fort Sam Houston, San Antonio, Texas, 18 February 1988.

Pruitt BA Jr: Opportunistic infections in severely injured patients. Presented to the Department of Surgery, University of Texas Health Science Center, Dallas, Texas, 20 February 1988.

Pruitt BA Jr: Current concepts of burn therapy. Presented at the Quarterly Trauma Conference, Uniformed Services University of the Health Sciences, Bethesda, Maryland, 25-26 February 1988.

Pruitt BA Jr: The diagnosis and treatment of opportunistic infections in injured man. Presented to the Department of Surgery, United States Navy Medical Center, Bethesda, Maryland, 26 February 1988.

Pruitt BA Jr: Current treatment of the burn wound. Presented at the Department of Surgery Grand Rounds, University of Nebraska Medical Center, Omaha, Nebraska, 26-27 February 1988.

Latona PS: Initial management of the burn patient. Presented to the Combat Medical Specialty Division, Fort Sam Houston, San Antonio, Texas, 4 March 1988.

Keenan JR: Initial management of burn patients. Presented to the Intensive Care Course, Brooke Army Medical Center, Fort Sam Houston, San Antonio, Texas, 10 March 1988.

Waymack JP: The effect of blood transfusions on immune function. Presented at the First International Congress on the Immune Consequences of Trauma Shock and Sepsis Symposium, Munich, Germany, 10 March 1988.

Pruitt BA Jr: Presentation as National Faculty Member to Advanced Burn Life Support Course. Presented to the Department of Surgery, University of North Carolina School of Medicine, Chapel Hill, North Carolina, 10-11 March 1988.

Pruitt BA Jr: Fluid resuscitation of the injured patient. Presented as part of the 1988 University of California Extension Course, Palm Springs, California, 13-17 March 1988.

Pruitt BA Jr: Management of the complications of IV therapy. Presented as part of the 1988 University of California Extension Course, Palm Springs, California, 13-17 March 1988.

Cioffi WG Jr: Early care and aeromedical transport of the thermally injured patient. Presented at the National Aerospace Conference, New Orleans, Louisiana, 16 March 1988.

Beverly ED: Wear and apparel of the military uniform. Presented at the Noncommissioned Officer Development Program, Fort Sam Houston, San Antonio, Texas, 17 March 1988.

Cioffi WG Jr: Electrical injury. Presented at the 42nd Annual Texas Job Training and Safety Conference, Corpus Christi, Texas, 17 March 1988.

Vaughan GM: Hypodipsia-hypernatremia. Presented at the Endocrine Combined Conference, University of Texas Health Science Center, San Antonio, Texas, 17 March 1988.

Summers TM: Psychosocial aspects of thermal injuries. Presented at the Tri-Service Reserve Burn Nursing Seminar, Portland, Oregon, 19 March 1988.

Duncan DJ: Pediatric burns: are they different? Presented at the Tri-Service Reserve Burn Nursing Seminar, Portland, Oregon, 20 March 1988.

Luster SH: Rehabilitation procedures in burn care. Presented at the 45th Station Hospital, Vancouver, Washington, 20 March 1988.

Burleson DG: Measurement of in vitro function of B lymphocytes from burned patients. Presented at the 20th Annual Meeting of the American Burn Association, Seattle, Washington, 24 March 1988.

Gutierrez RT: Physical therapy in burn care. Presented to the Advanced Physical Therapy Course, Wilford Hall USAF Medical Center, Lackland Air Force Base, San Antonio, Texas, 24 March 1988.

McManus AT: Oral nystatin does not alter the incidence of Candidemia in severely burned patients. Presented at the 20th Annual Meeting of the American Burn Association, Seattle, Washington, 25 March 1988.

Shimazu T: Carbon monoxide elimination process in acute and chronic CO poisoning: comparison by two-compartment analysis. Presented at the 20th Annual Meeting of the American Burn Association, Seattle, Washington, 25 March 1988.

Pruitt BA Jr: Ethics in burn care. Presented at the 20th Annual Meeting of the American Burn Association, Seattle, Washington, 26 March 1988.

Molter NC: USAISR Nursing Service. Presented to the Educators Tour, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 29 March 1988.

McManus WF: Surgical nutrition - indications and problems. Presented at the Gary P. Wratten Surgical Symposium, Bethesda, Maryland, 30 March 1988.

Summers TM: USAISR Nursing Service. Presented to the Educators Tour, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 4 April 1988.

Pruitt BA Jr: Changing concepts in burn therapy. Presented to the Controversies in Surgery Postgraduate Course, Harvard Medical School and Brigham and Women's Hospital, Boston, Massachusetts, 7-8 April 1988.

Pruitt BA Jr: Evolution and revolution in the management of the seriously burned. Presented as the Excelsior Surgical Society/Edward D. Churchill Lecturer at the 16th Annual Meeting of the American College of Surgeons, San Antonio, Texas, 11 April 1988.

Duncan DJ: Army practical nurses: are they prepared to perform as head nurses and practical nursing instructors expect them to perform? Presented at the Phyllis J. Verhonick Nursing Research Seminar, Washington, DC, 11 April 1988.

Pruitt BA Jr: Fluid resuscitation of injured patients. Presented to the Department of Surgery, Surgical Grand Rounds, New Jersey Medical School, Newark, New Jersey, 18 April 1988.

Pruitt BA Jr: Care of the burn wound. Presented at Hackensack Hospital, Hackensack, New Jersey, 19 April 1988.

Gutierrez RT: The role of physical therapists in a natural disaster. Presented at the Central District Meeting of the Texas American Physical Therapy Association, San Antonio, Texas, 19 April 1988.

Jordan BS: The posttrauma surgical reconstructive patient - the continuum of burn nursing care: current concepts in burn nursing. Presented at the University of New York, Elmira, New York, 20 April 1988.

Molter NC: Standards of nursing care for the large burn patient in the initial hours. Presented at the University of New York, Brockport, New York, 20 April 1988.

Beverly ED: Wear and apparel of the military uniform. Presented at the Noncommissioned Officer Development Program, Fort Sam Houston, San Antonio, Texas, 21 April 1988.

Jordan BS: The posttrauma surgical reconstructive patient - the continuum of burn nursing care: current trends in critical care. Presented at the American Association of Critical Care Nurses Annual Education Conference, Buffalo, New York, 21 April 1988.

Pruitt BA Jr: Current concepts in burn management. Presented at the Grand Rounds, Tulane University Medical Center, New Orleans, Louisiana, 23 April 1988.

Molter NC: Extended role of the nurse. Presented to the Military Postgraduate Critical Care Course, Washington, DC, 26 April 1988.

Duncan DJ: Initial management of burn victims. Presented to the Luling Emergency Medical Services, Luling, Texas, 28 April 1988.

Maly DW: Aeromedical transport of the burn victim. Presented to the Luling Emergency Medical Services, Luling, Texas, 28 April 1988.

Zelenka JP: Occupational therapy in burn care. Presented to Occupational Therapy Course (91L), Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 2 May 1988.

Foresman JL: Thermal injuries - physical therapy management. Presented to the Physical Therapy Specialist Course (91J), Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 4 May 1988.

Latona PS: Initial management of the burn victim. Presented to the United States Air Force Battlefield Nursing Course, Brooks Air Force Base, San Antonio, Texas, 4 May 1988.

Burleson DG: The effect of intravenous immune globulin administration on lymphocyte phenotype and function in burned patients. Presented at the 8th Annual Meeting of the Surgical Infection Society, San Francisco, California, 5 May 1988.

Pruitt BA Jr: Diagnosis and treatment of opportunistic infections in injured man. Presented as the Marion Donald Lecturer at the Annual Meeting of the Alabama Chapter of the American College of Physicians, Mobile, Alabama, 11-12 May 1988.

Pruitt BA Jr: Fluid resuscitation of surgical patients. Presented as the Marion Donald Lecturer at the Annual Meeting of the Alabama Chapter of the American College of Physicians, Mobile, Alabama, 11-12 May 1988.

Jordan BS: Wound management and complications of burn injury. Presented at Seton Medical Center, Austin, Texas, 13 May 1988.

Maly DW: Aeromedical transport of the burn victim. Presented at Seaton Medical Center, Austin, Texas, 13 May 1988.

McManus WF: Prehospital care of the burn patient. Presented at Seaton Hospital, Austin, Texas, 13 May 1988.

Summers TM: Psychosocial nursing and the burn patient. Presented at Seton Medical Center, Austin, Texas, 13 May 1988.

Pruitt BA Jr: Epidemiology, triage, and aeromedical transfer of burn patients. Presented to the Combat Casualty Management Course (C4A), Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 16 May 1988.

Pruitt BA Jr: Burn care as a military problem. Presented to the AMEDD Officers' Advanced Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 17 May 1988.

Molter NC: Acute pain management: clinical decision-making responsibilities for the professional nurse. Presented at the 39th Medical Surgical Congress, Garmish, Germany, 18 May 1988.

Molter NC: Extended role of the nurse. Presented at the 39th Medical Surgical Congress, Garmish, Germany, 18 May 1988.

Beverly ED: Wear and apparel of the military uniform. Presented at the Noncommissioned Officer Development Program, Fort Sam Houston, San Antonio, Texas, 19 May 1988.

Duncan DJ: Initial management of burn victims. Presented to the Physical Therapy Specialists Course (91J), Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 20 May 1988.

Gutierrez RT: Physical therapy management of burn patients. Presented to the Physical Therapy Specialists Course (91J), Academy of Health Sciences, Fort Sam Houston, Texas, 20 May 1988.

Luster SH: A checklist for identification of rehabilitation problems in burn patients. Presented at the Annual Meeting of the American Trauma Society, Arlington, Virginia, 20 May 1988.

Molter NC: Acute pain management: Clinical decision-making responsibilities for the professional nurse. Presented to the 2nd Aeromedical Evacuation Squadron, Rhein Main Air Force Base, Germany, 20 May 1988.

Molter NC: Aeromedical evacuation of burn victims. Presented to the 2nd Aeromedical Evacuation Squadron, Rhein Main Air Force Base, Germany, 20 May 1988.

Carlson DE: Nutritional needs of the burn patient. Presented at the Patients' Family Group Meeting, US Army Institute of

Surgical Research, Fort Sam Houston, San Antonio, Texas, 24 May 1988.

Duncan DJ: Initial management of burn victims. Presented to the Nursing Service Branch, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 25-27 May 1988.

Hollan E: Infection control procedures in the burn unit. Presented to the Nursing Service Branch, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 25-27 May 1988.

Jordan BS: General nursing care of the burn wound. Presented to the Nursing Service Branch, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 25-27 May 1988.

Jordan BS: Principles of hemodynamic monitoring in the burn unit. Presented to the Nursing Service Branch, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 25-27 May 1988.

McDaniel J: Documentation in the intensive care unit. Presented to the Nursing Service Branch, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 25-27 May 1988.

Summers TM: Communicating effectively. Presented to the Nursing Service Branch, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 25-27 May 1988.

Summers TM: Psychosocial aspects of thermal injuries. Presented to the Nursing Service Branch, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 25-27 May 1988.

Wright ML: Perioperative care of the burn patient. Presented to the Nursing Service Branch, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 25-27 May 1988.

Cioffi WG Jr: Aeromedical transport of the thermally injured patient. Presented to the Aerospace Medical Association, New Orleans, Louisiana, 1 June 1988.

Vaughan GM: Entrainment of both rise and fall of rat serum melatonin in cycling lighting. Presented at the 70th Annual Meeting of the Endocrine Society, New Orleans, Louisiana, 9 June 1988.

Gutierrez RT: Getting mobilized to El Salvador. Presented to the Physical Therapy Specialists Advanced Course (91J), Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 13 June 1988.

Gutierrez RT: Physical therapy and thermal injuries. Presented to the Physical Therapy Specialists Advanced Course (91J), Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 13 June 1988.

Pruitt BA Jr: Diagnosis and treatment of opportunistic infections in critically ill patients. Presented at the 8th Annual Teaching Day, St. Francis Hospital and Medical Center, Hartford, Connecticut, 15 June 1988.

Pruitt BA Jr: Fluid resuscitation of injured patients. Presented at the 8th Annual Teaching Day, St. Francis Hospital and Medical Center, Hartford, Connecticut, 15 June 1988.

DePew CL: Fluid and electrolyte pathophysiology. Presented to the Critical Care Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 15-16 June 1988.

Summers TM: Crisis and families. Presented to the Critical Care Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 15-16 June 1988.

Beverly ED: Wear and apparel of the military uniform. Presented at the Noncommissioned Officer Development Program, Fort Sam Houston, San Antonio, Texas, 16 June 1988.

Gutierrez RT: The role of physical therapy in El Salvador. Presented to United States Army Medical Specialist Corps, Fort Sam Houston, San Antonio, Texas, 17 June 1988.

DePew CL: Pacemakers. Presented to the Critical Care Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 28 June 1988.

Summers TM: Stress and crisis management. Presented to the Critical Care Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 1 July 1988.

Pruitt BA Jr: Diagnosis and treatment of opportunistic infections in burn patients. Presented as the Kirk Anderson Traber Memorial Lecturer, University of Texas Medical Branch and Shriners Burns Institute, Galveston, Texas, 6-7 July 1988.

Vaughan GM: The pineal and its relation to burn injury and human physiology. Presented to Anatomy Department, Mahidol University, Bangkok, Thailand, 18 July 1988.

Duncan DJ: Initial management of the burn victim. Presented to the Critical Care Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 18 July 1988.

Jordan BS: Standards of nursing care for the large burn victim in the initial 48 hours. Presented to the Critical Care Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 18 July 1988.

Beverly ED: Wear and apparel of the military uniform. Presented at the Noncommissioned Officer Development Program, Fort Sam Houston, San Antonio, Texas, 21 July 1988.

Vaughan GM: Daytime unresponsiveness of the human and hamster pineal to adrenergic stimulation. Presented at the Satellite Symposium of the 8th International Congress of Endocrinology, Hong Kong, 27 July 1988.

Vaughan GM: Entrainment of rat serum melatonin (MEL) rise and fall in cyclic lighting. Presented at the Satellite Symposium of the 8th International Congress of Endocrinology, Hong Kong, 28 July 1988.

Vaughan GM: The endocrine response to burn injury. Presented to the Departments of Medicine and Pathophysiology, First Medical College of the Peoples' Republic of China, 29 July 1988.

Duncan DJ: Management of burn victims in the theater of operations. Presented at the 2nd Annual Joint Military Trauma Symposium, United States Air Force Academy, Colorado Springs, Colorado, 3 August 1988.

Selzer RA: Management of burn victims in the theater of operations. Presented to the United States Air Force Battlefield Nursing Course, Brooks Air Force Base, San Antonio, Texas, 3 August 1988.

Luster SH: Occupational therapy in burn care. Presented to the Occupational Therapy Assistant Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 4 August 1988.

Pruitt BA Jr: Current treatment of the burn wound. Presented to the Department of Surgery, Ben Taub Hospital, Houston, Texas, 6 August 1988.

Luster SH: A checklist for identification of rehabilitation problems in burn patients. Presented at the Annual Army Medical Specialist Corps Symposium, Leesburg, Virginia, 8 August 1988.

Vaughan GM: Pituitary. Presented to the Endocrine Pathophysiology Sophomores, Department of Medicine, University of Texas Health Science Center, San Antonio, Texas, 8 August 1988.

Vaughan GM: Thyroid. Presented to the Endocrine Pathophysiology Sophomores, Department of Medicine, University of Texas Health Science Center, San Antonio, Texas, 8 August 1988.

Beverly ED: Liver disease. Presented to the Practical Nurse Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 16 August 1988.

Carlson DE: Nutritional needs of the burn patient. Presented at the Patients' Family Group Meeting, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 16 August 1988.

Beverly ED: Wear and apparel of the military uniform. Presented at the Noncommissioned Officer Development Program, Fort Sam Houston, San Antonio, Texas, 18 August 1988.

Pruitt BA Jr: Clinical and laboratory studies of acute inhalation injury. Presented at the Annual Meeting of the International Surgical Group, Reykjavik, Iceland, 21–22 August 1988.

Molter NC: USAISR Nursing Service. Presented to the Educators Tour, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 23 August 1988.

Pruitt BA Jr: Inhalation injury and airway management. Presented to the American Burn Life Support Instructor Course, Pittsburgh, Pennsylvania, 26–27 August 1988.

Pruitt BA Jr: Transfer and transport of burn patients. Presented to the American Burn Life Support Instructor Course, Pittsburgh, Pennsylvania, 26–27 August 1988.

Broberg R: Initial management of burn victims. Presented to the Combat Medical Specialty Division, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 1 September 1988.

McManus AT: Occurrence of *Pseudomonas aeruginosa* in seriously burned patients: a review of 950 patients (1983–1987). Presented at the International Symposium on Basic Research and Clinical Aspects of *Pseudomonas aeruginosa* infections, Copenhagen, Denmark, 1 September 1988.

Pruitt BA Jr: Burn wound excision and the null hypothesis. Presented at the Annual Meeting of the Halsted Society, Jackson Hole, Wyoming, 6–9 September 1988.

Keenan JR: Initial management of burn victims. Presented to the 32nd Aeromedical Evacuation Squadron, Kelly Air Force Base, San Antonio, Texas, 10 September 1988.

Dorsey CS: Role of the LVN on the burn flight team. Presented to the Practical Nurse Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 12 September 1988.

Dimmick DJ: Role of the LVN in the Clinical Data Division, US Army Institute of Surgical Research. Presented to the Practical Nurse Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 12 September 1988.

Pruitt BA Jr: Diagnosis and treatment of opportunistic infections in injured man. Presented to the Department of Surgery, University of North Carolina, Chapel Hill, North Carolina, 12-13 September 1988.

Pruitt BA Jr: Fluid resuscitation of injured man. Presented to the Department of Surgery, University of North Carolina, Chapel Hill, North Carolina, 12-13 September 1988.

Duncan DJ: Initial management of the burn victim. Presented to the School of Nursing, Baptist Memorial Hospital, San Antonio, Texas, 13 September 1988.

Duncan DJ: Standards of nursing care for the large burn victim in the initial 48 hours. Presented to the School of Nursing, Baptist Memorial Hospital, San Antonio, Texas, 13 September 1988.

Beverly ED: Wear and apparel of the military uniform. Presented at the Noncommissioned Officer Development Program, Fort Sam Houston, San Antonio, Texas, 13 September 1988.

Anderson SE: Standards of nursing care for the large burn victim in the initial 48 hours. Presented to the Nursing Service Branch, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 15 September 1988.

DePew CL: Documentation in the burn unit. Presented to the Nursing Service Branch, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 15 September 1988.

Duncan DJ: Initial management of the burn victim. Presented to the Nursing Service Branch, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 15 September 1988.

Hollan E: Infection control in the burn unit. Presented to the Nursing Service Branch, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 15 September 1988.

Jordan BS: General nursing care of the burn wound. Presented to the Nursing Service Branch, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 15 September 1988.

Keenan JR: Aeromedical transport of thermally injured patients. Presented to the Nursing Service Branch, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 15 September 1988.

Summers TS: Communicating effectively. Presented to the Nursing Service Branch, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 15 September 1988.

Summers TS: Psychosocial aspects of thermal injuries. Presented to the Nursing Service Branch, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 15 September 1988.

Wright ML: Perioperative management of the burn patient. Presented to the Nursing Service Branch, US Army Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 15 September 1988.

McManus WF: Excision of the burn wound - major burns. Presented at the Paul E. Hodgson Scientific Symposium, Department of Surgery, University of Nebraska College of Medicine, Omaha, Nebraska, 17 September 1988.

Cioffi WG Jr: Physiologic effects of smoke inhalation in an ovine model. Presented at the Tri-Service Conference on Pulmonary Injury, Denver, Colorado, 20 September 1988.

Cioffi WG Jr: The use of high frequency ventilation in the treatment of patients with inhalation injury. Presented at the Tri-Service Conference on Pulmonary Injury, Denver, Colorado, 20 September 1988.

Selzer RA: Management of burn victims in the theater of operations. Presented to the United States Air Force Battlefield Nursing Course, Brooks Air Force Base, San Antonio, Texas, 21 September 1988.

Pruitt BA Jr: Diagnosis and treatment of opportunistic infections in injured man. Presented to the Department of Surgery, Michigan State University, East Lansing, Michigan, 21-22 September 1988.

Waymack JP: The immunologic sequelae of transfusions. Presented at the Scottish National Blood Bank Headquarters, Edinburgh, Scotland, 22 September 1988.

Cioffi WG Jr: Initial care of the thermally injured patient. Presented at the Operational Emergency Medical Conference, Seattle, Washington, 24 September 1988.

Duncan DJ: General nursing care of the burn wound. Presented at the Emergency Medical Symposium: Burn Care, Naval Reserve Readiness Command, Seattle, Washington, 24-25 September 1988.

Duncan DJ: Pediatric burns: are they different? Presented at the Emergency Medical Symposium: Burn Care, Naval Reserve Readiness Command, Seattle, Washington, 24-25 September 1988.

Summers TM: Psychosocial aspects of thermal injuries. Presented at the Emergency Medical Symposium: Burn Care, Naval Reserve Readiness Command, Seattle, Washington, 24-25 September 1988.

McManus WF: Infections in burns - burn care: state-of-the-art. Presented to the Department of Surgery, University of Cincinnati, Cincinnati, Ohio, 30 September 1988.

Chu C-S: Multiple graft harvestings from deep partial-thickness scald wounds healed under the influence of weak direct current. Presented at the 48th Annual Meeting of the American Association for the Surgery of Trauma, Newport Beach, California, 6 October 1988.

Graves TA: Relationship of transfusion and infection in a burn population. Presented at the 48th Annual Meeting of the American Association for the Surgery of Trauma, Newport Beach, California, 6 October 1988.

DePew CL: Acid base balance. Presented to the Critical Care Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 19 October 1988.

Summers TM: Crisis and families. Presented to the Critical Care Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 19 October 1988.

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ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3S162787A874-00, APPLIED RESEARCH

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

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

1 January 1988 - 31 December 1988

INVESTIGATORS

Thomas B. Dougherty, MD, PhD, Major, MC
William F. McManus, MD, Colonel, MC
Basil A. Pruitt, Jr., MD, Colonel, MC

ABSTRACT

PROJECT NUMBER: 3S162787A874-00, APPLIED RESEARCH

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

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

PERIOD COVERED IN THIS REPORT: 1 Jan 88 through 31 Dec 88

INVESTIGATORS: Thomas B. Dougherty, MD, PhD, Major, MC
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During the period of this report, 467 anesthetics were administered to 161 patients, an average of 2.9 anesthetics per patient. The most commonly used anesthetic agent was isoflurane (59.5%) followed by narcotics (24.4%), ketamine (7.5%), enflurane (4.9%), and halothane (1.5%). Due to the nature and combinations of procedures now performed, regional anesthesia is no longer used.

ANESTHESIOLOGY

PREOPERATIVE PROCEDURES

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

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

Premedication. Routine medications such as cimetidine and cardiovascular medications are continued up to the time of surgery. Glycopyrrolate (Robinul®), from 0.005 mg/kg to a maximum dose of 0.2 mg/kg, is given intramuscularly 30 min prior to anesthesia or intravenously upon entering the operating room. No other premedications are routinely used with the exception of diazepam preceding ketamine anesthetic.

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

TYPES OF ANESTHESIA

The pattern of anesthetic administration has changed from previous years and involves a greater use of isoflurane, having replaced enflurane which was the primary anesthetic agent for the previous 3 yr. Ketamine, narcotics, and halothane are used, but to a much lesser extent (Table 1).

Isoflurane (Forane®). Isoflurane, which is an isomer of enflurane, is the most recent halogenated ether 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

TABLE 1. Pattern of Anesthesia Administration (1985-8)

Agent	1985		1986		1987		1988	
	Number	%	Number	%	Number	%	Number	%
Enflurane	279	71.9	272	66.3	129	27.8	23	4.9
Halothane	2	0.5	35	8.5	29	6.3	7	1.5
Isoflurane	35	9.0	23	5.6	196	42.3	278	59.5
Ketamine	52	13.4	63	15.4	57	12.3	35	7.5
Local	10	2.6	10	2.4	9	1.9	9	1.9
Nitrous oxide	0	0.0	0	0.0	4	0.9	1	0.2
Other*	10	2.6	7	1.7	39	8.4	114	24.4
TOTAL	**388**	**100.0**	**410**	**100.0**	**463**	**100.0**	**467**	**100.0**

*This category includes mainly narcotics after 1986.

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 or ketamine provides a smooth anesthetic induction that is significantly more rapid than enflurane plus those three intravenous agents. Isoflurane is presently the most commonly used anesthetic agent at this Institute.

Enflurane (Ethrane®). Enflurane is a halogenated ether which provides a relatively smooth anesthetic induction and good muscle relaxation. Unfortunately, the anesthetic induction can be somewhat prolonged in certain healthy adult patients. Biotransformation amounts to 2 to 2.5% of an inhaled dose, which perhaps accounts for the few clinical toxic effects observed. Plasma fluoride levels in hypermetabolic burn patients during and after enflurane administration have been measured and found not to be in the toxic range.

Halothane (Fluothane®). Halothane is an halogenated alkane that has met with only limited use over the last 6 yr. Biotransformation can account for as much as 25% of an inhaled dose. Halothane hepatitis, although rare, fortunately has not been reported in burn patients. Since the successful introduction of enflurane 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. Halothane hepatitis has not been reported to be an issue in the pediatric population.

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

Ketamine. This agent is used both intramuscularly and intravenously to produce its characteristic dissociative state with preservation of basal functions and laryngeal reflexes plus stimulation of the cardiovascular system. Unfortunately, ketamine shares with its parent compound, phencyclidine, the production of a high incidence of unpleasant hallucinogenic side effects. 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. Pronounced blepharospasm prevents its use in eye cases. All ketamine anesthetics, other than in children, are preceded by intravenous administration of diazepam (0.15-0.2 mg/kg) or midazolam (0.05 mg/kg).

Muscle Relaxants. Succinylcholine has not been used for any purpose at this Institute for more than a decade. On the other hand, nondepolarizing muscle relaxants (vecuronium bromide, pancuronium bromide, and atracurium besylate) have been used in 86% of the operative cases over the past year.

Regional Anesthetics. 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.

MONITORING TECHNIQUES

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

The Dinamap™ 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. Usually, blood pressure is monitored at two sites. Direct arterial lines are used when necessary.

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

Body Temperature. Skin, rectal, nasopharyngeal, or esophageal temperatures are continually monitored. Because of the greatly increased evaporative losses in burn patients, hypothermia is a serious problem. Several methods are employed to maintain body temperature during anesthesia. Ambient temperatures were maintained between 85 and 90°F. Maintaining the room temperature above 88°F appears most effective in preventing patient cooling. Anesthetic gases are heated and humidified and radiant heat lamps are used when necessary. Disposable K-thermia™ heating blankets are also helpful and are most effective when used on children. Scrub solutions, intravenous fluids, and blood products are all warmed prior to use.

RESULTS

Complications. There was one intraoperative death in the operating room during this reporting period. The patient was a 42-year-old male who sustained a 31% total body surface area burn 11 days earlier. This patient was scheduled for excision and grafting on the morning of 18 February 1988. The patient was ventilator-dependent and had demonstrated marginal cardiac reserve at the time of surgery. He was monitored invasively with a pulmonary artery catheter and arterial pressure line in addition to the usual monitors in the operating room. Anesthesia was induced in the patient uneventfully and he was stable during the first 90 min of the surgery, which consisted of excision of burns of the chest to fascia with the patient in the supine position. The mean arterial pressure (torr) remained in the 80s, the pulmonary capillary wedge pressure was approximately 12 torr, and cardiac output was about 7.0 l/min. The patient's cardiac output then fell rather dramatically to 4.0 l/min in the face of seemingly adequate blood pressure, fluid and blood replacement, and pulse oximetry. In spite of treatment with inotropic support (dopamine hydrochloride and dobutamine hydrochloride), the patient's condition continued to deteriorate, with a decrease in heart rate and blood pressure. No pulse was palpable following treatment with atropine and epinephrine. CPR was initiated and the advanced cardiac life support protocol was followed without success, despite placement of a transvenous pacemaker and continued fluid resuscitation. The patient was pronounced dead after an hour of CPR. An autopsy revealed that multiple pulmonary emboli were the cause of death.

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* (1986-8)

Monitor/Parameter	1986		1987		1988	
	Number	%	Number	%	Number	%
Pulse oximeter (hemoglobin saturation)	370	90.2	444	95.9	465	99.6
Inspired oxygen concentration	356	86.8	442	95.4	461	98.7
Temperature	397	96.8	443	95.7	460	98.5
End-tidal carbon dioxide	356	86.8	441	95.2	459	98.3
Pulmonary function	332	81.0	391	84.4	423	90.6
Arterial line	38	9.3	75	16.2	138	29.6
Swan-Ganz catheter	9	2.2	26	5.6	29	6.2
Central venous pressure	9	2.2	29	6.3	14	3.0

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

TABLE 3. Overall Anesthetic Patient Data (1971-88)

Year	Number of Patients	Number of Patients Anesthetized	% of All Patients	Total Anesthestics Given	Average Anesthetics Per Patients Anesthetized
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 (1984-8)

Procedure	1984		1985		1986		1987		1988	
	Number	%	Number	%	Number	%	Number	%	Number	%
Excision	323	41.0	304	43.4	303	38.3	397	44.7	421	45.6
Autograft	371	47.1	304	43.4	372	47.0	389	43.8	395	42.7
Orthopedic	30	3.8	19	2.7	29	3.7	27	3.0	36	3.9
Eye and lid	18	2.3	9	1.3	19	2.4	9	1.0	11	1.2
Intra-abdominal	5	0.6	12	1.7	4	0.5	7	0.8	6	0.6
Chrondrectomy	4	0.5	0	0.0	1	0.1	5	0.6	2	0.2
Plastic	5	0.6	9	1.3	5	0.6	5	0.6	12	1.3
Other	31	3.9	44	6.3	58	7.3	50	5.6	41	4.4
TOTAL	**787**	**100.0**	**701**	**100.0**	**791**	**100.0**	**889**	**100.0**	**924**	**100.0**

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION: DA315017
2. DATE OF SUMMARY: 89 02 16
REPORT CONTROL SYMBOL: DD-DR&E(AR) 636

3. DATE PREV SUM'RY: 88 10 01
4. KIND OF SUMMARY: H
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	62787A	3S162787A874	DA	161
b. CONTRIBUTING			AD	
c. CONTRIBUTING	DA LRRDAP,	FY89-01		

11. TITLE *(Precede with Security Classification Code)* (U) A Two-Stage Technique Versus a One-Stage Technique for Excision and Grafting: A Prospective Randomized Trial

12. SUBJECT AREAS
06 05 Medicine and Medical Research

13. START DATE: 88 08
14. ESTIMATED COMPLETION DATE: 89 02
15. FUNDING ORGANIZATION: DA
16. PERFORMANCE METHOD: C

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

18. RESOURCES ESTIMATE

FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS *(In thousands)*
88	0.1	1
89	0.1	2

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

20. PERFORMING ORGANIZATION
a. NAME: US Army Institute of Surgical Research
b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-5012
c. NAME OF PRINCIPAL INVESTIGATOR: WAYMACK, J P
d. TELEPHONE NUMBER *(include area code)*: 512-271-7267
f. NAME OF ASSOCIATE INVESTIGATOR *(if available)*: DOUGHERTY, T B
g. NAME OF ASSOCIATE INVESTIGATOR *(if available)*: SCOTT, C L

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

22. KEYWORDS *(Precede EACH with Security Classification Code)* (U) Excision; (U) Skin Grafts; (U) Metabolism; (U) Hemorrhage; (U) Volunteers: (U) Adults; (U) RAII

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

23. (U) Early excision followed by immediate skin grafting is widely considered to be a preferred method of burn wound closure. However, the disadvantages of this technique include significant blood loss with need for large volume blood transfusions, lengthy anesthesia time, and excessive metabolic demands. A recently described two-stage technique for excision and grafting of burn wounds allows a period of stabilization, during which blood volume replacement and temperature correction occurs. On the following day, autografting is performed. This study will compare the two-stage excision technique with the conventional one-stage technique in terms of incidence of sepsis, metabolic demands, functional and cosmetic results, duration of hospital stay, and cost in burned soldiers. A literature search was performed and indicated no duplication of effort.

24. (U) One hundred patients with burns requiring excision of > 12% of the total body surface area will be studied. Patients will be randomized into pairs to undergo two-stage or one-stage excision and grafting of their burn wounds. Initial excision of burns will be performed during the first postburn week.

25. (U) 8810 - 8909. This project has been terminated at the request of the primary investigator. No patients were enrolled in the study.

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

ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3S162787A874-00, APPLIED RESEARCH

PROJECT TITLE: A Two-Stage Technique for Excision and Grafting Versus a One-Stage Technique for Excision and Grafting of Burn Wounds: A Prospective Randomized Study

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

1 October 1988 - 16 February 1989

INVESTIGATORS

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William F. McManus, MD, Colonel, MC
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ABSTRACT

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PERIOD COVERED IN THIS REPORT: 1 Oct 88 through 16 Feb 89

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

Early excision followed by immediate skin grafting is widely considered to be a preferred method of burn wound closure. However, the disadvantages of this technique include significant blood loss with need for large volume blood transfusions, lengthy anesthesia time, and excessive metabolic demands. A recently described two-stage technique for excision and grafting of burn wounds allows a period of stabilization, during which blood volume replacement and temperature correction occurs. On the following day, autografting is performed. This study was designed to compare the two-stage excision technique with the conventional one-stage technique in terms of incidence of sepsis, metabolic demands, functional and cosmetic results, duration of hospital stay, and cost.

This study was terminated at the request of the primary investigator. No patients were enrolled in the study.

A TWO-STAGE TECHNIQUE FOR EXCISION AND GRAFTING VERSUS A ONE-STAGE TECHNIQUE FOR EXCISION AND GRAFTING OF BURN WOUNDS: A PROSPECTIVE RANDOMIZED STUDY

In reference to the timing and method of excision and skin grafting of the burn wound, the surgeon has a variety of excellent options available. Aggressive early excision followed by immediate skin grafting has been widely accepted as the preferred method in burn treatment centers (1-4). This technique may decrease sepsis, diminish metabolic demands, improve functional and cosmetic results, and result in decreased hospital stay and cost. The disadvantages of this technique can include significant blood loss, resuscitation with large blood transfusions, lengthy anesthesia time, and excessive metabolic demands.

A recently described two-stage technique for excision and grafting of burn wounds allows a period of stabilization, during which time blood volume replacement and temperature correction occurs (5). The patient returns to the operating room the following day for autografting. Advocates of this technique claim to minimize metabolic stress and reduce the hemorrhage which accompanies burn wound excision.

The objective of this study is to evaluate a two-stage technique for excision and grafting by comparison to the conventional one-stage technique.

MATERIALS AND METHODS

Study Design. Patients will receive standard appropriate therapy for their burn and will be randomized into pairs to undergo a two-stage technique for excision and grafting or a one-stage technique for excision and grafting of their burn wounds. All patients will receive preoperative vancomycin and amikacin and four doses each of vancomycin and amikacin in the postoperative period. Patients in the two-stage protocol will not receive antibiotics beyond those ordered by the physician following the first operation. These patients will have an index card on the front of their chart identifying them as participants in the two-stage protocol so that they will not receive a second course of antibiotics. This will result in coverage for the initial 16 h following the second surgery. This should not affect their risk of infection since bacterial seeding appears to occur following excision on burn eschar, not harvesting of donor sites. No other modifications will be made in the care of either group of patients.

A method of excision will be standardized in both groups by utilizing the Padgett™ dermatome, the Goulian™ knife, and/or any other surgical instruments as deemed appropriate by the attending surgeon. Split-thickness skin grafts will be harvested using the Padgett™ dermatome. Patients will be enrolled in the study as

sequential pairs with treatment randomly allocated between the paired patients. In the first group, immediately upon appreciation of adequate hemostasis, split-thickness skin grafts will be applied to the excised areas. In the second group, the tangentially excised areas will be dressed and the patients will be allowed an overnight period of stabilization prior to returning to the operating room the following day for autografting. If the two-stage technique for excision and grafting proves to be either very effective of detrimental, the sequential analysis will allow the study to be completed with significantly fewer patients.

Number of Patients. Up to 100 patients will be enrolled in this study, with an early cutoff by closed and sequential analysis possible.

Selection of Patients. Patients admitted to the US Army Institute of Surgical Research will be eligible for enrollment in the study.

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

1. Patients hospitalized for burn injury.

2. Male or female patients ≥ 18 and ≤ 80 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.

3. Patients requiring excision of > 12% of the total body surface area.

4. Concomitant injury or preexisting disease will not contraindicate enrollment in the study.

Patient Exclusion. Patients with the following characteristics will be excluded from the study:

1. Patients < 18 or > 80 yr old.

2. Patients who are pregnant or nursing.

3. Patients who have burns requiring excision of < 12% of the total body surface area.

4. Patients who have head and/or neck burns but have no other burns requiring excision of > 12% of the total body surface area.

Patient Procedures. In all patients, initial excision of burns will be performed during the first postburn week unless contraindicated due to other medical problems. Assessment and

determination of a normal fluid and electrolyte balance and normal RBC volume will be made prior to surgery. Operations will be designed to cover no more than 20% of the patient's burn. Operative procedures will begin when body temperature is normal and hematocrit is ≥ 30%. Prior to transport of the patient to he operating room, the operating room will be preheated. Heat lamps and mattresses will be used intraoperatively to maintain body temperature. General anesthesia will be used in all cases. Excision of burn wounds will be performed using the Padgett™ electric dermatome, Goulian™ knife, and/or any variety of hand-held dermatomes and knives.

One-Stage Technique. In patients randomized to the one-stage technique, thrombin and/or epinephrine-soaked sponges followed by hot saline packs will be applied following burn wound excision. Lactated Ringer's solution will be infused subcutaneously to facilitate harvesting of autograft. Autograft thickness will be determined by the staff surgeon based on the patient's overall condition. The dermatome will be utilized for obtaining split-thickness autografts, which will be meshed to a ratio of 1.5:1 and occasionally 3:1 when adequate donor sites are limited. Skin grafts will be secured in position using a stapling apparatus (US Surgical Corporation, Norwalk, CT) or chromic sutures. Autograft dressings will consist of wet fine-mesh gauze covered with bulky dressings. Donor sites will be dressed with dry fine-mesh gauze or Biobrane® dressing. Patients will be returned to Ward 14A or 14B for full recovery from anesthesia and wound care.

Two-Stage Technique. In patients randomized to the two-stage technique, excised wounds will be covered with bulky gauze dressings soaked in 5% mafenide acetate solution. After controlling bleeding with hot compresses, dressings will be applied and secured in position with Kerlix™ dressings (Kendall, Boston, MA) and elastic bandages for extremity wounds and secured with netting for torso wounds. Patients will then be returned to Ward 14A or 14B for full recovery from anesthesia and wound care. Attention will be directed to restoration of euthermia by the use of heat lamps, heat shields, and thermal blankets if necessary. Transfusions will be used to maintain an hematocrit ≥ 30%. Irrigating catheters will be utilized to prevent desiccation of excised wounds. The following day, patients will be returned to the operating room for autograft coverage of the previously excised burns. The dermatome will be utilized for obtaining split-thickness autografts. A subcutaneous infusion of lactated Ringer's solution will be used to facilitate harvesting. Bleeding will be controlled with hot saline packs, suture ligation, or electrocautery where indicated. It is anticipated that bleeding will be minimal since hemostasis should have been achieved by coagulation during the preceding 24 h. Split-thickness skin grafts will be meshed to a ratio of 1.5:1 and occasionally 3:1 when adequate donor sites are limited. Skin grafts will be secured in

position using a stapling apparatus or chromic sutures. Autograft dressings will consist of wet fine-mesh gauze covered with bulky dressings. Donor sites will be dressed with dry fine-mesh gauze or Biobrane® dressing. Patients will be returned to Ward 14A or 14B for full recovery from anesthesia and wound care.

Preoperative Data. Preoperative data collection will include hematocrit, electrolytes, creatinine, arterial blood gases, total and 3° burn sizes, age, and past history.

Intraoperative Data. Intraoperative data collection will include blood loss, anesthesia time, blood and blood products received, lowest mean arterial blood pressure, peak pulse rate, area of excision, area grafted, whether or not the patient required postoperative intubation, and the type of anesthetic agents used. In the immediate postoperative period, patient temperature, arterial blood gases, and hematocrit will be obtained.

Postoperative Data. Postoperative data collection will include percent total body surface area covered and percent graft take. Infection and respiratory status based on incidences of pneumonia, tracheobronchitis, and atelectasis will be recorded. Morbidity, mortality, and hospital stay will be recorded as well.

Statistical Analysis. In evaluating the data from the one-stage versus the two-stage procedure, a superior result will be considered to be any of the following:

1. Statistically significant decrease in the number of blood transfusions required.

2. Significant increase in graft "take".

3. Significant increase in the amount of burn excised per procedure.

4. Significant decrease in respiratory complications.

5. Significant decrease in time to wound closure.

RESULTS

No patients were enrolled in this study.

DISCUSSION

This study was terminated at the request of the primary investigator.

PRESENTATIONS/PUBLICATIONS

None.

REFERENCES

1. Burke JF: The benefits of prompt excision. **J Trauma** 19:924-, 1979.

2. Burke JF, Bondoc CC, and Quinby WC: Primary burn excision and immediate grafting: a method shortening illness. **J Trauma** 14:389-95, 1974.

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4. Canizaro PC, Sawyer RB, and Switzer WE: Blood loss during excision of third-degree burns. **Arch Surg** 88:800-3, 1964.

5. Warden GD, Saffle JR, and Kravitz M: A two-stage technique for excision and grafting of burn wounds. **J Trauma** 22:98-103, 1982.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
				DAOG6971	1 Oct 89	DD-DR&E(AR) 636
3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
1 Oct 88	D. Chg	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62787A	3S162787A874	FB	162
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP,	FY90-01		

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

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 Basil A. Pruitt

a. DATE EFFECTIVE		b. RESOURCES ESTIMATE		
b. CONTRACT/GRANT NUMBER		a. FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS *(In thousands)*
c. TYPE	d. AMOUNT	89	1.7	130
e. KIND OF AWARD	f. CUM/TOTAL	90	1.7	137

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME: US Army Institute of Surgical Research	a. NAME: US Army Institute of Surgical Research
b. ADDRESS *(include zip code)*: Fort Sam Houston, San Antonio, Texas 78234-5012	b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-5012
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR: MC MANUS, W F
d. TELEPHONE NUMBER *(Include area code)*: 512-221-2720	d. TELEPHONE NUMBER *(Include area code)*: 512-221-3301
21. GENERAL USE: FINA 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) Burn Injury; (U) Topical Therapy; (U) Mafenide Acetate; (U) 5% Mafenide Acetate Solution; (U) Volunteers;

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

22. (Continued) (U) Adults; (U) Children; (U) RA II

23. (U) A DTIC literature search was conducted under DTIC request number W6R17I dated 19 October 1989 for the work report database and request number W6R23L dated 19 October 1989 for the work unit database. The proposed effort will not result in the duplication of effort. The objective of this work is to 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.

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

25. (U) 8801 - 8812. One hundred and sixty-eight patients were treated with 5% aqueous mafenide acetate soaks. Twenty-two of these patients exhibited mild cutaneous atopy. This low incidence of mild side effects of 5% aqueous mafenide acetate and its continued clinical effectiveness speak for the continued use of this valuable therapeutic agent. For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1989.*

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

ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3S162787A874-00, APPLIED RESEARCH

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

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

1 October 1988 - 30 September 1989

INVESTIGATORS

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

ABSTRACT

PROJECT NUMBER: 3S162787A874-00, APPLIED RESEARCH

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

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

PERIOD COVERED IN THIS REPORT: 1 Oct 88 through 30 Sep 89

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

During this reporting period, 5% aqueous mafenide acetate dressings have continued to be an efficacious treatment modality in the care of the burn wound. One hundred and sixty-five patients were treated with 5% aqueous mafenide acetate dressings, employed either for final debridement of a wound or following application of meshed cutaneous autograft to prevent desiccation of tissue exposed in the interstices of such grafts. An 11.5% incidence of skin rash (atopy) was noted as the only adverse reaction. The clinical results achieved by the use of 5% aqueous mafenide acetate solution strongly support its continued use.

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

During this reporting period, the evaluation of 5% aqueous mafenide acetate solution for topical treatment of the burn wound has continued at this Institute where it was used in 165 of 196 patients (84.2%). 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.

Nineteen patients (11.5%) 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: DAOG6970	2. DATE OF SUMMARY: 1 Oct 89	REPORT CONTROL SYMBOL: DD-DR&E(AR) 636
3. DATE PREV SUM'RY: 1 Oct 88	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	62787A	3S162787A874	CI	163
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP,	FY90-01		

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: 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)*
89	1.7	132
90	1.7	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: VAUGHAN, G M
d. TELEPHONE NUMBER *(Include area code)*: 512-221-2720	d. TELEPHONE NUMBER *(Include area code)*: 512-221-5416
21. GENERAL USE: FINA 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) Pineal; (U) Indoles; (U) Catecholamines; (U) Volunteers; (U) Adults; (U) Lab Animals: (U) Hamsters; (U) Rats;

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

22. (U) RA II

23. (U) A DTIC literature search was conducted under DTIC request number W6R11I dated 19 October 1989 for the technical report database and request number W6R13L dated 19 October 1989 for the work unit database. The proposed effort will not result in the duplication of effort. The objective of this work is to characterize alteration of neuroendocrine function in burned patients in order to improve survival.

24. (U) To assess photic control of the melatonin rhythm and the daytime loss of sympathetic responsiveness of the pineal in murine models for the sympathetic unresponsiveness of critical injury.

25. (U) 8810 - 8909. In Syrian hamsters (the model for human pineal control), pineal adrenergic responsiveness (AR) is absent during most of the day. Early morning hamster pineal AR was assessed with a standard bolus injection of isoproterenol that raises pineal melatonin content in the dark phase. Injection at 50 min after the end of the usual 10-h dark phase, either in light or extension of darkness, raised daytime pineal melatonin almost to the same level as seen after injection during the nocturnal "sensitive" period. Extension of the sensitive period into the first part of the light phase (but not acutely affected by light) indicates that AR is controlled very differently from endogenous melatonin production which is acutely lowered by light at night to daytime values. A new antibody, iodinated melatonin, and a new technique that achieves detectability near the low daytime serum level and an ED_{50} near the peak nocturnal level will permit further investigation of AR mechanisms. For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1989.*

DD FORM 83 MAR 1498 EDITION OF MAR 88 IS OBSOLETE. * U.S.G.P.O.: 1988-491-803/30329

ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3S162787A874, APPLIED RESEARCH

PROJECT TITLE: STUDIES OF THE NEUROENDOCRINE ABNORMALITIES IN BURN INJURY: Syrian Hamster Pineal Sympathetic Responsiveness in the Early Light Phase

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

1 October 1988 - 30 September 1989

INVESTIGATORS

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

ABSTRACT

PROJECT NUMBER: 3S162787A874, APPLIED RESEARCH

PROJECT TITLE: STUDIES OF THE NEUROENDOCRINE ABNORMALITIES IN BURN INJURY: Syrian Hamster Pineal Sympathetic Responsiveness in the Early Light Phase

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

PERIOD COVERED IN THIS REPORT: 1 Oct 88 through 30 Sep 89

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

A rise of pineal melatonin (MEL) content occurs 2 h after isoproterenol (ISO) is injected in the second half of the 10-h dark phase. For this response, because of a ceiling effect of endogenous catecholamine neurotransmission at night, elimination of high MEL synthesis at that time by brief light exposure just before the injection of ISO is important. Such agonists are much less effective in most of the light phase in this species. Using adult males and MEL RIA, we asked if nocturnal responsiveness continues after the endogenously determined fall of high pineal MEL synthesis and content. The fall occurs before onset of light. Hamsters injected once with ISO (1 mg/kg SC) at 1 h into the light phase had a rise of pineal MEL content 2 h later to 5.5-fold that after vehicle (CON) injection ($P < 0.001$). If injection was delayed to 4 h into light, pineal MEL (maximal 2 h post-ISO) was only 1.7-fold ($P < 0.01$) that in CON. Extending darkness through the morning protocol did not significantly alter the results. Once well established, pineal responsiveness (PR) is controlled differently from the signal for endogenous MEL synthesis (EMS) itself, PR persisting after the fall of EMS. PR remains even in early light, and then wanes without a dominant acute influence of light. Activation of a part of the postreceptor cascade in EMS may not be required for the presence of PR.

SYRIAN HAMSTER PINEAL SYMPATHETIC RESPONSIVENESS IN THE EARLY LIGHT PHASE

The Syrian hamster pineal gland may provide a model for sympathetic unresponsiveness (SU), which state characterizes the normal pineal during the light phase in both Syrian hamsters and humans (1). This phenomenon (daytime pineal SU) is potentially of interest, since cardiovascular SU occurs in the terminal stages of critical illness, major trauma, and severe burn injury, when support with sympathomimetics is no longer possible. The pineal of normal humans and hamsters responds to beta-adrenergic stimulation delivered via specific adrenergic innervation to the pineal during each night, producing a nocturnal rise in melatonin (MEL) synthesis and serum levels of MEL. However, the sympathetic stimulation that accompanies psychologically adverse experiences, insulin hypoglycemia, exercise, painful diagnostic procedures, burn injury, isoproterenol (ISO) infusions, and pheochromocytoma has not produced any consistent elevation of circulating or excreted MEL during the daytime in human subjects (2,3).

Similarly, in Syrian hamsters, insulin hypoglycemia (4) as well as systemic injections of (5-9) and pineal incubations with ISO (10) disclosed daytime pineal SU in Syrian hamsters, as did in vivo and in vitro use of norepinephrine with desmethylimiprimine (11). Responses were sought within a 4-h time frame after an injection or during incubation. However, in these hamster studies, the sympathetic beta-agonist given or applied in the second half of the night was able to raise pineal MEL synthesis, which documented nocturnal responsiveness. At night, the animals were exposed to light briefly (20-30 min) just before injection (or before taking the pineals for incubation) in order acutely to lower pineal MEL synthesis which is normally high during the second half of darkness in this species maintained in a regular 14-h light and 10-h dark phase schedule.

In mammals generally, the nocturnal MEL surge is driven by a rise in sympathetic transmission at night via the nerve fibers entering the pineal. In rats, general or systemic sympathetic stimulation even during the day can raise the low daytime pineal MEL content and synthesis (4,10,12). However, in humans and Syrian hamsters, the pineal response so far has seemed mostly restricted to a time roughly corresponding to the normal rise in pineal MEL content and synthesis at night. Interestingly, in most species, even though darkness is necessary for the nocturnal MEL surge to occur, and light exposure at night can prevent it or eliminate it after it begins (9), pineal MEL synthesis and content normally falls before the expected time of lights-on (13).

In the Syrian hamster, the apparently appropriate model for humans (1), the approximate coincidence of pineal responsiveness and the endogenous MEL surge might suggest a similar or common

control over both. However, part of the demonstration of nocturnal responsiveness in this species involved brief 20- to 30-min exposure of the animals to light, which suppressed the endogenous MEL surge but allowed an exogenously stimulated one. Further, separate control of the endogenous MEL surge (through the signal via the pineal sympathetic innervation) on the one hand, and pineal sympathetic responsiveness on the other, was shown by observation of an approximate 4-fold response of pineal MEL (similar to the one in intact animals) after nocturnal injection of norepinephrine and desmethylimiprimine in hamsters previously subjected to superior cervical ganglionectomy (8). This procedure interrupts the postganglionic sympathetic fibers projecting to the pineal, eliminates the endogenous nocturnal MEL surge (14), and does not introduce a pineal response to ISO injection during the day (5). This indicates that the onset of responsiveness is controlled separately from the signal for the MEL surge. Less is known for the offset.

In previous testing at night, the brief period of light, reducing high pineal MEL just before use of an agonist, might be viewed as an artificial interference. This light exposure with the attendant use of the catechol agonist occurred during the time when MEL is otherwise normally elevated and responsiveness might be expected despite the "artificial" lowering of MEL. In the present case, we injected ISO well after the endogenously determined fall of the nocturnally high pineal MEL had occurred.

MATERIALS AND METHODS

Young adult male Syrian hamsters were maintained (4-5 per clear plastic cage with corncob bedding), at least 2 wk in a light:dark cycle of 14:10 (darkness 2000-0600 h) at 25°C with food and water available ad libitum. Procedures in darkness were done in the presence of two 25-W incandescent lamps shielded by Kodak No. 1A red filters which prevent the light from affecting pineal function.

Fourteen groups of 8-10 hamsters each were injected on the back with ISO (1 mg/kg SC) or a physiologic saline vehicle (NaCl) or left uninjected. ISO or NaCl were injected at 0650 h, with samples taken 2 h later. Other groups were injected at 1000 h and sampled 2 h after NaCl injection or 1, 2, or 3 h after ISO injection. Uninjected controls were sampled at 0945 h. On another day, this sequence of events was repeated in darkness extended from the previous night by preventing the lights from turning on at the usual time (0600 h). Sampling was by guillotine decapitation with collection of trunk blood and prompt extirpation and freezing (on dry ice) of the pineal gland. Pineals were kept at -60°C until sonication and dilution in 1 ml phosphate buffered saline (pH 7) with 0.1% gelatin and 100 mg/l thimerosal, with subsequent storage at -60°C until assay of MEL with the Rollag antibody (13). The least detectable value was 10 pg/pineal.

Data were analyzed by student's t test with Bonferroni correction and by ANOVA with use of a VAX-3400 computer.

RESULTS

Figure 1 shows that ISO injection at approximately 1 h after the expected time of lights-on produced a significantly elevated pineal MEL content (vs. NaCl) 2 h later, whether or not the lights were allowed to come on, i.e., in the presence or absence of light. Neither NaCl nor ISO groups differed (light vs. dark). Previous experience has documented that pineal MEL falls to daytime levels prior to 0600 h on this schedule (13).

Uninjected controls at 3.75 h into light (or extended darkness) had the expected low daytime levels. In light or darkness, among the four groups uninjected or receiving NaCl at 4 h into the usual light phase, there were no differences in pineal MEL content. Values from uninjected and NaCl-injected animals were pooled (separately for light and dark) to provide a control estimation for comparison with respective light or dark values obtained in the groups injected with ISO at 4 h into the usual light phase. Two h after this ISO injection, in light or darkness, the relatively small rise in pineal MEL was significant, as it was 1 h after ISO in darkness (Fig 1).

In light, 2 h after the later ISO injection, pineal MEL was lower ($P < 0.01$) than that seen 2 h after the earlier injection. Also in dark, the 2-h post-ISO mean was lower ($P < 0.001$) after the later than after the earlier injection (Fig 1). Thus, the response 2 h after injection was markedly attenuated after ISO given at 4 h into the expected light phase (though it was present) compared to the response after ISO given at 1 h into the usual light time, whether or not novel darkness extended from the previous night throughout the duration of the study.

A 2-way ANOVA (with injection time and light:dark as main effects) analyzing pineal MEL 2 h after ISO revealed the effect of injection time ($P < 0.001$) and a small effect of light versus dark ($P < 0.05$) with use of log-transformed data, suggesting the possibility of a greater response to ISO in extended darkness. However, use of untransformed data and the usual (or even the Brown-Forsythe, not assuming equal variances) ANOVA method showed the effect of injection time, but not of light versus dark. When a 2-way ANOVA was restricted to post-ISO data after the later injection and included sampling time after injection and light:dark as main effects, post-ISO MEL varied with sampling time ($P < 0.05$), but not with light versus darkness, whether or not log-transformed data were used.

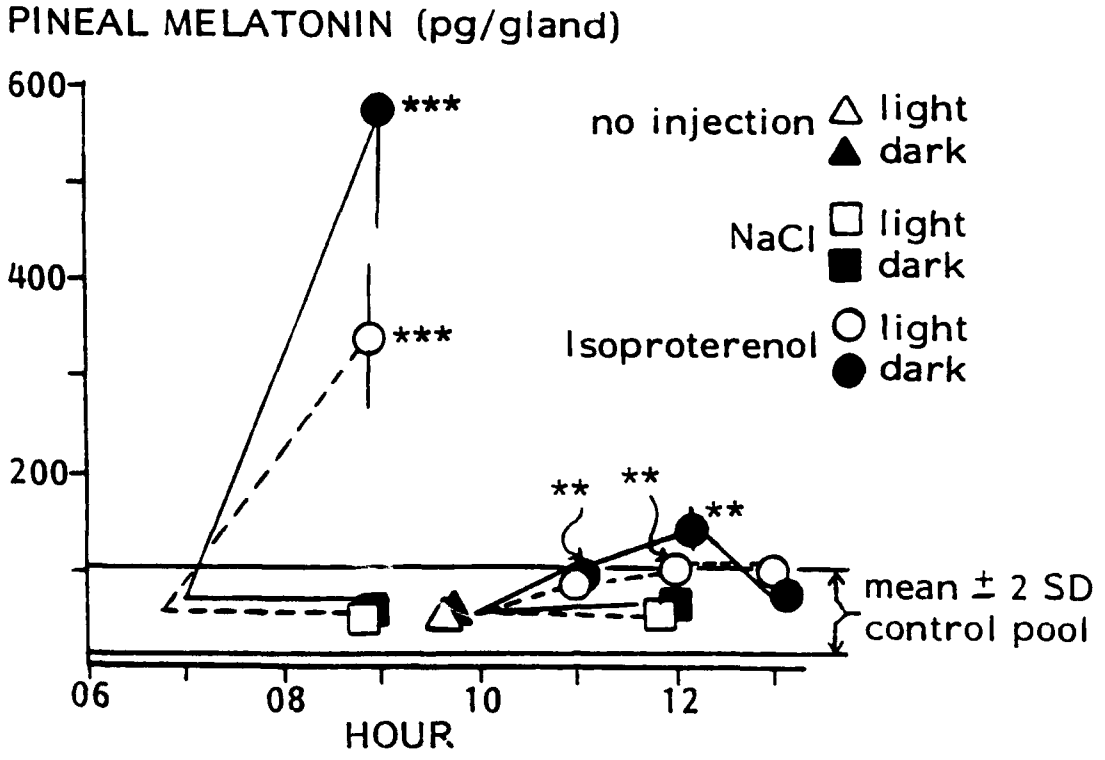

FIGURE 1. Mean ± SE for pineal melatonin content in Syrian hamsters in the morning after prior adaptation to a schedule of 14:10 h (light:dark), with lights-on at 0600 h (light, open symbols). • indicates values from animals for which nocturnal darkness was extended throughout the time of the study. Lines extend from the time of the injection of NaCl or ISO. The control pool refers to all uninjected and NaCl-injected animals. **P < 0.01 vs. subpool of NaCl-injected (taken at 1200 h) and uninjected animals, for light or dark, respectively. ***P < 0.001 vs. NaCl-injected animals (taken at 0900 h).

DISCUSSION

Figure 2 combines the means from the present data with those from previous data (9,13) in order to facilitate comparisons visually among responses 2 h after ISO injection at the end of the light phase, later during the time of the nocturnal MEL surge, and in the first part of the usual light phase in male Syrian hamsters with reference to the normal fluctuation of pineal MEL on a schedule of 14:10 h (light:dark). Previous results (8), not specifically addressing the early light phase, showed that after a single injection in a time period restricted essentially to the second half of darkness, a response to agonists (including ᵀSO) occurred and was maximal at 2 h postinjection.

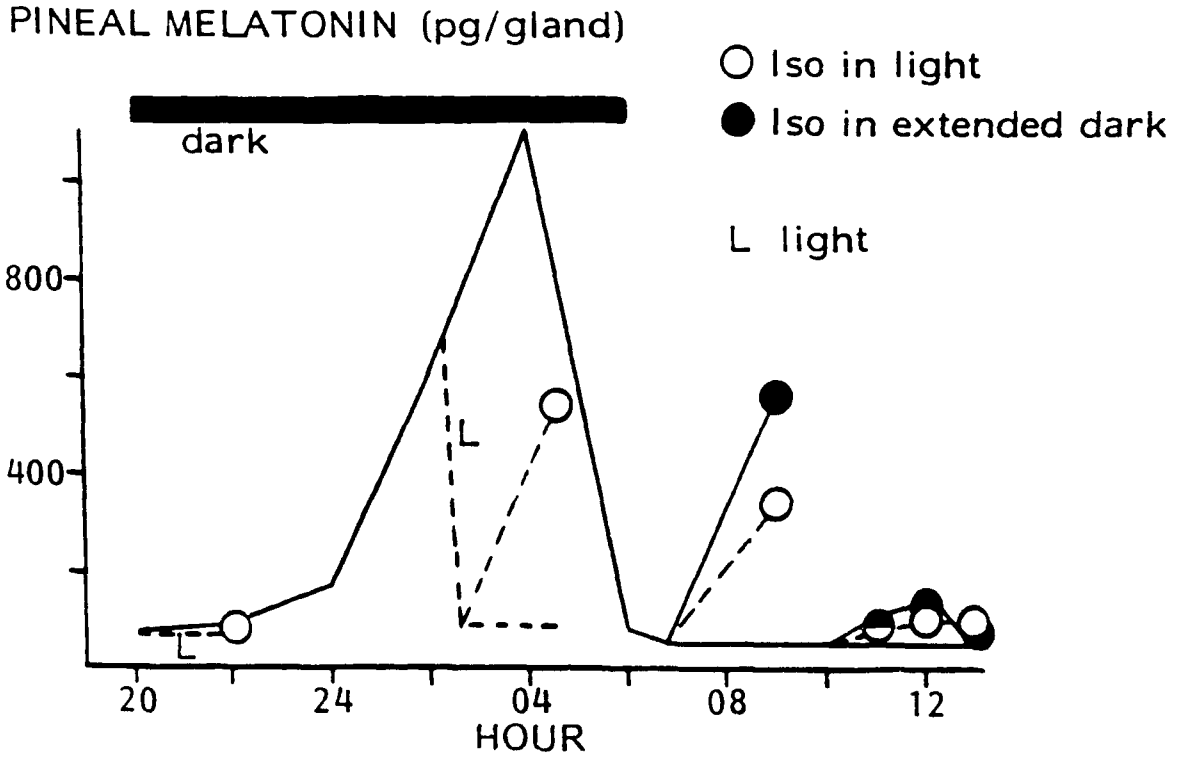

FIGURE 2. Display of data from Figure 1 in relation to data from Vaughan et al (9,13) showing the normal endogenous MEL curve from 2000 h to 1300 h, the response to isoproterenol (ISO) injected at the end of the light phase (2000 h), and the response of MEL to light (L) in the second half of darkness followed by its response to ISO. The horizontal bar near the top indicates the time of darkness.

We now show that the response to this ISO stimulus occurs in the early light phase. At 1 h into light, the stimulus is nearly as effective as in the dark phase and is much less but still effective at 4 h into light. By the middle of the day and later, agents with beta-adrenergic activity appear ineffective (5-11) when responses to various doses in vivo or in vitro are sought for up to 4 h after injection or incubation. This differs considerably from the situation in rats, in which responses can be obtained even in the second half of the light phase (10-12), with stimuli that are ineffective in Syrian hamsters.

In hamsters, neither acute light-induced interruption of the ongoing nocturnal surge of the endogenous sympathetic signal for MEL synthesis, nor the normal fall-off of this signal without ensuing light, nor the fall-off followed by normal morning light exposure eliminates the pineal response to ISO. This indicates that control of the termination of the surge of endogenous MEL synthesis (EMS) is exerted independently of the control for pineal

responsiveness. The latter is terminated later and appears much less influenced by light exposure of the animals. Light exposure can immediately (within 20 min) terminate an EMS surge (Fig 2). The present in vivo responsiveness to ISO observed even after spontaneous EMS termination plus normal morning light exposure is corroborated by a previous observation of an in vitro MEL response to 4 h of 10^{-4} M norepinephrine incubation of pineals taken at 45 min into the normal light phase. The response was of the same magnitude as that seen in pineals taken at night (after acute light exposure), but was markedly diminished in those taken at 2.75 h into light (11). Persistence of responsiveness after light-induced or spontaneous fall of EMS suggests that control of adrenergic responsiveness may not normally be exerted through sympathetic innervation to the pineal, which does control EMS. This notion is corroborated by onset of nocturnal responsiveness to norepinephrine even after superior cervical ganglionectomy (8), though this procedure does block the EMS surge (14). However, ganglionectomy did not lead to supersensitivity (5,8), again suggesting that responsiveness itself may be independent of sympathetic input at the level of the pinealocyte.

However, if hamsters are deprived of darkness (by exposure to light) for the first 6 h or more of the night, then a response to agonist (by 4 h) does not develop or is attenuated (8,11) or may be delayed to 6-8 h after multiple injections (15). Thus, with consideration also of the results after ganglionectomy which involved nocturnal darkness before injection and a response 2 h later, light exposure (but not just absence of the nocturnal sympathetic input) may inhibit the onset of sympathetic responsiveness.

This day/night fluctuation in adrenergic responsiveness in the pineal apparently depends upon a change at a postreceptor stage in the response cascade, because 4-h incubations with forskolin, which bypasses the receptor and directly stimulates adenylcyclase, raised MEL synthesis only in pineals taken during the time of sensitivity to ISO (16). Further, the alteration in pineal sensitivity may occur at a point beyond cAMP. In pineal incubations with a phosphodiesterase inhibitor, forskolin produced the same elevation of cAMP in pineals whether taken in the second half of light or the second half of darkness (17), MEL synthesis rising only in the latter.

Though a prolonged (8-h) incubation with 50 μM forskolin of hamster pineals taken at the end of the light phase produced a small rise in MEL synthesis, a similar incubation begun at 1 h into the light phase resulted in an 8-fold greater rise (18). This corroborates the present findings of extension of the sensitive period into early light in terms of its postreceptor dependence.

Two recent reports show an advancement in the time of the pineal MEL surge after multiple ISO or forskolin injections in the

light phase (15,19). This rise apparently could be brought forward into the end of the light phase (19). Exposure of animals to light from the beginning of the night did not prevent a rise in pineal MEL by 6 h after beginning two-hourly injections of ISO at the expected onset of darkness (15). Thus, prolonged systemic beta-adrenergic stimulation may activate whatever factor produces pineal responsiveness. It is not yet clear how this effect of exogenous sympathetic agonist may be related to the normal activation of responsiveness in consideration of the previously mentioned development and recession of pineal responsiveness without sympathetic input, observed inhibition of the spontaneous onset of responsiveness by light, and the apparent postreceptor and postcyclase level of the determination of responsiveness. However, three possible explanations are considered.

A lag time for buildup of messenger RNA (mRNA) after onset of normal sympathetic input from the beginning of the night, before MEL synthesis responds in the second half of the night has been hypothesized to explain the normal onset of sensitivity. A similar mechanism was proposed to explain the response to catechol after acute light exposure of the animals in the second half of darkness and the response after prolonged exogenous adrenergic stimulation in the normal or extended light phase (18). This apparently would not explain the nocturnal response seen 2 h after injection with prior interruption of pineal sympathetic input by superior cervical ganglionectomy. Another difficulty with this hypothesis is that after acute light exposure given well into the dark phase, MEL synthesis does not rise again unless the exogenous agonist is applied at that time. That implies that any buildup of postreceptor and postcyclase cascade elements from endogenous adrenergic stimulation prior to the procedure is not present without re-induction by the exogenous stimulus. More impressively, the normal falloff of the MEL surge begins at about 2 h before lights-on and is essentially complete by the time of lights-on, indicating absence of active mRNA and dependent elements in the cascade by then. Our present observations of a near normal 2-h response to ISO given 1 h into light and a small response initiated after an injection 3 h later, without a major influence of light, suggest control of responsiveness by a mechanism separate from the sympathetic input at the level of the pinealocytes.

Alternatively, it is possible to hypothesize that beta-adrenergic influence at other sites (perhaps including distant or local pineal vascular endothelial cells) may stimulate release of other agents, e.g., IL 1, TNF, endothelin, nitric oxide, arachodonate metabolites) that might act (with a delay) on pineal cells to induce postreceptor postcyclase adrenergic responsiveness persisting past the time of catechol stimulation of the other sites. Perhaps greater general sympathetic activation with the onset of nocturnal motor activity (perhaps blocked by extension of daytime light) could provide a rise in catecholamines at an appropriate site in darkness that would provide the stimulus for

producing the intermediate agent(s) from such a site to induce adrenergic responsiveness in pineal cells even in superior cervical ganglionectomized animals.

Another possibility is that glucagon might suppress pinealocyte catecholamine responsiveness. With little eating during the light phase, plasma glucagon might be relatively high. The onset of darkness is associated with initiation of feeding which probably reduces the need for glucagon to maintain constant circulating glucose concentration. An eventual fall in plasma glucagon (from the inhibitory effects both of the increased glucose availability and the rise of insulin) could allow responsiveness to develop by the time it usually does. With onset of relative fasting later in the night or during the day, glucagon would rise and reduce pineal responsiveness. Prolonged ISO injection during the day may stimulate glycogenolysis and/or gluconeogenesis as well as insulin secretion, all perhaps depressing glucagon, allowing eventual pineal responsiveness to develop. Another version of this possibility is that insulin might promote pinealocyte sympathetic responsiveness. Insulin secretion is stimulated by food intake and ISO administration. This general possibility, related to meals and pancreatic secretion would seem unlikely with respect to humans, whose food intake occurs mainly during the day rather than during the night when the sympathetics stimulate the nocturnal MEL surge. These and other hypotheses to explain the so far enigmatic rhythm of pineal adrenergic responsiveness in Syrian hamsters and humans remain to be tested.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
				DA314707	1 Oct 89	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
1 Oct 88	D. Chg	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62787A	3S162787A874	DA	165
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP	FY90-01		

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	9209	DA	C. In-House

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

a. DATE EFFECTIVE		18. RESOURCES ESTIMATE: a. FISCAL YEARS	a. PROFESSIONAL WORK YEARS	b. FUNDS *(In thousands)*
b. CONTRACT/GRANT NUMBER				
c. TYPE	d. AMOUNT	89	0.4	7
e. KIND OF AWARD	f. CUM/TOTAL	90	0.4	8

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	f. 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; (U) Peptides; (U) Growth Hormone; (U) Wound Healing; (U) Pharmacology; (U) Volunteers; (U) Adults; (U) RA II

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

23. (U) A DTIC literature search was conducted under DTIC request number W6R00J dated 19 October 1989 for the technical report database and request number W6R03I dated 19 October 1989 for the work unit database. The proposed effort will not result in the duplication of effort. The objective of this work is to evaluate the effects and safety of recombinant human growth hormone when given to accelerate the rate of wound healing in burned patients.

24. (U) 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 was 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) 8810 - 8909. Ten patients have been enrolled in this study to date, with 2 patients enrolled during this reporting period. Addenda have been approved. Upon completion of patient entry, data will be analyzed for the effect of recombinant human growth hormone on wound healing in thermally injured patients. For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1989.*

DD FORM 83 MAR 1498 EDITION OF MAR 88 IS OBSOLETE. * U.S.G.P.O. 1988-491-003/503.

ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3S162787A874-00, APPLIED RESEARCH

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

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

1 October 1988 - 30 September 1989

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ABSTRACT

PROJECT NUMBER: 3S162787A874-00, APPLIED RESEARCH

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

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

PERIOD COVERED IN THIS REPORT: 1 Oct 88 through 30 Sep 89

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This double-blinded, randomized, placebo-controlled, multicenter study was designed to determine whether the administration of recombinant human growth hormone could accelerate wound healing in thermally injured patients. Preliminary data have suggested that the administration of growth hormone can promote anabolism in surgical patients. The administration was accompanied by accelerated wound healing. Burn patients in this study were randomized to receive either 5 or 10 mg/day of recombinant human growth hormone or a placebo, with administration commencing on the day of their first surgery. The rate of donor site healing was used as an index of the effect of growth hormone on wound healing in these patients.

At the end of this reporting period, 10 patients from this Institute had been enrolled in the 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. Again, this lack of response may be secondary to inadequate drug dosing or the fact that therapy was initiated at various times following resuscitation. No untoward effects secondary to administration of recombinant human growth hormone were noted in this group of patients.

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

The development of genetic enegineering 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 is 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 efficacy of this hormone on clinical indications.

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 acclerated wound healing and an apparent decrease in morbidity and hospitalization time.

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' donor graft sites. However, healing of the primary burn, duration of hospitalization, and mortality will also be evaluated.

MATERIALS AND METHODS

Study Design. This is a multicenter, randomized, double-blind study. Patients are 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 are randomized to treatment or control groups by the Biostatistics and Data Management Division of Genentech, Inc. (South San Francisco CA). Groups are balanced for age, cause, and extent of burn.

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

Number of Patients. Up to 100 patients will be entered into 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 is used. A 25% reduction in healing time, 3-4 days, is 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 provides 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).

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.

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

1. Male or female patients $\geq$ 18 and $\leq$ 80 yr old. Female patients must have been surgically sterilized, be postmenopausal (> 45 yr old and the lack of menstrual periods for > 1 yr), or have 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 is the "test wound" and is 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 who have undergone successful resuscitation without major complication.

5. Patients who are 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 are eligible and may require mechanical ventilation, but a satisfactory PO_2 and PCO_2 will be required 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 are eligible.

Exclusion Criteria. Patients with the following characteristics are excluded from the study:

1. Patients < 18 or > 80 yr old.

2. Patients who are 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 have had complications undergoing resuscitation.

6. Patients who are 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 which require specific therapy.

10. Patients with associated injuries to the chest or abdomen which require 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 requiring chronic glucocorticoid or nonsteroidal anti-inflammatory drugs.

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

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

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

Wound Care. Graft donor sites evaluated as part of this study are on the anterior upper thigh, buttock, or upper arm. These are the usual and preferred sites for taking skin for grafting. Skin is taken from the site with a dermatome set at 10/1000s of an inch in thickness and full width. Two designated dermatomes are used exclusively for patients enrolled in this study. In addition, skin from the donor site is harvested by only two investigators. Only the first harvest of a donor site is used for this study. Fine-mesh gauze is applied to all donor sites. Bed cradles are used to assure that bed sheets do not displace the fine-mesh gauze

and the patient is positioned so that the donor site is exposed to the air.

Laboratory Studies. Laboratory studies (see Table 1 for the study plan flow chart) are performed weekly and include complete blood count, 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 are 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 are measured. A 3-ml sample is 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 are then shipped to Genentech, Inc., via Federal Express as soon as possible after the sample is drawn.

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

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

Nutrition. Near constant nutritional intake is provided beginning on the first postoperative day. This provides at least 80% of energy and protein requirements (calculated by standard formulae). The nutrients are 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 can be provided, but they are not more than 25% of the estimated total requirements. Carbohydrate intake does not exceed 6 mg/kg/min (about 600 g/day for the usual patient). Nutrients are provided by the enteral or enteral-parenteral routes throughout the study and the route is altered according to the clinical course of the patient. Intake is monitored by the hospital dietitian and nutrition is supervised by a single nutritionist.

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.

End Points.

Donor sites. On the third postoperative day, the wound is examined by a trained evaluator. The donor site wound is measured and photographed using a standard camera and distance. Using sterile technique, each of the four corners of the fine-mesh gauze is gently lifted to determine if the dressing is adherent to the underlying skin. This is done using sterile forceps and minimal tension. At the end of the examination, the unattached fine-mesh

gauze is trimmed away with scissors and the dressing rephotographed. This examination procedure is performed each day until the fine-mesh gauze is completely removed. Complete removal indicates complete wound healing. Using the measurements and photographs, the fraction of the wound covered with fine-mesh gauze (unhealed) is plotted as a function of time. The time to 90% wound closure is compared between treatment and control groups.

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

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

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

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

Statistical Analyses. Mortality (survival time) and healing time of the donor graft site are the primary end points. Differences between treatment and control groups will be 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 donor graft site for patients who do not die will also be 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 are secondary end points, the analysis of which will be similar to that for healing time of the donor graft site. Nutritional intake and infection rates will also be secondary end points for which appropriate comparisons will be made between treatment and control groups.

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

RESULTS

Ten patients from this Institute and 34 patients overall have been enrolled in this study to date. 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 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 treatment group and 8-14 days for the control group. Again, this lack of response may be secondary to inadequate drug dosing or the fact that therapy was initiated at various times following resuscitation. No untoward effects secondary to administration of recombinant human growth hormone were noted in this group of patients.

DISCUSSION

Because of inconsistent and varied responses to growth hormone administration as indexed by donor site healing and IGF-1 response, the design of this study was changed. In an attempt to assure adequate dosing of recombinant growth hormone, the dose was changed so that it is now indexed to body weight (0.2 mg/kg). In addition, instead of starting treatment on the day of first grafting, patients begin treatment as early as possible following resuscitation and stabilization, with treatment continuing for the duration of hospitalization. When 100 patients have been enrolled in the study, the data will analyzed for the effect of recombinant human growth hormone on wound healing in thermally injured patients.

PRESENTATIONS/PUBLICATIONS

None.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION DA312333	2. DATE OF SUMMARY 1 Oct 89	REPORT CONTROL SYMBOL DD-DRA&E(AR) 636
3. DATE PREV SUM'RY 1 Oct 88	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	62787A	3S162787A874	CA	166
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP,	FY90-01		

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

12. SUBJECT AREAS
0605 Medicine and Medical Research

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
8703	9009	DA	C. In-House

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

a. DATE EFFECTIVE		18. RESOURCES ESTIMATE		
b. CONTRACT/GRANT NUMBER		a. FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
c. TYPE	d. AMOUNT	89	0.1	8
e. KIND OF AWARD	f. CUM/TOTAL	90	0.1	8

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	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) High Frequency Ventilation; (U) Inhalation Injury; (U) Thermal Injury; (U) Pneumonia; (U) Morbidity;

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

23. (U) A DTIC literature search was conducted under DTIC request number W6Q15K dated 19 October 1989 for the technical report database and request number W6Q18M dated 19 October 1989 for the work unit database. The proposed effort will not result in the duplication of effort. The objective of this work is to evaluate the use of high frequency ventilation in the treatment of respiratory failure in patients with inhalation injury with respect to incidence of pneumonia, time of initial onset and duration of pneumonitis, total duration of ventilatory support, and the age- and burn size-adjusted mortality.

24. (U) Initial high frequency ventilation support will be established by adjustment of the driving pressure, rate, and inspiratory-expiratory ratio. A chest roentgenogram will be obtained and sputum or endotracheal secretions will be examined by culture and Gram stain. It will then be determined how high frequency ventilation affects outcome after inhalation injury with respect to several well defined factors as compared to an historical cohort using conventional ventilation. The incidence of pneumonia will be related to burn size by appropriate burn-size stratification within the study group.

25. (U) 8810 - 8909. Twenty-seven patients have been enrolled in this study, 9 during this reporting period. After the entry of 50 patients, the effects of high frequency ventilation on the outcome after inhalation injury with respect to several well-defined factors, to include mortality and pulmonary morbidity, will be compared to an historical cohort. For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1989.*

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ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3S162787A874-00, APPLIED RESEARCH

PROJECT TITLE: High Frequency Ventilation in Patients with Inhalation Injury

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

1 October 1988 - 30 September 1989

INVESTIGATORS

William G. Cioffi, Jr., MD, Major, MC
Theresa A. Graves, MD, Captain, MC
William F. McManus, MD, Colonel, MC
Basil A. Pruitt, Jr., MD, Colonel, MC

ABSTRACT

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

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High frequency ventilation is being assessed as a therapeutic modality for the treatment of patients with inhalation injury. This study is designed to compare the incidence of pneumonia and the effect on mortality of high frequency ventilation in patients with an inhalation injury when compared to an historical cohort. To date, 27 patients have been enrolled in this study, 9 during this reporting period. There have been 8 deaths in this patient group, which compares to a predicted number of 8 (95% CL 2-12). Eight patients developed pneumonia, slightly less than predicted. Although the present results do not indicate a significant survival advantage with the use of high frequency ventilation, the incidence of pneumonia for this group of patients appears to be decreased.

HIGH FREQUENCY VENTILATION IN PATIENTS WITH INHALATION INJURY

Inhalation injury continues to be a significant occurrence in the thermally injured patient (1). With the advent of topical chemotherapy, burn wound sepsis has been greatly reduced. However, this has been replaced by bacterial pneumonia as the primary cause of infectious morbidity and mortality stemming from the initial burn injury. Inhalation injury has been shown to predispose to the development of bacterial pneumonia, with 38% of patients with inhalation injury developing pneumonia as compared to 8.8% of those patients without inhalation injury (2). Furthermore, in this most recent analysis, it has been shown that inhalation injury complicated by pneumonia results in a maximum 60% increase in mortality as opposed to a maximum 20% increase in mortality when inhalation injury alone is added to burn mortality.

The pathophysiology of inhalation injury is the subject of active research. Extensive tracheobronchial injury is known to result from the initial insult, with sloughing of respiratory mucosa and resultant impairment of the normal mucociliary clearance mechanism of the tracheobronchial tree. In addition, disruption of the alveolar capillary membrane results in a protein-rich plasma exudate in the terminal airway which, coupled with impaired transport, serves as a setup for bacterial overgrowth and consolidated pneumonia. Injury to Type II pneumocytes with impairment of surfactant production contributes significantly to the pathologic process.

Current treatment modalities for progressive respiratory insufficiency resulting from inhalation injury involve support with conventional volume-cycled positive pressure ventilators to promote alveolar ventilation. Supplemental oxygen and standard methods of tracheobronchial toilet are also employed. Additionally, systemic antimicrobial treatment, as dictated by results of cultures and sputum microbiology, are administered to patients who develop clinical pneumonia.

Unfortunately, the outcome observed with conventional mechanical ventilatory management is usually one of ever worsening pneumonia. A documented spiral of pulmonary dysfunction ensues with progressive pulmonary shunting and carbon dioxide retention, necessitating ever increasing minute ventilation. Difficulty in arterial oxygenation necessitates administration of toxic levels of oxygen. Decreasing pulmonary compliance results in excessive peak airway pressures to maintain alveolar ventilation. This adds significant barotrauma which may cause further airway damage.

A means of pulmonary support which would more effectively aid in the recovery from the initial insult would be of great clinical benefit. An alternative to the current method of ventilatory support which has proven to be of some benefit in other settings

requiring artificial ventilation has been the high frequency ventilator (3-4). This technique is approved for use where large pulmonary air leaks preclude adequate ventilation with conventional techniques, e.g., bronchopleural fistulas (5). This method has also been shown to be efficacious during operative procedures where a quiet operative field is of benefit (6), to prevent deoxygenation during tracheal suctioning (7), in the treatment of aspiration (8), while performing endotracheal tube changes (9), and during laryngoscopy (10).

High frequency ventilation (HFV) has been reported to be effective in a number of clinical and laboratory trials (11-12). This technique employs tidal volumes less than physiologic dead space (1-5 ml/kg) at rapid respiratory rates (1-15 hertz) as compared to conventional ventilation which employes high tidal volumes at low frequencies. The utility of this method of ventilatory support is still being defined, but several potential advantages over conventional ventilation exist in the burn patient. For a given level of PEEP, peak airway pressure is significantly less for HFV than conventional ventilation, thus conceivably reducing the risk of barotrauma (2,13-15). Also, smaller fluctuations in peak airway pressure and mean airway pressure associated with HFV may have less effect on hemodynamic function. Oxygenation is also improved, presumably secondary to increased diffusion, which permits use of lower levels of inspired oxygen. Additionally, recruitment of collapsed alveoli is prompted by the oscillatory nature of the inspired breaths in association with the use of PEEP. Limited clinical experience with HFV in burn patients has shown that clearance of secretions from the tracheobronchial tree is dramatically increased when a patient is converted from conventional ventilation to HFV. Increased turbulence of flow has been postulated as the responsible mechanism. Conventional ventilation, on the other hand, delivering large tidal volumes at high pressures only drives these secretions farther into lower airways, exacerbating the pneumonitic process. The ability to ventilate burned patients with inhalation injury at lower peak and mean airway levels, lower levels of PEEP, and lower FIO_2 while at the same time enhancing clearance of secretions and improving recruitment of collapsed alveoli should at the very least limit the compounding damage normally superimposed by conventional ventilation on the initial insult. Whether this will allow the lung to recover more quickly and reduce the incidence of pulmonary complications is unknown.

The objective of this study is to evaluate the effect of HFV in the treatment of respiratory failure in patients with inhalation injury with respect to incidence of pneumonia, time of initial onset and duration of pneumonitis, total duration of ventilatory support, and the age- and burn size-adjusted mortality.

MATERIALS AND METHODS

Patient Population. Up to 50 patients will be enrolled in this study based on eligibility and informed consent.

Criteria for Admission to the Study. Patients admitted to the US Army Institute of Surgical Research with evidence of inhalation injury are offered the opportunity to participate in this study.

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

1. Male or female patients ≥ 18 yr old. Female patients are previously surgically sterilized or 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 admitted to the Institute within 48 h postburn. Patients aeromedically transferred who were previously on conventional ventilatory support are eligible for entry if the total duration of ventilatory support was < 48 h.

3. Patients with a history of inhalation injury confirmed by xenon lung scan or bronchoscopy. All patients with a positive xenon lung scan have a bronchoscopy.

4. Patients with a clinical need for ventilatory support as deemed appropriate by current clinical practice.

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

1. Any patient < 18 yr old.

2. Any patient admitted to the Institute > 48 h postinjury.

3. Any patient without confirmed inhalation injury.

4. Any pregnant patient.

5. Any patient being treated with antibiotics for diagnosed pneumonia. Administration of prophylactic antibiotics does not exclude entry into the study.

Procedure Prior to Patient Entry. Any patient deemed eligible based on the above criteria have the following documented prior to initiation of the study:

1. A history and physical examination.

2. A chest roentgenogram within 24 h.

3. Arterial blood gases within 6 h.

4. Sputum culture and microscopic examination within 24 h.

5. Informed consent.

High Frequency Percussive Ventilator. High frequency percussive ventilation (HFPV) is delivered by a high frequency pulse generator (Bird Space Technologies, Percussionaire Corporation, Sand Point, ID). Gas from the high frequency pulse generator is delivered through a nongated sliding venturi connected to a standard endotracheal tube. The venturi entrained humidified gas from a fresh bias flow provided from the ventilator. The system combines a series of high frequency sub-dead space volume breaths with a variable I:E ratio. Initially, the I:E ratio for the subtidal volume breaths is 1:1. Periodic interruption of high frequency pulsatile flow is programmed to allow return of airway pressure to baseline CPAP. The ratio of the duration of the percussive phase to the duration of baseline CPAP is adjusted as a means of manipulating oxygenation and CO_2 elimination. Additionally, peak airway pressure can be adjusted to maintain adequate CO_2 elimination. The frequency of the sub-dead space breaths is maintained between 200-600 breaths/min. FIO_2 and PEEP are adjusted to maintain an O_2 saturation > 90%.

Study Design. Initial HFV support is established with a high frequency, flow interrupter-type ventilator. No other modifications are made in the patient's care. Fluid resuscitation is managed as clinically indicated and nutritional support is provided to meet increased metabolic demands. Excision and grafting of the burn wound is carried out as clinically indicated. As is the routine in patients with purulent sputum and physical findings suggestive of pneumonia, a chest roentgenogram is obtained and sputum or endotracheal secretions are examined by culture and Gram stain. Patients exhibiting clinical signs and symptoms of respiratory tract infection and characteristic pneumonic infiltrates on chest roentgenogram receive antibiotics, with modifications in therapy based on clinical and microbiologic criteria.

The recent experience with inhalation injury in 373 patients during a 5-yr period at this Institute was reviewed (3). The specific contribution of inhalation injury and pneumonia to age- and burn size-dependent patient mortality was defined in this population using multiple logistic regression analysis. In addition, postburn day of diagnosis of pneumonia in patients with inhalation injury and those without inhalation injury was defined. In the present study, we will determine how HFV affects outcome after inhalation injury with respect to these well defined factors in comparison to the historical cohort using conventional ventilation. The incidence of pneumonia will be related to burn

size by appropriate burn size stratification within the study group.

RESULTS

Twenty-seven patients were enrolled into this prospective study of the use of HFPV in patients with inhalation injury from March 1987 to September 1988. The predicted mortality was 8 patients, with an actual mortality of 8 patients. Eight patients developed pneumonia, slightly less than the predicted incidence.

DISCUSSION

When inhalation injury accompanies cutaneous burns, mortality is disproportionately higher than that predicted by the extent of cutaneous injury alone (16). This synergistic effect is most apparent in patients with burns associated with a 40-60% mortality. Inhalation injury alone results in a maximum 20% increase in mortality. The incidence of bacterial pneumonia is greatly increased by the presence of inhalation injury, with 38% of patients with inhalation injury developing pneumonia as compared to 8.8% of burn patients without inhalation injury. When inhalation injury was moderate to severe, i.e., diagnosed by bronchoscopy, 48% developed pneumonia. The addition of pneumonia to inhalation injury results in a maximum 60% increase in mortality for this group of patients (2).

The pathophysiology of inhalation injury is complex and not fully understood. Extensive tracheobronchial injury may result in sloughing of the respiratory tract mucosa and impairment of the normal mucocilliary clearance mechanism. Slough of the mucosa results in cast formation which obstructs moderate-sized airways, leading to distal atelectasis, or a ball-valve effect, leading to distal air trapping and increased barotrauma. Disruption of endothelial and epithelial integrity permits exudation of protein-rich plasma into terminal airways which serves as media for bacterial overgrowth and subsequent development of pneumonia. Injury to Type II pneumocytes impairs surfactant production and contributes significantly to the pathologic process. The parenchymal changes are associated with hypoxemia and hypercapnia due to the shift of the V/Q relationship to the left, i.e., an increase in lung segments with V/Q ratios > 0 but < 1. This change in V/Q has been adequately documented in animal models of inhalation injury (17).

The goal of any new treatment regimen for inhalation injury should be the reversal of these pathophysiologic changes. A second and equally important goal is that the treatment causes no further injury. For patients with moderate to severe injury who require mechanical ventilation, current conventional ventilators do not accomplish either of these goals. Clearance of secretions is not enhanced, and the complications of high inflation pressures and

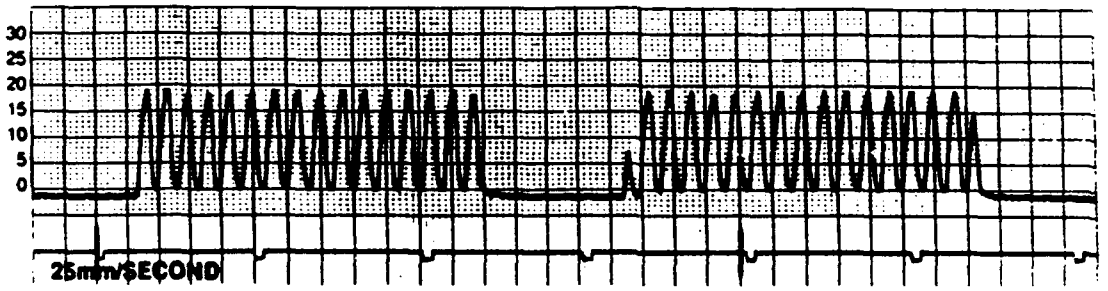
high FIO_2 compound the existing injury. The goal of ventilator therapy should be adequate oxygenation on a nontoxic FIO_2 and CO_2 clearance at the lowest possible inspiratory pressures, with maintenance of functional residual capacity above closing volume. Better clearance of secretions and a shift of the V/Q mismatch back to the right are also desirable. Various reports have concluded that high frequency ventilation may accomplish all of these goals (13,18-23).

The major characteristics of HFV include a ventilatory frequency > 60 breaths/min, tidal volumes of less than dead space, lower peak airway pressures than conventional ventilation, positive endotracheal pressures throughout the ventilatory cycle, lower transpulmonary pressures than in conventional ventilation, increased FRC, possibly less circulatory interference than with conventional ventilation, and more efficient pulmonary gas distribution than with conventional ventilation (20). These characteristics of HFV should make it the ideal form of ventilatory support for patients with significant inhalation injury but it should be noted that each of these reported advantages has been refuted in various reports (24-26).

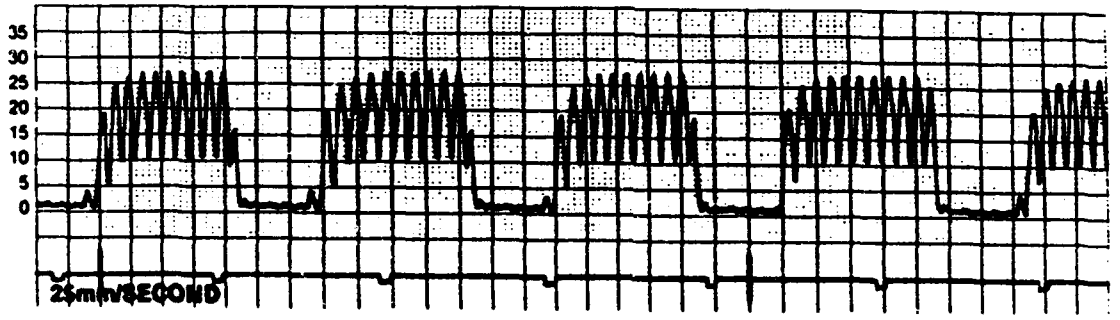
The safety of HFV has been documented in several reports. Its use in postoperative general surgery and cardiac surgery patients has demonstrated no untoward hemodynamic effects (27). Peak airway pressures and mean airway pressures are reduced and several studies have demonstrated decreased pulmonary shunt flow with the use of this type of ventilatory support. Two recent reports have documented the effectiveness of short-term salvage use of HFV in patients with ARDS (19,20). Oxygenation was improved and adequate CO_2 clearance was maintained in each patient.

Carlon et al (11) reported a series of 309 patients who were randomized to high frequency jet ventilation or conventional ventilation, respectively. Mortality from underlying disease was quite high in that study; however, the pulmonary dysfunction was not severe. Mean ventilator time was 2.5 days. Peak airway pressures were lower in the HFV group as compared to the conventional ventilation group. There was a 4% incidence of barotrauma in each gorup. Overall outcome did not differ between the two groups. Standard high frequency jet ventilation at a rate of 100 breaths/min and an I:E ratio of 1:2 was used in each patient. The only manipulated variable was driving pressure. In an attempt to standardize the ventilatory support for each patient, the versatility of high frequency jet ventilation was not utilized, i.e., frequency and I:E ratios were not altered to obtain the best possible result.

HFPV is an exceptionally versatile form of HFV in which high frequency subtidal breaths are superimposed upon conventional ventilation (Fig 1). This ventilator combines the entrainment



A



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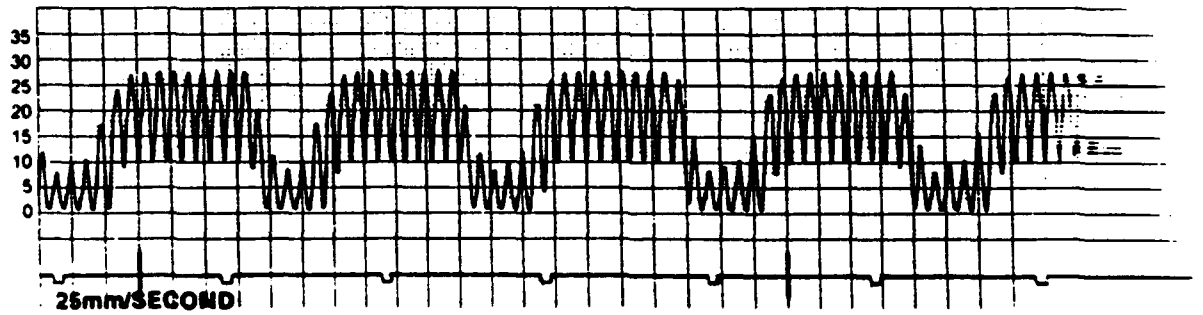

C

FIGURE 1. Representative wave form tracings from proximal airway using HFPV. A, Standard wave form with periodic interruption of pulsatile flow. Note reverse I:E ratio. B, Standard wave form with higher inspiratory pressures and increased convective gas flow. C, Standard wave form with institution of oscillatory PEEP during period of interruption of pulsatile flow.

mechanism of high frequency jet ventilation with the ability to manipulate airway pressure in a phasic manner.

The results from the prospective use of HFV in this study are favorable. The degree of pulmonary insult was moderate to severe in all patients as assessed bronchoscopically. Using data collected from an historical cohort treated at this Institute between 1980-5, one would predict an approximate 40% mortality for this group of patients (2). Additionally, approximately one-half of the patients should have developed pneumonia. In actuality, all patients survived and only 8 patients developed pneumonia during their hospital course. Although no firm conclusions can be drawn because of the small number of patients, a favorable trend is noted. To determine whether HFV significantly improves survival, approximately 50 study patients will be needed.

The results of this study support but do not confirm the opinion that HFV is effective in the treatment of patients with severe ARDS. Although it is unlikely that HFV represents a panacea for all forms of respiratory failure, the results suggest that HFV will be useful in the treatment of patients with severe inhalation injury. These patients were different than those with severe ARDS in that the insult was acute in nature, and given the proper circumstances, should heal in 10-21 days. If during this period of spontaneous airway repair one can provide adequate ventilatory support, not increase the risk of barotrauma, and at the same time facilitate clearance of airway secretions and debris resulting from the injury, one may be able to decrease the morbidity and mortality associated with inhalation injury.

PRESENTATIONS

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

PUBLICATIONS

Cioffi WG, Graves TA, McManus WF, and Pruitt BA Jr: High-frequency percussive ventilation in patients with inhalation injury. **J Trauma** 29(3):350-4, March 1989.

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undergone cardiac operations. **J Thorac Cardiovasc Surg** 89:269-74, 1985.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
				DA313318	1 Oct 89	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
1 Oct 88	D. Chg	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62787A	3S162787A874	EC	167
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP,	FY90-01		

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

12. SUBJECT AREAS
0605 Medicine and Medical Research

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
8710	9009	DA	C. In-House

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

a. DATE EFFECTIVE		c. RESOURCES ESTIMATE		
b. CONTRACT/GRANT NUMBER		a. FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS *(In thousands)*
c. TYPE	d. AMOUNT	89	1.0	25
e. KIND OF AWARD	f. CUM/TOTAL	90	1.0	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 LUSTER, S H
d. TELEPHONE NUMBER *(Include area code)* 512-221-2720	d. TELEPHONE NUMBER *(Include area code)* 512-221-5957
21. GENERAL USE FINA MILITARY/CIVILIAN APPLICATION: M	f. NAME OF ASSOCIATE INVESTIGATOR *(If available)* PATTERSON, P E g. NAME OF ASSOCIATE INVESTIGATOR *(If available)* CIOFFI, W G

22. KEYWORDS *(Precede EACH with Security Classification Code)* (U) Metacarpophalangeal Joint Stiffness; (U) Dynamic Flexion Splint; (U) Force Quantification; (U) Volunteers:

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

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

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

25. (U) 8810 - 8909. A prototype stiffness monitoring device capable of recording changes in reactive force and joint angle was designed and tested. The device was then redesigned to facilitate data collection in the clinical setting. Technical problems were encountered when testing the second prototype and it was returned to the contractor for repair. Further technical testing and initiation of the normal finger and comparative treatment studies are pending return of the device. For technical reports, refer to they *US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1989.*

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

ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3S162787A874-00, APPLIED RESEARCH

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

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

1 October 1988 - 30 September 1989

INVESTIGATORS

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ABSTRACT

PROJECT NUMBER: 3S162787A874-00, APPLIED RESEARCH

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

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

PERIOD COVERED IN THIS REPORT: 1 Oct 88 through 30 Sep 89

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An electronic device capable of measuring finger joint stiffness has been developed and used to evaluate the effects of dynamic flexion splinting on the recovery of joint motion in patients with burned hands. The device locates an angle of primary (greatest) resistance and the reactive torque at that angle for a selected joint. Using the device, 4 subjects with stiff hands were measured before and after dynamic splinting treatments. During the 3-day treatment period, there were statistically significant differences in the angle of primary resistance ($P < 0.0001$) and reactive torque ($P < 0.001$). This initial trial suggests that finger stiffness can be quantified in terms of reactive torque as well as joint excursion; dynamic rubber-band flexion splinting does alter joint condition, allowing increased motion; the amount of initial joint stiffness may be an indicator of treatment outcome; and increasing treatment time may not enhance outcome.

QUANTIFICATION OF DYNAMIC SPLINT FORCES ON METACARPOPHALANGEAL FUNCTION RECOVERY

Stiffness of hand joints with functional impairment is a frequent complication following burn injury. In addition to tissue damage from the original injury, prolonged edema and subsequent immobilization following skin grafting contribute to loss of motion. While protection of the extensor hood mechanism of the proximal interphalangeal joints is of major importance in the management of the severely burned hand (1), metacarpophalangeal (MCP) joint mobility must also be maintained. Loss of motion at these joints can severely limit hand function, as they serve to position the distal phalanges to perform full opening or closing of the hand as well as all other manipulative motions. Following burn injury, the MCP joints are subject to the development of stiffness from scarring over the collateral ligaments and adhesions of the dorsal synovial pouch, extensor tendon, and volar plate (2). Maintaining and, if necessary, regaining MCP joint motion is one of the primary goals of rehabilitation of the burned hand.

Dynamic splinting is commonly used in burned hand rehabilitation (3-6). This type of splinting uses an outrigger, rubber bands, and finger slings to apply moderate force to a joint for an extended period of time. Because human tissues are viscoelastic, they respond to the forces applied by a dynamic splint in specific ways (7). If force is applied for a short period of time, the tissue will display an elastic response in which it will be elongated but recover its initial shape when the force is released. If the force is of low magnitude and prolonged, the tissue will display a plastic response and remain elongated after removal of the force. This elongation is thought to be a result of cell proliferation and reorientation in the stressed tissue (8).

Elongation of the tissues surrounding an impaired joint allows increased joint motion which can be measured with a goniometer. This angular measurement alone, however, does not describe the stiffness of the joint. Joint stiffness is a measure of the joint's resistance as it is moved through a given range-of-motion. Currently, there is no reliable objective method to measure joint stiffness in the hand. The treatment outcome of splinting and other rehabilitation procedures is therefore generally measured in terms of joint excursion without reference to the force required to achieve that excursion. An additional problem concerns the selection of the force levels applied with dynamic splints. Splinting force levels are based primarily on subjective judgment of the therapist as modified by the need to avoid local tissue ischemia resulting from finger-sling pressure. Splinting procedures continue to be more an art than a science. The ability to quantify the stiffness of a joint and surrounding tissue could

lead to a better understanding of the effects of dynamic splinting and to the development of objectively based treatment protocols.

The objectives of this study are to develop an instrument to quantify joint stiffness in burned hands and to measure the effects of different treatment protocols on recovery of range of motion in impaired MCP joints.

MATERIALS AND METHODS

A device capable of concurrently producing and measuring forces similar to those applied to a phalanx by a dynamic splint was designed and constructed. The complete system consists of a dorsal thermoplastic wrist control splint that supports an electromagnetically controlled hydraulic piston linked to a finger "cradle" (Fig 1), a single-board microcomputer configured to control the position and rate of movement of the piston, and a lap-top computer that serves as a master controller and provides data logging and analysis functions. The system (Fig 2) was configured to apply a torque at a constant 90° angle to the proximal phalanx which would match the reactive torque being generated by the involved joint. Because the joint tissue components are viscoelastic, the loading rate was kept constant (0.5°/sec) for all applications. As the phalanx was moved through its range-of-motion, the resulting curve of reactive torques (measured in inch pounds) and their associated angular locations were recorded. The first measurement logged was the angle at which initial resistance was encountered. The computer then calculated the rate of change (torque/°) and located the point at which the largest change occurred. This point was called the point of primary resistance and, along with its corresponding reactive torque, was selected to characterize joint stiffness. Figure 3 shows the shape of the torque and angle curve and location of the angles of initial and primary resistance for a noninjured MCP joint.

Four male patients with a total of 20 stiff MCP joints following burn injury participated in the initial trial of the device. After obtaining informed consent, each patient's joints were measured with the device and, on the basis of initial values of primary resistance, each joint was assigned to either a high or low stiffness group. The division of the stiffness groups was arbitrarily defined by the midpoint of the range of initial of resistance values. The joints were evenly assigned to the groups so that the half with the highest angle, i.e., greater motion before reaching their point of primary resistance, were placed in the low stiffness group and the remainder to the high stiffness group. The patients within each of the stiffness groups were then randomly assigned to one of two treatment groups. Each group received dynamic flexion splinting of the MCP joints with a ventral rubber-band-powered dynamic splint exerting 400 g of force on each joint. Group I received 1 h of treatment per day and Group II

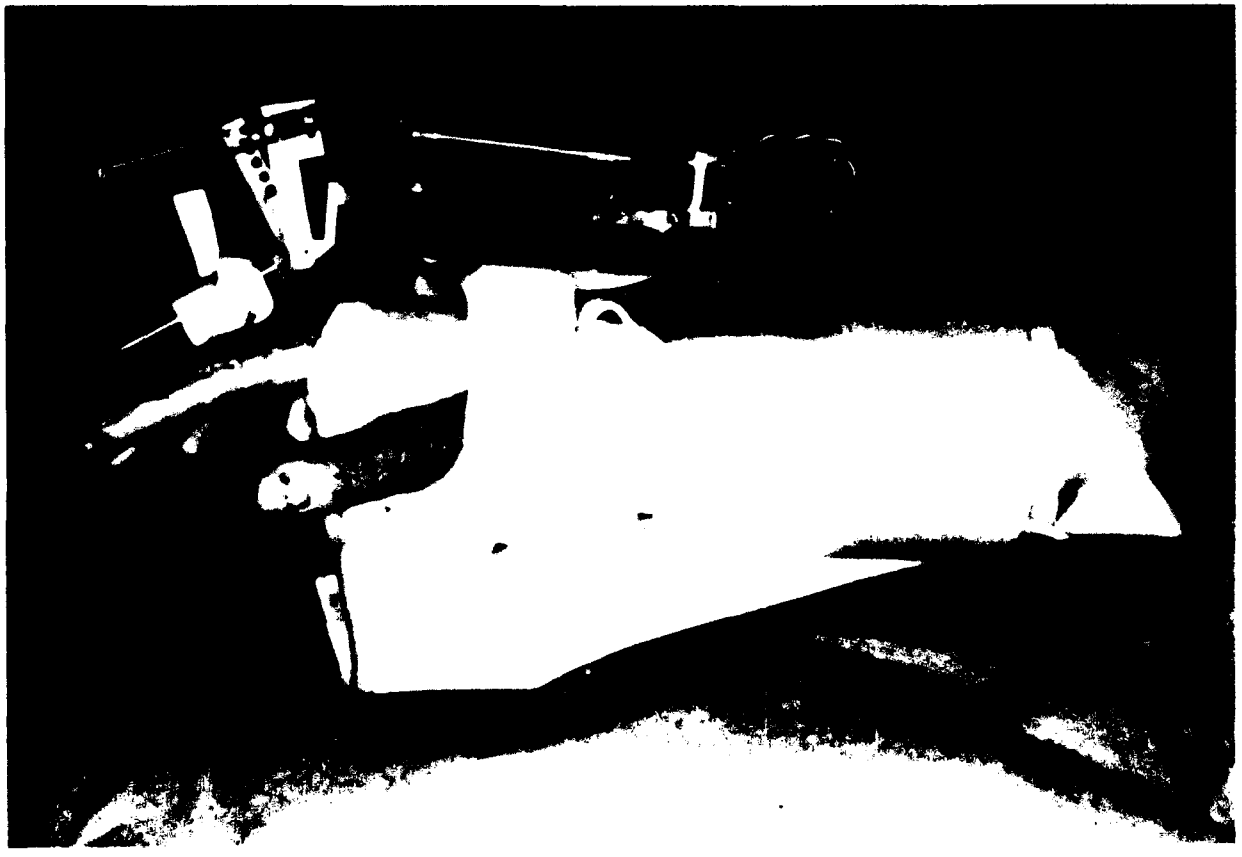

FIGURE 1. Electronic finger joint stiffness measurement device.

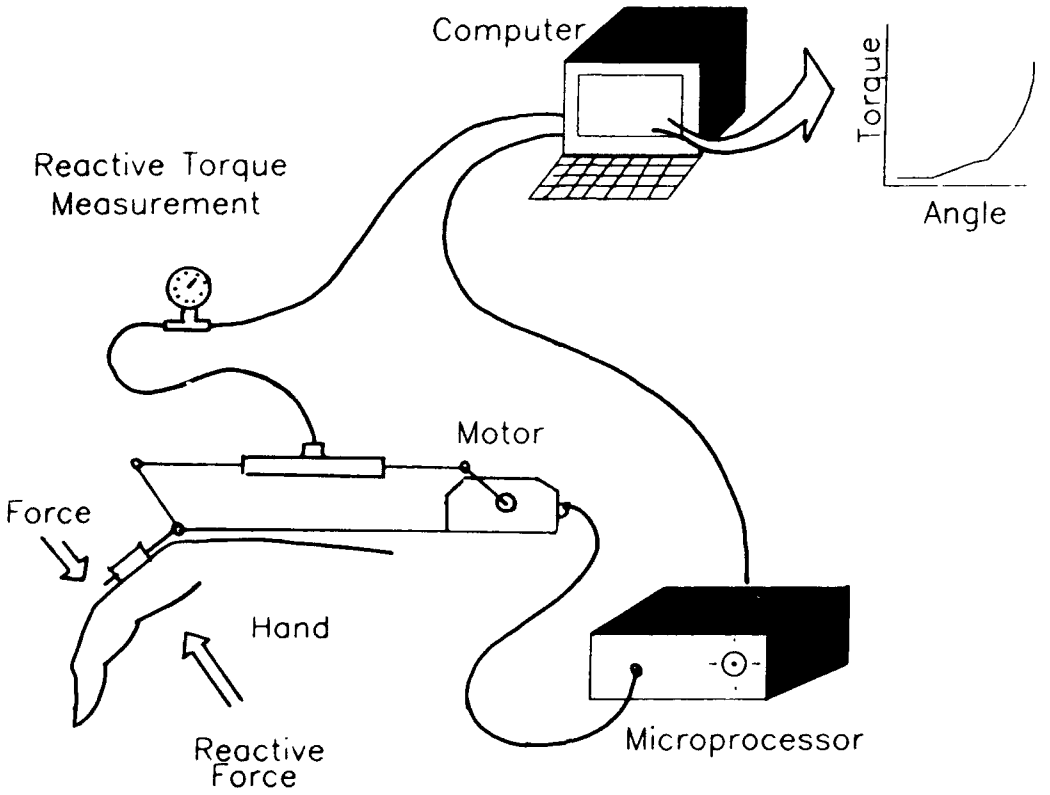

FIGURE 2. Mechanical and electronic components of the finger joint stiffness measurement device. A microprocessor controls an electric motor which applies force to a finger through a hydraulic piston system which concurrently measures the resistance of the joint. A computer calculates the angle of the joint from the motor actuator arm, logs the data, and generates a torque and angle curve for the measured joint.

received 1 h of treatment twice daily. Each patient received 3 days of treatment; measurements of primary resistance were taken before and after each session. On day 6, after 2 days of no splinting, a fourth measurement was taken to assess the effects of discontinuing treatment.

Statistical Analysis. Two-way ANOVA of treatment days and the variables, angle of initial resistance, angle of primary resistance, and reactive torque was performed.

RESULTS

Figure 4 shows the plots of the primary resistance points for the 20 fingers for treatment days 1-3 compared with day 6. The shifting of the points to the right over the three treatment days

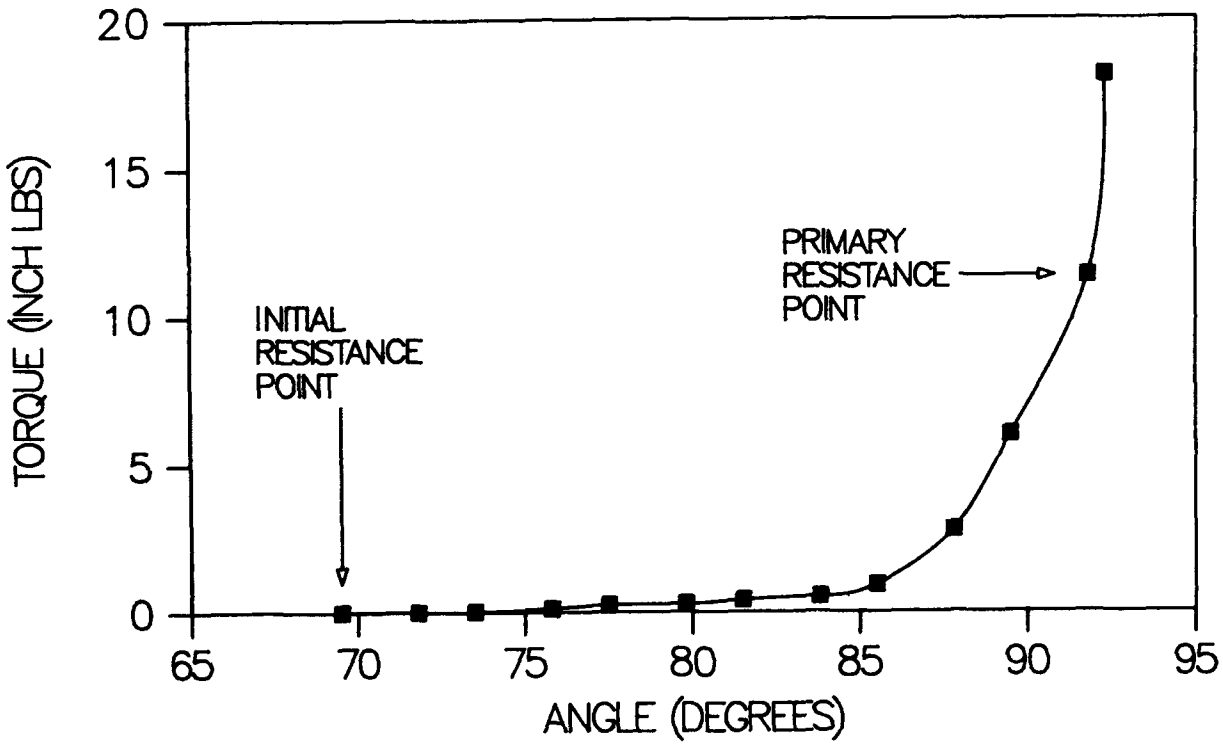

FIGURE 3. Noninjured finger torque and angle curve showing points of initial and primary resistance.

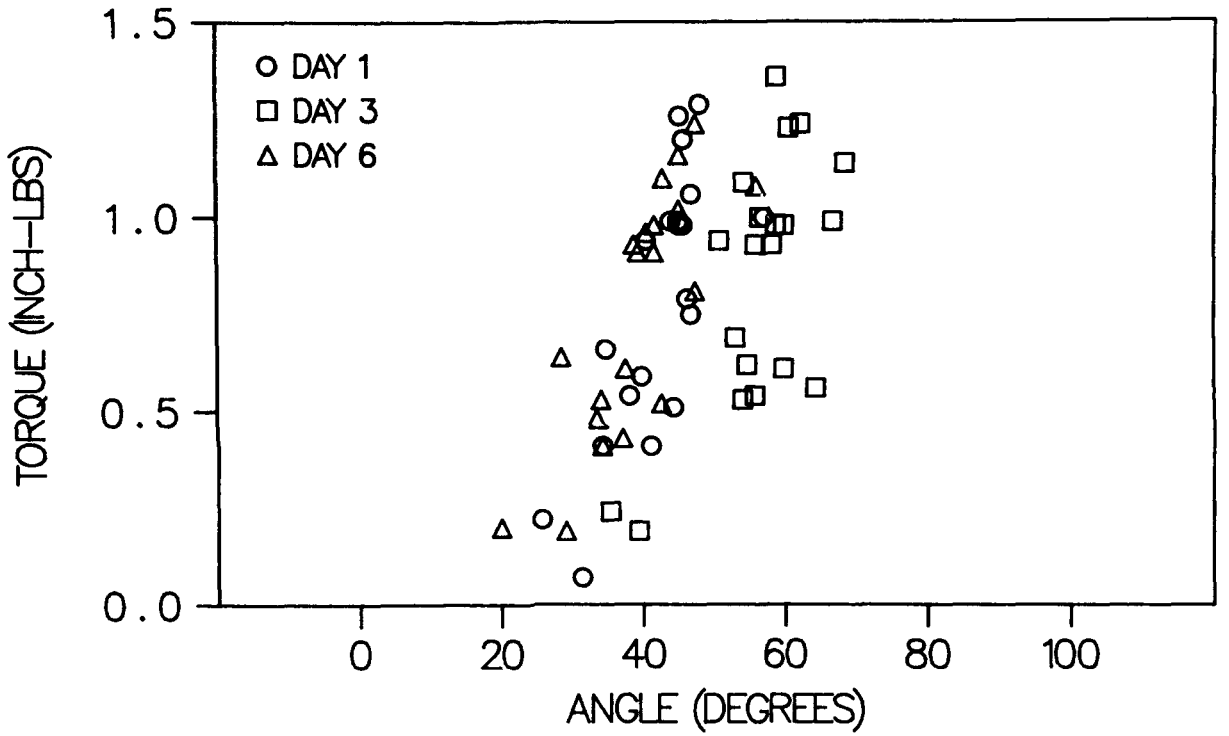

FIGURE 4. Plots of primary resistance points for 20 injured fingers for days 1-6.

suggests a decrease in joint stiffness. On day 6, however, the points of primary resistance shifted back to the left, indicating a return to pretreatment levels of stiffness. The ANOVA among treatment days and angles of initial and primary resistance and reactive torque demonstrated significant differences. There was a mean increase of 6.8° in the initial angle of resistance between treatment days 1 and 3, followed by a decrease of 2.3° after 2 days of no splinting ($P < 0.0001$). The mean angle of primary resistance increased by 15°, then decreased by 3° ($P < 0.0001$). Mean reactive torque increased by 2.3 inch pounds between days 1 and 3, then increased an additional 1.7 inch pounds between days 3 and 6 ($P < 0.001$). Figure 5 compares the change of mean values of the three variables over the treatment period. There was an increase in all three variables over the treatment days. There was also a change in the relationship between the angle of primary resistance and reactive torque. On day 1, the angle of primary resistance was less than reactive torque. On day 2, they were equal and, by day 3, the angle of primary resistance was greater. A Duncan multiple-range test comparing the high and low stiffness groups for differences in treatment days, angular position of initial resistance, reactive torque, and length of treatment time was performed. There were statistically significant differences between the low and high stiffness groups in angular position of primary resistance ($P < 0.05$) and reactive torque ($P < 0.0001$). There were no significant differences between the 1- and 2-h treatment groups.

DISCUSSION

The results of this initial trial of a finger joint stiffness measurement device showed that splinting effects can be measured in terms of joint stiffness (reactive torque) as well as range of joint motion. Normal joints demonstrate minimal reactive torque through the majority of their excursion but a rapid increase in torque at the end of the range. Impaired joints show an increase in torque much earlier in the range of motion. In treating impaired joints, the desired treatment effect would therefore be to decrease reactive torque during the maximal range-of-motion. Data from the three treatment days suggest that while dynamic flexion splinting of the MCP joints does increase joint range-of-motion, reactive torque also increases. It is, however, interesting to compare the rate of change. The angle of primary resistance increased at a faster rate than reactive torque. It is possible that this was an early indication of the viscous tissue in which the elongation of joint structures allowed increased excursion without matching reactive torques. The loss of joint motion following the termination of treatment on day 3 supports previous clinical observations that short-term treatment effects are generally temporary. The significant differences in posttreatment joint stiffness between the low and high stiffness groups further suggest that the initial stiffness of a joint may be an indicator of treatment outcome. While an optimum duration of treatment was

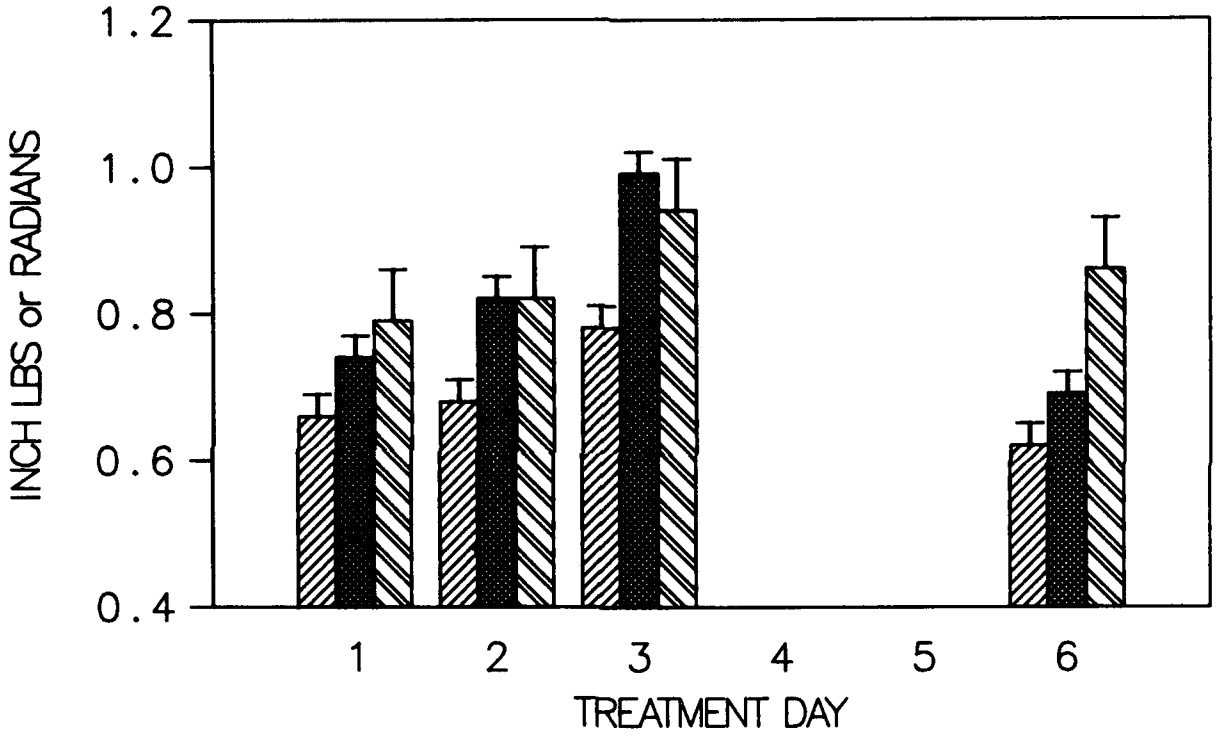

FIGURE 5. Change in mean values of initial and primary resistance points and reactive torque for days 1-6. ▨ Angle of initial resistance (radian, = 57°) ± SEM, ■ angle of primary resistance (radian, = 57°) ± SEM, and ▧ reactive torque (inch pounds) ± SEM.

not defined, the lack of significant change in joint stiffness as the result of treatment frequency does suggest that increased treatment time may not necessarily enhance outcome.

While this trial of the splint-monitoring device was limited in subject number and study duration, the results suggest that the study of hand joint function and postinjury recovery may be enhanced by analysis of reactive torque as well as range of joint motion. The device will be used to document joint motion characteristics of noninjured and injured joints, outcome comparisons of a variety of hand treatment modalities and, ultimately, the dynamic splint force levels and treatment durations required for optimum hand rehabilitation.

PRESENTATIONS/PUBLICATIONS

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

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION: DA314853	2. DATE OF SUMMARY: 1 Oct 89	REPORT CONTROL SYMBOL: DD-DR&E(AR) 636
3. DATE PREV SUM'RY: 1 Oct 88	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	62787A	3S162787A874	F	169
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP.	FY90-01		

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

12. SUBJECT AREAS: 0605 Medicine and Medical Research 0615 Pharmacology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
8806	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)
89	0.8	32
90	0.8	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): 512-221-4440
21. GENERAL USE: FINA 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; (U) Immunosuppression; (U) Colony-Stimulating Factor; (U) Granulocytes; (U) Macrophages;

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

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

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

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

25. (U) 8810 - 8909. Ten patients were enrolled in this study during this reporting period. No significant untoward effects of human recombinant granulocyte macrophage colony-stimulating factor were noted in these patients. Analysis of granulocyte function data is now being performed. For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1989.*

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

ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3S162787A874-00, APPLIED RESEARCH

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

US ARMY INSTITUTE OF SURGICAL RESEARCH
FORT SAM HOUSTON
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1 October 1988 - 30 September 1989

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Arthur D. Mason, Jr., MD
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ABSTRACT

PROJECT NUMBER: 3S162787A874-00, APPLIED RESEARCH

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

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We studied the effects of granulocyte-macrophage colony-stimulating factor in burn patients. Serial measurements of granulocyte oxidative function were obtained in treated patients and in a group of controls matched for age and total burn size. The administration of granulocyte-macrophage colony-stimulating factor resulted in a 50% increase in mean leukocyte counts. Both groups showed significant baseline increases in granulocytic cytosolic oxidative function. Treated patients showed normal stimulated cytosolic oxidative function, which was significantly depressed when compared with that of untreated patients ($P < 0.05$). Myeloperoxidase activity was increased in treated patients during the first postburn week but then declined to normal levels. Untreated patients had a significant increase in myeloperoxidase activity for the first 3 weeks following injury. Untreated patients exhibited a significant decrease in superoxide activity during the second 3 weeks following injury ($P < 0.01$). Treated patients demonstrated normal superoxide activity.

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

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

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

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

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

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

from the administration of CSFs. Our study was designed to determine the safety of the administration of human rGM-CSF in patients with thermal injury.

MATERIALS AND METHODS

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

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

Drug Administration. Patients were administered 3 or 10 μg/kg of human rGM-CSF intravenously over 4-h period. Treatment began within 5 days of injury and continued for a minimum of 2 wk or until a Grade 3 or 4 toxic reaction developed. All potential adverse effects were recorded on the following scale: 1 = mild, 2 = moderate, 3 = severe, and 4 = life-threatening. Any patient who experienced a Grade 3 or 4 toxic reaction that was deemed attributable to the human rGM-CSF received no further treatment until the adverse reaction resolved. The patient was then retreated at a dosage not more than 50% of the original dose. Recurrence of the same toxic reaction necessitated withdrawal from the study. In patients who exhibited a WBC count > 50,000/cm3, subsequent doses of the lymphokine was withheld until the WBC count decreased to < 30,000/cm3. Administration was then resumed at a dose of 30-50% of the original dose.

In vitro Testing. CBCs were obtained daily from each patient. In vitro granulocyte function tests were performed twice daily during treatment up to 3 wk following cessation of the lymphokine. Granulocytes were isolated from heparinized whole blood by Ficoll-Hypaque gradients. Cells passing through the gradient were recovered from the cell pellet. Contaminating RBCs were removed by

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

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

Statistical Analysis. Differences between groups were analyzed with use of the t test and ANOVA, with post-hoc testing, when appropriate, with use of the BMDP statistical package (University of California, Berkeley, CA).

RESULTS

Ten patients with a mean age of 28.6 yr and mean burn size of 37% were enrolled in the study. Individual patient data, including the dose of human rGM-CSF, and the duration of treatment are outlined in Table 1. Two patients, both with inhalation injury, died, for a mortality rate of 20%. Fourteen patients with thermal injury with a mean age of 30.5 yr and mean burn size of 36%, admitted during the same time period, were used as nonrandomized controls for comparison of oxidative metabolism data. There was no statistical difference between the two groups of patients with respect to age, burn size, and mortality rate, although a greater proportion of untreated patients had inhalation injury (Table 2). Grades 1 and 2 adverse effects were common. Seven patients complained of pruritus, 4 demonstrated pyrexia with human rGM-CSF administration, 2 complained of back pain, and 1 experienced pleuritic chest pain. Acute parostitis and a subcutaneous abscess, which required incision and drainage, occurred in 1 patient each.

Blood Count Data. Patients receiving human rGM-CSF demonstrated a significant increase in total WBC count during the second postburn week as compared to the first, third, fourth, fifth, sixth, and seventh postburn weeks (Fig 1). One patient receiving 10 μg/kg of human rGM-CSF had a WBC count > 50,000/mm^3 during the second postburn week. The lymphokine administration was discontinued for 2 days, during which time the WBC count decreased to 26,000/mm^3, and treatment was resumed at 3 μg/kg/day. The majority of treated patients demonstrated a relative decrease in their WBC counts during the third postburn week despite continued administration of human rGM-CSF. Compared to the untreated patients with thermal injury, the patients receiving human rGM-CSF exhibited a significant elevation in their WBC counts only during the second postburn week. The percentage of granulocytes was not different between treated and untreated patients with thermal injury during the first three weeks. However, on cessation of the human rGM-CSF administration, a significant decrease in the percentage of granulocytes was noted in the treated patients compared with the untreated burn patients (63.5% vs. 80.9%) (Fig 2). The percentage of PMN cells was not different during treatment but decreased significantly during the fourth postburn week compared with untreated patients (43.3% vs. 71.2%), accounting for the difference in the granulocyte percentages (Fig 2). No statistically significant differences between treated and untreated patients were noted in the percentage of monocytes, lymphocytes, myelocytes, or band forms either during or after treatment, although patients receiving the cytokine tended to have an increased percentage of band forms and myelocytes during treatment.

Dichlorofluorescein Oxidation. No significant difference in baseline unstimulated cytosolic oxidative activity was noted between the two patient groups although both were significantly higher than values for unburned controls (Table 3). Patients

TABLE 1. Patient Demographics*

Patient Number	Age (Yr)	Sex	Total Burn Size (%)	3° Burn Size (%)	Dosage (µg/kg)	Duration of Treatment (Days)
1	24	Male	36	32	3	17
2	45	Male	39	5	3	17
3	24	Male	24	18	10	17
4	27	Male	20	17	10**	12
5	24	Male	35	-	3	12
6	35	Female	23	23	3	11
7	23	Male	45	42	3	17
8	22	Male	54	44	3	5
9	21	Female	42	8	3	29
10	41	Male	52	10	3	2

*GM-CSF administration was stopped on days 5 and 2 in patients 8 and 10, respectively, because of worsening pulmonary status. Both patients had abnormal xenon 133 lung scan but normal bronchoscopic findings. The degradation in pulmonary function was not temporally related to the administration of GM-CSF. **Dosage decreased to 3 µg/kg during course of study.

TABLE 2. Comparison of Treated and Untreated Groups*

Group	Age (Yr)	Total Burn Size (%)	Inhalation Injury (+/-)	Mortality (Dead/Total)
Treated	28.6 ± 2.7	37.1 ± 3.8	2/10 (20%)	2/10 (20%)
Untreated	30.6 ± 3.0	36.2 ± 3.0	6/14 (43%)	0/14 (0%)

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

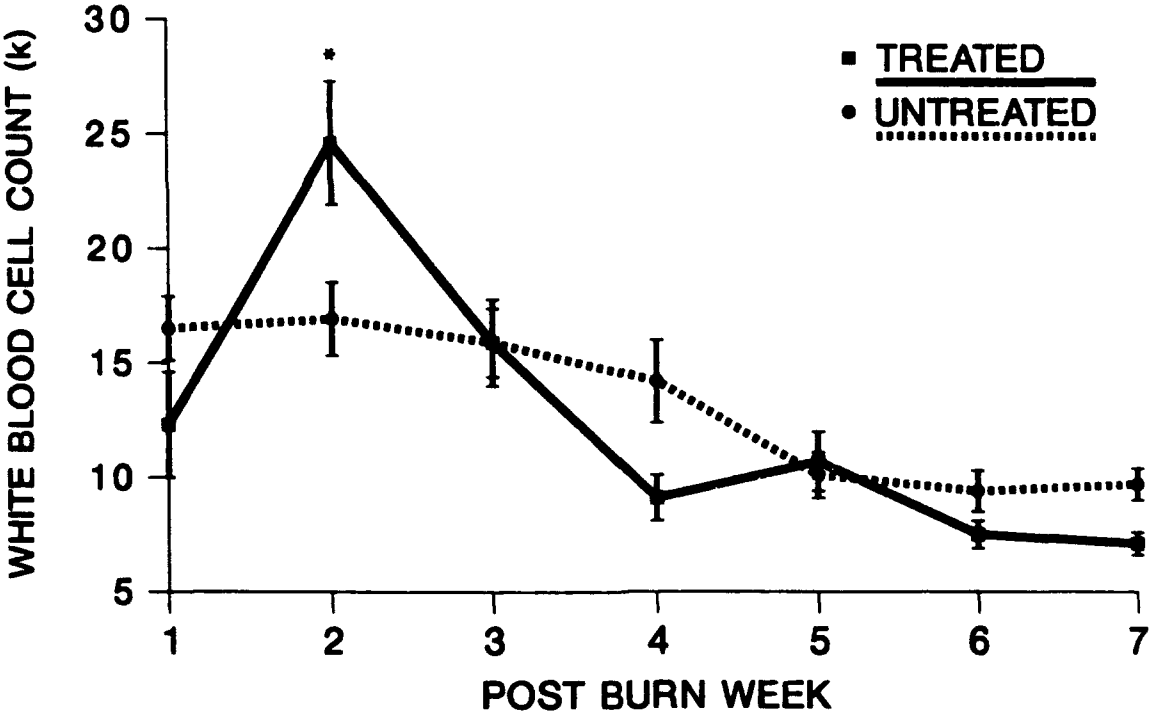

FIGURE 1. WBC counts during the first 7 postburn weeks are shown for treated (solid line) and untreated (broken line) patients. The only significant difference was detected during the second postburn week ($P < 0.05$). Vertical bars indicate SD.

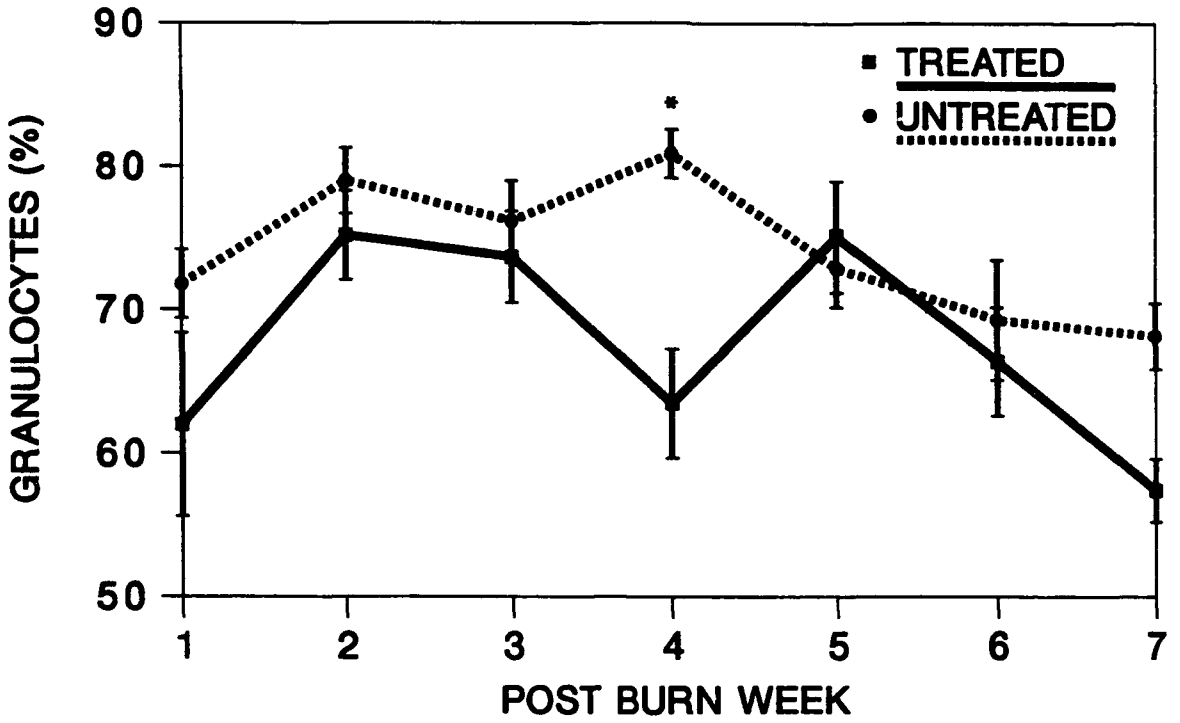

FIGURE 2. The percentages of granulocytes for treated (solid line) and untreated (broken line) patients are displayed for the first 7 postburn weeks. No differences between groups were evident, except during postburn week 4, when treated patients had significantly fewer granulocytes than untreated patients. The percentage of lymphocytes increased in treated patients, but the difference between groups was not significant. Vertical bars indicate SD.

receiving human rGM-CSF exhibited a significant decrease in maximal cytosolic oxidative activity compared with untreated patients with thermal injury (92.9% vs. 114.7% of control values, $P < 0.01$) during the 3 weeks of treatment. Upon cessation of cytokine administration, the peak cytosolic oxidative activity of treated patients increased slightly to 97% of that for controls, whereas untreated patients remained elevated at 113% of control values (Table 4).

Chemiluminescence. During the first 7 days following injury, both treated and untreated burn patients exhibited a significant increase in luminol chemiluminescence compared with healthy controls. This increase was independent of the type of stimulation employed to activate the granulocytes, as the response to opsonified zymosan and PMA were essentially identical (Table 5). After 1 week, oxidation of luminol following stimulation by opsonified zymosan and PMA decreased to control values for the

TABLE 3. Cytosolic Peroxidase Activity for Postburn Days 0–21 (Mean ± SD)

Group	PTUR	PTSR
Untreated	0.243 ± 0.01	1.150 ± 0.05
Treated	0.249 ± 0.01	0.929 ± 0.06

PTUR indicates the ratio of the mean log fluorescence for unstimulated patients' cells to stimulated control subjects' cells (normal, 0.16). Both patient groups were significantly different from controls ($P < 0.05$). PTSR indicates the ratio of the mean log fluorescence for stimulated patients' cells to stimulated control subjects' cells (normal, 1.0). Untreated patients were significantly different from treated patients and controls ($P < 0.05$).

TABLE 4. Cytosolic Peroxidase Activity for Postburn Days 22–42 (Mean ± SD)*

Group	PTUR	PTSR
Untreated	0.223 ± 0.02	0.972 ± 0.05
Treated	0.227 ± 0.01	1.140 ± 0.04

*Values are for the 3 weeks after cessation of human rGM-CSF. PTUR indicates the ratio of the mean log fluorescence for unstimulated patients' cells to stimulated control subjects' cells (normal, 0.16). The PTUR for both patient groups remained elevated compared with controls ($P < 0.05$). PTSR indicates the ratio of the mean log fluorescence for stimulated patients' cells to stimulated control subjects' cells (normal, 1.0). The PTSR for untreated patients remained significantly different from treated patients and controls ($P < 0.05$).

treated patients but remained elevated for the untreated patients. During the third postburn week (the last week of therapy with human rGM-CSF), luminol chemiluminescence remained unchanged in the untreated patients and increased significantly in the treated patients in response to PMA but not opsonified zymosan administration. After 21 days, luminol chemiluminescence began to decrease in untreated patients but remained significantly elevated in treated patients in response to PMA administration.

The oxidation of DBA, an indicator of extracellular superoxide production, normally declines as time after injury progresses. In

TABLE 5. Chemiluminescence Data (Mean ± SD)

Group (n)	LOZ	LPMA	DPMA
	Postburn Days 0–7		
Control (137)	2344 ± 179	2482 ± 158	14371 ± 944
Untreated (14)	4060 ± 686	6635 ± 784	11076 ± 1803
Treated (11)	6355 ± 3047*	7523 ± 4513*	10932 ± 4956
	Postburn Days 8–14		
Control (137)	2344 ± 179	2482 ± 158	14371 ± 944
Untreated (24)	4298 ± 912*	5330 ± 763**	12036 ± 1670
Treated (15)	2302 ± 750	2989 ± 861	14248 ± 4934
	Postburn Days 15–21		
Control (137)	2344 ± 179	2482 ± 158	14371 ± 944
Untreated (22)	4779 ± 987*	4919 ± 678*	13110 ± 2170
Treated (14)	3649 ± 593	3943 ± 632***	19812 ± 4904
	Postburn Days > 21		
Control (137)	2344 ± 179	2482 ± 158	14371 ± 944
Untreated (74)	3120 ± 319	4009 ± 897	8781 ± 9611**
Treated (44)	3217 ± 469	5141 ± 1166***	16791 ± 2422

LOZ indicates opsonified zymosan-stimulated luminol chemiluminescence; LPMA, phorbol myristate acetate (PMA)-stimulated luminol chemiluminescence; and DPMA, PMA-stimulated dimethyl biacridinium dinitrate chemiluminescence. *Significant difference compared with controls ($P < 0.01$). **Significant difference compared with treated patients ($P < 0.01$). ***Significant difference compared with controls ($P < 0.05$).

this group of untreated patients with thermal injury, the mean luminescence value was 87% of the control patients' mean value during the first three weeks and 61% of the control value ($P < 0.01$) during the second three-week period following injury (Table 5). Patients treated with human rGM-CSF failed to show a decrement in oxidation of DBA, with luminescence values similar to those for controls during the first 2 weeks following injury and 137% of those for controls during the third and final week of treatment. The DBA chemiluminescence remained significantly elevated at 117% of control values when administration of human rGM-CSF was discontinued.

DISCUSSION

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

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

The in vitro effect of GM-CSF on WBCs isolated from healthy volunteers has been well documented. Although GM-CSF has little effect on WBC function alone, it appears to "prime" the cell for increased oxidative function when activated in vitro by physiologic chemoattractants, such as PMA, FMLP, C5a, leukotriene B_4, and opsonified zymosan (38). Chemotaxis, cytotoxic and phagocytic activity, superoxide production, and degranulation are all increased by prior incubation with GM-CSF (39).

Few data exist concerning the effect of parenteral GM-CSF on various WBC functions in patients with documented functional defects. Defects in granulocyte phagocytosis and bactericidal capacity in two patients with AIDS were reversed with the parenteral administration of GM-CSF (38). Reductions in phagocytic capacity, nitroblue tetrazolium reduction, and migration were

restored to normal by administration of GM-CSF in one patient with chronic idiopathic neutropenia (35).

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

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

The level of DBA chemiluminescence, which primarily indexes superoxide anion production, was significantly affected by the administration of human rGM-CSF. During the first 3 weeks after injury, granulocytes from untreated patients showed normal to slightly decreased PMA-stimulated chemiluminescence when DBA was used as a probe. During the subsequent 3 weeks, this defect was exaggerated. In patients receiving GM-CSF, DBA chemiluminescence was only slightly depressed during the first postburn week, normal during the second postburn week, and supranormal during the third week of drug administration. After discontinuation of GM-CSF administration, DBA chemiluminescence remained normal and did not decrease in contrast to that in the untreated patients. The maintenance of DBA chemiluminescence following cessation of GM-CSF administration indicates that the effect of the cytokine is not direct, because the half-life of circulating neutrophils is significantly less than 1 day.

The administration of parenteral GM-CSF to patients with thermal injury but without inhalation injury appears to be safe and resulted in the expected increase in circulating numbers of

granulocytes. Whether this compound can be safely administered to patients with inhalation injury cannot be answered from our study. Although both patients with inhalation injury who received GM-CSF died, deterioration in the status of these patients was not temporally related to its administration. A more complex question is whether or not the effect of parenteral administration of GM-CSF on WBC function is beneficial. Restoration of superoxide production by stimulated cells has the potential for both beneficial and adverse effects. An increase in extracellular superoxide may lead to an increase in capillary permeability due to endothelial injury from adherent WBCs. The reduction in myeloperoxidase activity might also be viewed as detrimental to the patient, as this enzyme plays an important role in the bactericidal capabilities of the phagocyte. The effect of these changes on morbidity and mortality cannot be determined from our nonrandomized trial of GM-CSF in patients with limited thermal injury. Our results caution against the extrapolation of data obtained through the in vitro incubation of normal cells with GM-CSF. Future studies concerning the effect of parenteral administration of GM-CSF on WBC function in healthy subjects as well as its effects on pulmonary function in lung injury in animal models will be important to define the in vivo effects and the potential beneficial or detrimental effects when administered to injured patients.

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PRESENTATIONS/PUBLICATIONS

None.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
				DA315359	1 Oct 89	DD-DRAE(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
1 Oct 88	D. Chg	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62787A	3S162787A874	DA	170
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP,	FY90-01		

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

12. SUBJECT AREAS

0605 Medicine and Medical Research 0613 Microbiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
8610	9009	DA	C. In-House

17. CONTRACT/GRANT MILITARY RELEVANCY CERTIFIED APPROVED BY

a. DATE EFFECTIVE		c. RESOURCES ESTIMATE		
b. CONTRACT/GRANT NUMBER		a. FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS *(In thousands)*
c. TYPE	d. AMOUNT	89	1.5	105
e. KIND OF AWARD	f. CUM/TOTAL	90	1.5	110

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: BUESCHER, T M
d. TELEPHONE NUMBER *(Include area code)*: 512-221-2720	d. TELEPHONE NUMBER *(Include area code)*: 512-221-4440
21. GENERAL USE: FINA	f. NAME OF ASSOCIATE INVESTIGATOR *(If available)*: CIOFFI, W G
MILITARY/CIVILIAN APPLICATION: M	g. NAME OF ASSOCIATE INVESTIGATOR *(If available)*: MASON, A D

22. KEYWORDS *(Precede EACH with Security Classification Code)* (U) Healing; (U) Keratinocytes; (U) Cell Cultures; (U) Skin; (U) Skin Graft; (U) Frozen Skin; (U) Volunteers;

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

23. (U) A DTIC literature search was conducted under DTIC request number W6K05C and W6J57D dated 20 October 1989 for the technical report database and request number W6K07C dated 20 October 1989 for the work unit database. The proposed effort will not result in the duplication of effort. The objective of this work is to 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 patients.

24. (U) 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) 8810 - 8909. Nine applications have been performed on 7 patients. Due to poor results with cultured keratinocytes obtained from the current contractor, an addendum is being developed to change to a local contractor for the growth of keratinocyte sheets. For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1989.*

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

ANNUAL RESEARCH PROGRESS REPORT

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

PROJECT TITLE: Evaluation of in vitro Cultivated Keratinocytes as Epithelial Autografts for the Closure of Burn Wounds

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

1 October 1988 - 30 September 1989

INVESTIGATORS

Teresa M. Buescher, MD, Captain, MC
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ANNUAL RESEARCH PROGRESS REPORT

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

PROJECT TITLE: Evaluation of in vitro Cultivated Keratinocytes as Epithelial Autografts for the Closure of Burn Wounds

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

PERIOD COVERED IN THIS REPORT: 1 Oct 88 through 30 Sep 89

INVESTIGATORS: Teresa M. Buescher, MC, MD, Captain, MC
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Two patients have been enrolled in this study to date. Upon completion of the enrollment of 10 patients, the safety and efficacy of the cultured keratinocytes will be data will be analyzed.

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

The extent of the skin loss caused by a burn is the major determinant of outcome. Several techniques have been developed to effect closure of the burn wound. These include autografting as well as temporary coverage with allograft skin, heterograft skin, or artificial skin substitutes. The goal of burn wound care is to achieve timely permanent closure of the open wound. At this time, the only adequate permanent cover is autograft since all other biological membranes are temporary and artificial skin substitutes require eventual grafting with the patient's own epidermis. When the surface area and the depth of the burn are so extensive that the patient's available donor sites are insufficient to provide skin grafts to cover the wound in a timely fashion, a new source of autograft is desirable. Human keratinocytes can be cultured to produce confluent epithelial sheets. These cells can be grown from a relatively small initial sample of the patient's unburned epidermis and can be expanded, over a period of weeks, to a size sufficient to cover the entire body surface area. The use of cultured autologous epithelium in burn patients has been reported by a few institutions (2–3). It has been employed as an adjunct to conventional therapy and has resulted in successful closure of massive burns in small numbers of patients who might have otherwise succumbed to their injury. Physicians working at the Mayo Clinic in Rochester, Minnesota, have recently reported successful use of cultured autologous epithelial grafts in a patient who sustained a 99% total body surface area burn (5). This is the first report of the use of cultured keratinocytes grown in a defined medium without the use of a feeder layer of lethally irradiated mouse 3T3 cells in the culture flasks (1). The lack of this feeder layer removes the potential risk of transmission of viral diseases from the cells in the medium to the cultured cells.

The objective of this study is to determine the suitability of cultured autologous epithelium for the closure of burn wounds. Wounds covered with cultured epithelium will be compared with similar wounds covered with fresh autograft. Investigation will also be undertaken to determine a more efficient means of applying the cultured epithelium to the wound bed and reducing the incidence of graft loss due to bacterial colonization of the recipient bed. Prior investigators have employed 75-cm^2 flasks or 5-cm diameter disks to grow the cultured epithelium (2–5). After separating the epithelium from the culture bed, the epithelium routinely shrinks to one-third of the original size. Efforts will be made to adhere the cultured epithelium to an overlying antimicrobial carrier in such a way that this contraction will not occur, thereby producing larger sheets of epithelium. Additional efforts will be made to enlarge the epithelial sheets by growing them in larger flasks (150 cm^2) or in roller bottles. Bacterial colonization of the recipient bed has impaired the graft take of cultured epithelium more than

would be expected with autograft (4-6). Evaluation of the recipient wound beds will be made to determine the best way to prepare wounds for grafting with cultured epithelium.

MATERIALS AND METHODS

Number of Patients. Thirty patients will be enrolled in this study. Properly signed and witnessed volunteer agreement affidavits are obtained from each patient prior to beginning the study.

Selection of Patients.

Inclusion Criteria. The following patients will be eligible for entry into the study:

1. Patients hospitalized for burn injury.

2. Male or female patients ≥ 18 yr old and ≤ 65 yr old. Female patients must have been 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.

Exclusion Criteria. The following patients will be excluded from the study:

1. Patients < 18 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 with the consent of the physicians caring for the patient. 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 routine within the Institute. The cultured epithelial grafts are covered by the backing recommended by the supplier with the consent of the physicians caring for the patient. They 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 to areas grafted with fresh autograft. Additional grafting procedures are undertaken as needed to close the patient's burn wounds. Cultured epithelium is used at these later graftings if the patient's own donor sites are insufficient to cover the recipient bed. Healed cultured grafts are examined with regard to fragility. Punch biopsies from areas of adherent cultured epithelium are taken once the patient's burn wounds are fully healed. Patients enrolled in this study are followed after discharge from the hospital to determine any incidence of late sequelae such as contractures and breakdown of the grafted wounds.

Photographs are obtained of the burn wound prior to grafting, at the first dressing change, and weekly thereafter during the period of hospitalization. Additional photographs are obtained at

any follow-up outpatient visits which take place. At these times, the graft viability is evaluated by quantitatively estimating the percentage of the grafted area which is covered by viable graft and by qualitatively evaluating the durability of the grafts and their tendency to ulcerate. The quantitative scale ranges from 0-10, 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 3 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 are examined for evidence of surviving dermal elements.

Sterile Techniques: The skin biopsy is harvested using sterile technique after using 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

Two patients have been enrolled in this study to date.

DISCUSSION

Upon completion of the enrollment of 10 patients, the safety and efficacy of the cultured keratinocytes will be data will be analyzed.

PRESENTATIONS/PUBLICATIONS

None.

REFERENCES

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
				DA318017	1 Oct 89	DD-DRAR(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
16 Feb 89	D. Chg	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62787A	3S162787A874	DA	172
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP,	FY90-01		

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

12. SUBJECT AREAS
0605 Medicine and Medical Research

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

17. CONTRACT/GRANT MILITARY RELEVANCY CERTIFIED 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)*
89	0.1	15
90	0.1	16

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME: US Army Institute of Surgical Research	a. NAME: US Army Institute of Surgical Research
b. ADDRESS *(Include zip code)*: Fort Sam Houston, San Antonio, Texas 78234-5012	b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-5012
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR: WAYMACK, J P
d. TELEPHONE NUMBER *(Include area code)*: 512-221-2720	d. TELEPHONE NUMBER *(Include area code)*: 512-271-7267
21. GENERAL USE: FINA MILITARY/CIVILIAN APPLICATION: M	f. NAME OF ASSOCIATE INVESTIGATOR *(If available)*: MC MANUS, A T g. NAME OF ASSOCIATE INVESTIGATOR *(If available)*: BURLESON, D G

22. KEYWORDS *(Precede EACH with Security Classification Code)* (U) Tumor Necrosis Factor; (U) Burn Injury; (U) Infection; (U) Immune System; (U) Volunteers; (U) Adults; (U) RA II

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

23. (U) A DTIC literature search was conducted under DTIC request number W6K33C dated 20 October 1989 for the technical report database and request number W6K34C dated 20 October 1989 for the work unit database. The proposed effort will not result in the duplication of effort. The objective of this work is to determine the significance of TNF levels in burn patients and whether or not 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 in burned patients.

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

25. (U) 8810 - 8909. This project was approved by the USAISR Research Council and US Army Institute of Surgical Research Human Use Committee during the second quarter of fiscal year 1989. Six patients with major burn injuries have been enrolled in the study to date. Serial plasma samples were drawn and are currently being stored at -70°C for later analysis by ELISA. For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1989.*

DD FORM 63 MAR 1498 EDITION OF MAR 68 IS OBSOLETE. * U.S.G.P.O. 1988 -491-003/50329

ANNUAL RESEARCH PROGRESS REPORT

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

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

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

16 February 1989 - 30 September 1989

INVESTIGATORS

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William F. McManus, MD, Colonel, MC
Rey F. Guzman, BS, Sergeant
Basil A. Pruitt, Jr., MD, Colonel, MC

ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3S162787A874-00, 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: 16 Feb 89 through 30 Sep 89

INVESTIGATORS: Albert T. McManus, PhD
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Burn patients have a significant infection diathesis due in part to the loss of the normal epithelial skin barrier and to 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 patients' 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 their 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.

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 will be offered the opportunity to participate in this study.

Patient Inclusion. Patients meeting the following criteria will be considered eligible for entry into the study:

1. Male or female patients ≥ 18 yr old.

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

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

Patient Exclusion. Patients with the following characteristics will be excluded from enrollment in the study:

1. Patients < 18 yr old.

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

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

4. Patients with toxic epidermal necrolysis syndrome.

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

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

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

Data Analysis Plan. Data will be analyzed comparing TNF levels in infected versus noninfected patients. This will include

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

FIGURE 2. Data collection sheet.

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 for any correlations.

RESULTS

This project was approved by the USAISR Research Council on 10 January 1989 and the US Army Institute of Surgical Research Human Use Committee on 13 February 1989.

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, and Falk W: Tumor necrosis factor: a cytokine involved in toxic effects of endotoxin. **Rev Infect Dis** 9:S602-6, 1987.

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6. Tracey KJ, Beutler B, Lowry SF, et al: Shock and tissue injury induced by recombinant human cachectin. **Science** 234:470-4, 1986.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION: DAOG6972	2. DATE OF SUMMARY: 1 Oct 89	REPORT CONTROL SYMBOL: DD-DR&E(AR) 636
3. DATE PREV SUM'RY: 1 Oct 88	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	F	301
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP	FY90-01		

11. TITLE (Precede with Security Classification Code) (U) Studies of Infection and Microbiologic Surveillance of Troops with Thermal Injury

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 WORKYEARS	b. FUNDS (In thousands)
89	0.5	282
90	0.5	296

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: FINA MILITARY/CIVILIAN APPLICATION: M	f. NAME OF ASSOCIATE INVESTIGATOR (if available) g. NAME OF ASSOCIATE INVESTIGATOR (if available)

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Pseudomonas; (U) Klebsiella; (U) Staphylococcus; (U) Wound Infection; (U) Antibiotic Resistance;

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

22. (Continued) (U) Sepsis; (U) Topical Chemotherapy; (U) Volunteers; (U) Adults; (U) Children; (U) Lab Animals; (U) Rats; (U) Guinea Pigs; (U) Mice; (U) RA II

23. (U) A DTIC literature search was conducted under DTIC request number W6L05K dated 19 October 1989 for the technical report database and request number W6L09M dated 19 October 1989 for the work unit database. The proposed effort will not result in the duplication of effort. The objective of this work is to perform epidemiologic studies, study the response of significant species to topical chemotherapy modalities, and determine the relationship of antibiotic usage to sepsis control.

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

25. (U) 8801 - 8812. During calander year 1988, microbiologic surveillance was carried out on 220 of the 223 admitted and discharged burn patients. More than 9,794 isolates were identified from 11,788 specimens. Gram-negative organisms represented less than 33% of isolates. *Klebsiella pneumoniae* was the most common Gram-negative isolate. The most common blood isolate was *Staphylococcus aureus*. *Pseudomonas aeruginosa* was recovered from the blood of only 3 patients. No *Pseudomonas aeruginosa* wound infections were identified. For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1989*.

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

ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3M161102BS14-00, BASIC RESEARCH

PROJECT TITLE: Studies of Infection and Microbiologic Surveillance of Troops with Thermal Injury

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

1 January 1988 - 31 December 1988

INVESTIGATORS

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Jack R. Henderson, PhD
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ABSTRACT

PROJECT NUMBER: 3M161102BS14-00, BASIC RESEARCH

PROJECT TITLE: Studies of Infection and Microbiologic Surveillance of Troops with Thermal Injury

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

PERIOD COVERED IN THIS REPORT: 1 Jan 88 through 31 Dec 88

INVESTIGATORS: Albert T. McManus, PhD
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During calendar year 1988, 220 burned patients were cultured and 9,794 isolates were identified. A relatively low colonization frequency (< 34%) 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 36.6% 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 1988. Data were collected from admission through disposition. This is the fifth report that is based on calendar year rather than fiscal year. This change more nearly aligns culture results with the annual research progress report produced by the Clinical Division for the same patient population.

AUTOMATED MICROBIOLOGY DATA BASE

The microbiology data base now contains complete surveillance data for > 1,400 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 1988 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 1988 (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 220 patients admitted during 1988 were cultured. Species isolated and number of patients yielding each species are

TABLE 1. In vitro Sensitivity Panels (1988)

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.

presented in Table 2. Because of the decreased host resistance of the patient population, no organism is considered "normal" flora and all isolated organisms are reported to the physician. A summary of the 10 most common isolates is presented in Table 3. The table contains 79% 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 7,267 organisms were recovered from respiratory system specimens. The majority of these were sputum cultures

TABLE 2. Distribution by Organism (1988)

Organism	Number of Isolates	Number of Patients Colonized	Organism	Number of Isolates	Number of Patients Colonized
Acinetobacter anitratus	108	6	*Neisseria* species	2	2
Acinetobacter lwoffii	2	2	*Neisseria mucosa*	561	130
Acinetobacter species	1	1	*Neisseria subflava*	3	2
Aeromonas hydrophila	2	2	*Propionibacterium acnes*	3	3
Alcaligenes faecalis	4	4	*Proteus mirabilis*	232	35
Alcaligenes xylosoxydans	1	1	*Proteus vulgaris*	30	8
Aspergillus species	12	20	*Providencia stuartii*	1	1
Bacillus	30	29	*Pseudomonas aeruginosa*	636	67
Bacteroides species	2	1	*Pseudomonas fluorescens*	1	1
Branhamella catarrhalis	9	20	*Pseudomonas maltophila*	92	9
Candida albicans	138	30	*Pseudomonas putida*	1	1
Candida rugosa	192	25	*Pseudomonas* species	3	3
Candida tropicalis	24	5	*Pseudomonas stutzeri*	2	2
Citrobacter freundii	31	13	*Serratia marcescens*	111	15
Citrobacter diversus	27	16	*Staphylococcus aureus*	1,711	151
Clostridium difficile	1	1	*Staphylococcus epidermidis*	495	142
Clostridium species	1	1	*Staphylococcus saprophyticus*	166	60
Corynebacterium species	2	2	*Staphylococcus* species	11	11
Enterobacter aerogenes	236	36	Alpha Streptococcus	8	9
Enterobacter agglomerans	10	9	Beta Streptococcus	3	3
Enterobacter cloacae	196	45	Beta Streptococcus, Not Group A, B, or D	115	41
Enterobacter sakazakii	2	1	Group A nonhemolytic beta Streptococcus	1	1
Enterobacter species	5	5	Group D Streptococcus, not *Enterococcus*	264	104
Escherichia coli	401	86	Group D Enterococcus	417	78
Flavobacterium species	3	3	Nonhemolytic Streptococcus	4	4
Gram-negative rod	1	1	Nonhemolytic Streptococcus, not Group D	983	198
Haemophilus influenzae	22	8	*Streptococcus pneumoniae*	20	11
Klebsiella oxytoca	12	11	*Streptococcus viridans*	1,690	211
Klebsiella ozaenae	4	3	*Trichosporon beigelii*	2	2
Klebsiella pneumoniae	565	87	True fungi species (other)	116	49
Klebsiella species	3	3	Yeast species (other)	4	4
Micrococcus luteus	1	1			
Morganella morganii	25	7			
Neisseria lactamicas	1	1			
Neisseria meningitidis	3	2			

Total Number of Isolates = 9,794

Total Number of Cultured Patients = 220

TABLE 3. Ten Most Frequent Isolates (1988)

Organism	Number of Patients Colonized	% Patients	Number of Isolates	% Total Isolates
Streptococcus viridans	211	95.9	1,690	17.3
Nonhemolytic Streptococcus, not Group D	198	90.0	983	10.0
Staphylococcus aureus	151	68.6	1,711	17.5
Staphylococcus epidermidis	142	64.5	495	5.1
Neisseria mucosa	130	59.1	561	5.7
Group D Streptococcus, not *Enterococcus*	104	47.3	264	2.7
Klebsiella pneumoniae	87	39.5	565	5.8
Escherichia coli	86	39.1	401	4.1
Group D Enterococcus	78	35.6	417	4.3
Pseudomonas aeruginosa	67	30.5	636	6.5

Total Number of Patients Cultured = 220
Total Number of Isolates = 9,794

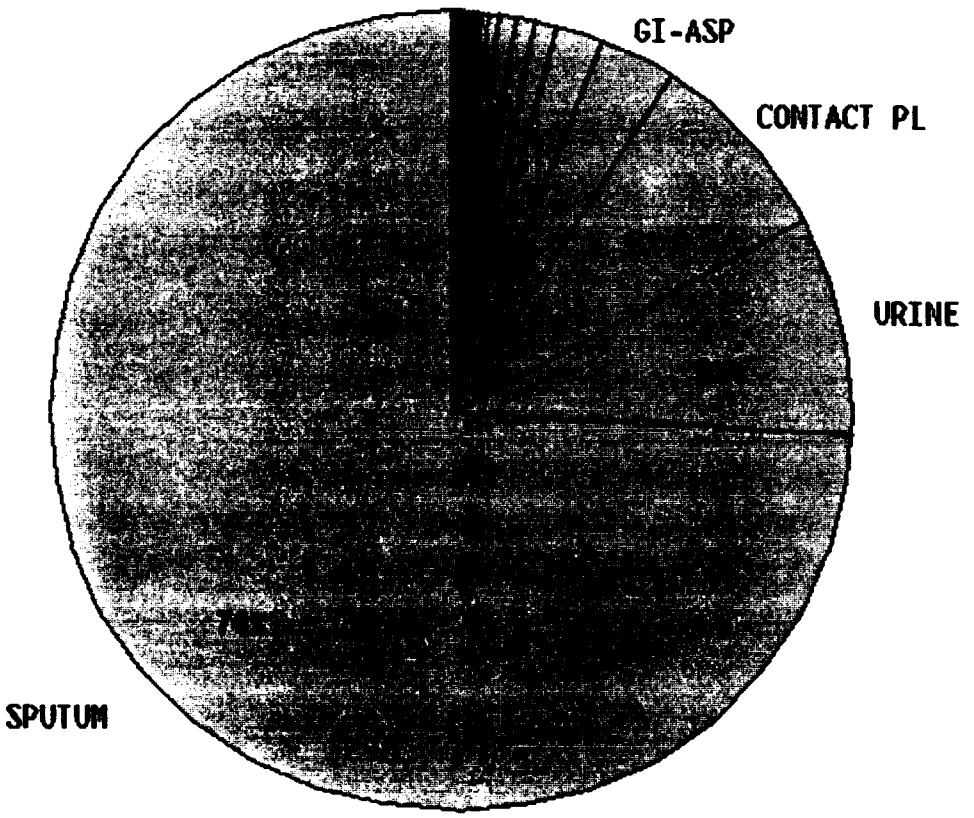

FIGURE 1. Display of the relative frequency of specimen sources yielding isolates in 1988.

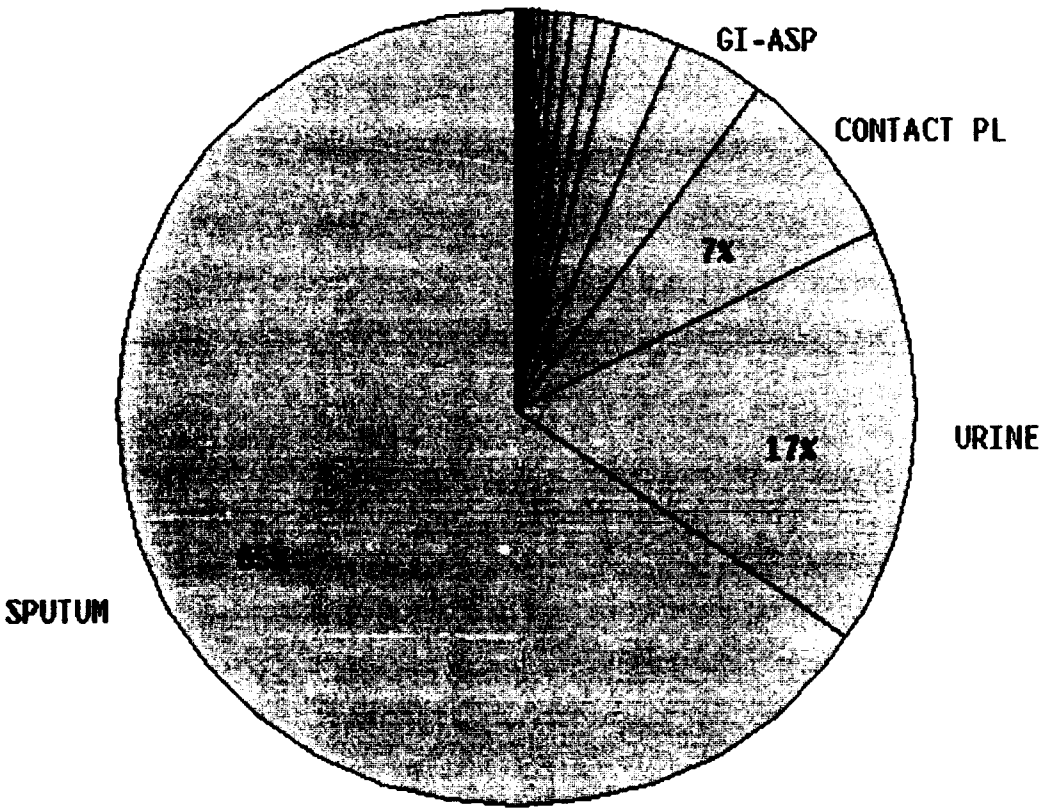

FIGURE 2A. Display of the relative frequency of specimen sources yielding Gram-negative organisms.

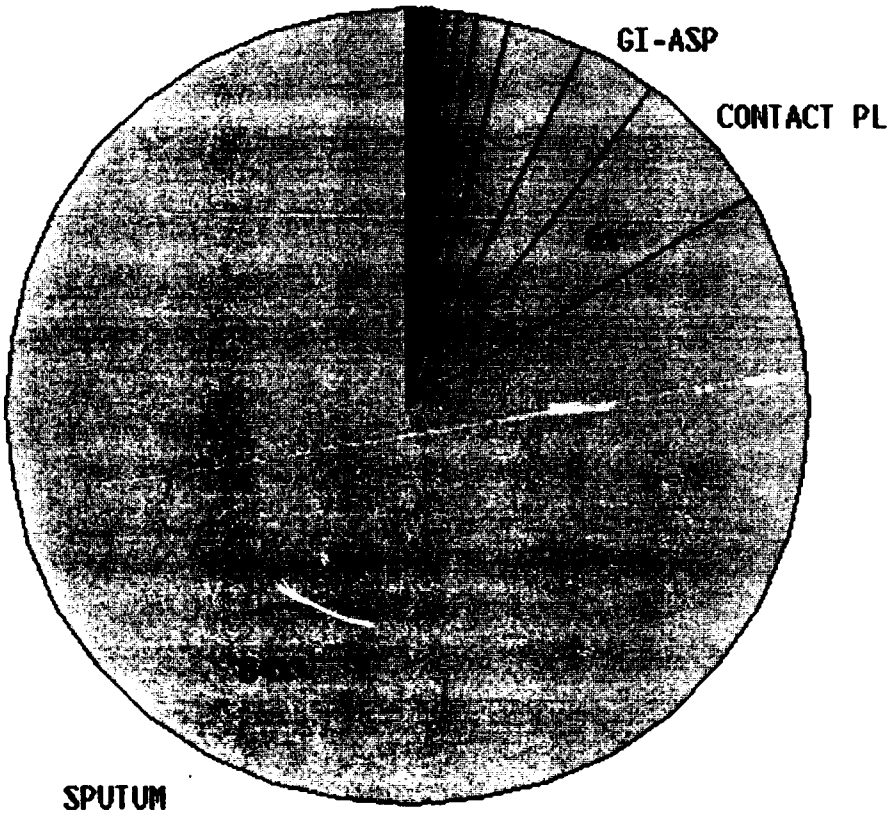

FIGURE 2B. Display of the relative frequency of specimen sources yielding Gram-positive organisms.

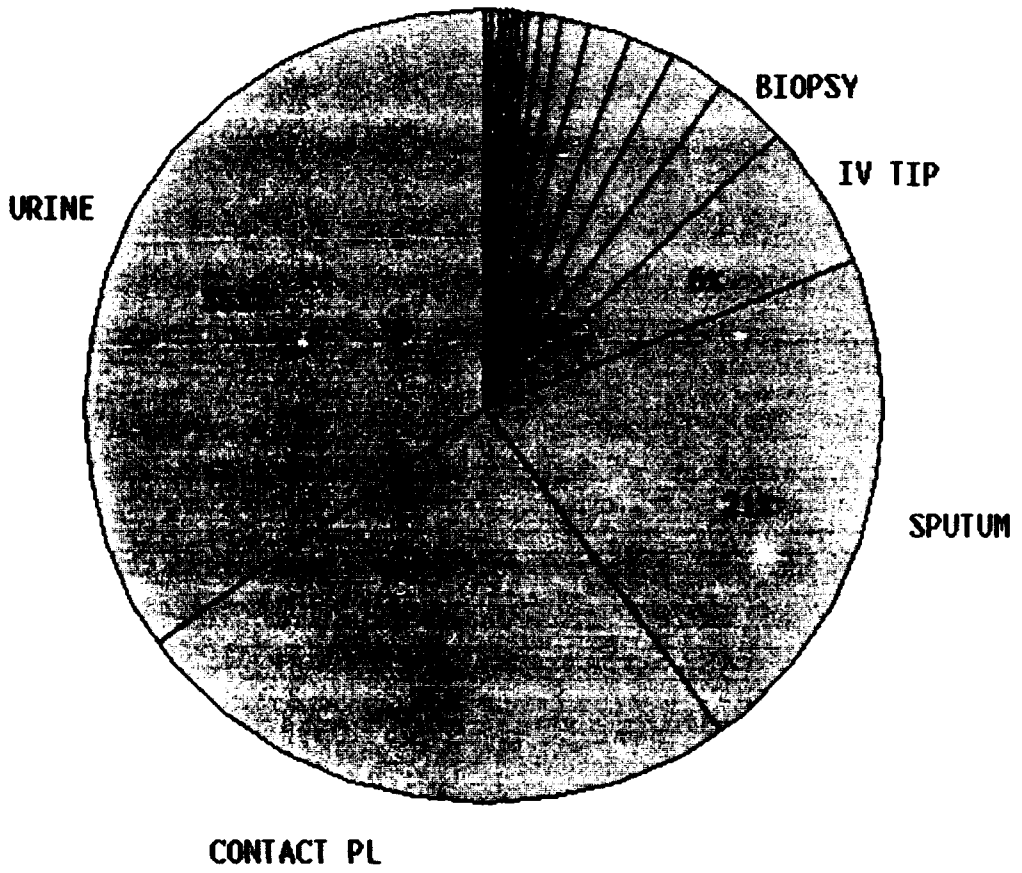

FIGURE 2C. Display of the relative frequency of specimen sources yielding yeast-like organisms.

collected in the surveillance program. The 10 most frequent species are presented in Table 4, which represents 79.7% of the respiratory isolates. Of particular note is the continued decline of Gram-negative isolates. *Pseudomonas aeruginosa* was not in the top 10 organisms, with only 24 of the 213 patients colonized. This frequency was not significantly different from previous years.

FLORA RECOVERED FROM WOUND SURFACE SPECIMENS

A total of 1,722 contact plate surface cultures were taken and 762 isolates were made. Relative frequencies of isolated species are presented in Figure 3. Subsurface flora, as measured by biopsy specimens, was measured in 386 biopsies taken from 91 patients. Organisms were recovered from 32 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 19 biopsies taken from 11 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 217 patients yielded 867 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 135 patients for a total of 1,379 cultures. The principal organisms recovered are listed in Table 8. Positive cultures were obtained from 32 patients and 74 isolates were made from 74 positive cultures. Forty-four cases of bacteremia were noted. A case of bacteremia was defined as isolation of an organism once or more than once with a 30-day period.

Intravenous catheter tips were cultured from 81 patients. Isolations were made from 49 patients and 185 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 4,978 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.

TABLE 4. Ten Most Frequent Isolates from Respiratory Sources (1988)

Organism	Number of Patients Colonized	% Patients	Number of Isolates	% Total Isolates
Streptococcus viridans	209	98.1	1,615	22.2
Nonhemolytic Streptococcus, not Group D	197	92.5	920	12.7
Staphylococcus aureus	138	64.8	1,409	19.4
Neisseria mucosa	129	60.6	546	7.5
Staphylococcus epidermidis	102	47.9	294	4.0
Group D Streptococcus, not *Enterococcus*	98	46.0	253	3.5
Klebsiella pneumoniae	43	20.2	315	4.3
Group D Enterococcus	40	18.8	211	2.9
Staphylococcus saprophyticus	40	18.8	120	1.7
Beta hemolytic Streptococcus, not Group A, B, or D	40	18.8	111	1.5

Total Number of Patients Cultured = 213
Total Number of Isolates = 7,267

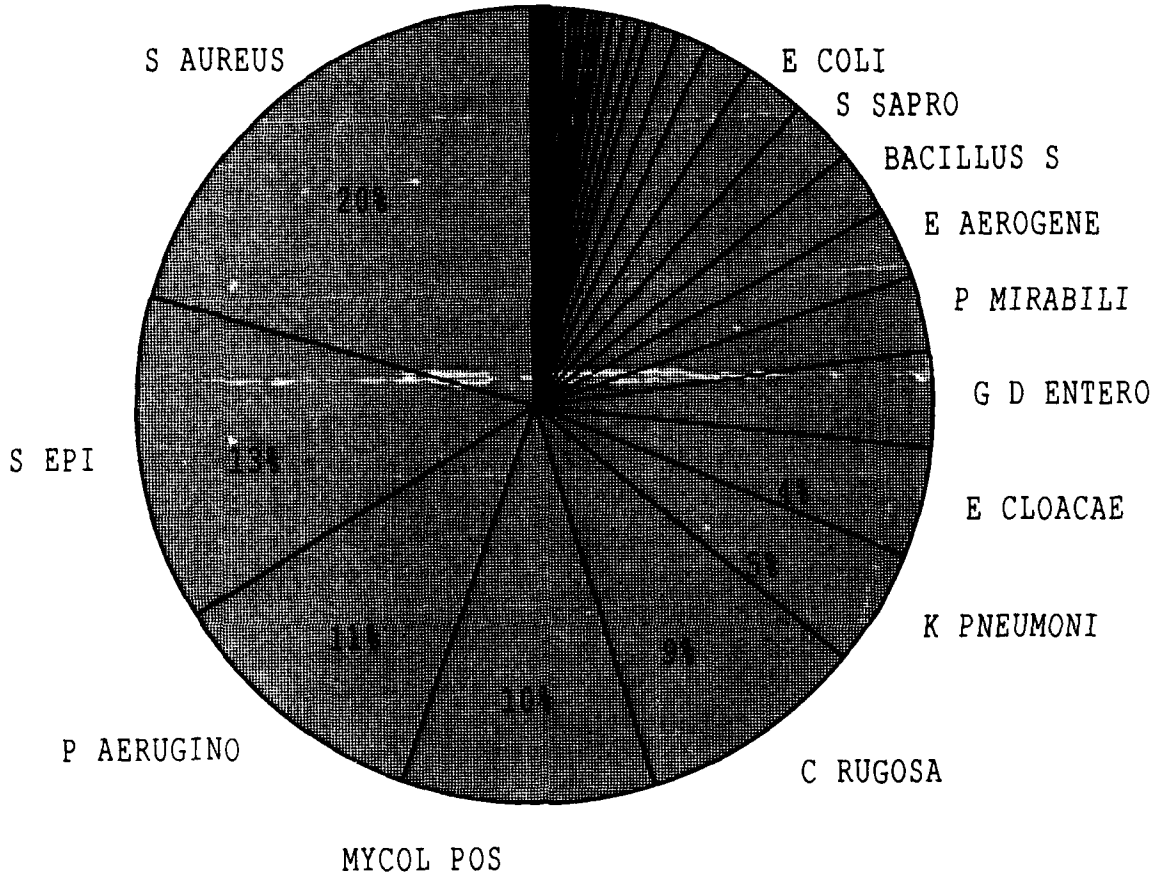

FIGURE 3. Display of the relative frequency of organism types isolated from surface wound cultures.

TABLE 5. Principal Organisms Recovered in Biopsy Specimens (1988)

Organism	Number of Patients Colonized	% Patients	Number of Isolates	% Total Isolates
Filamentous fungi	19	20.9	49	34.3
Escherichia coli	7	7.7	10	7.0
Candida rugosa	6	6.6	18	12.6
Pseudomonas aeruginosa	6	6.6	10	7.0
Group D Enterococcus	5	5.5	8	5.6
Staphylococcus epidermidis	5	5.5	5	3.5
Staphylococcus aureus	4	4.4	5	3.5
Candida albicans	3	3.3	5	3.5
Enterobacter cloacae	3	3.3	5	3.5
Bacillus species	3	3.3	4	2.8

Total Number of Patients Biopsied = 91
Total Number of Isolates = 143
Biopsies Taken = 386

TABLE 6. Ten Most Frequent Organisms from Urinary Specimens (1988)

Organism	Number of Patients Colonized	% Patients	Number of Isolates	% Total Isolates
Klebsiella pneumoniae	58	26.7	157	18.1
Escherichia coli	57	26.3	147	17.0
Pseudomonas aeruginosa	41	18.9	97	11.2
Group D Enterococcus	32	14.7	58	6.7
Proteus mirabilis	29	13.4	76	8.8
Streptococcus epidermidis	23	10.6	30	3.5
Staphylococcus aureus	19	8.8	27	3.1
Nonhemolytic Streptococcus, not Group D	19	8.8	21	2.4
Candida rugosa	15	6.9	69	8.0
Streptococcus viridans	15	6.9	16	1.8

Total Number of Patients Cultured = 217
Total Number of Isolates = 867

TABLE 7. Ten Most Frequent Organisms from Urinary Specimens with ≥ 10^5 cfu (1988)

Organism	Number of Patients Colonized	% Patients	Number of Isolates	% Total Isolates
Klebsiella pneumoniae	39	42.4	79	18.4
Pseudomonas aeruginosa	31	33.7	60	14.0
Escherichia coli	31	33.7	69	16.0
Proteus mirabilis	18	19.6	29	6.7
Group D Enterococcus	16	17.4	28	6.5
Candida albicans	11	12.0	33	7.7
Nonhemolytic Streptococcus, not Group D	11	12.0	11	2.6
Enterobacter cloacae	10	10.9	17	4.0
Streptococcus viridans	9	9.8	10	2.3
Candida rugosa	8	8.7	33	7.7

Total Number of Patients Cultured = 92
Total Number of Isolates = 430

TABLE 8. Principal Organisms Found in Blood Cultures (1988)

Organism	Number of Patients	% Patients Cultured	% Cases	Number of Isolates	% Total Isolates
Staphylococcus aureus	12	9.0	23.1	28	31.5
Staphylococcus epidermidis	7	5.3	13.5	11	12.4
Klebsiella pneumoniae	5	3.8	9.6	12	13.5
Group D Enterococcus	4	3.0	7.7	5	5.6
Pseudomonas aeruginosa	3	2.3	5.8	7	7.8
Escherichia coli	3	2.3	5.8	3	3.4
Propionibacterium acnes	3	2.3	5.8	3	3.4
Candida albicans	2	1.5	3.8	6	6.7
Candida rugosa	2	1.5	3.8	2	2.2
Enterobacter cloacae	2	1.7	3.8	5	4.9

Total Number of Patients Cultured = 133 **Total Number of Cultures = 1,230**
Total Number of Isolates = 89 **Total Number of Patient Positives = 30**

TABLE 9. Ten Most Frequent Organisms from Intravenous Catheters (1988)

Organism	Number of Patients Colonized	% Patients	Number of Isolates	% Total Isolates
Staphylococcus epidermidis	24	29.6	31	16.6
Group D Enterococcus	14	17.3	18	9.7
Klebsiella pneumoniae	11	13.6	17	9.2
Staphylococcus aureus	10	12.3	15	8.1
Candida rugosa	9	11.1	16	8.6
Escherichia coli	7	8.6	15	8.1
Enterobacter cloacae	6	7.4	12	6.5
Pseudomonas aeruginosa	6	7.4	16	8.6
Candida albicans	4	4.9	5	2.7
Proteus mirabilis	4	4.9	5	2.7

Total Number of Patients Cultured = 81
Total Number of Isolates = 185

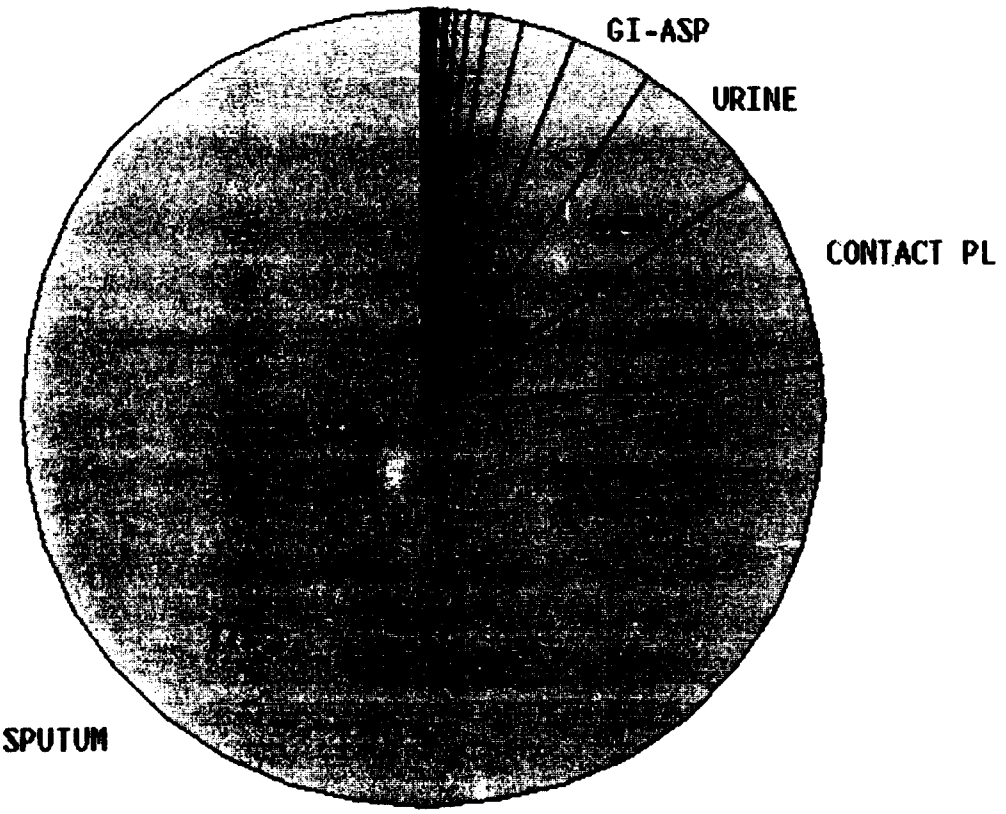

FIGURE 4. Display of the relative frequency of sources yielding organisms tested for in vitro sensitivity to antibiotics in 1988.

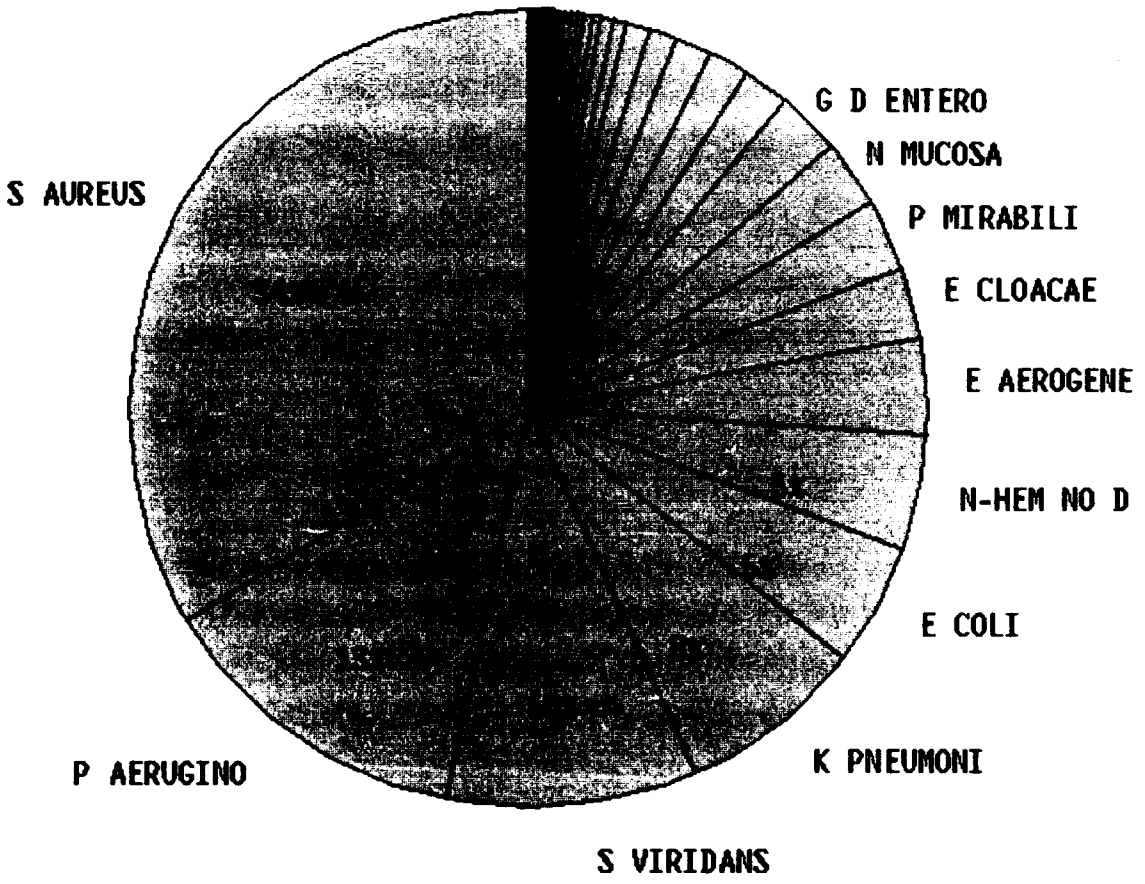

FIGURE 5. Display of the relative frequency of organisms tested for in vitro sensitivity to antibiotics in 1988.

Gentamicin resistance was again used as a plasmid surveillance marker. Testing was done on 3,722 isolates. Figure 6 displays the relative frequency of tested organisms. Figure 7 displays the frequency of resistant species. *Staphylococcus aureus* represented 94% of the gentamicin-resistant isolates. Only 132 Gram-negative isolates of 1,978 strains tested were resistant to gentamicin (6.7%). This is 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 28% of isolates and these strains were isolated from 83 patients. The resistant strains are multiply resistant, with expression of gentamicin, erythromycin, oxacillin, and streptomycin resistance. Multiply resistant *Staphylococcus aureus* and gentamicin-sensitive strains are displayed separately in Table 10 and histograms are shown in Figure 9.

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

Klebsiella pneumoniae. A total of 375 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.

Acinetobacter anitratus. The sources of isolation for tested strains are presented in Figure 16. The results of in vitro antibiotic testing are presented in Table 13. Histogram displays of the distributions of zone sizes for selected antibiotics are presented in Figure 17.

Group D Enterococcus. 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.

Proteus mirabilis. The sources of isolation for tested strains are presented in Figure 20. The results of in vitro antibiotic testing are presented in Table 15. Histogram displays of the

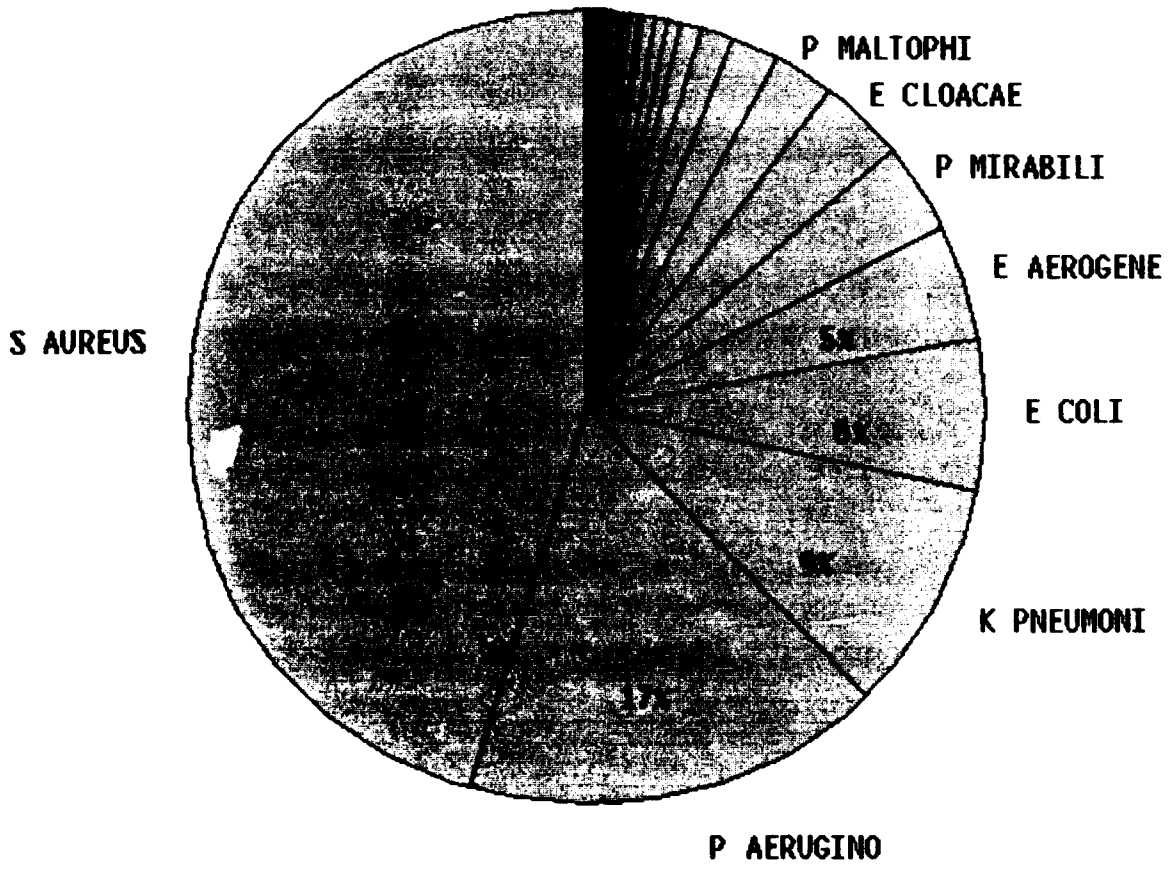

FIGURE 6. Display of the relative frequency of organisms tested for in vitro sensitivity to gentamicin in 1988.

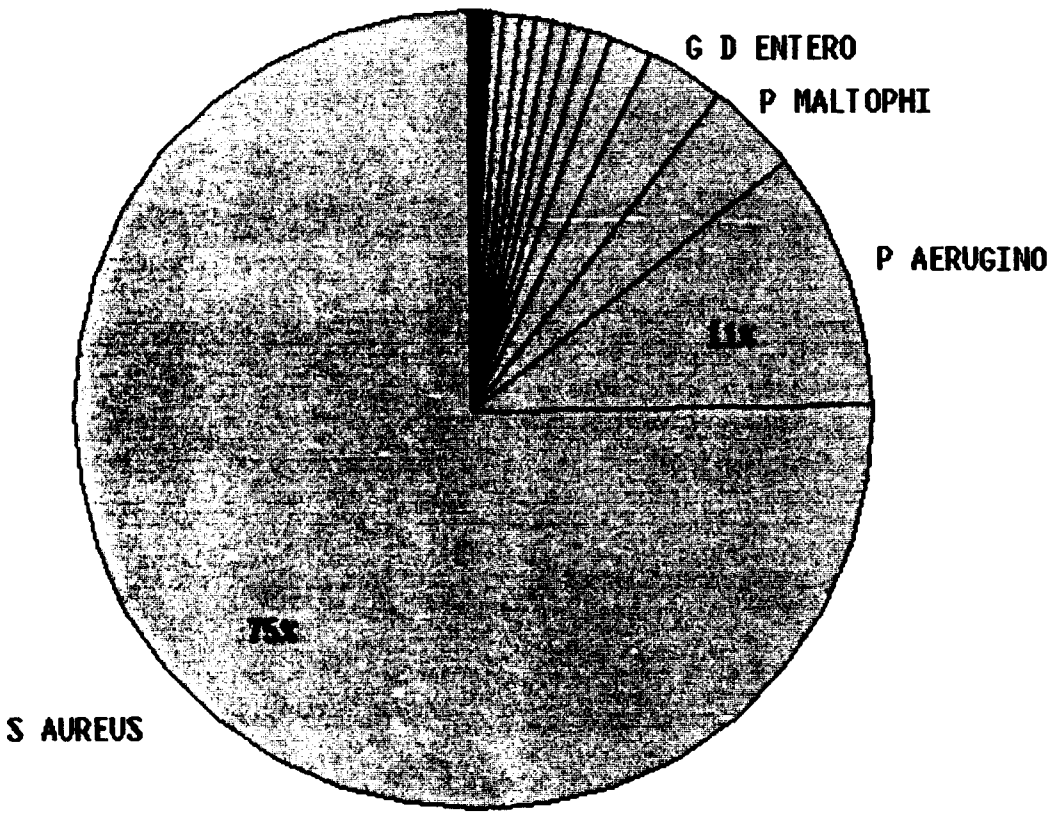

FIGURE 7. Display of the relative frequency of gentamicin-resistant organisms isolated in 1988.

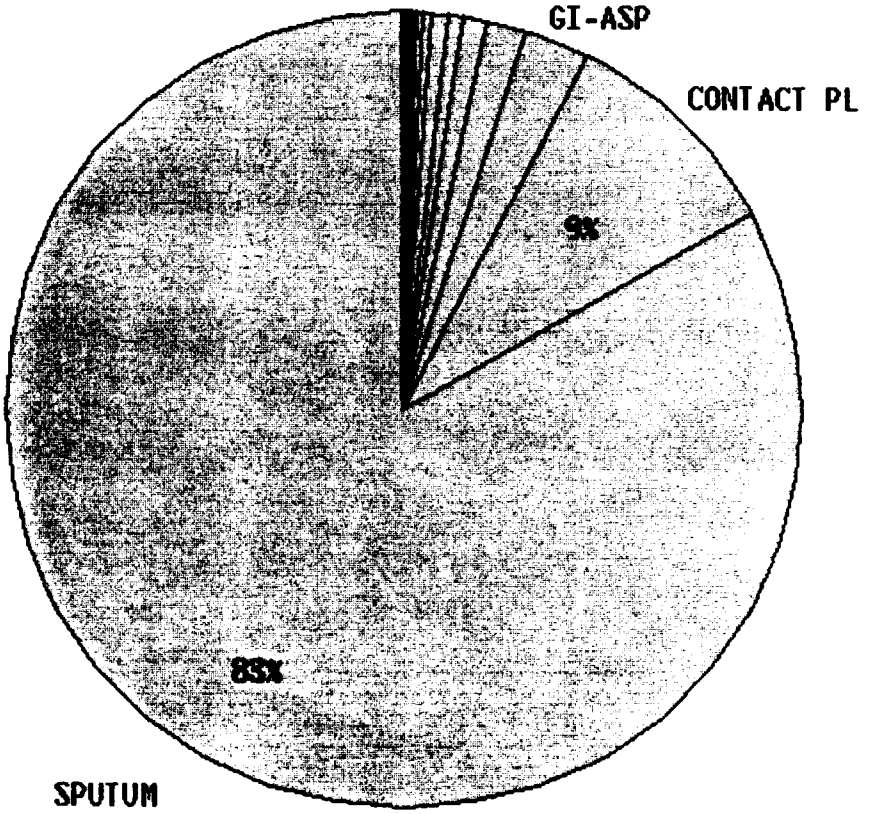

FIGURE 8. Display of the relative frequency of sources yielding *Staphylococcus aureus* tested for in vitro sensitivity to antibiotics in 1988.

TABLE 10A. Antibiotic Sensitivity Data for *Staphylococcus aureus* Sensitive to Gentamicin (1988)

Antibiotic	RESISTANT		INTERMEDIATE		SENSITIVE		Total
	%	Number	%	Number	%	Number	Number
Amikacin	-	-	0.93	11	99.07	1,178	1,189
Ampicillin	5.30	63	8.25	98	86.45	1,027	1,188
Cefoperazone	2.02	24	16.30	194	81.68	972	1,190
Cefotaxime	4.29	51	3.54	42	92.17	1,095	1,188
Cefsulodin	-	-	-	-	100.00	1	1
Ceftazidime	-	-	-	-	100.00	1,187	1,187
Ceftriaxone	-	-	-	-	100.00	1,183	1,183
Cephalothin	0.84	10	1.26	15	97.90	1,165	1,190
Chloramphenicol	0.08	1	0.17	2	99.75	1,186	1,189
Clindamycin	6.31	75	-	-	93.69	1,114	1,189
Erythromycin	15.66	186	1.43	17	82.91	985	1,188
Gentamicin	-	-	-	-	100.00	1,190	1,190
Imipenem-cilastatin sodium	-	-	-	-	100.00	1,190	1,190
Kanamycin	-	-	-	-	100.00	3	3
Methicillin	-	-	-	-	100.00	1	1
Mezlocillin	5.72	68	30.11	358	64.17	763	1,189
Moxalactam	7.23	86	30.70	365	62.07	738	1,189
Oxacillin	5.63	67	3.95	47	90.42	1,076	1,190
Penicillin	63.19	752	25.13	299	11.68	139	1,190
Piperacillin	6.48	77	29.04	345	64.48	766	1,188
Streptomycin	0.42	5	2.52	30	97.06	1,154	1,189
Sulfadiazine	2.27	27	8.17	97	89.55	1,063	1,187
Tetracycline	12.02	143	1.26	15	86.72	1,032	1,190
Ticarcillin	-	-	-	-	100.00	2	2
Tobramycin	6.48	77	0.08	1	93.44	1,111	1,189
Vancomycin	-	-	0.17	2	99.83	1,188	1,190

TABLE 10B. Antibiotic Sensitivity Data for *Staphylococcus aureus* Resistant to Gentamicin (1988)

Antibiotic	RESISTANT %	RESISTANT Number	INTERMEDIATE %	INTERMEDIATE Number	SENSITIVE %	SENSITIVE Number	Total Number
Amikacin	0.61	3	8.78	43	90.61	444	490
Ampicillin	75.00	369	11.18	55	13.82	68	492
Cefoperazone	14.43	71	75.20	370	10.37	51	492
Cefotaxime	19.92	98	75.61	372	4.47	22	492
Ceftazidime	-	-	-	-	100.00	492	492
Ceftriaxone	-	-	-	-	100.00	491	491
Cephalothin	7.33	36	4.07	20	88.59	435	491
Chloramphenicol	0.41	2	0.41	2	99.19	488	492
Clindamycin	2.64	13	-	-	97.36	479	492
Erythromycin	17.28	85	0.81	4	81.91	403	492
Gentamicin	98.98	487	1.02	5	-	-	492
Imipenem-cilastatin sodium	-	-	0.20	1	99.80	491	492
Methicillin	50.00	1	-	-	50.00	1	2
Mezlocillin	83.94	413	7.32	36	8.74	43	492
Moxalactam	88.21	434	11.38	56	0.41	2	492
Oxacillin	89.21	438	8.15	40	2.65	13	491
Penicillin	97.76	481	2.24	11	-	-	492
Piperacillin	84.35	415	6.10	30	9.55	47	492
Streptomycin	86.12	422	0.20	1	13.67	67	490
Sulfadiazine	64.43	317	22.36	110	13.21	65	492
Tetracycline	15.65	77	0.41	2	83.94	413	492
Tobramycin	93.29	459	5.89	29	0.81	4	492
Vancomycin	-	-	-	-	100.00	491	491

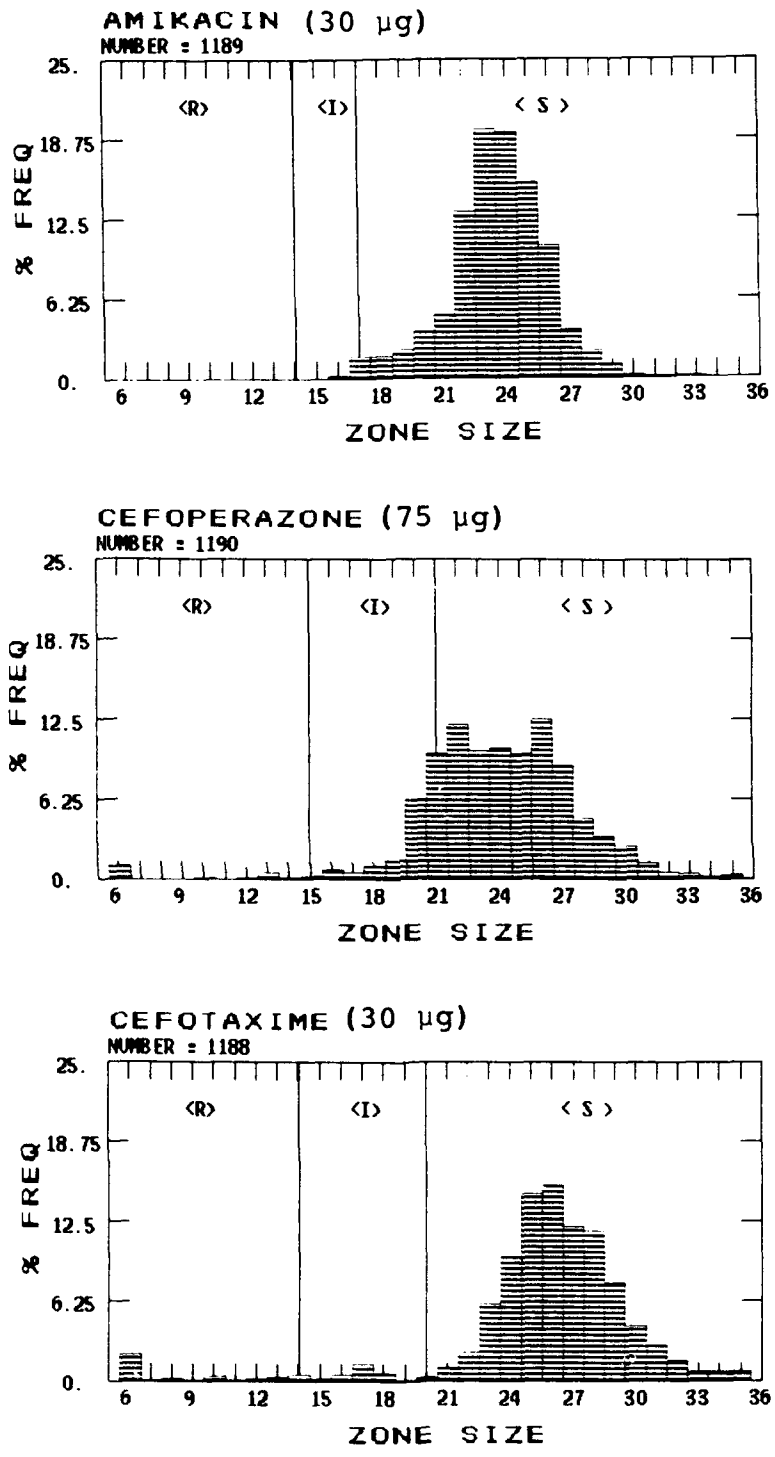

FIGURE 9A. Histogram display of the distribution of zones of inhibition of growth of multiply-resistant *Staphylococcus aureus.*

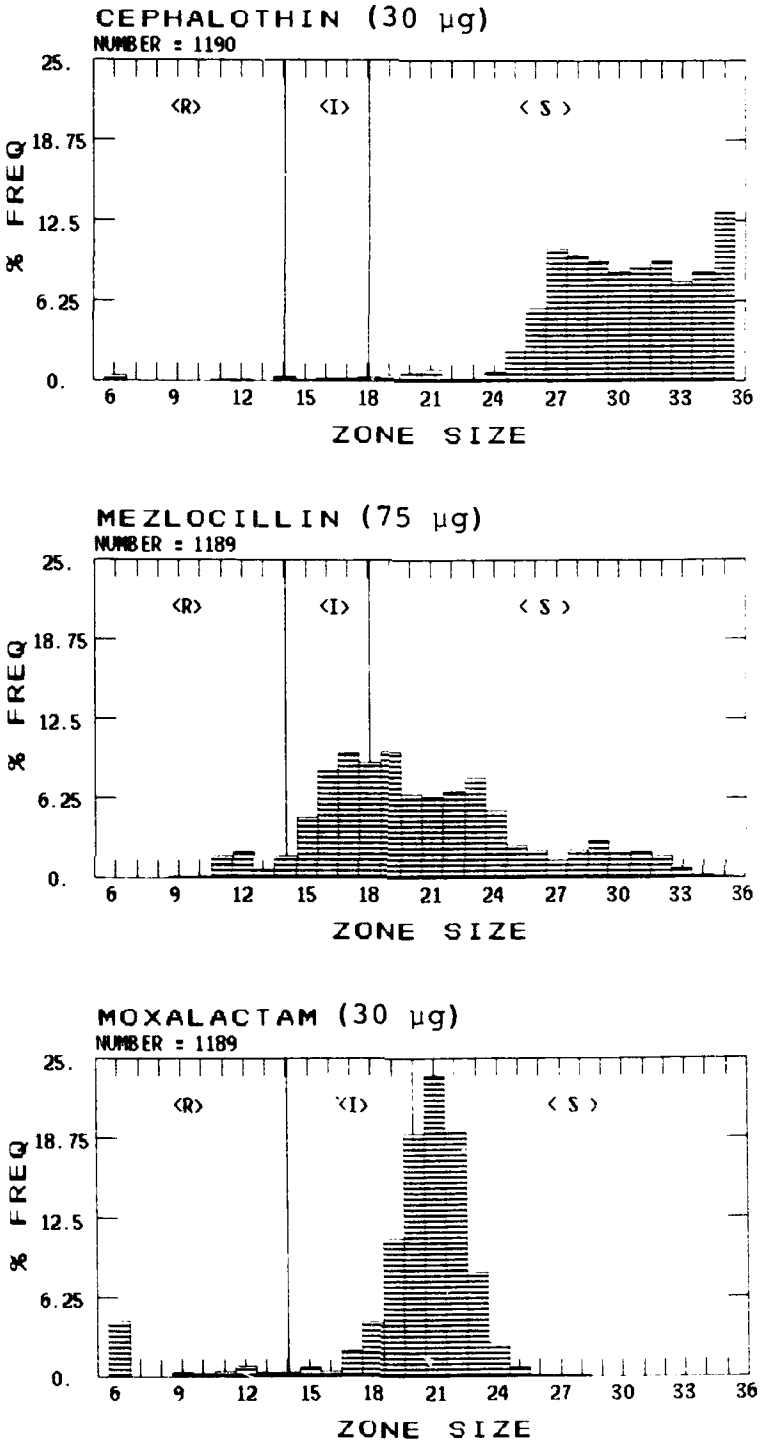

FIGURE 9A. Histogram display of the distribution of zones of inhibition of growth of multiply-resistant *Staphylococcus aureus* (continued).

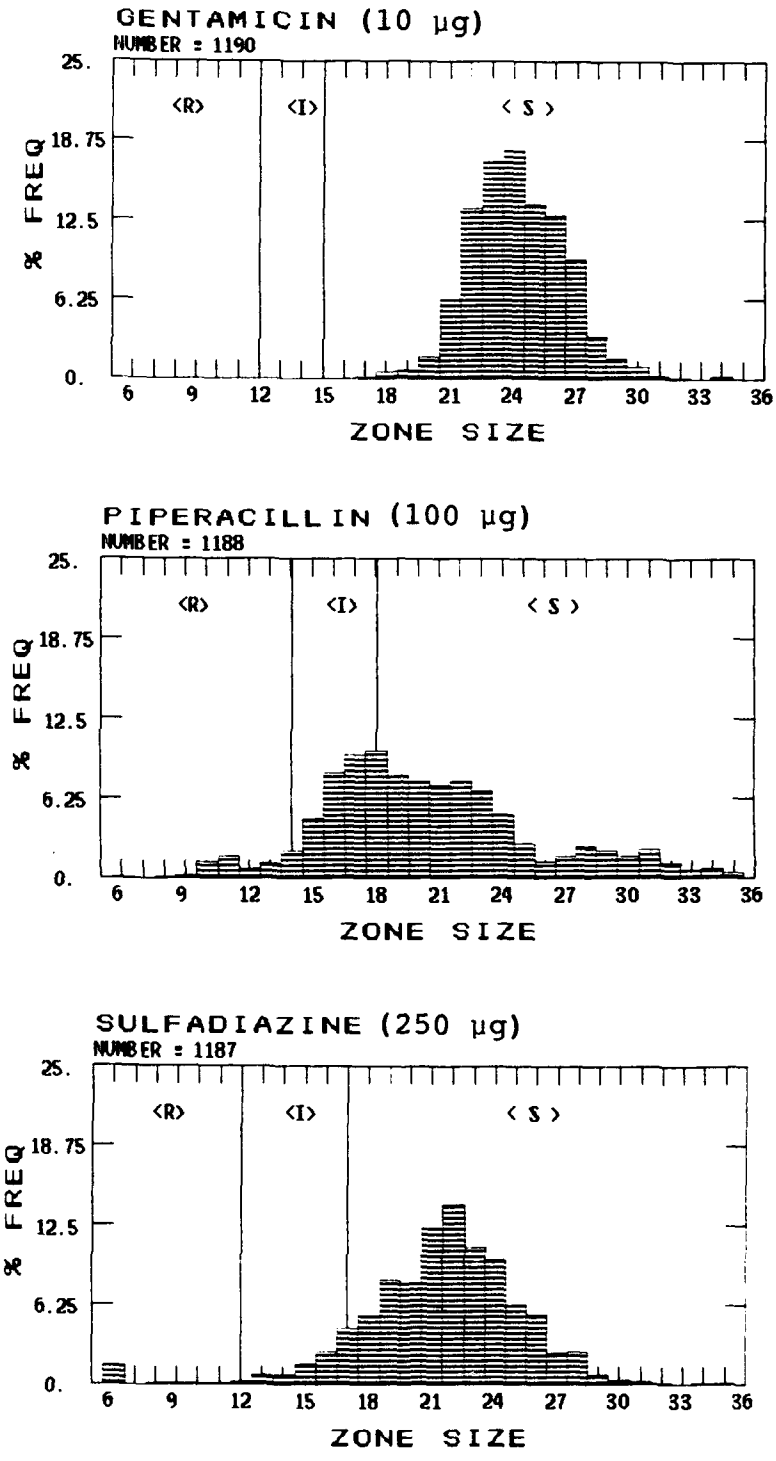

FIGURE 9A. Histogram display of the distribution of zones of inhibition of growth of multiply-resistant *Staphylococcus aureus* (continued).

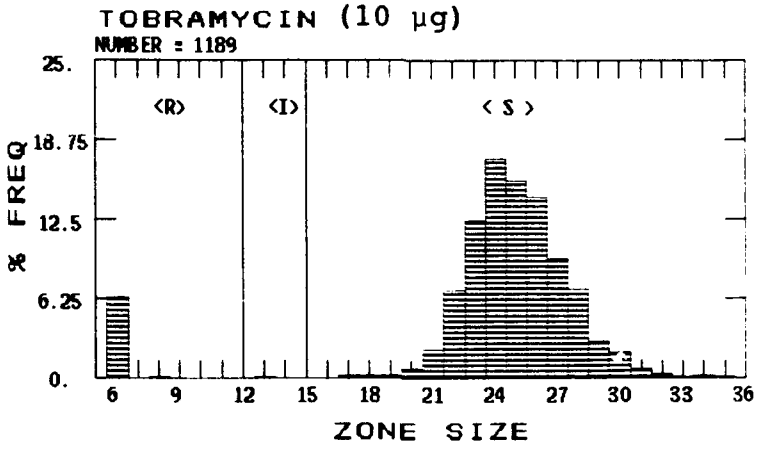

FIGURE 9A. Histogram display of the distribution of zones of inhibition of growth of multiply-resistant *Staphylococcus aureus* (continued).

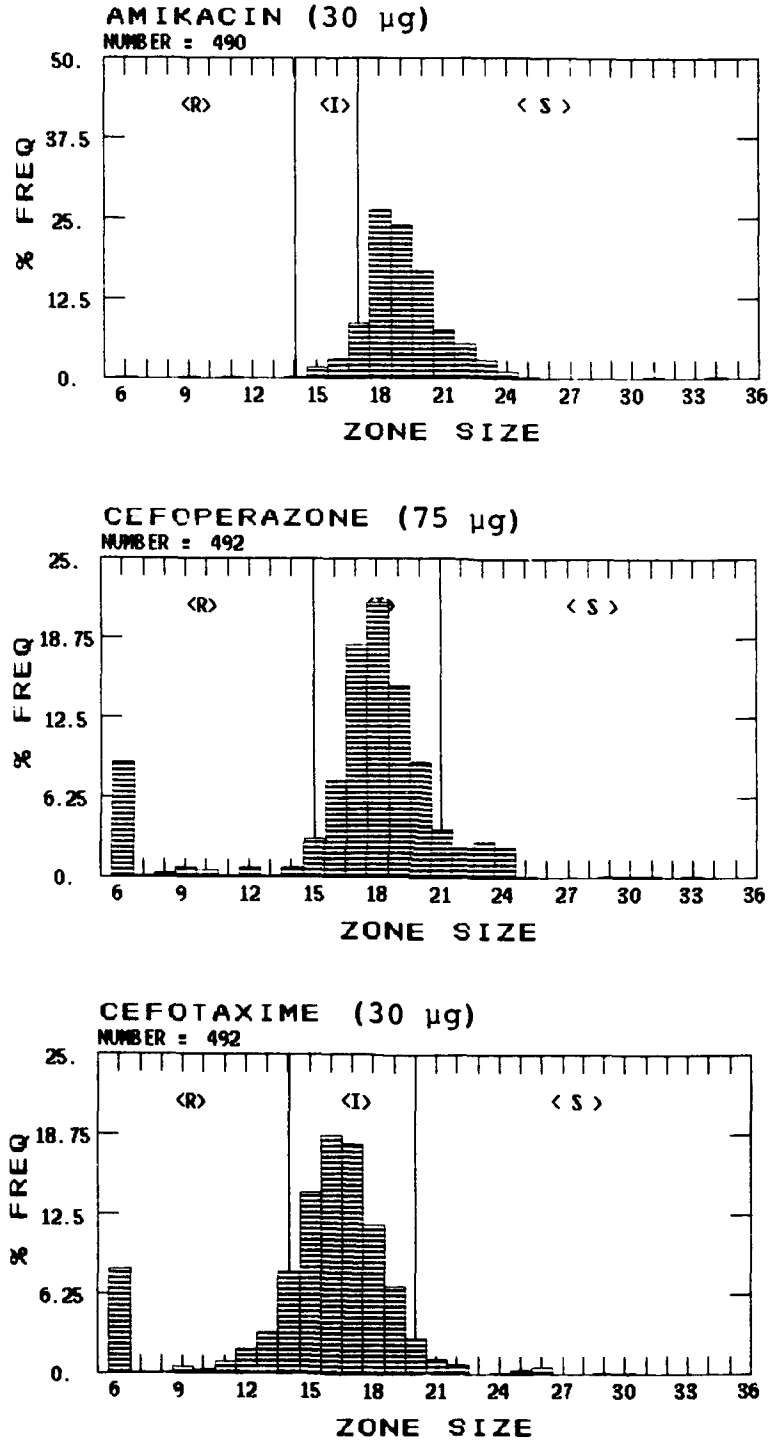

FIGURE 9B. Histogram display of the distribution of zones of inhibition of growth of multiply-sensitive *Staphylococcus aureus*.

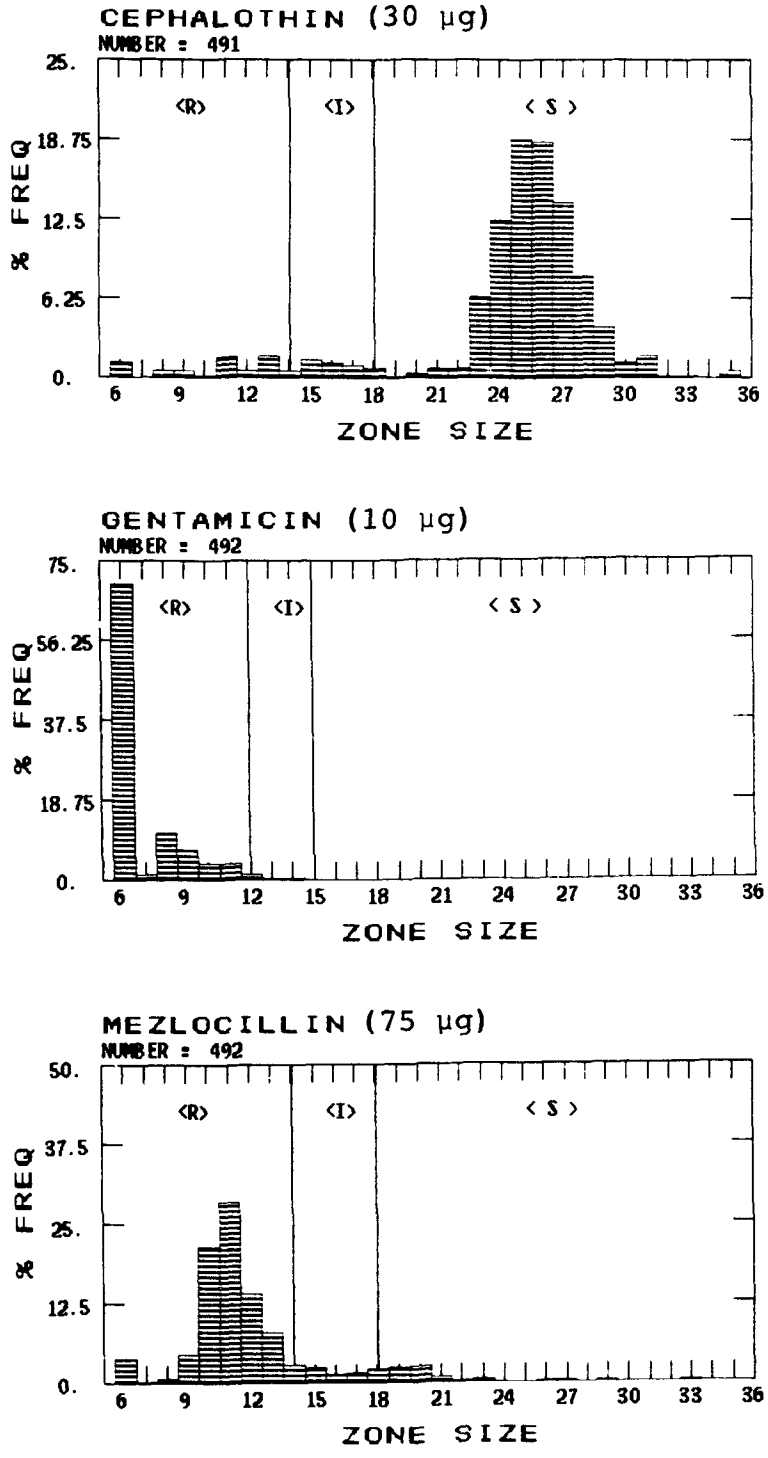

FIGURE 9B. Histogram display of the distribution of zones of inhibition of growth of multiply-sensitive *Staphylococcus aureus* (continued).

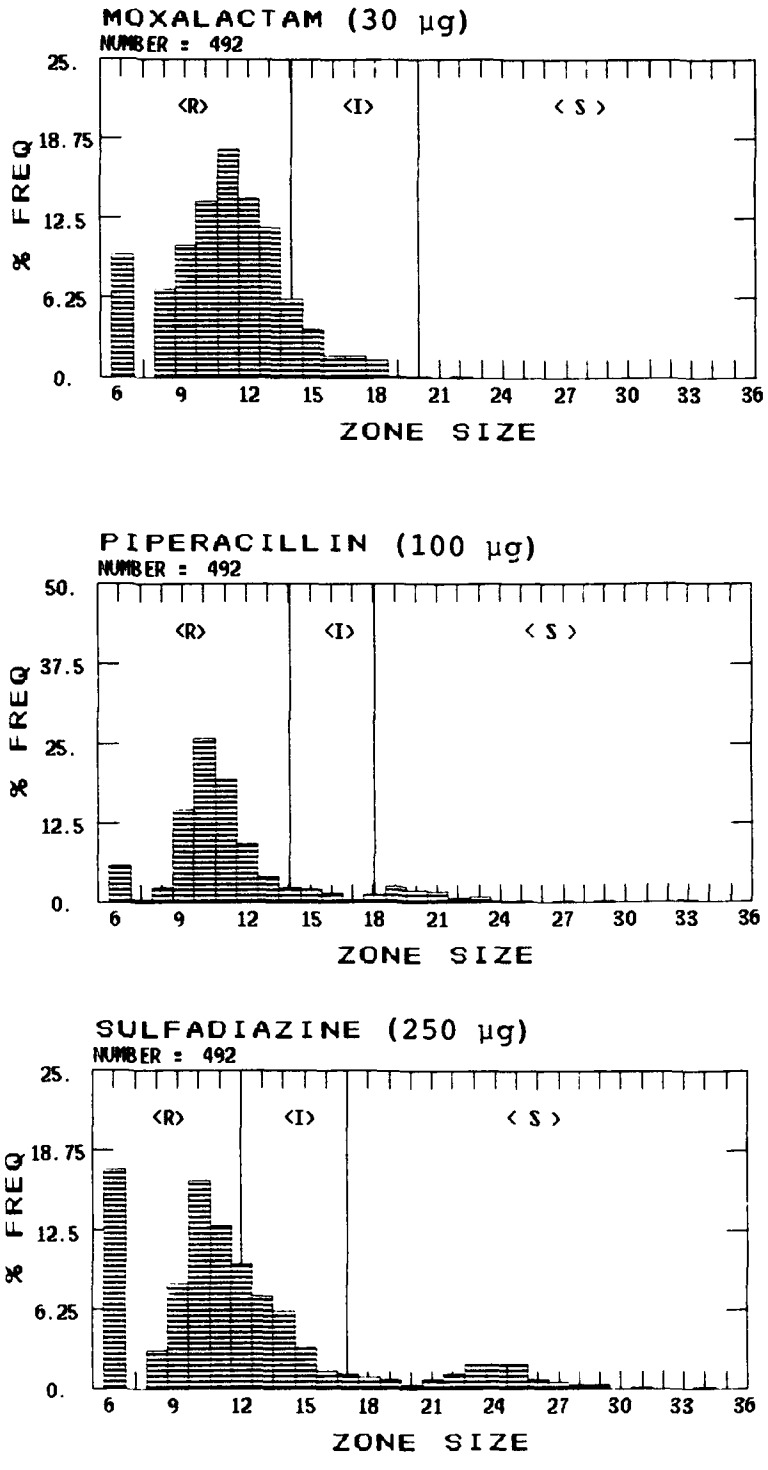

FIGURE 9B. Histogram display of the distribution of zones of inhibition of growth of multiply-sensitive *Staphylococcus aureus* (continued).

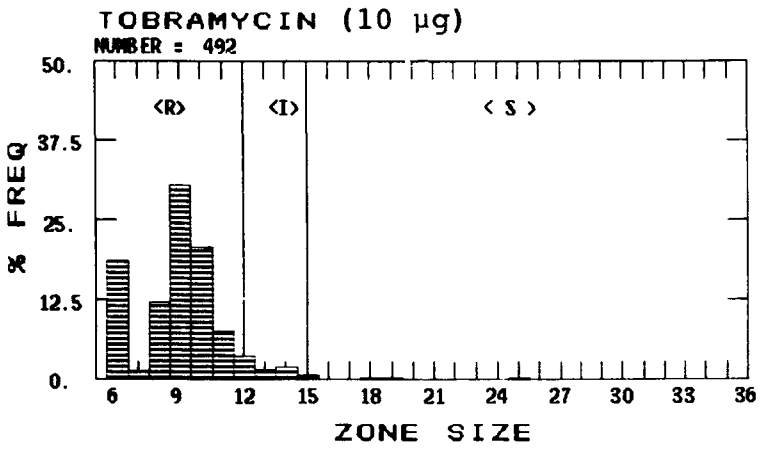

FIGURE 9B. Histogram display of the distribution of zones of inhibition of growth of multiply-sensitive *Staphylococcus aureus* (continued).

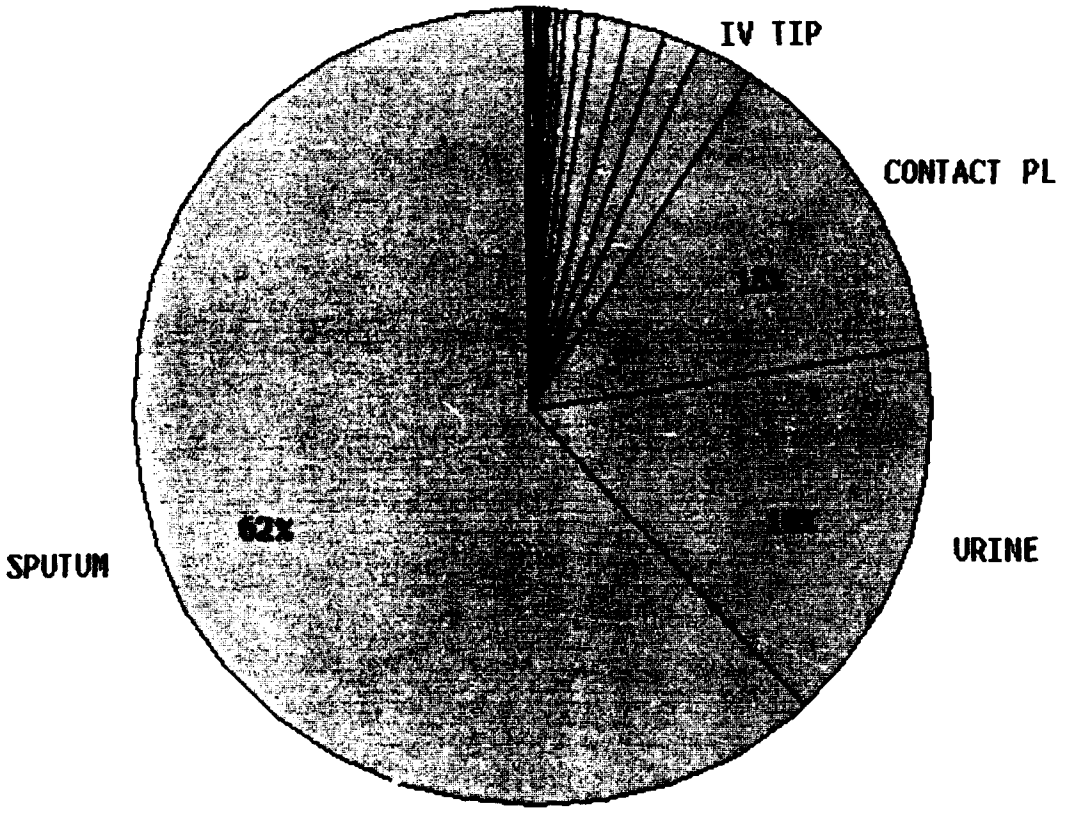

FIGURE 10. Display of the relative frequency of sources yielding *Pseudomonas aeruginosa* tested for in vitro sensitivity to antibiotics in 1988.

TABLE 11. Antibiotic Sensitivity Data for *Pseudomonas aeruginosa* (1988)

Antibiotic	RESISTANT		INTERMEDIATE		SENSITIVE		Total
	%	Number	%	Number	%	Number	Number
Amikacin	8.86	56	9.81	62	81.33	514	632
Azlocillin	20.22	128	4.58	29	75.20	476	633
Aztreonam	8.37	53	39.81	252	51.82	328	633
Cefoperazone	6.01	38	21.20	134	72.78	460	632
Cefotaxime	48.66	308	45.66	289	5.69	36	633
Cefsulodin	22.22	2	0.00	0	77.78	7	9
Ceftazidime	0.00	0	0.00	0	100.00	633	633
Ceftriaxone	0.00	0	0.00	0	100.00	631	631
Chloramphenicol	85.62	542	10.74	68	3.63	23	633
Colistin	0.63	4	0.00	0	99.37	629	633
Gentamicin	10.78	68	15.21	96	74.01	467	631
Imipenem-cilastatin sodium	1.26	8	0.47	3	98.26	622	633
Kanamycin	99.05	627	0.47	3	0.47	3	633
Mezlocillin	31.28	198	20.70	131	48.03	304	633
Moxalactam	33.33	211	55.13	349	11.53	73	633
Netilmicin	9.79	62	3.32	21	86.89	550	633
Norfloxacin	0.00	0	2.21	14	97.79	619	633
Piperacillin	11.08	70	8.54	54	80.38	508	632
Sulfadiazine	30.02	190	22.43	142	47.53	301	633
Tetracycline	75.99	481	20.70	131	3.32	21	633
Ticarcillin	11.06	70	18.01	114	70.93	449	633
Tobramycin	11.69	74	2.84	18	85.47	541	633
TIM-85	15.24	96	12.38	78	72.38	456	630

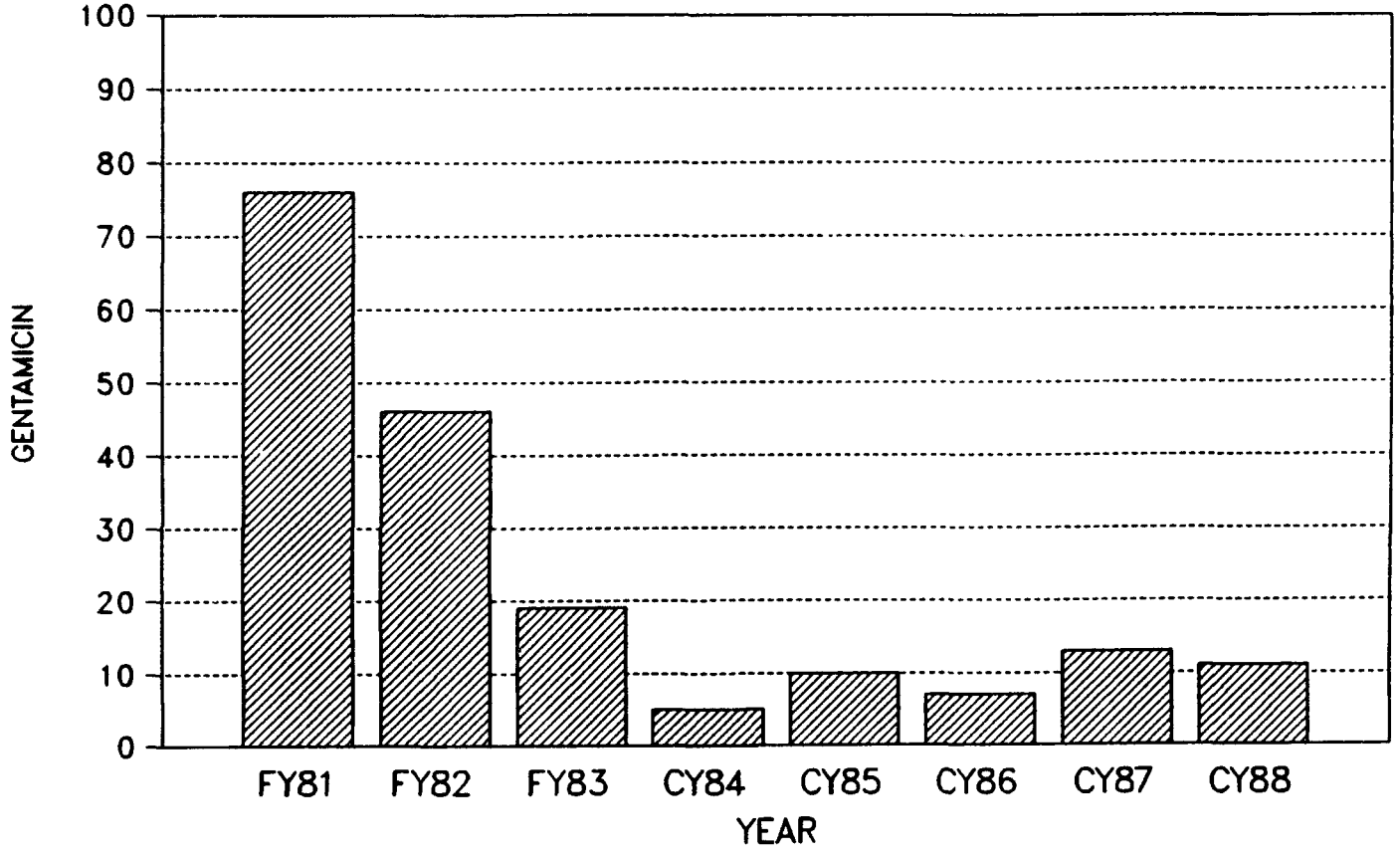

FIGURE 11. Relative frequency (%) of *Pseudomonas aeruginosa* resistant to gentamicin for fiscal years 1981-3 and calendar years 1984-8.

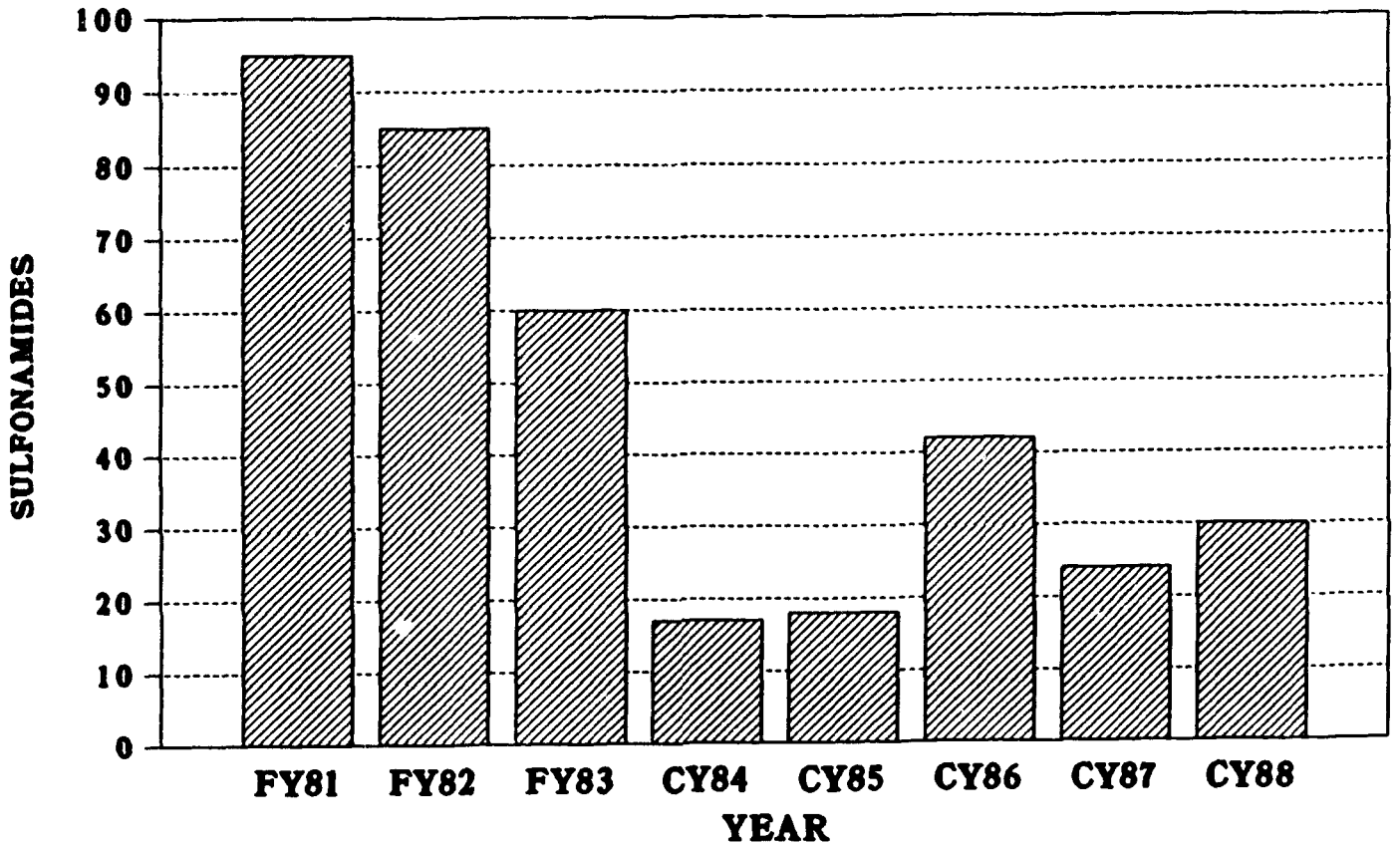

FIGURE 12. Relative frequency (%) of *Pseudomonas aeruginosa* resistance to sulfonamides for fiscal years 1981-3 and calendar years 1984-8.

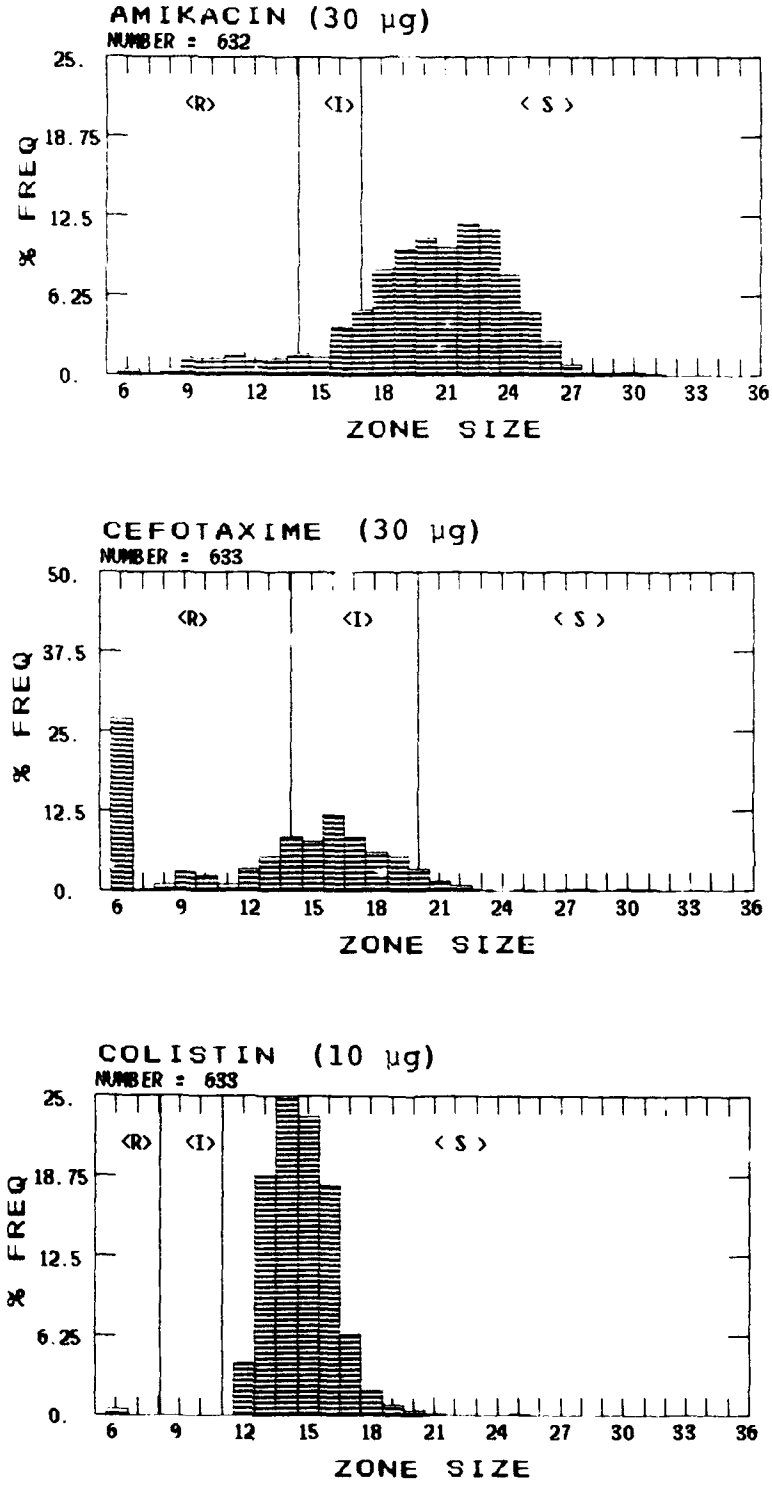

FIGURE 13. Histogram display of the distribution of zones of inhibition of growth of *Pseudomonas aeruginosa*.

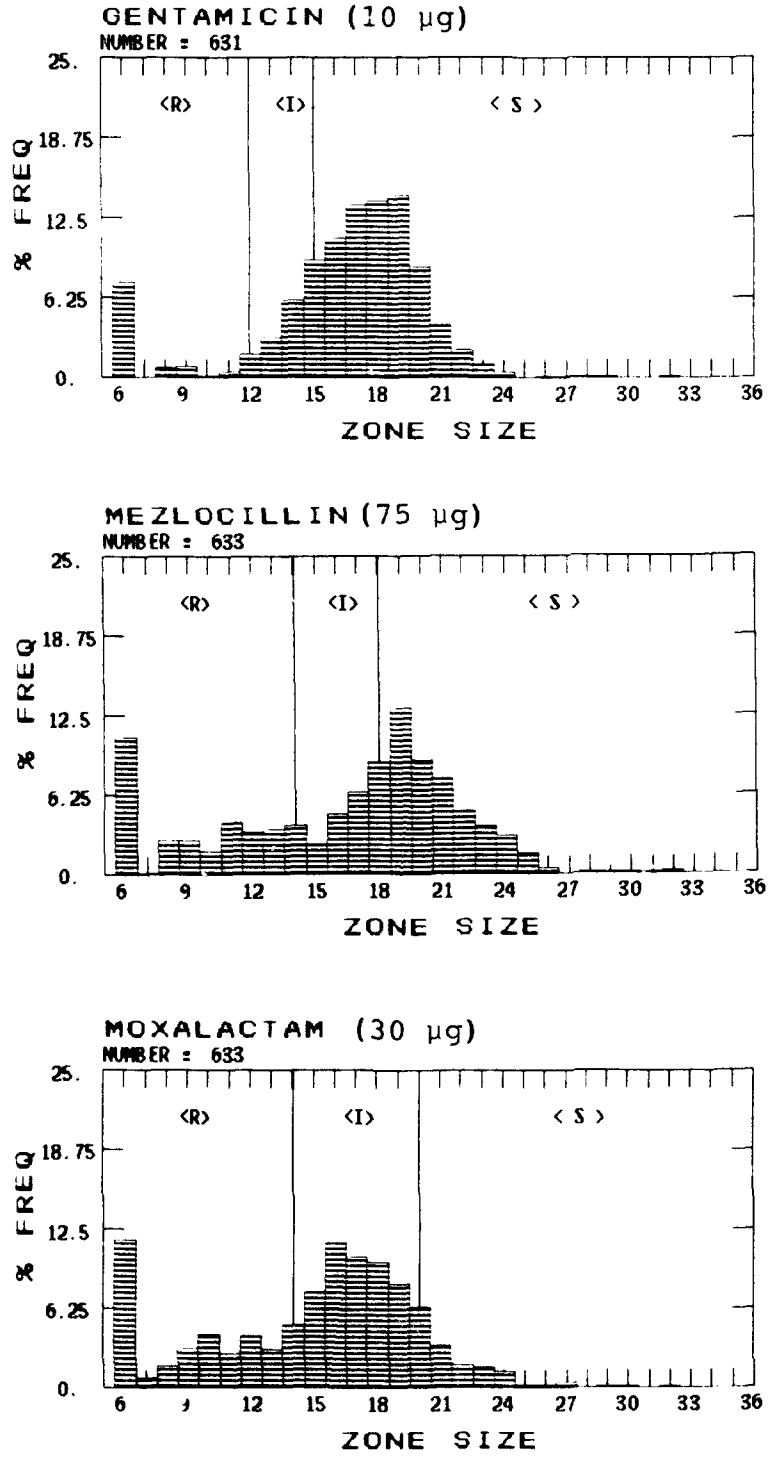

FIGURE 13. Histogram display of the distribution of zones of inhibition of growth of *Pseudomonas aeruginosa* (continued).

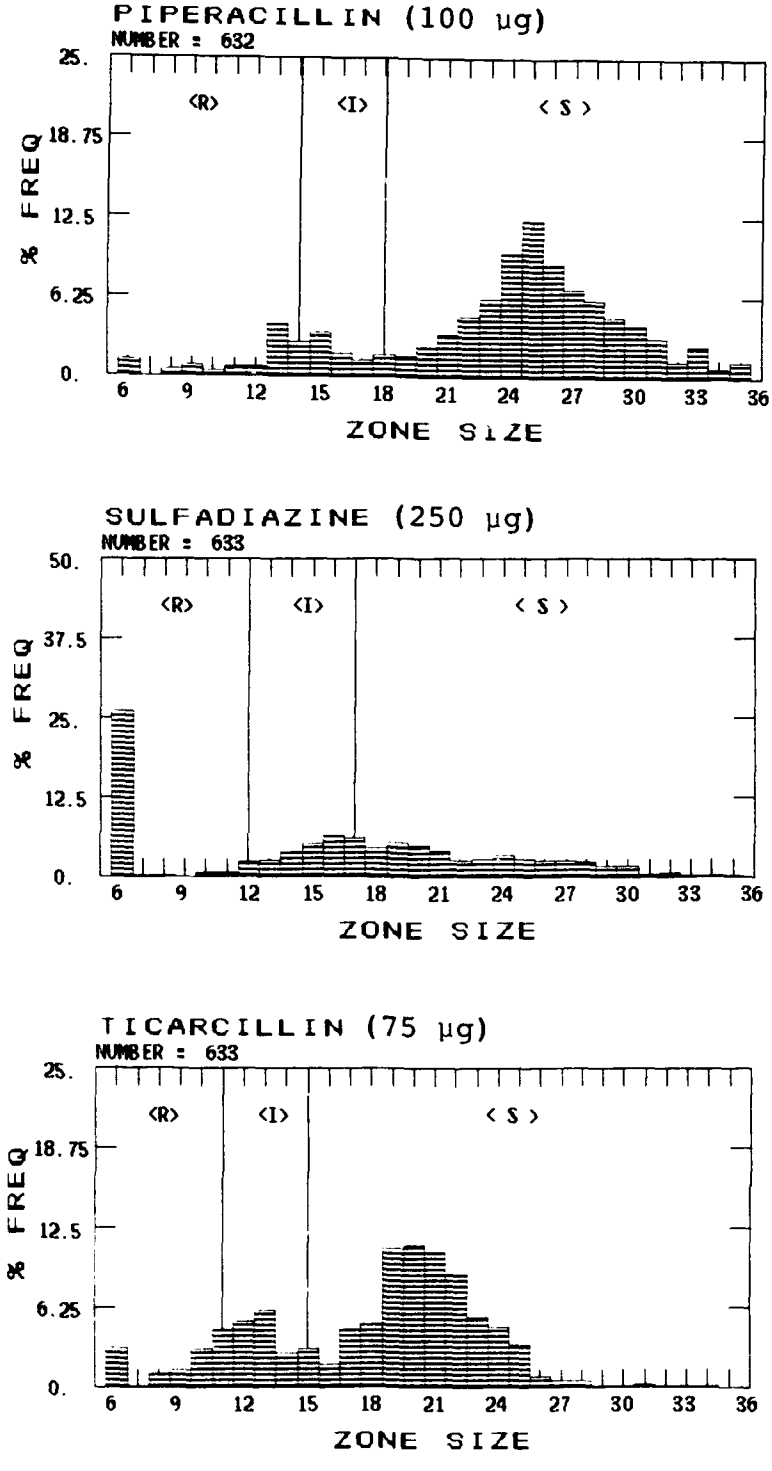

FIGURE 13. Histogram display of the distribution of zones of inhibition of growth of *Pseudomonas aeruginosa* (continued).

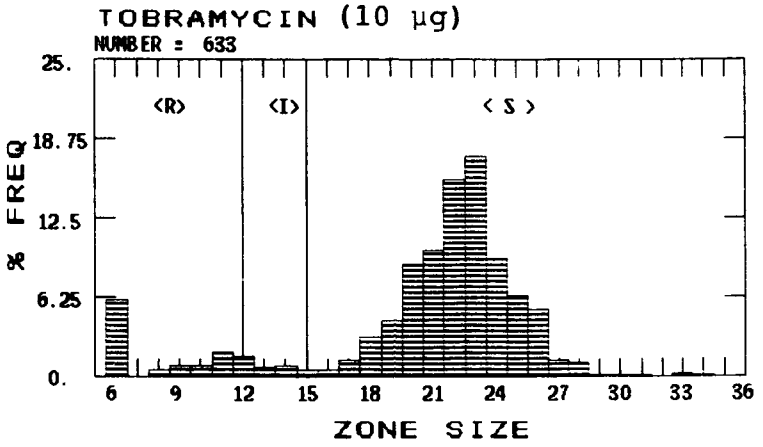

FIGURE 13. Histogram display of the distribution of zones of inhibition of growth of *Pseudomonas aeruginosa* (continued).

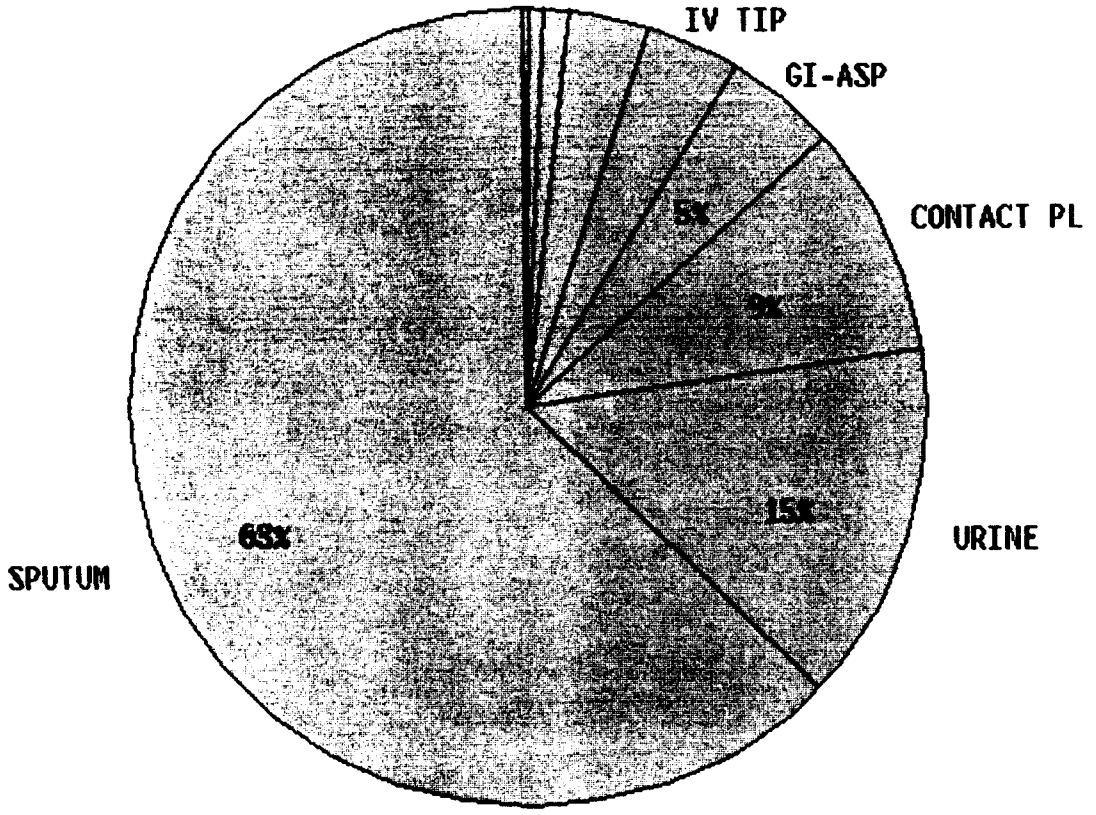

FIGURE 14. Display of the relative frequency of sources yielding *Klebsiella pneumoniae* tested for in vitro sensitivity to antibiotics in 1988.

TABLE 12. Antibiotic Sensitivity Data for *Klebsiella pneumoniae* (1988)

Antibiotic	RESISTANT %	RESISTANT Number	INTERMEDIATE %	INTERMEDIATE Number	SENSITIVE %	SENSITIVE Number	Total Number
Amikacin	-	-	0.85	39	9.15	348	351
Ampicillin	79.71	279	10.57	37	9.71	34	350
Aztreonam	0.85	3	0.85	39	8.29	345	351
Cefamandole	1.42	5	1.14	49	7.44	342	351
Cefoperazone	0.57	2	2.85	10	96.58	339	351
Cefotaxime	0.28	1	-	-	99.72	350	351
Cefoxitin	0.57	2	3.70	13	95.73	336	351
Ceftazidime	-	-	-	-	100.00	351	351
Ceftriaxone	-	-	-	-	100.00	351	351
Chloramphenicol	3.13	11	1.14	49	5.73	336	351
Gentamicin	1.42	5	-	-	98.58	346	351
Imipenem-cilastatin sodium	-	-	-	-	100.00	350	350
Kanamycin	1.43	5	3.72	13	94.84	331	349
Mezlocillin	3.70	13	17.95	63	78.35	275	351
Nalidixic acid	2.29	8	3.14	11	94.57	331	350
Netilimicin	1.14	4	-	-	98.86	347	351
Norfloxacin	2.31	8	-	-	97.69	339	347
Piperacillin	3.13	11	3.99	14	92.88	326	351
Streptomycin	3.15	11	11.17	39	85.67	299	349
Sulfadiazine	26.00	91	1.43	5	72.57	254	350
Tetracycline	1.71	6	5.70	20	92.59	325	351
Ticarcillin	80.34	282	11.11	39	8.55	30	351
Trimethoprim	3.14	11	0.57	2	96.29	337	350
Trimeth & sulfa	2.85	10	1.71	6	95.44	335	351

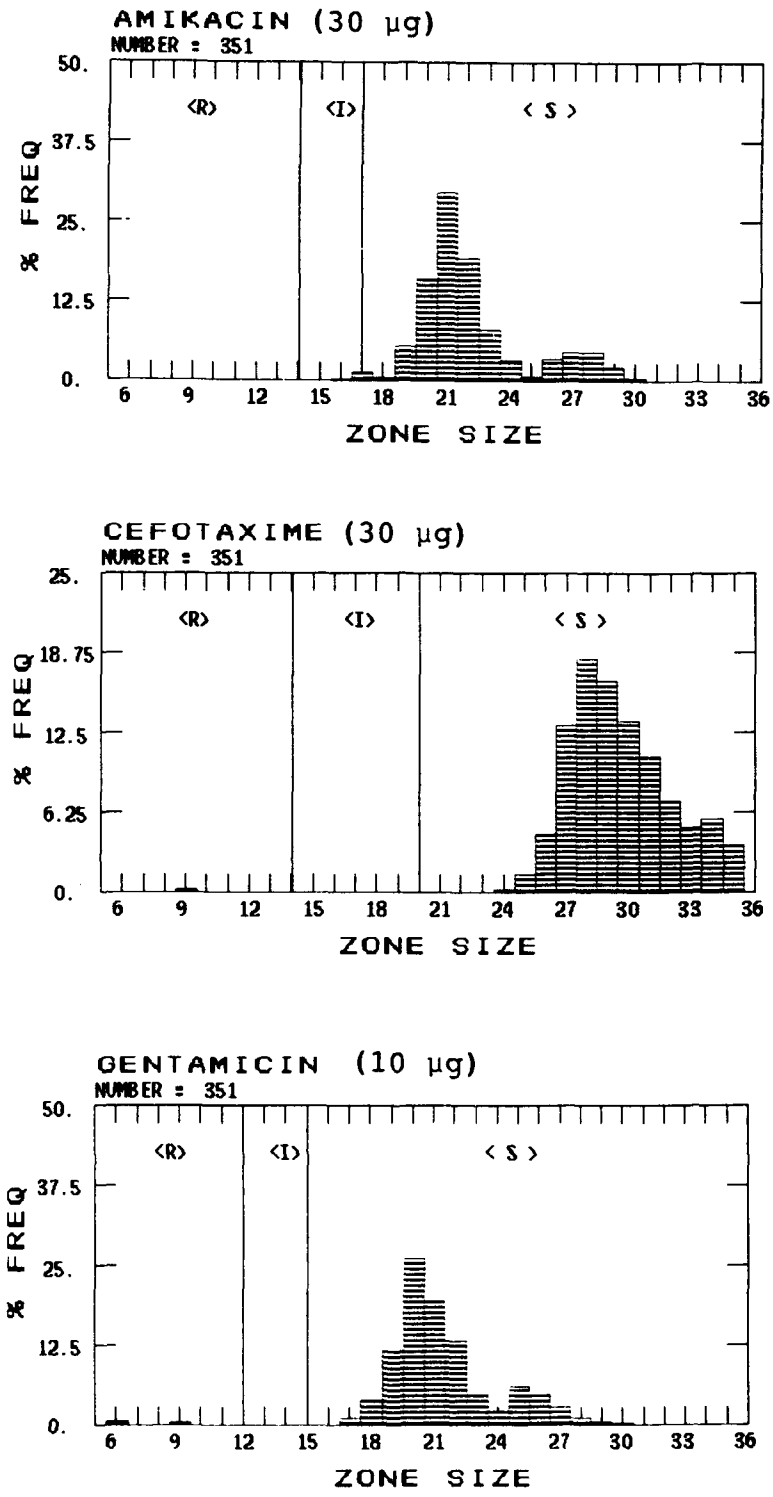

FIGURE 15. Histogram display of the distribution of zones of inhibition of growth of *Klebsiella pneumoniae*.

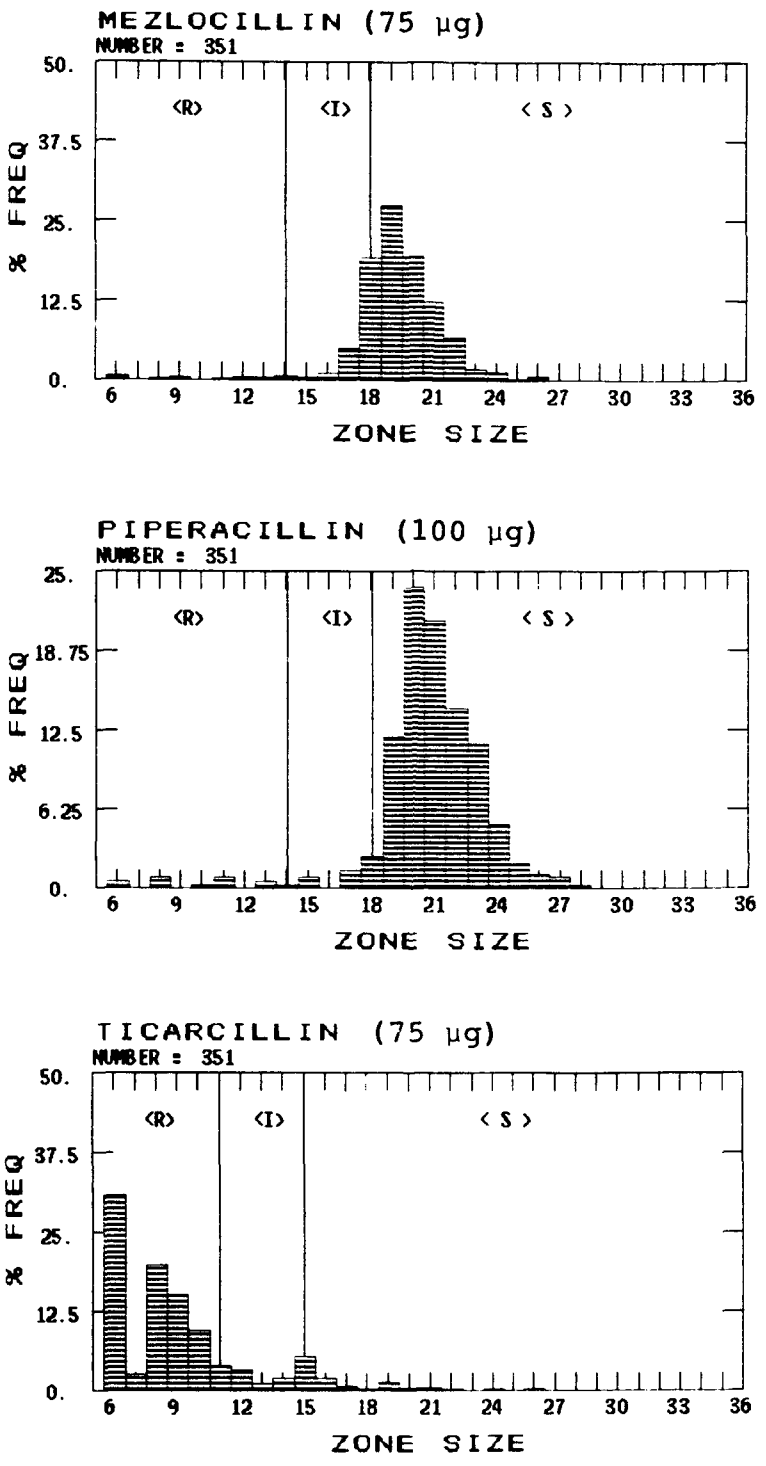

FIGURE 15. Histogram display of the distribution of zones of inhibition of growth of *Klebsiella pneumoniae* (continued).

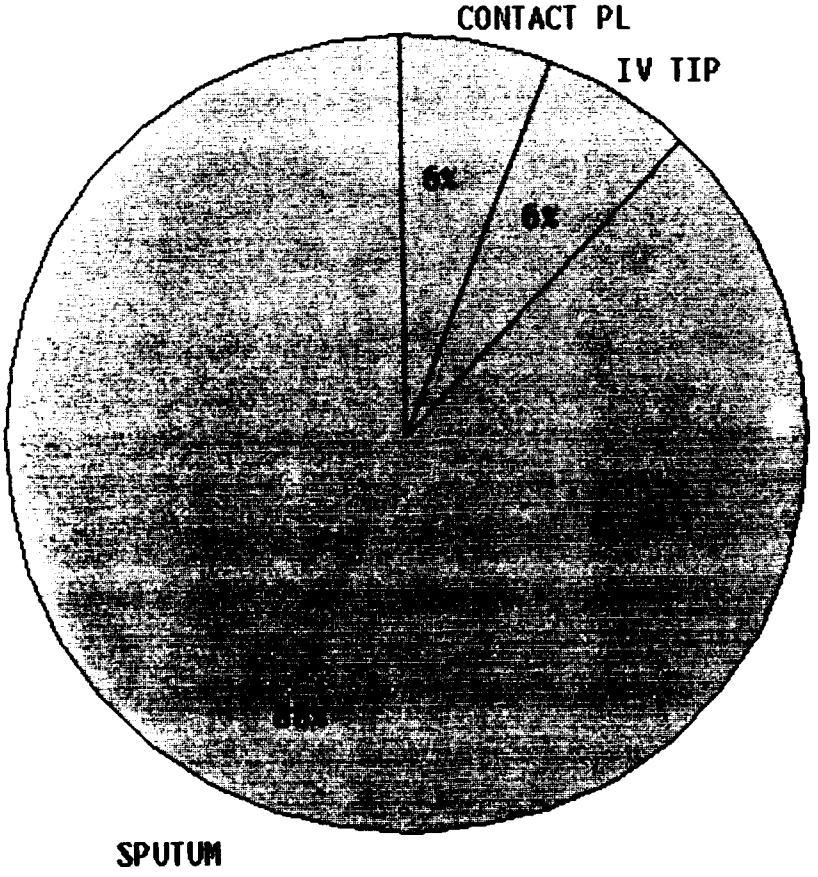

FIGURE 16. Display of the relative frequency of sources yielding *Acinetobacter anitratus* tested for in vitro sensitivity to antibiotics in 1988.

TABLE 13. Antibiotic Sensitivity Data for *Acinetobacter anitratus* (1988)

Antibiotic	RESISTANT		INTERMEDIATE		SENSITIVE		Total
	%	Number	%	Number	%	Number	Number
Amikacin	14.89	7	10.64	5	74.47	35	47
Azlocillin	50.00	1	50.00	1	-	-	2
Aztreonam	44.68	21	53.19	25	2.13	1	47
Cefoperazone	63.83	30	29.79	14	6.38	3	47
Cefotaxime	27.66	13	65.96	31	6.38	3	47
Ceftazidime	-	-	-	-	100.00	47	47
Ceftriaxone	-	-	-	-	100.00	47	47
Chloramphenicol	82.98	39	2.13	1	14.89	7	47
Colistin	-	-	-	-	100.00	2	2
Gentamicin	23.40	11	-	-	76.60	36	47
Imipenem-cilastatin sodium	4.26	2	2.13	1	93.62	44	47
Kanamycin	8.51	4	10.64	5	80.85	38	47
Mezlocillin	19.15	9	55.32	26	25.53	12	47
Moxalactam	50.00	1	50.00	1	-	-	0
Netilimicin	23.40	11	-	-	76.60	36	47
Norfloxacin	21.28	10	10.64	5	68.09	32	47
Piperacillin	4.26	2	29.79	14	65.96	31	47
Sulfadiazine	2.13	1	-	-	97.87	46	47
Tetracycline	10.64	5	27.66	13	61.70	29	47
Ticarcillin	-	-	2.13	1	97.87	46	47
TIM-85	-	-	-	-	100.00	2	2
Tobramycin	50.00	1	-	-	50.00	1	2

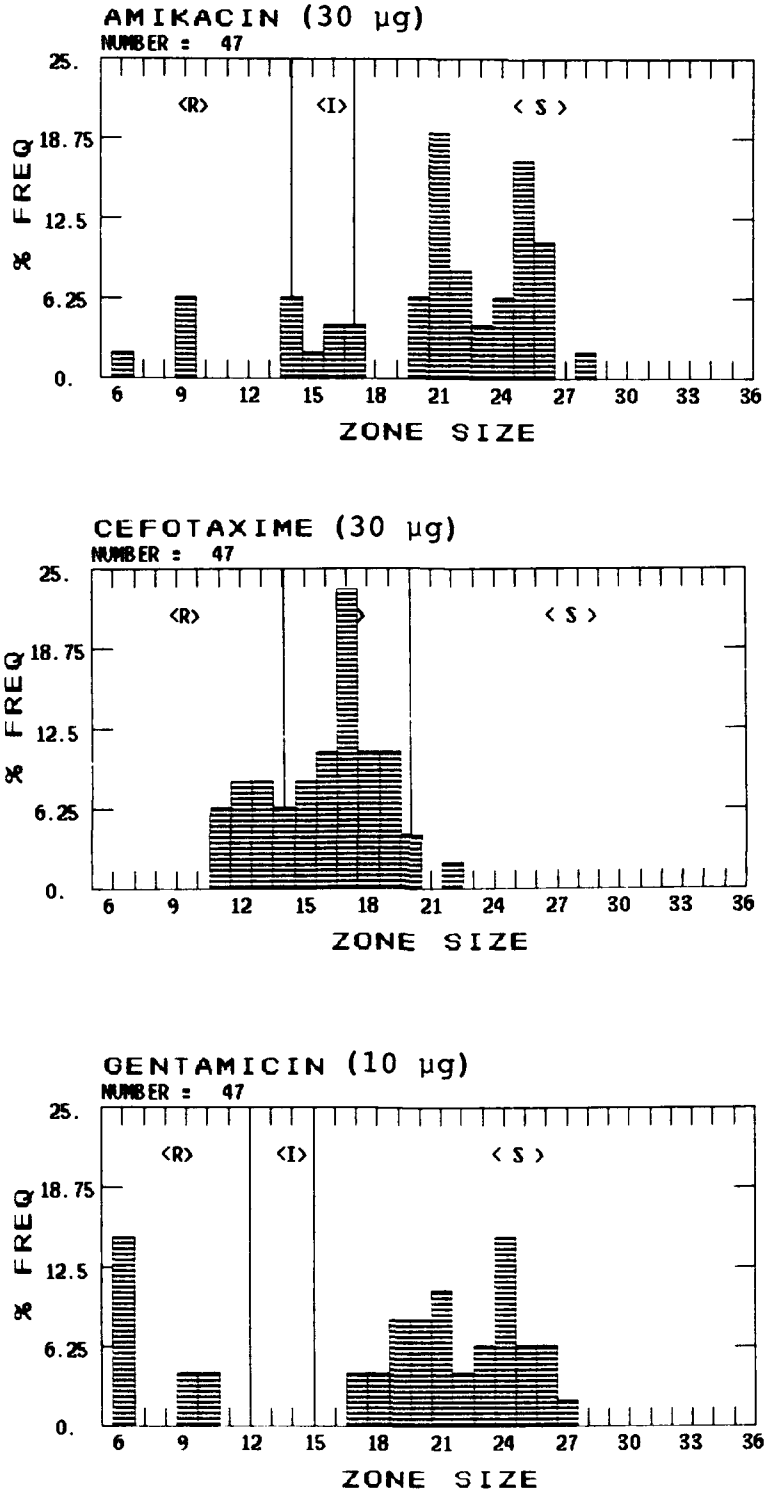

FIGURE 17. Histogram display of the distribution of zones of inhibition of growth of *Acinetobacter anitratus*.

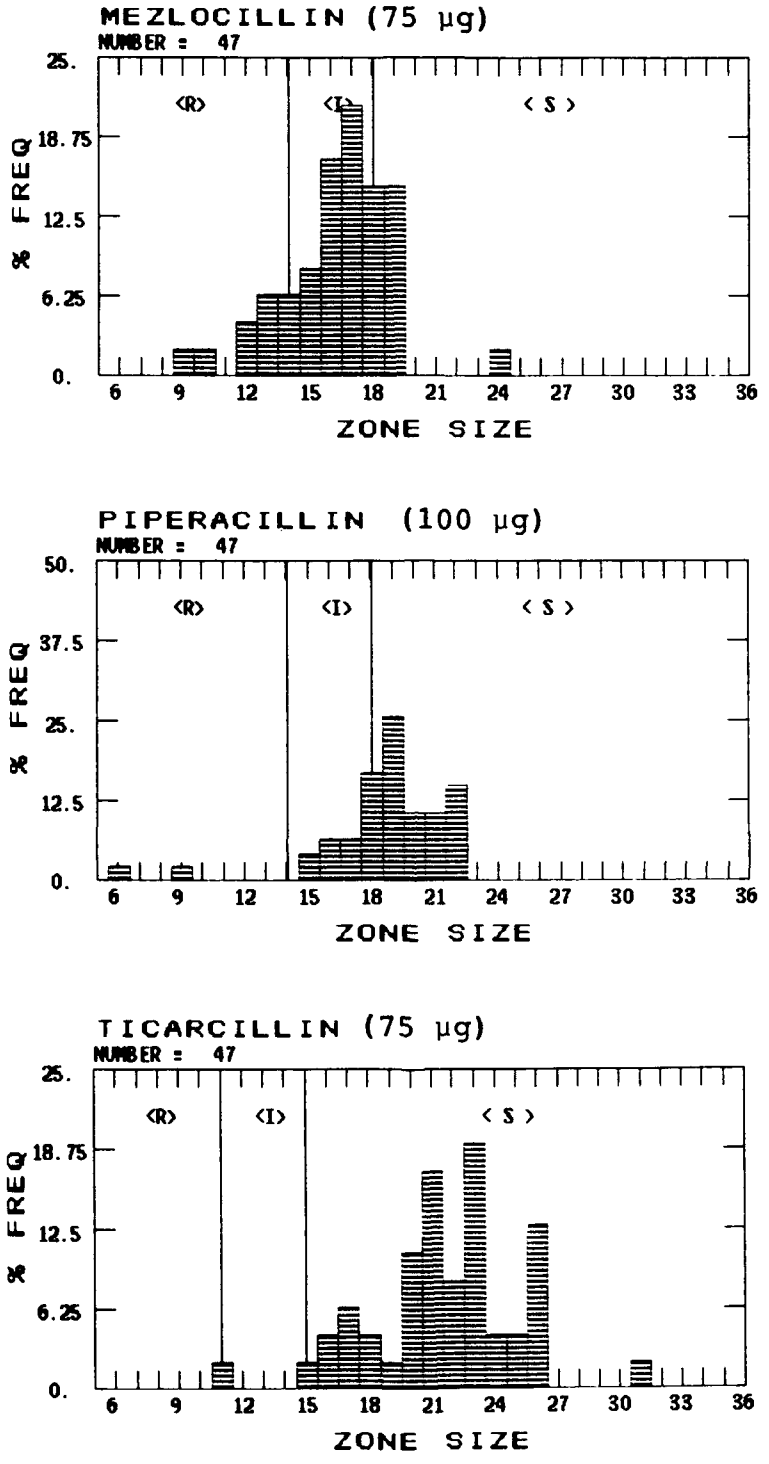

FIGURE 17. Histogram display of the distribution of zones of inhibition of growth of *Acinetobacter anitratus* (continued).

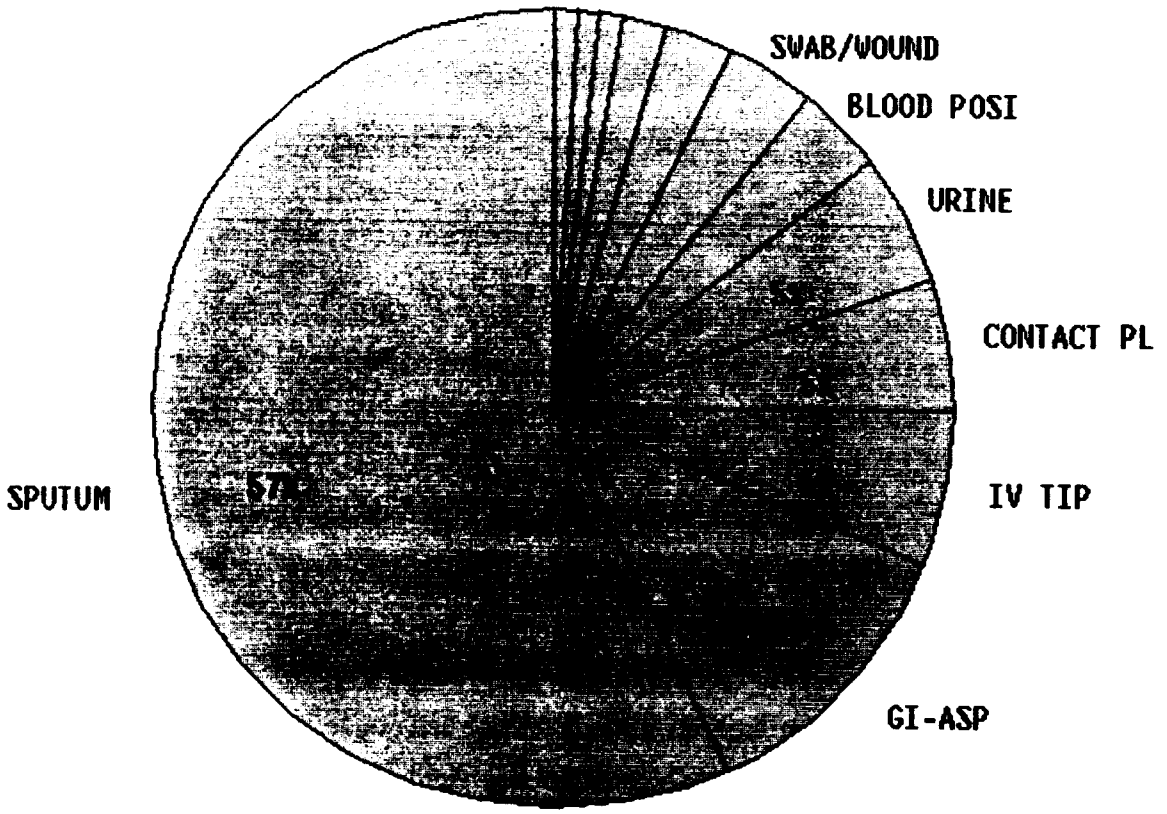

FIGURE 18. Display of the relative frequency of sources yielding Group D Enterococcus tested for in vitro sensitivity to antibiotics in 1988.

TABLE 14. Antibiotic Sensitivity Data for Group D Enterococcus (1988)

Antibiotic	RESISTANT %	RESISTANT Number	INTERMEDIATE %	INTERMEDIATE Number	SENSITIVE %	SENSITIVE Number	Total Number
Amikacin	93.33	28	6.67	2	-	-	30
Ampicillin	-	-	-	-	100.00	30	30
Cefoperazone	3.33	1	96.67	29	-	-	30
Cefotaxime	83.33	25	16.67	5	-	-	30
Ceftazidime	-	-	-	-	100.00	30	30
Ceftriaxone	-	-	-	-	100.00	30	30
Cephalothin	73.33	22	23.33	7	3.33	1	30
Chloramphenicol	6.67	2	3.33	1	90.00	27	30
Clindamycin	96.55	28	-	-	3.45	1	29
Erythromycin	43.33	13	30.00	9	26.67	8	30
Gentamicin	66.67	20	26.67	8	6.67	2	30
Imipenem-cilastatin sodium	-	-	-	-	100.00	30	30
Mezlocillin	-	-	-	-	100.00	30	30
Moxalactam	96.67	29	3.33	1	-	-	30
Oxacillin	100.00	29	-	-	-	-	29
Penicillin	86.67	26	13.33	4	-	-	0
Piperacillin	-	-	-	-	100.00	30	30
Streptomycin	96.55	28	3.45	1	-	-	29
Sulfadiazine	100.00	30	-	-	-	-	30
Tetracycline	66.67	20	3.33	1	30.00	9	30
Tobramycin	80.00	24	10.00	3	10.00	3	30
Vancomycin	-	-	-	-	100.00	30	30

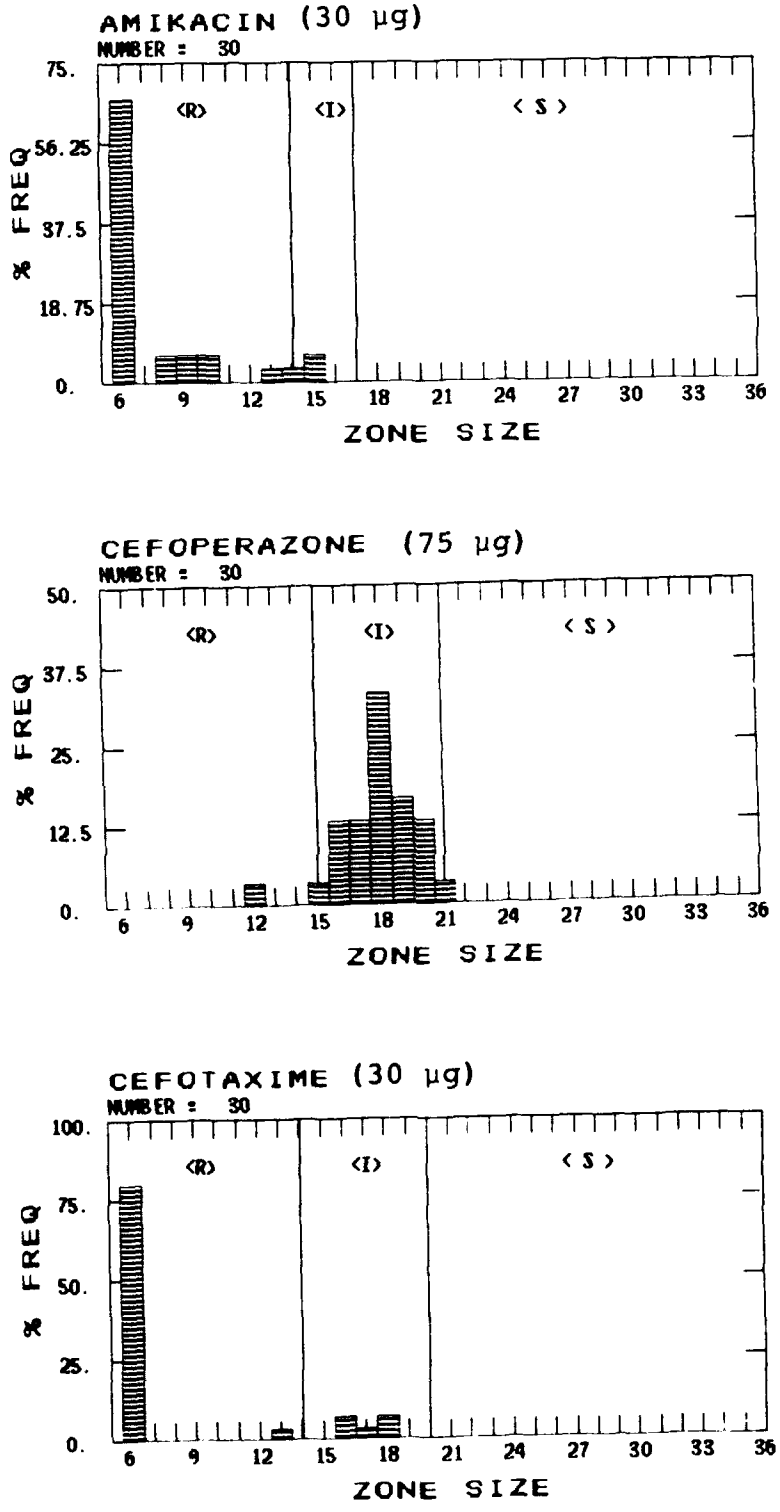

FIGURE 19. Histogram display of the distribution of zones of inhibition of growth of Group D Enterococcus.

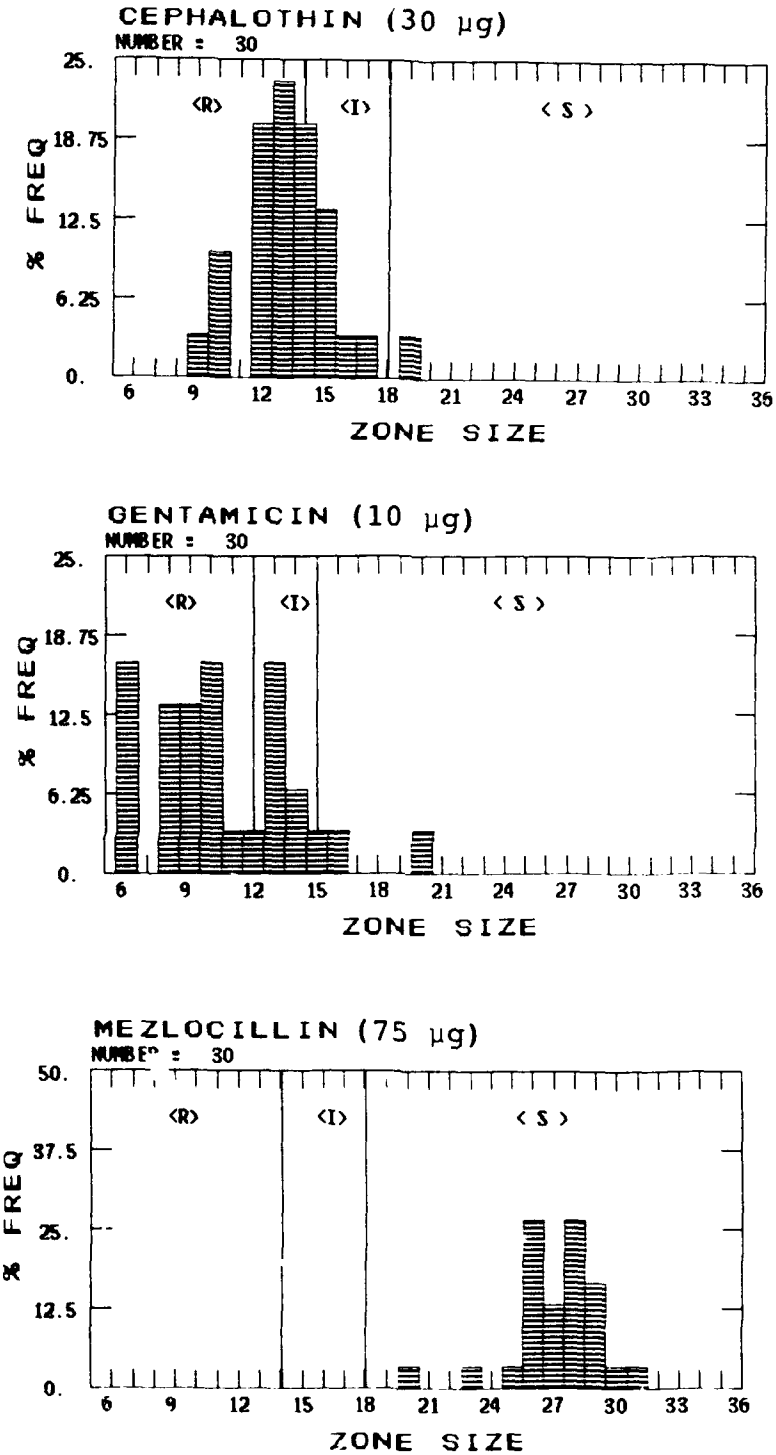

FIGURE 19. Histogram display of the distribution of zones of inhibition of growth of Group D Enterococcus (continued).

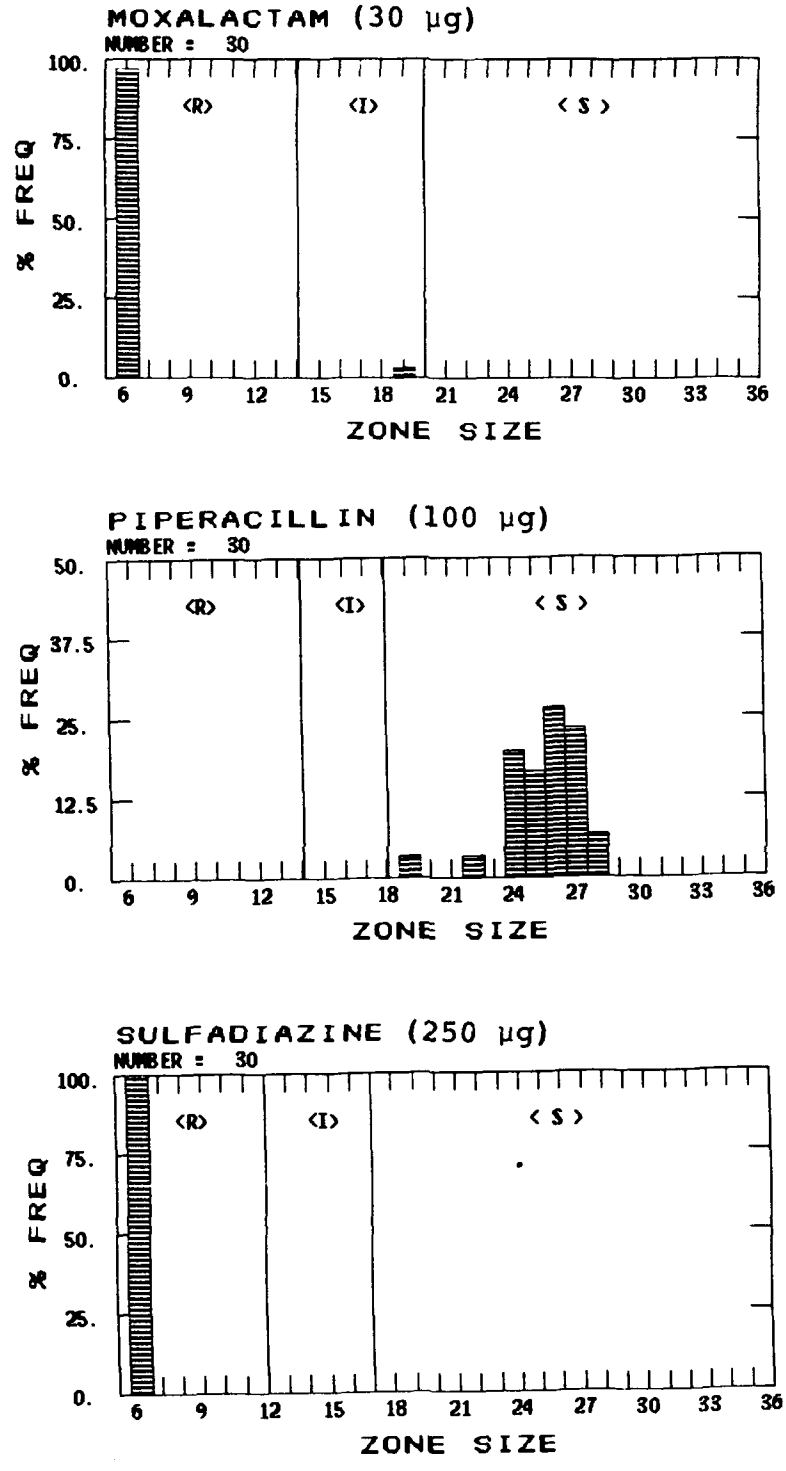

FIGURE 19. Histogram display of the distribution of zones of inhibition of growth of Group D Enterococcus (continued).

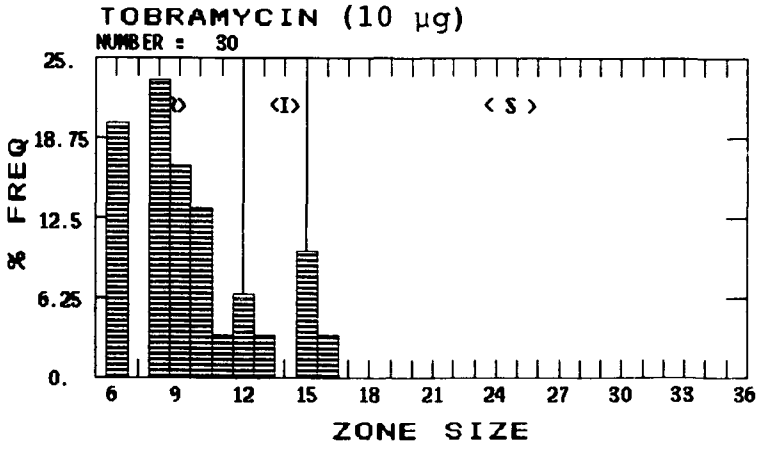

FIGURE 19. Histogram display of the distribution of zones of inhibition of growth of Group D Enterococcus (continued).

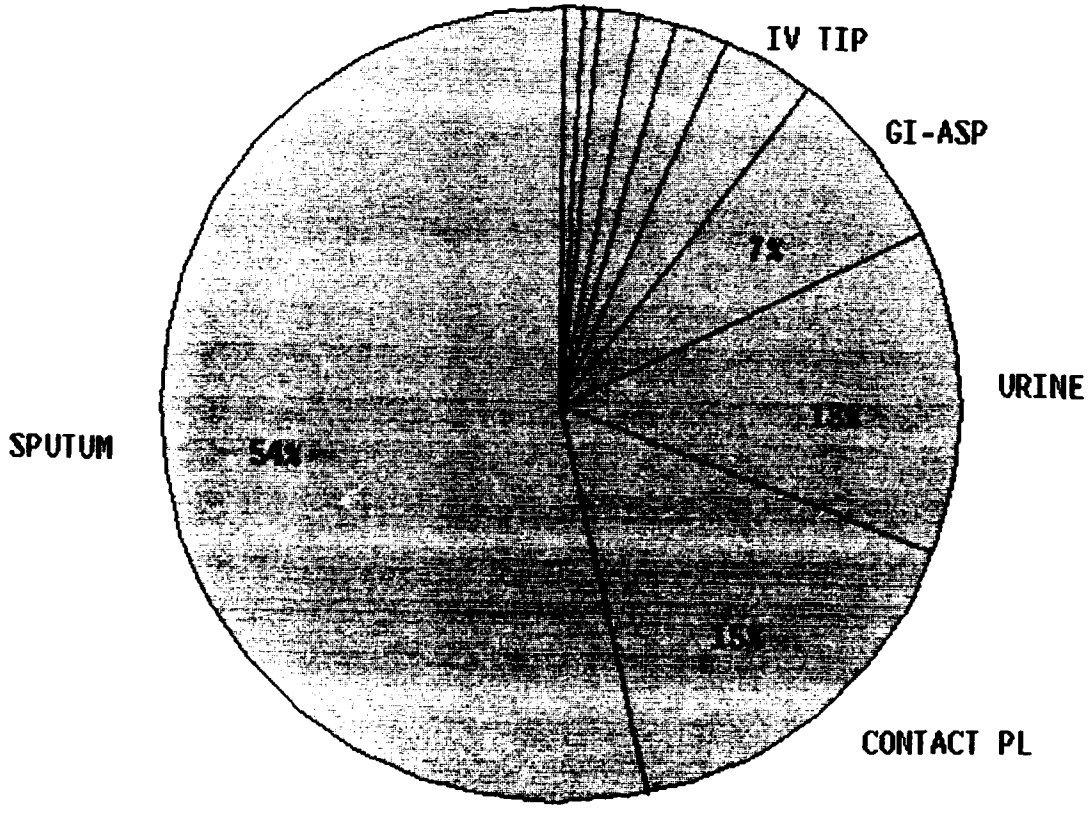

FIGURE 20. Display of the relative frequency of sources yielding *Proteus mirabilis* tested for in vitro sensitivity to antibiotics in 1988.

TABLE 15. Antibiotic Sensitivity Data for *Proteus mirabilis* (1988)

Antibiotic	RESISTANT		INTERMEDIATE		SENSITIVE		Total
	%	Number	%	Number	%	Number	Number
Amikacin	-	-	-	-	100.00	134	134
Ampicillin	4.48	6	-	-	95.52	128	134
Aztreonam	-	-	8.27	11	91.73	122	133
Cefamandole	2.24	3	1.49	2	96.27	129	134
Cefoperazone	-	-	0.75	1	99.25	133	134
Cefotaxime	-	-	0.75	1	99.25	133	134
Cefoxitin	0.75	1	-	-	99.25	133	134
Ceftazidime	-	-	-	-	100.00	134	134
Ceftriaxone	-	-	-	-	100.00	134	134
Chloramphenicol	-	36	28.57	38	71.43	95	133
Gentamicin	-	-	-	-	100.00	134	134
Imipenem-cilastatin sodium	-	-	-	-	100.00	134	134
Kanamycin	-	-	-	-	100.00	134	134
Mezlocillin	-	-	-	-	100.00	134	134
Nalidixic Acid	-	-	0.75	1	99.25	133	134
Netilimicin	-	-	-	-	100.00	134	134
Norfloxacin	0.75	1	-	-	99.25	132	133
Piperacillin	-	-	-	-	100.00	134	134
Streptomycin	-	-	2.99	4	97.01	130	134
Sulfadiazine	-	-	2.24	3	97.76	131	134
Tetracycline	97.01	130	1.49	2	1.49	2	134
Ticarcillin	-	-	-	-	100.00	134	134
Trimethoprim	0.75	1	24.63	33	74.63	100	134
Trimeth & Sulfa	-	-	-	-	100.00	134	134

distributions of zone sizes for selected antibiotics are presented in Figure 21.

PRESENTATIONS/PUBLICATIONS

REFERENCES

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.

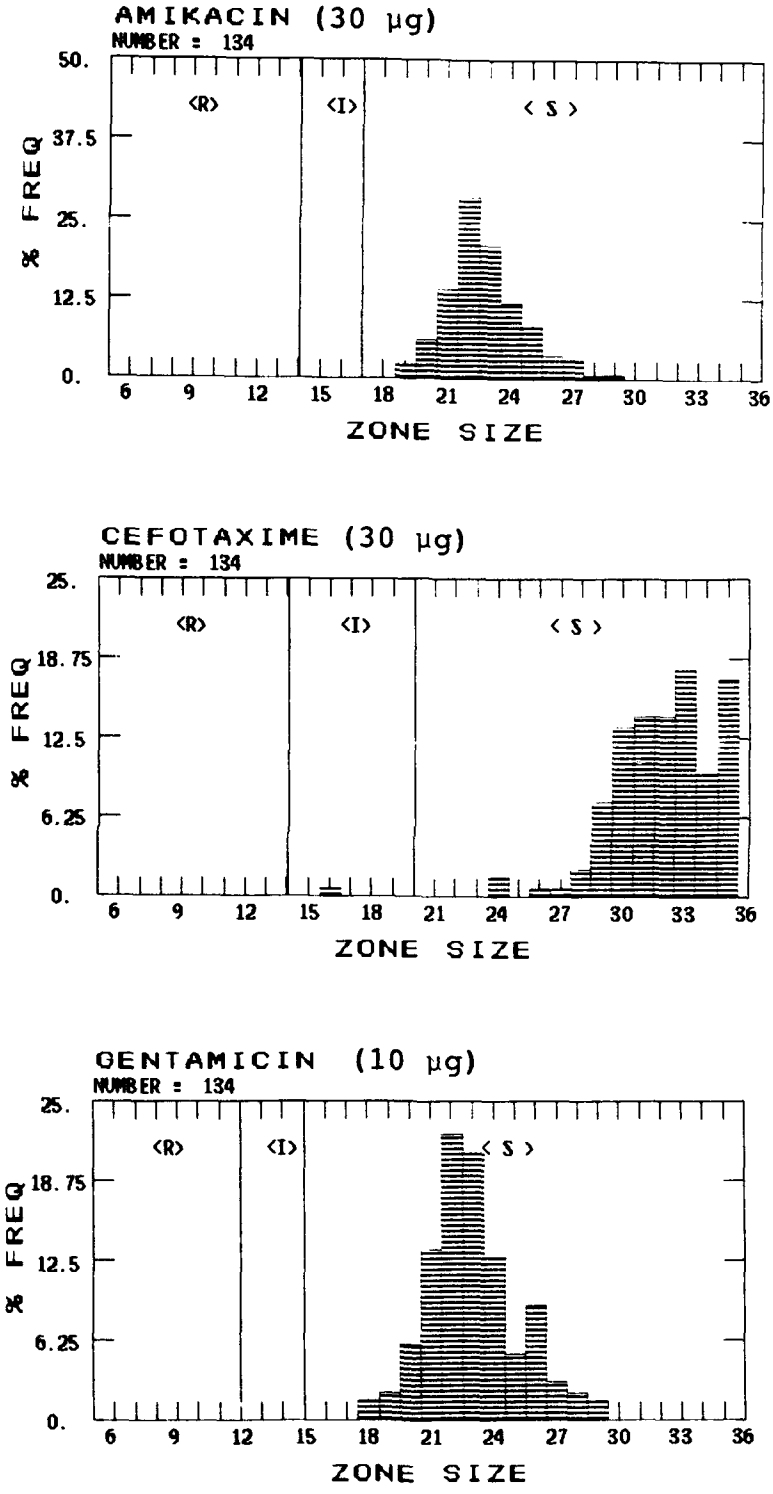

FIGURE 21. Histogram display of the distribution of zones of inhibition of growth of *Proteus mirabilis*.

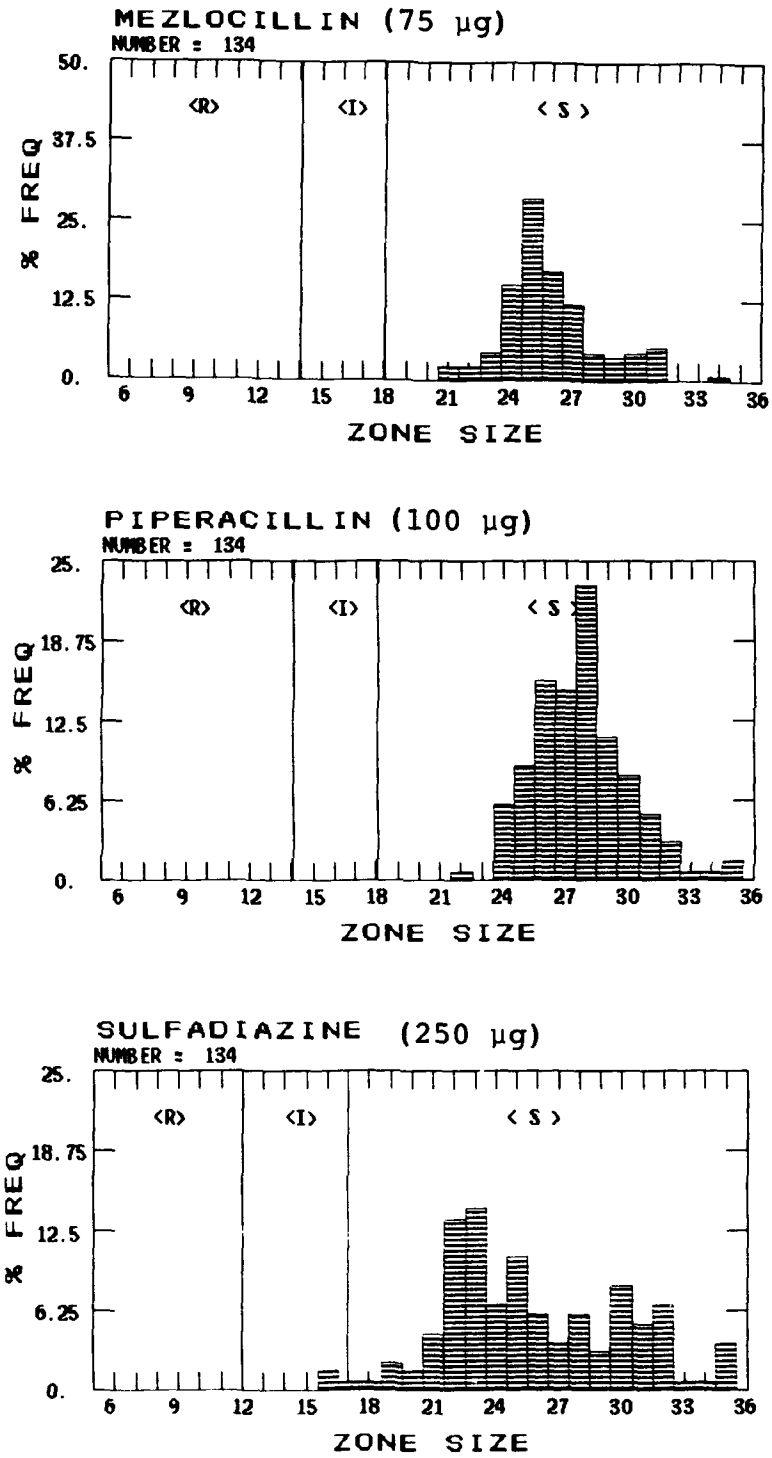

FIGURE 21. Histogram display of the distribution of zones of inhibition of growth of *Proteus mirabilis* (continued).

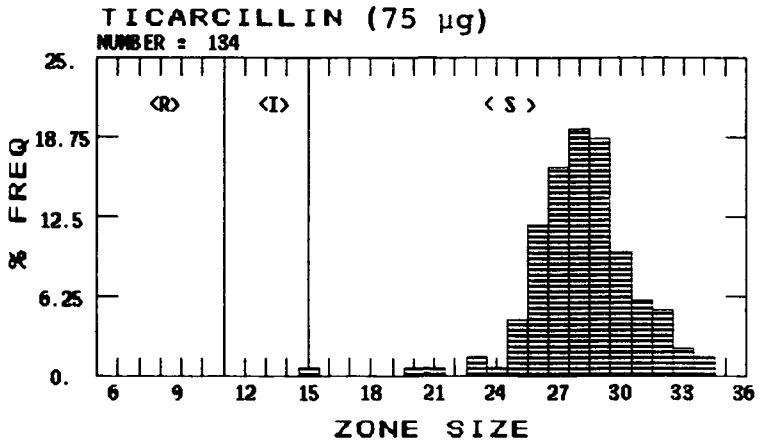

FIGURE 21. Histogram display of the distribution of zones of inhibition of growth of *Proteus mirabilis* (continued).

ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3M161102BS14-00, BASIC 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

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

1 October 1988 - 30 September 1989

INVESTIGATORS

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

ABSTRACT

PROJECT NUMBER: 3M161102BS14-00, BASIC RESEARCH

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

PERIOD COVERED IN THIS REPORT: 1 Oct 88 through 30 Sep 89

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

This study is designed to evaluate the efficacy of imipenem-cilastatin sodium for prophylactic activity against bacterial pneumonias in burned patients with inhalation injury. Patients enrolled in the study receive standard appropriate therapy for their burns and are randomized in pairs to receive or not receive prophylaxis with imipenem-cilastatin sodium. Patient pairs are then 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.

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 problem in the care of the burn patient. It increases the mortality of burn injury and causes the largest effect in burns of moderate size. In the most recent review, inhalation injury was found in 35% of patients and bacterial pneumonia in 19% (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 is 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 will be entered into 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 will be offered the opportunity to participate in a study of prophylactic administration of imipenem-cilastatin sodium. Patients having the following will be considered 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 will be selected for participation in the study if they meet the following criteria:

1. The patient is ≥ 18 yr old.

2. The diagnosis of inhalation injury is confirmed by bronchoscopic examination.

3. The patient has an expected probability of survival of 20-80% based on an age and burn size predictor model.

4. The patient is not subject to any preexisting disease which would contraindicate administration of imipenem-cilastatin sodium.

5. The patient or representative signs the appropriate informed consent.

6. The patient's treatment can begin by 72 h postburn.

Patient Exclusion. Patients with the following characteristics will be excluded from participation in the study:

1. Patients < 18 yr old.

2. Patients who are pregnant or nursing.

3. Patients who have a prior history of renal dysfunction.

4. Patients who are hypersensitive to thienamycin or who have 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 a hepatic coma.

Study Design. Patients will receive standard appropriate therapy for their burn and will be randomized in pairs to receive or not receive prophylaxis with imipenem-cilastatin sodium. Imipenem-cilastatin sodium at 500 mg every 6 h will be started between 36 and 72 h postburn and administered for 240 h (10 days). No other modifications will be made in the care of either group of patients. Sputum cultures and other cultures will be collected according to the usual protocol for all patients. Respiratory status will be carefully monitored according to the usual procedures in all patients and documented appropriately.

Patients in both groups will be carefully watched for the development of pulmonary infection. Tracheobronchitis and pneumonia will be diagnosed by the usual criteria (Table 1) and will be treated with antibiotics when judged appropriate by the primary physician. Cellulitis diagnosed by clinical criteria and treated with penicillin will not be grounds for exclusion from the study. Patient pairs will be excluded from the study if either patient develops a deep tissue infection requiring treatment with antibiotics or succumbs without pneumonia in the first 10 days.

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 not consistent with pneumonia.
4. Positive sputum culture (confirmatory, but not essential for diagnosis).

Patient pairs will 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 will be entered into the study as sequential pairs with treatment randomly allocated between the paired patients. This will allow sequential analysis with a 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 can be estimated to be 82 patients per group (Fig 1). If imipenem-cilastatin sodium therapy proves to be either very effective or detrimental, the sequential analysis

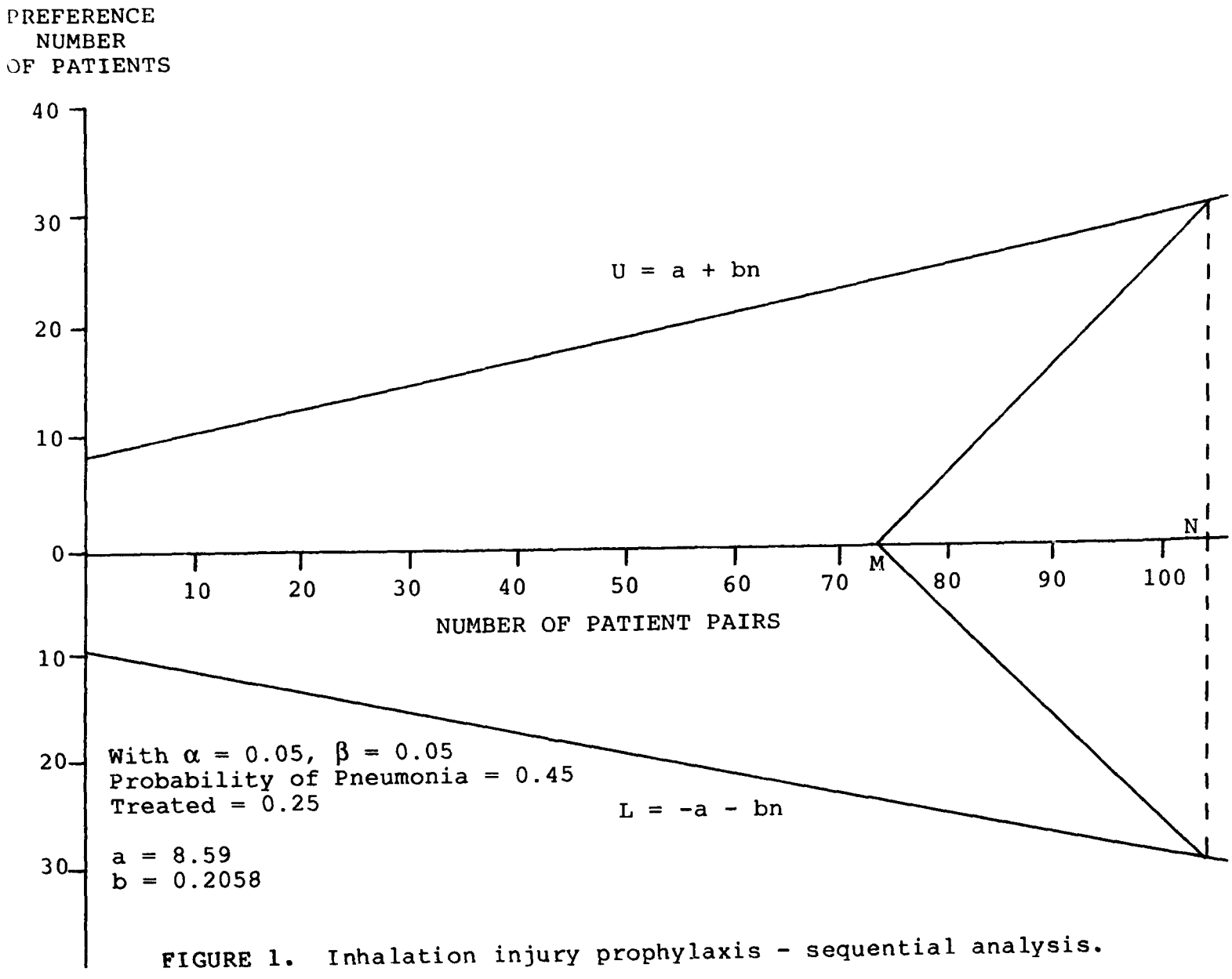

FIGURE 1. Inhalation injury prophylaxis - sequential analysis.

will allow the study to be completed with significantly fewer patients.

RESULTS

Because the population for this study conflicts with another study, no patients have been entered into this study to date.

DISCUSSION

When 50 patients have completed the study, the data will be analyzed as to the prophylactic activity of imipenem-cilastatin sodium against bacterial pneumonias.

PRESENTATIONS/PUBLICATIONS

None.

REFERENCES

1. Shirani KZ, Pruitt BA Jr, and Mason AD Jr: The influence of inhalation injury and pneumonia on burn mortality. **Ann Surg** 205:82-7, 1987.

2. Levine BA, Petroff PA, Slade CL, et al: Prospective trials of dexamethasone and aerosolized gentamicin in the treatment of inhalation injury in the burned patient. **J Trauma** 18:188-93, 1978.

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
				DA314656	1 Oct 89	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
1 Oct 88	D. Chg	U	U		CX	

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS14	F	302
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP,	FY90-01		

11. TITLE *(Precede with Security Classification Code)* (U) Investigation of the Physiologic and Immunologic Effects of Prostaglandin E in Septic and Traumatized Rats

12. SUBJECT AREAS
0605 Medicine and Medical Research 0615 Pharmacology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
8805	9009	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)*
89	0.5	30
90	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: WAYMACK, J P
d. TELEPHONE NUMBER *(include area code)*: 512-221-2720	d. TELEPHONE NUMBER *(include area code)*: 512-271-7267
21. GENERAL USE: FINA MILITARY/CIVILIAN APPLICATION: M	f. NAME OF ASSOCIATE INVESTIGATOR *(if available)*: MASON, A D g. NAME OF ASSOCIATE INVESTIGATOR *(if available)*: MC MANUS, A T

22. KEYWORDS *(Precede EACH with Security Classification Code)* (U) Prostaglandin; (U) Physiological Effects; (U) Immunosuppression; (U) Pharmacology; (U) Trauma; (U) Septicemia; (U) Lab

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

22. (Continued) (U) Animals: (U) Rats; (U) RA II

23. (U) A DTIC literature search was conducted under DTIC request number W6K44L dated 20 October 1989 for the technical report database and request number W6K45D dated 20 October 1989 for the work unit database. The proposed effort will not result in the duplication of effort. The objective of this work was to determine the physiologic and immunologic effects of the elevations in prostaglandin E levels seen following trauma, sepsis, and tumor growth, to determine whether the net effect of the elevation in prostaglandin E levels of these disease states was beneficial or detrimental, and to determine whether pharmacologic manipulation of the prostaglandin E level was beneficial in terms of immunologic or physiologic function.

24. (U) Healthy adult Lewis rats were administered varying doses of the long-acting prostaglandin E derivative, 16,16-dimethyl-prostaglandin E, prior to receiving a number of physiologic challenges. Initial evaluations centered on the ability of prostaglandin E to alter resistance to mortality following challenge with endotoxin.

25. (U) 8810 - 8909. Prostaglandin E (PGE) was shown to enhance survival in mouse *Escherichia coli* peritonitis models and to improve survival in endotoxin shock models. These effects may have been secondary to decreased TNF production following infusion of PGE. PGE has not been shown to have a significant effect on T cell subsets or on immunologic function in tumor-bearing rats. For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1989.*

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

ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3M161102BS14-00, BASIC RESEARCH

PROJECT TITLE: Investigation of the Physiologic and Immunologic Function of Prostaglandin E in Septic and Traumatized Rats

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

31 January 1989 - 30 September 1989

INVESTIGATORS

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

ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3M161102BS14-00, BASIC RESEARCH

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PERIOD COVERED IN THIS REPORT: 31 Jan 89 through 30 Sep 89

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Burn injuries have been shown to impair immune function. One of the hypotheses for the etiology of the immunosuppression is that burn injuries result in an elevation of prostaglandin E (PGE) levels which then impair leukocyte function. We evaluated the effect of PGE levels on immune function in multiple animal models utilizing T cell subset levels for our immunologic measurements. Elevations in PGE levels were achieved by administering 16,16-dimethyl-prostaglandin E (dPGE) and reductions by administering indomethacin. The animal models included burned rats, burned-septic rats, and nonburned rats. Neither indomethacin nor dPGE administration resulted in alterations in any of the T-cell subset populations in our models.

INVESTIGATION OF THE PHYSIOLOGIC AND IMMUNOLOGIC FUNCTION OF PROSTAGLANDIN E IN SEPTIC AND TRAUMATIZED RATS

Traumatic injures have been demonstrated to result in a suppression of the body's normal immune system (1,2). The postinjury immunosuppression involves nearly the entire immune system (3). Its exact etiology has not been completely elucidated; however, the most frequently espoused explanation for the development of the immunosuppression is that the injury results in the release of potent immunosuppressive metabolites (4). One of the most extensively investigated of these metabolites is prostaglandin E (PGE) (5,6).

It has been demonstrated that PGE impairs immune function in in vitro leukocyte culture models (7). In these models, PGE was added to WBC cultures and various leukocyte functions were assayed. Additional support for an immunosuppressive effect of PGE has been obtained through the use of animal models in which cyclooxygenase inhibitors were administered to traumatized animals (8,9). In these studies, the administration of cyclooxygenase inhibitors prevented and/or corrected posttraumatic immunosuppression and increased survival rates following bacterial challenges.

More recently, it has been reported that the parenteral administration of a long-acting PGE derivative, 16,16-dimethyl-prostaglandin E (dPGE) (10), resulted in an enhanced rate of survival in nontraumatized rats subjected to an *Escherichia coli* peritonitis (11). This improved survival appears due, at least in part, to an enhanced resistance to endotoxin shock in the dPGE-treated rats (12). Finally, it has been reported that dPGE administration decreases the rate of release of TNF in response to endotoxin challenge (12).

The discrepancies in the above studies may be due to differences between the effect of PGE in vitro and in vivo. If this is the case, previous work demonstrating that PGE decreases T lymphocyte helper/suppressor ratios in in vitro models may not accurately reflect the in vivo effect of PGE (7). Our current study attempts to delineate the in vivo effects of PGE on T-lymphocyte subsets by administering the cyclooxygenase inhibitor, indomethacin, or dPGE to normal, traumatized, and traumatized, septic rats.

MATERIALS AND METHODS

Animals. Seventy-two adult male Lewis rats weighing ±250 g were used for these studies. The animals were housed in individual stainless steel hanging cages and allowed food and water ad libitum. The animals were observed for a minimum of 1 week prior to entry into the study to exclude the possibility of any preexisting diseases.

Drugs. The dPGE was generously supplied by the Upjohn Company (Kalamazoo, MI) and the indomethacin by the Merck Sharpe & Dohme Corporation (Columbus, OH). Both drugs were diluted with sufficient normal saline to achieve final concentrations such that each dose of drug administered to the animals was in a final volume of 1 ml. The drugs were administered by intraperitoneal injection through a 25-ga needle.

Animal Models. The three animal models were chosen to reflect three common clinical situations:

To evaluate the effect of elevated PGE levels in nontraumatized animals, 9 animals received twice daily injections of saline for 4 days and 9 animals received twice daily injections of 80 μg/kg dPGE.

To evaluate the effect of elevated PGE levels in traumatized animals, the animals were anesthetized with sodium pentobarbital (35 mg/kg IP). The hair was clipped from the backs and the animals were placed in an asbestos-coated template which exposed 30% of the total body surface area. The template was immersed in a 95°C water bath for 10 sec. This model has been shown to result in an uniform full-thickness burn injury which is painless to the animal due to the destruction of nerve endings by the burn. The animals were resuscitated with lactated Ringer's solution (5 ml IP) immediately following scald injury. Nine animals were pretreated with 4 mg/kg indomethacin, which was continued on a twice daily basis for 4 days. This dosage of indomethacin had been previously demonstrated to have no major adverse effects in Lewis rats (13). Nine animals were pretreated with 1 ml saline, which was continued on a twice daily basis. Finally, 9 animals were pretreated with 80 μg/kg dPGE, which was continued on a twice daily basis.

To evaluate the effect of elevated levels of PGE in traumatic-septic situations, the animals received scald injuries as previously described. Following scald injury, the burn wounds were seeded with 1×10^8 *Pseudomonas aeruginosa* (Strain 1244). The *Pseudomonas aeruginosa* organisms had been cultured the night before in trypticase soy broth, washed 3X in normal saline, and resuspended in sufficient saline to achieve a final concentration of 1×10^8 cfu/ml. One milliliter of this suspension was painted onto the wound immediately following burn injury. This model had been previously demonstrated to result in septic changes in Lewis rats within 4 days of burn injury (14). Nine animals were pretreated with 4 mg/kg indomethacin, which was continued on a twice daily basis for 4 days. Nine animals were pretreated with saline and 9 animals were pretreated with 80 μg/kg dPGE, which were continued on a twice daily basis.

Lymphocyte Subset Analysis. Four days after infliction of burn injury, the animals were anesthetized with sodium pentobarbital (35

mg/kg IP). The animals underwent celiotomy and blood was obtained by vena cava puncture. The spleens were removed, bivalved, and lavaged with HBSS to harvest the splenocytes. Both blood and splenocyte samples underwent leukocyte separation by centrifugation on a Ficoll-Hypaque density gradient. The resulting lymphocyte preparations were stained with standard anti-lymphocyte monoclonal antibody preparations. These were washed once and then reacted with affinity-purified, fluorescein-labeled goat anti-mouse IgG as a second-step reagent.

Fluorescein-labeled cells were analyzed on a standard flow cytometer. For each sample, 5,000 cells were assayed and the number of cells labeled by the monoclonal antibodies specific for pan-T (OX-19), helper-inducer (W-25), or suppressor-cytotoxic (OX-8) cell surface markers was determined. Each sample had a negative control using a monoclonal antibody of the same isotype (IgG_1) to human T cells (anti-Leu3) run to determine the cutoff point. The positive cutoff was set at a point defining the upper 2% or less of the background control and the number of background control cells subtracted from each sample. Nonlymphoid cell contamination was monitored by analyzing forward and 90° light scatter. Cells with scattered-light intensities that were outside limits established for normal lymphocytes were removed (gated) from analysis.

All data were presented as mean ± SE of the mean. Comparison between groups was performed using ANOVA.

RESULTS

In the nonburned animal groups, the administration of dPGE failed to alter the percentage of pan-T cells, helper T cells, suppressor T cells, or the helper-suppressor T lymphocyte ratio of either blood lymphocytes or splenocytes (Table 1). There were significant differences between blood and splenic lymphocyte subpopulations. In the saline treatment groups, there was a higher percentage of pan ($P = 0.0019$), helper ($P = 0.0004$), and suppressor ($P = 0.02$) T lymphocytes among blood lymphocytes as compared to splenic lymphocytes. The helper-suppressor T cell ratio was also noted to be significantly higher among blood T lymphocytes when compared to splenic T lymphocytes ($P = 0.0013$). When analyzing lymphocytes from dPGE-treated animals, there again was an increased percentage of pan-T cells ($P = 0.014$) and helper T lymphocytes ($P = 0.016$) among blood lymphocytes as compared to the splenic lymphocytes. The differences in suppressor T cells and the helper-suppressor T cell ratio were not significant.

The analysis of T cell subpopulations in burned animals failed to demonstrate any measurable effect of either indomethacin or dPGE treatment (Table 2). Although neither drug was able to significantly alter the percentage of pan, helper, or suppressor T cells or the helper-suppressor cell ratio compared to saline-treated controls, there were noted to be significant

TABLE 1. Percentage of Pan, Helper, and Suppressor T Cells and Helper/Suppressor Ratios in Normal Rats Treated with Saline or dPGE

Group	Pan	Helper	Suppressor	Helper/ Suppressor
		Splenic Lymphocytes		
Saline	51.9 ± 3.4	31.4 ± 3.2	14.8 ±0.9	2.1 ± 0.2
dPGE	55.3 ± 5.2	38.8 ± 5.4	14.3 ±1.1	2.7 ± 0.3
		Blood Lymphocytes		
Saline	81.7 ± 3.7	65.0 ± 2.6	16.8 ±1.1	3.9 ± 0.2
dPGE	82.2 ± 2.7	61.3 ± 1.7	18.2 ±1.9	3.9 ± 0.5

TABLE 2. Percentage of Pan, Helper, and Suppressor T Cells and Helper/Suppressor Ratios in Burned Rats Treated with Saline, dPGE, or Indomethacin

Group	Pan	Helper	Suppressor	Helper/ Suppressor
		Splenic Lymphocytes		
Saline	82.7 ± 0.9	62.3 ± 0.5	17.6 ± 0.8	3.6 ± 0.2
dPGE	78.2 ± 1.6	57.0 ± 1.7	18.3 ± 0.6	3.2 ± 0.1
Indomethacin	81.3 ± 1.5	59.8 ± 1.8	18.6 ± 0.5	3.2 ± 0.1
		Blood Lymphocytes		
Saline	86.9 ± 1.7	67.5 ± 1.3	16.3 ± 1.4	4.4 ± 0.4
dPGE	87.1 ± 1.5	65.9 ± 1.3	18.1 ± 2.0	3.9 ± 0.4
Indomethacin	87.2 ± 1.2	67.9 ± 3.1	22.7 ± 4.5	3.4 ± 0.3

differences in T cell subpopulations between blood and splenic lymphocytes. In saline-treated burned animals, the blood helper T lymphocyte percentage was greater than the splenic helper T lymphocyte percentage ($P = 0.0113$). In dPGE-treated animals, the blood pan-T cell population was significantly greater than the splenic pan-T cell population ($P = 0.0043$), as were the helper ($P = 0.0033$) and helper-suppressor T lymphocyte ratios ($P = 0.0414$). Burned animals treated with indomethacin also demonstrated significantly higher T cell subpopulations among blood lymphocytes as compared to splenic lymphocytes. These differences

were noted in the pan-T cell subpopulations (P = 0.0081) and the helper T cell populations (P = 0.0476).

Neither indomethacin nor dPGE administration were found to alter lymphocyte subpopulations in burned-infected animals (Table 3). There were noted to be significantly lower percentages of pan-T lymphocytes and helper T lymphocytes in the spleens as compared to blood in the indomethacin treatment group (P = 0.0331 and P = 0.0218, respectively).

TABLE 3. Percentage of Pan, Helper, and Suppressor T Cells and Helper/Suppressor Ratios in Burned-Infected Rats Treated with Saline, dPGE, or Indomethacin

Group	Pan	Helper	Suppressor	Helper/ Suppressor
		Splenic Lymphocytes		
Saline	72.7 ± 2.0	51.5 ± 3.7	19.6 ± 0.7	2.8 ± 0.2
dPGE	64.8 ± 3.9	52.1 ± 2.7	18.8 ± 0.8	2.8 ± 0.2
Indomethacin	63.9 ± 3.9	48.2 ± 3.4	16.9 ± 1.0	2.9 ± 0.2
		Blood Lymphocytes		
Saline	76.6 ± 3.6	60.9 ± 4.7	17.2 ± 0.9	3.7 ± 0.5
dPGE	76.4 ± 1.7	60.1 ± 2.8	18.6 ± 1.9	3.5 ± 0.5
Indomethacin	80.2 ± 2.5	62.2 ± 1.4	17.5 ± 1.7	3.7 ± 0.4

DISCUSSION

Previously published papers on the effects of PGE in trauma and burn patients suggested that serum PGE levels are increased following such injuries and that PGE was immunosuppressive. The date which demonstrated an immunosuppressive effect of PGE were primarily obtained from in vivo models in which PGE was added to leukocyte cell cultures and the leukocytes assayed for various WBC functions. Utilizing such in vitro models, Chouaib et al (7) demonstrated that PGE was a potent inducer of suppressor T lymphocytes. There has been little in vivo data available to confirm these in vitro findings due to the extremely short half-life of PGE following parenteral administration.

To circumvent this problem, Zapata-Sirvent and Hansbrough (15) attempted to assay indirectly the immunologic effects of elevated PGE levels by administering cyclooxygenase inhibitors to burned mice. They reported that the administration of either ibuprofen or indomethacin to burned mice increased the number of helper T

lymphocytes and the helper-suppressor ratio while it decreased the number of suppressor T lymphocytes as compared to burned, untreated mice.

Our current study failed to demonstrate such a beneficial effect on the number of helper or suppressor T lymphocytes or the helper-suppressor T lymphocyte ratio in either burned or burned-septic rats administered indomethacin. There would appear to be several possible explanations for this discrepancy. Our model was a different animal species (rats) with a more extensive burn size (30%). Also in our study, the animals were sacrificed after 4 days of drug treatment while in the prior study, the mice were sacrificed after 2 weeks of drug treatment. This difference in time sequence may be important since Zapata-Sirvent and Hansbrough reported that the decrease in helper-suppressor ratio was not apparent in mice by postburn day 4. However, Burleson et al reported that a significant decrease in the helper-suppressor ratio could be identified in burned-septic rats as early as 2 days following burn injury (16).

Our current study also failed to demonstrate any measurable immunologic effect of dPGE administration on the percentage of helper, suppressor, or pan-T lymphocytes or on the helper-suppressor T-lymphocyte ratios. This discrepancy between our in vivo data and earlier in vitro studies emphasizes the limitations of trying to extrapolate cell culture data to predict the physiologic and immunologic effects of parenteral drug treatments.

Although our study failed to demonstrate any statistically significant effects of either drug treatment on lymphocyte T cell subset populations, it did demonstrate significant differences in the T lymphocyte subpopulations between blood and splenic lymphocytes. In nonburned, burned, and burned-septic animals, it was frequently found that blood lymphocytes had a higher percentage of pan-T and helper T cells and a higher helper-suppressor T-lymphocyte ratio when compared to splenic lymphocytes. This finding is consistent with previous work which demonstrated that the spleen has a high proportion of suppressor lymphocytes which can impair immune function and protect against allograft rejection (17). Such a finding emphasizes the limitations of utilizing data obtained from splenic cells in an attempt to estimate total body immune function.

PRESENTATIONS

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

PUBLICATIONS

Waymack JP, Guzman RF, Burleson DG, McManus AT, Mason AD Jr, and Pruitt BA Jr: Effect of prostaglandin E in multiple experimental models: VI. Effect on T-cell subsets. **Prostaglandins** 38(3):345-53, September 1989.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION DAOG6968	2. DATE OF SUMMARY 1 Oct 89	REPORT CONTROL SYMBOL DD-DRA&E(AR) 636
3. DATE PREV SUM'RY 1 Oct 88	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	F	303
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP,	FY90-01		

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	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
7610	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	89	5.0	263
e. KIND OF AWARD	f. CUM/TOTAL	90	5.0	276

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 FINA MILITARY/CIVILIAN APPLICATION M	f. NAME OF ASSOCIATE INVESTIGATOR *(If available)* g. NAME OF ASSOCIATE INVESTIGATOR *(If available)*

22. KEYWORDS *(Precede EACH with Security Classification Code)* (U) Tissue-Spreading Factors; (U) Infection; (U) Immunostimulants; (U) Virulence Factors; (U) Plasmids; (U) Antibiotic

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

22. (U) Effects; (U) Volunteers: (U) Adults; (U) Children; (U) RA II.

23. (U) A DTIC literature search was conducted under DTIC request number W6K56A dated 20 October 1989 for the technical report database and request number W6K56F dated 20 October 1989 for the work unit database. The proposed effort will not result in the duplication of effort. The objective of this work is to 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.

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

25. (U) 8701 - 8712. The mechanisms of resistance to the antimicrobial action of phosphanilic acid and sulfonamides have been examined using DNA probes specific for two plasmid-mediated dihydropteroate synthetase genes (R1drd19 and GS04). Examination of sulfonamide-resistant burn isolates were 97.9% reactive (95/97) with the probes. None of the sulfonamide-sensitive control strains reacted with the probes. Phosphanilic acid cross-resistance was 24.7% (24/97). Transfer of sulfonamide resistance plasmids showed that only plasmids with the GS04-specific probe co-transferred phosphanilic acid resistance. For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1989.*

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ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3M161102BS14-00, BASIC RESEARCH

PROJECT TITLE: Alteration of Host Resistance in Burned Soldiers

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

1 October 1988 - 30 September 1989

INVESTIGATORS

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Virginia C. English, MS
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Aldo H. Reyes, SSG
Arthur D. Mason, Jr., MD
Basil A. Pruitt, Jr., MD, Colonel, MC

ABSTRACT

PROJECT NUMBER: 3M161102BS14-00, BASIC RESEARCH

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

PERIOD COVERED IN THIS REPORT: 1 Oct 88 through 30 Sep 89

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A clinical trial and in vitro evaluation of the parenteral antibiotic ceftazidime as monotherapy in infected burn patients is in progress. Twenty-one patients have been enrolled into the study. Sensitivity of Gram-negative organisms to ceftazidime has remained high (> 93%) during the trial. A beta-lactamase capable of inactivating ceftazidime and all other tested cephalosporins has been recovered from several treated patients. The characteristics and spread of this enzyme are being investigated.

Experimental Parenteral Agents. The in vitro activity of three newly approved antibiotics is presented in Table 1. A clinical trial of ceftazidime as monotherapy for infected burn patients is in progress. Ceftriaxone sodium and aztreonam have not been used clinically to date.

TABLE 1. Activity of Experimental Antibiotics for Fiscal Year 1989

Fiscal Year	1986	1987	1988	1989
		Ceftazidime[a]		
Resistant	63 (6.4%)	80 (6.7%)	480 (15.4%)	630 (21.0%)
Sensitive	928	1,106	2,633	2,367
		Ceftriaxone Sodium[a]		
Resistant	157 (16.8%)	167 (14.4%)	843 (37.0%)	737 (24.6%)
Sensitive	928	1,106	2,633	2,367
		Aztreonam[b]		
Resistant	1 (0.0%)	81 (7.1%)	540 (33.9%)	616 (37.6%)
Sensitive	928	1,106	2,633	2,367

( ) = Percent resistant. [a]Against all flora except oxacillin-resistant *Staphylococcus aureus*. [b]Against Gram-negative aerobic flora.

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

Experimental Topical Agents. Five-percent mafenide acetate was examined for in vitro activity against *Pseudomonas aeruginosa* isolated from 39 burn patients. Agar dilution minimal inhibitory concentration (MIC) assays were done on 193 strains. The mean MIC was 0.225 g/100 ml. The median MIC was 0.156 g/100 ml. Data comparing fiscal years 1988 and 1989 are presented in Table 2. The increase in strains with MIC of 0.6258 g/100 ml was not significantly different from the previous reporting period.

TABLE 2. Minimal Inhibitory Concentration for *Pseudomonas aeruginosa* Strains to Mafenide Acetate

Mafenide Acetate Concentration (g/100 ml)	Number of Strains Fiscal Year 1988	Number of Strains Fiscal Year 1989
0.019	10	24
0.039	16	11
0.078	36	26
0.156	39	50
0.312	42	59
0.625	13	23
1.250	2	-
Total Number of Strains	**158**	**193**

The therapeutic effects of p-aminobenzoic acid (PABA) and p-aminobenzoic acid/mafenide acetate (PABA/MA) as antipseudomonal agents were tested using the thermally injured rat burn model. These results are presented in Tables 3 through 6. PABA was found not to have significant topical antimicrobial activity.

Two experimental liquid burn dressings (basic formula and basic formula with 3% DMSO) were tested for therapeutic effects using the thermally injured rat burn model infected with *Pseudomonas aeruginosa* (Strain 1244) at 24 h postburn. Immediately following burn injury, the wounds were covered with sterile gauze held in place with surgical staples. The experimental liquid burn dressing was applied liberally to the gauze covering the burn wound twice daily for 10 days beginning at 1 or 24 h postburn. The results are presented in Table 7.

TABLE 3. Examination of PABA in *Pseudomonas aeruginosa*-Infected Rats (Dead/Total)

Group	1st Study	2nd Study	Total	Mortality (%)
Control	10/10	10/10	20/20	100
Silver sulfadiazine	1/10	-	1/10	10
Mafenide acetate	10/10	2/10	12/20	60
7.6% PABA	7/10	10/10	17/20	85
3.8% PABA	9/10	10/10	19/20	95
1.5% PABA	10/10	9/9	19/19	100

Serologic Types of *Pseudomonas aeruginosa* Isolated from Burn Patients. *Pseudomonas aeruginosa* isolates from 14 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. A total of 51 strains were typed. Data are presented as the total number of patients with each serotype and the total number of isolates per serotype in Figure 1. Serotypes 01 and 04 were the predominant types identified.

Modification of the Standard Infected, Burned Animal Model for Delayed Infection Studies. Delayed inoculation studies were continued this year in order to better simulate a clinical infection in the burned animal model. Inoculation of the burned rat was delayed 5 days postburn. Delayed inoculation was carried out with two clinical isolates and the standard challenge (Table 8). The standard challenge strain and the better of the two clinical isolates were titered (see Tables 9 and 10) to determine optimum concentration of Pseudomonas aeruginosa to establish an LD_{100}. The delayed infection studies showed that it is possible to infect burned rats several days after burn injury, although on a limited basis.

Surveillance of Clinically Isolated Candida species for Resistance to Nystatin. Clinically isolated and identified Candida species were tested for susceptibility to nystatin using the agar dilution method for determining MIC values. A test range of 0.007-1000 µg/ml nystatin was used. A total of 213 isolates from 36 burn patients were tested during this fiscal year. Table 11 lists the organisms isolated. Table 12 lists the MIC for nystatin of tested *Candida albicans* and *Candida rugosa* strains. These two

TABLE 4. Examination of Carrier Cream on Effect of PABA and Mafenide Acetate in *Pseudomonas aeruginosa*-Infected Rats (Dead/Total)

			Aquaphor		Unibase	
Group	Control	Mafenide Acetate	11% Mafenide Acetate	10% PABA	11% Mafenide Acetate	10% PABA
1	5/5	2/10	8/10	4/10	0/10	9/10
2	5/5	0/10	10/10	8/10	1/10	8/9
Total	**10/10**	**2/20**	**18/20**	**12/20**	**1/20**	**17/19**
Mortality (%)	**100**	**10**	**90**	**60**	**5**	**90**

species accounted for over 97% of the isolates. Using a 8 µg/ml break point, all *Candida rugosa* and 98% of *Candida albicans* were sensitive to nystatin. A display of *Candida albicans* MIC values is presented in Figure 2.

PRESENTATIONS/PUBLICATIONS

None.

TABLE 5. Examination of PABA/MA in *Pseudomonas aeruginosa*-Infected Rats (Dead/Total)

Group	1st Study	2nd Study	Total	Mortality (%)
Control	-	10/10	10/10	100
MA	5/10	5/10	10/20	50
5% MA	5/10	7/9	12/19	63
5% PABA	10/10	10/10	20/20	100
5% PABA/5% MA (Unibase)	9/10	10/10	19/20	95
5% PABA/5% MA (Aquaphor)	9/10	-	9/10	90

TABLE 6. Examination of PABA/MA in *Pseudomonas aeruginosa*-Infected Rats (Dead/Total)

Group	1st Study	2nd Study	Total	Mortality (%)
Control	11/11	10/10	21/21	100
MA	5/13	10/10	15/23	65
5% PABA/10% MA (Unibase)	9/13	9/10	18/23	78
10% PABA/5% MA (Unibase)	12/13	10/10	22/23	96

TABLE 7. Examination of Experimental Liquid Burn Dressings in *Pseudomonas aeruginosa*-Infected Rats

Group	Dead	Total	Mortality (%)
Control	6	6	100
Mafenide acetate	0	7	0
Basic formula			
0 h postburn	10	10	100
24 h postburn	9	10	90
Basic Formula + 3% DMSO			
0 h postburn	9	10	90
24 h postburn	8	10	80

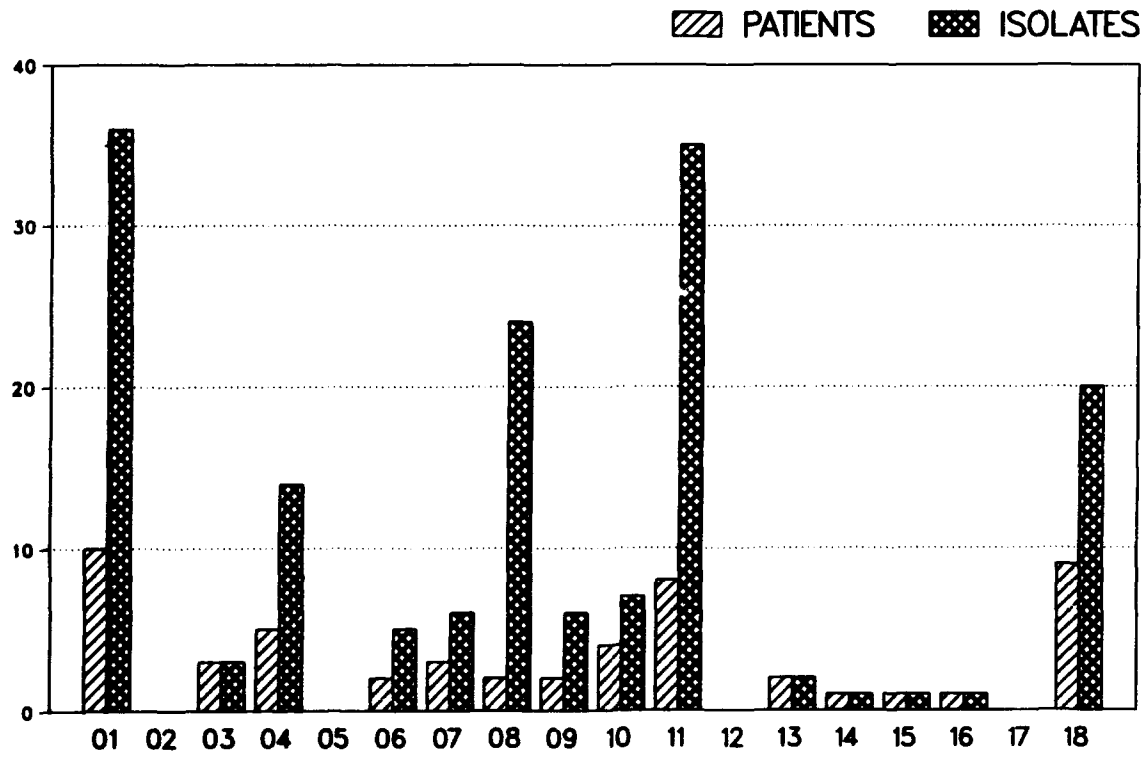

FIGURE 1. Frequency of Pseudomonas serotypes.

TABLE 8. Delayed Inoculation (5 Days Postburn) with Virulent Clinical Isolates in Burned Rats

Pseudomonas Strain	Dead	Total	Mortality (%)
1244	14	18	77.8
881111020	14	18	77.8
881211013	15	18	83.3

TABLE 9. Titration of *Pseudomonas aeruginosa* (Strain 1244) at 5 Days Postburn in Burned Rats

cfu/ml	10^0	10^1	10^2	10^3	10^4	10^5	10^6	10^7	10^8	10^9
Dead/Total	1/6	0/5	1/5	1/5	1/5	1/6	4/6	4/6	1/5	4/5
Mortality (%)	17	0	20	20	20	17	68	68	20	80

TABLE 10. Titration of *Pseudomonas aeruginosa* (Strain 881211013) at 5 Days Postburn in Burned Rats

cfu/ml	10^0	10^1	10^2	10^3	10^4	10^5	10^6	10^7	10^8	10^9
Dead/Total	0/5	2/5	0/5	0/5	2/5	5/5	4/5	4/5	5/5	4/5
Mortality (%)	0	40	0	0	40	100	80	80	100	80

TABLE 11. Clinically Isolated Candida species Tested for Nystatin Susceptibility

Candida species	Number of Isolates
Candida albicans	152
Candida rugosa	56
Candida tropicalis	3
Candida parapsilosis	2
TOTAL NUMBER OF ISOLATES	**213**

TABLE 12. MIC of Nystatin for *Candida albicans* and *Candida rugosa*

Nystatin Concentration (µg/ml)	*Candida albicans* (n=152)	*Candida rugosa* (n=56)
2	90	3
4	38	27
8	21	26
1000	3	-

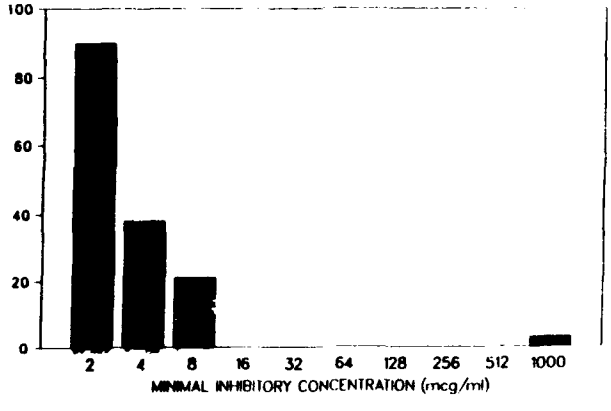

FIGURE 2. Frequency distribution of MIC values for Candida albicans against nystatin.

ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3M161102BS14-00, BASIC RESEARCH

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

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

1 October 1988 - 30 September 1989

INVESTIGATORS

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

ABSTRACT

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Several fluorescent substances are present in the supernatant of acid-precipitated blood from burned patients. We have previously reported that PCA supernatants of sera from infected, but not from uninfected, burned rats contain a fluorescent substance with maximum emission at 420 nm at 355 nm excitation (355 ex/420 em). In this study of similarly prepared sera from burned patients, several substances were resolved by reverse-phase HPLC. One of these fluorescent components had HPLC retention time and fluorescent characteristics identical to those of neopterin. The identification of this component as neopterin was verified by thermospray MS. Serum neopterin concentrations were then determined in supernatants of patient serum samples having various levels of 355 ex/420 em fluorescence. A correlation was found between the concentrations of neopterin determined by HPLC and the levels of fluorescence found in the PCA supernatants. These findings suggest that neopterin, which is a useful indicator of infection in other clinical settings, may also be an indicator of infection in burned patients.

CHARACTERIZATION OF BIOCHEMICAL INDICATORS OF INFECTION IN THE THERMALLY INJURED

Infection poses a serious threat to all severely burned patients and is a persistent obstacle to successful therapy. Prompt diagnosis of sepsis is crucial for timely treatment and for patient survival. Systemic changes induced by burn injury, such as leukocytosis and fever, hamper the early detection of sepsis and make its diagnosis more difficult. Rapid biochemical detection of sepsis, if reliable, could provide diagnostic verification more promptly than is currently possible with standard microbiologic techniques.

Abnormal levels of hormones (1,2), acute-phase proteins (3,4), and fluorescent substances (5,6) in blood and plasma occur in the presence of inflammation and/or infection in human burn patients and animal burn models. Their presence in blood and plasma reflects a metabolic response to both trauma and infection. Though biochemical species specific to the invading species are desirable, the spectrum of microbes to be differentiated, and their low levels in the blood early in infection, limit the likelihood of developing such indices. As an alternative, a biochemical change in serum or blood specific to infection would permit separate identification of this metabolic response and might have diagnostic utility.

We have purified and biochemically characterized substances from the blood of burned patients in which fluorescence with maximum emission at 420 nm at 355 nm excitation (355 ex/420 em) was detected in PCA supernates. These substances offer some promise as specific indicators of the presence of infection.

MATERIALS AND METHODS

Measurement of Fluorescent Indicators. One milliliter of anticoagulated blood was mixed with 4 ml of cold (4°C) PCA (0.8 M). After incubation for 10 min, the mixture was centrifuged at 4°C for 10 min at 3,000 g. The supernatant was recentrifuged at 20,000 g for 30 min. The clear supernatant was transferred to another tube and fluorescence was then measured using a spectrofluorometer (SLM Instruments, Inc., Urbana, IL) at 355 ex/420 em. The fluorometer was standardized by using a calibration standard (fluorescence intensity block).

HPLC Determination of the 355 ex/420 em Factor in Serum. Serum (100 µl) was deproteinized by incubating at 100°C in an oil bath for 20 min after the addition of 200 µl of 0.2 M potassium phosphate buffer (pH 4.5). The mixture was then centrifuged at 20,000 g for 20 min and the supernatant was injected directly on HPLC. HPLC was performed on an Hewlett-Packard™ liquid chromatograph (Model 1090) with a Biophase™ ODS reverse phase 4.6 X 250-mm column (Bioanalytical Systems, Inc., West Lafayette,

IN). The mobile phase consisted of 0.05 M ammonium acetate at pH 7.0. The column temperature was maintained at 45°C and the flow rate was 1.0 ml/min. The HPLC was equipped with a Kratos™ fluorescence detector (Model 980) with a 25-µl flow cell. The excitation monochronometer was set at 350 nm and the emission cutoff filter was at 389 nm. The retention time for standard pterins (Sigma® Chemical Company, St. Louis, MO) was determined using 10 µl of a standard solution of pterins (10 ng/ml). The amount of each fluorescent substance present was measured on a Hewlett-Packard integrator (Model 3392A).

Mass Spectral Analysis of the 355 ex/420 em Factor. Serum supernatants were purified by ion exchange chromatography after heat denaturation and separation of the serum proteins by a modification of the method of Stea et al (6). The supernatants were passed through a cation exchange membrane (Bio-Rad Laboratories, Richmond, CA), washed with distilled water, and eluted directly onto an anion exchange membrane (Bio-Rad Laboratories) with 3 ml of 0.1N ammonium hydroxide. The anion exchange membrane was washed with distilled water and the sample eluted with 3 ml of 1N formic acid. The ion exchange eluate was concentrated under a stream of nitrogen. A portion (1 µl) of the concentrate was injected into the HPLC and the column effluent directly into the thermospray module of the MS. Mass spectral analysis was performed on a Hewlett-Packard Model 5988 TS-LC/MS with a thermospray LC-MS interface. The HPLC column was a C-18 reverse phase, the flow rate was 1 ml/min, and the elution buffer was 0.1M ammonium acetate buffer.

RESULTS

The optimal chromatographic conditions described in the **MATERIALS AND METHODS** section were determined for chromatography of the 355 ex/420 em factor. The chromatogram obtained for a mixture of pterins under these conditions is shown in Figure 1. Neopterin had a retention time of 5.1 min and biopterin, 9.4 min. A patient sample that had a relatively high fluorescence at 355 ex/420 em after PCA precipitation was deproteinized by heat and analyzed by HPLC. The chromatogram obtained is compared with a sample extracted from a normal control in Figure 2. Three of the peaks in the patient sample had retention times similar to neopterin, iso-xanthopterin, and biopterin, respectively (Fig 1), while none of the same peaks were present in the control sample.

Since the fluorescence characteristics and HPLC chromatography of one of the substances were similar to those of neopterin, mass spectral analysis was performed on the partially purified sample in order to verify the identity of the peak corresponding to neopterin. Because the sensitivity of the MS was less than that of the fluorescence detector, a pool of serum samples (3 ml) from several patients with high 355 ex/420 em fluorescence was deproteinized and the supernatant purified by ion exchange

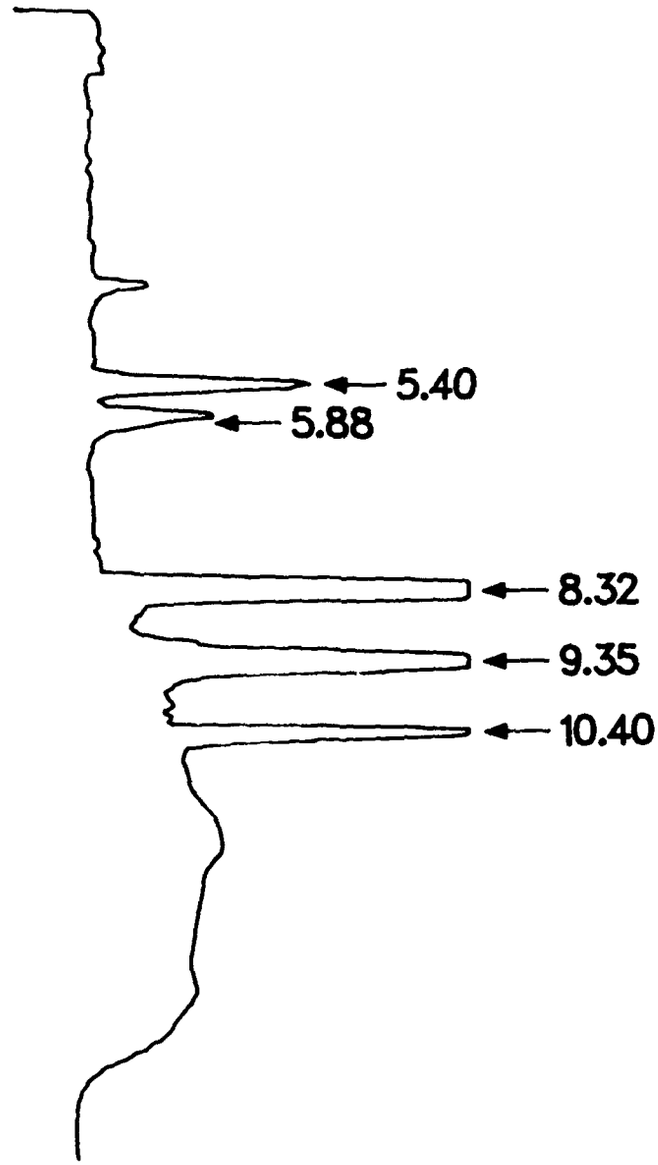

FIGURE 1. Chromatogram of neopterin and five other pterins. A 5-μl aliquot of a standard solution containing 10 µg/ml of pterin-6-carboxylic acid (4.0 min), neopterin (5.4 min), xanthopterin (5.88 min), iso-xanthopterin (8.32 min), biopterin (9.35 min), and 6-methyl pterin (10.4 min) was chromatographed under the conditions described in the text.

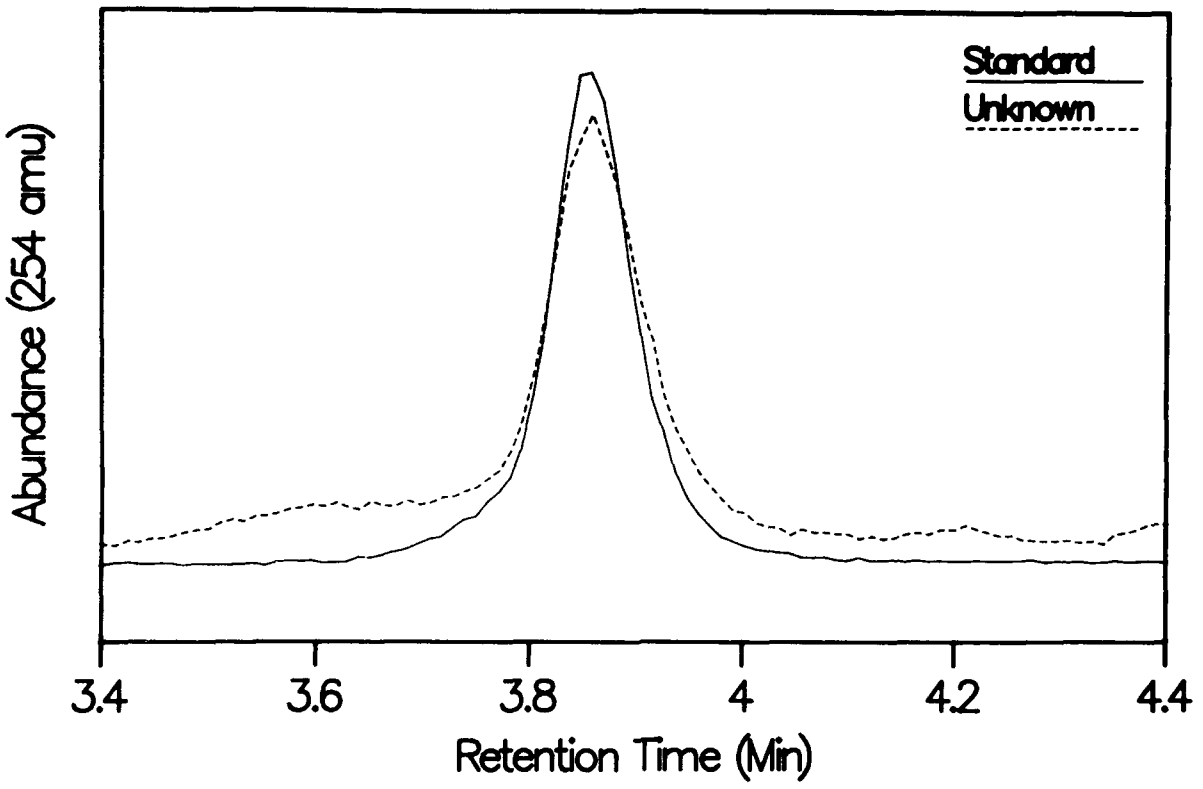

FIGURE 2. Comparison of HPLC chromatograms of partially purified patient sera and standard neopterin using mass spectral single ion monitoring for detection. Effluent from HPLC separation of the ion exchange-purified pool of patient sera was directed into the thermospray module of a MS. The parent ion (M+1 = 254 amu) for standard neopterin was monitored specifically to detect neopterin.

chromatography. The eluate from the ion exchange column was concentrated and repeatedly chromatographed by HPLC. The eluate of the peak corresponding to neopterin was collected after each injection. The collections were pooled and concentrated by evaporation. Confirmation of the identity of the unknown peak as neopterin was obtained by comparison of the unknown peak with that of a known neopterin standard. Since the fluorescence detector and the MS could not be run simultaneously, the column eluate was diverted to the MS and compounds separated on the HPLC column were detected by monitoring for the parent ion (254 amu). Specific monitoring for the parent ion of neopterin (m+1, 254 amu) revealed the presence of neopterin (Fig 3) in the HPLC-purified samples.

The chromatograms for five patients selected with high levels of 355 ex/420 em factor are shown in Figure 3. All of the patients experienced infection during their hospital course. Samples from

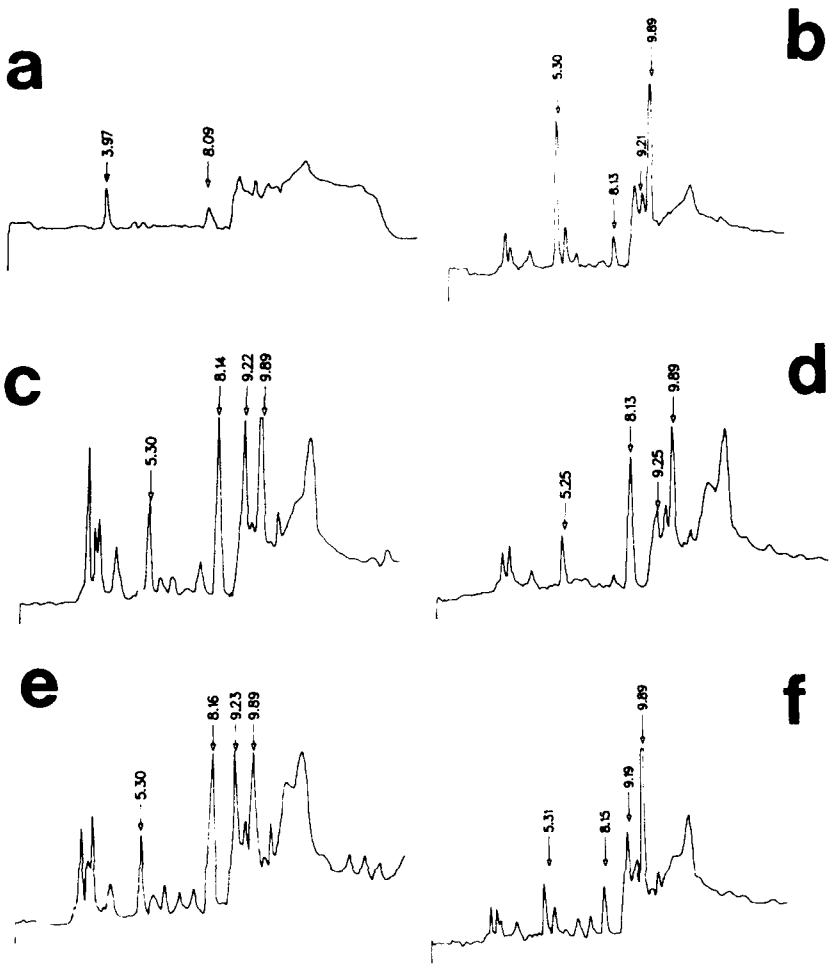

FIGURE 3. Comparison of HPLC chromatograms of patient samples (b-f) with high fluorescence readings to an unburned control sample (a). Five patient samples with high values for the 355 ex/420 em factor were chromatographed by HPLC with a fluorescence detector. Peaks are most consistently present at 5.3 min, 8.1 min, 9.2 min, and 9.9 min in the patient samples.

4 of the 5 patients were taken < 11 days after a clinically defined infection. Neopterin has a retention time of 5.3 min under the conditions employed when these chromatograms were obtained. All 5 patients had levels of neopterin above that found in the control. In addition to neopterin, three other fluorescent substances were present consistently in the PCA supernatants. These fluorescent substances had retention times of approximately 8.1, 9.2, and 9.9 min. The 8.1- and 9.2-min retention times corresponded to the retention times for standard solutions of iso-xanthopterin and biopterin, respectively. Further studies are being conducted to identify these substances. Combined, the neopterin and the other

three recurring peaks account for an average of 72.1% of the fluorescence measured during the first 10 min of analysis by HPLC under these conditions.

The PCA fluorescence was originally observed in the presence of infection in burned animals. Since the fluorescent spectra of neopterin shift to shorter wavelengths at low pH, the direct contribution of neopterin fluorescence to the total fluorescence in PCA supernatants may be quite small. A number of human serum samples were analyzed to determine whether neopterin concentration was correlated with the level of fluorescence in the PCA supernatants. The relative amounts of material corresponding to the neopterin HPLC retention time (using neopterin as a standard) were determined by integration of the HPLC peaks in randomly selected patient samples. This result was plotted against the log of the fluorescence of the same unpurified supernatants at 355 ex/420 em. Figure 4 depicts the correlation between neopterin concentration and the log of 355 ex/420 em fluorescence in plasma. The correlation coefficient (R) was 0.91, (R^2 = 0.83), suggesting that although neopterin may not contribute significantly to the 355 ex/420 em fluorescence in PCA supernatants, it is present in increased concentration under the same conditions that cause 355 ex/420 em fluorescence to be elevated.

DISCUSSION

We have consistently found four fluorescent components in patient sera. These substances have fluorescent characteristics similar to nucleotide derivatives such as the pterins. Three of the components co-purify with and are chromatographically similar to pterin standards. We have established the chemical identity of one of these substances as neopterin.

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) and tuberculosis (10). Neopterin has been used as a prognostic indicator for certain kinds of cancer (11) and as a measure of transplant rejection (12). 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 correlation of serum neopterin levels in human burn patients with the levels of fluorescence previously identified as potential indicators of infection in burned animals points to its potential as an indicator of infection in burned patients. It is relatively easy to identify in body fluids by HPLC and a RIA has also been developed (13). These facts, combined with its demonstrated utility as an indicator of infection in other

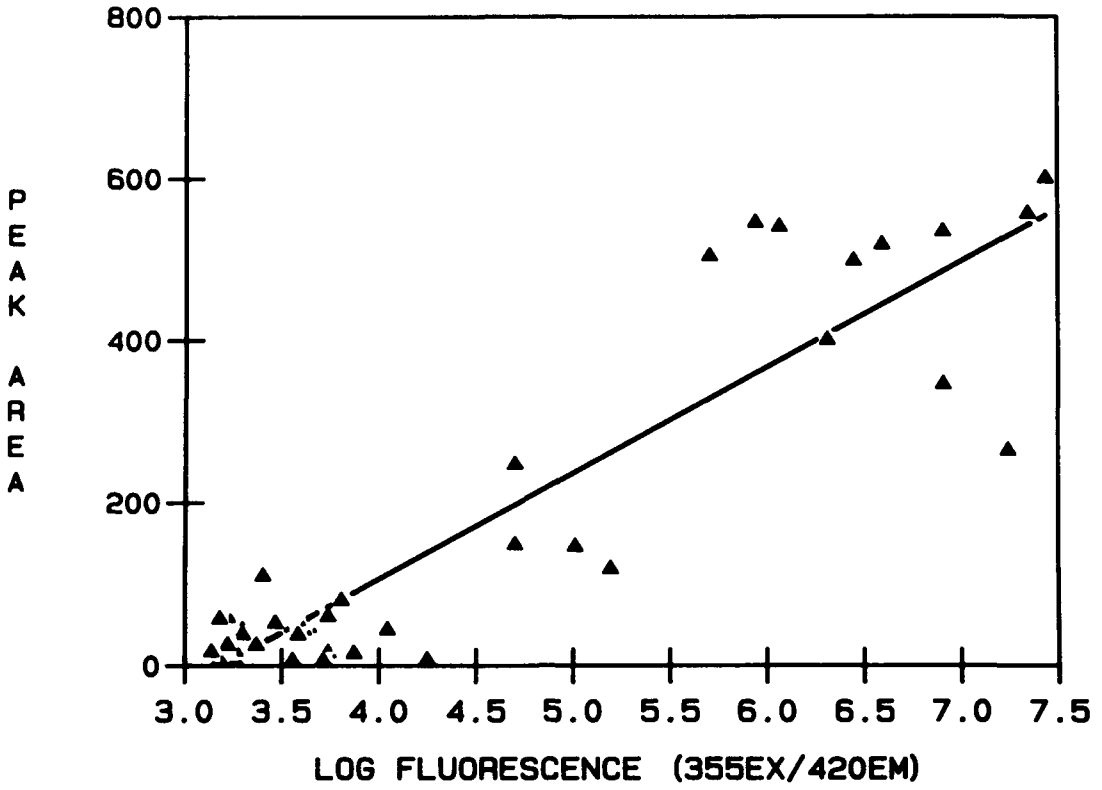

FIGURE 4. Correlation of neopterin levels with levels of 355 ex/420 em log fluorescence in serum samples from burned patients. Supernatant fluorescence of PCA-precipitated serum samples was determined and a separate aliquot of the same sample was used for neopterin determination. Neopterin was quantified by integrating peak areas obtained during HPLC analysis. The log of the 355 ex/420 em fluorescence is plotted on the X axis and the HPLC-determined neopterin concentration for the same sample is plotted on the Y axis.

conditions, portrays its potential usefulness as an indicator specific to infection in burned patients. Studies are underway to determine if there is a correlation between serum neopterin levels and the presence of infection.

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ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3M161102BS14-00, BASIC RESEARCH

PROJECT TITLE: ALTERATION OF HOST DEFENSE IN BURNED SOLDIERS: An Unexpected and Unexplained Mode of Antimicrobial Action of Phosphanilic Acid (PA) and Para-Aminobenzoic Acid (PABA) - Probable Similarity to the Mode of Action of Mafenide Acetate

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INVESTIGATORS

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We have previously demonstrated that PA was a weak inhibitor of dihydropteroate synthase. This observation supported the fact that *in vitro* resistance to the activity of sulfonamide agents often showed cross-resistance with PA. Further investigations into the antipseudomonal activities of PA have shown that much higher concentrations of the agent were required to inhibit dihydropteroate synthase in cell lysates than were required to inhibit the growth of intact organisms. This observation suggests that a second and more sensitive site of action for PA may exist.

AN UNEXPECTED AND UNEXPLAINED MODE OF ANTIMICROBIAL ACTION OF PHOSPHANILIC ACID (PA) AND PARA-AMINOBENZOIC ACID (PABA) - PROBABLE SIMILARITY TO THE MODE OF ACTION OF MAFENIDE ACETATE

We recently presented direct evidence at the enzyme level that PA was a weak inhibitor of dihydropteroate synthase (1,2). Through indirect evidence gained from studies with whole bacterial cells, previous workers had suggested this mode of antimicrobial action of PA (3-5). PA is a structural analog of PABA as well as of sulfanilic acid and the sulfonamides. Chemically, PA is PABA phosphate. Thus, PA has a phosphate group in the position occupied by a sulfonate group in the "sulfa" drugs and by a carboxyl group in PABA.

MATERIALS AND METHODS

Organisms. The two experimental organisms used in these studies were *Escherichia coli*, a clinical isolate from a urine specimen, and *Pseudomonas aeruginosa* (ATCC 27317). The latter organism is considered to be the "standard virulent strain" of *Pseudomonas aeruginosa* and is also referred to as Strain 1244.

Media. Unless otherwise indicated, the organisms were grown in a chemically defined basal salt medium (6) supplemented, in final concentration, with 20 mM glucose (BSG).

Minimal Inhibitory Concentration (MIC) Determinations. The tube serial dilution technique was used to determine MIC using BSG as the test medium.

Preparation of Cell-Free Extracts. Cells of *Pseudomonas aeruginosa* and *Escherichia coli* were grown overnight at 37°C in trypticase soy broth (BBL Microbiology Systems, Cookeysville, MD) on a reciprocating shaker. The cells were harvested by centrifuging, washed once with 10 mM phosphate buffer (pH 7), and then suspended in two volumes of the same buffer. The cells were ruptured by two passes through a French pressure cell at 18,000 psi. DNase and RNase were added (final concentration, 100 μg/ml) to the broken cell suspensions. Intact cells were removed by centrifuging at 36,000 g for 30 min at 4°C. The supernatant was then centrifuged at 100,000 g for 1 h at 4°C.

The supernatant from the latter centrifugation was brought to 55% saturation with $(NH_4)_2SO_4$ by drop-wise addition of a saturated $(NH_4)_2SO_4$ solution at 0-4°C. The precipitated proteins were sedimented by centrifuging at 35,000 g for 45 min at 4°C. The sediments were then dissolved in 10 mM phosphate buffer (pH 7) and dialyzed with gentle agitation for 24 h against the same buffer at 0-4°C. The extracts were stored in 0.5-ml samples at -70°C until needed.

Protein concentrations were determined by the Coomasie blue technique using the Bio-Rad™ protein assay kit (Bio-Rad Laboratories, Richmond, CA). Bovine serum albumin was used as the standard.

Assay for the Effect of Various Agents on Dihydropteroate Synthase. In a total volume of 200 μl, the following reagents, in final concentration, were used to measure the activity of dihydropteroate synthase: Tris-HCl buffer (pH 8.3), 40 mM; MgCL2, 5 mM; dithiothreitol, 5 mM; 2-amino-4-hydroxy-6-hydroxymethyl-7,8-dihydropteridine-pyrophosphate (H_2PtCH_2OPP), 20 μM; [ring-UL-^{14}C]PAB, 20 μM; cell extract, 0.5 mg protein/ml; and potassium phosphanilate and sodium sulfadiazine in final concentration.

The reactions were incubated for 5 min in 1.5-ml microfuge tubes in a 37°C water bath. The reactions were started by the addition of the cell extracts and were stopped by placing the incubation tubes in ice.

A 100-μl sample from each reaction was added to an area of 2 X 3 cm on Whatman™ 3MM chromatography paper. Ascending chromatography was run in 0.1 M phosphate buffer (pH 7). After developing, the origins, i.e., 2 X 3-cm areas, were cut out, placed in minivials with 6 ml of Packard™ Opti-fluor scintillation fluid (Packard Instrument Company, Inc., Downers Grove, IL), and their radioactivities determined. In this procedure, the ^{14}C-labelled dihydropteroate remained at the origin while the unreacted ^{14}C-PAB migrated with an R_f = 0.8.

Reagents. DNase, RNase, and [ring-UL-^{14}C]PAB were purchased from the Sigma Chemical Company (St. Louis MO). Phosphanilic acid was purchased from Raylo Chemicals (Edmonton, Alberta, Canada). All other reagents were purchased from commercial sources in their highest state of purity.

RESULTS

That PA inhibited dihydropteroate synthase was not surprising since this enzyme is the intracellular target of the sulfa drugs that, being structural analogs of PABA, compete with PABA for an active site on dihydropteroate synthase (7,8). Dihydropteroate synthase condenses 2-amino-4-hydroxy-6-hydroxymethyl-7,8-dihydropteridine-pyrophosphate with PABA to form dihydropteroic acid. Dihydropteroic acid is a precursor of tetrahydrofolic acid, the coenzymatic form of folic acid. The overall antimicrobial effect of the sulfonamides, therefore, is the inhibition of folic acid synthesis.

What was surprising was that a considerably higher concentration of PA was required to inhibit dihydropteroate synthase in cell-free extracts than was required to inhibit intact

bacterial cells, particularly those of *Pseudomonas aeruginosa* as compared to *Escherichia coli*. These data are shown in Tables 1 and 2. Sulfanilamide was used as a control for the data shown in Table 1 because it is a weak inhibitor of dihydropteroate synthase.

These results suggested, therefore, that PA had a second mode of action other than inhibition of dihydropteroate synthase. Two possibilities come to mind, i.e., PA inhibited some aspect of bacterial growth and metabolism other than inhibition of dihydropteroate synthase or PA was concentrated within the bacterial cell.

Another surprise was that PABA itself was inhibitory under the conditions of this study (Table 1). At the concentration used, PABA was effective in overcoming the inhibitory effect of sulfanilamide but not that of PA for *Pseudomonas aeruginosa* (Table 1). Instead, PABA appeared to enhance the antimicrobial activity of PA against *Pseudomonas aeruginosa*. These results suggested that the inhibitory effects of PABA and PA may have been due to the same mechanism and that an additive effect was seen when the two agents were used simultaneously.

Moreover, mafenide acetate, to which *Pseudomonas aeruginosa* is especially sensitive, has been shown not to inhibit dihydropteroate synthase (9,10). Chemically, mafenide acetate is para-aminomethylbenzene sulfonamide, which means that it too is a structural analog, not only of PABA, but of PA and the sulfa drugs as well.

DISCUSSION

We speculate that the alternative mechanism of action of PA is shared by mafenide acetate and by PABA. While our study methods and results did not permit us to discern this alternative mode of action, certain comparisons can be made. Lipophilic agents, such as benzoic acid and salicylic acid, long have been used as food preservatives. Their mechanism of action is considered to be due to their effect on the bacterial cell membrane (11-13). Specifically, these lipophilic agents are thought to uncouple electron transport phosphorylation, i.e., oxidative phosphorylation, thus interfering with energy generation and, as a consequence, bacterial cellular physiological mechanisms in general.

Mafenide acetate, PA, and PABA are also highly lipophilic. It is tempting to speculate, therefore, that PA, PABA, and mafenide acetate (and possibly the sulfa drugs as well) have an effect on the bacterial cell membrane similar to that of the lipophilic food preservatives. That bacterial cells may concentrate PA intracellularly cannot be ruled out. Thus, which of the two alternative modes of action listed above is correct, if indeed

either is correct, will have to await further studies designed to provide an answer to this question.

TABLE 1. Minimal Inhibitory Concentrations (MIC) of PA and Sulfanilamide in the Absence and Presence of PABA and MIC of PABA alone for *Pseudomonas aeruginosa* in Basal Salts Supplemented with 20 mM Glucose (BSG)

Agent	MIC (mM)[a]	
	Pseudomonas aeruginosa[b]	*Escherichia coli*[c]
Sulfanilamide	1.161 (3.483)	1.161
Sulfanilamide + 1.142 mM PABA	> 5.805[d]	2.322
Phosphanilate	0.117 (0.585)	> 0.585[e]
Phosphanilate + 1.142 mM PABA	< 0.059[f] (0.176)	> 5.85[d]
PABA	2.855 (2.855)	1.142

[a]The tube serial dilution technique was used in which the concentration of the agents were increased by increments of 100 μg/ml from 100 to 1,000 μg/ml or by increments of 10 μg/ml from 10 to 100 μg/ml. However, the results were recorded in molar quantities to facilitate comparison of data with Table 2.
[b]The nonparenthetical numbers were results after 24 h incubation while the parenthetical numbers were results after 48 h.
[c]*Escherichia coli* grew slowly on BSG and turbidity did not occur until in excess of 24 h incubation. Thus, the results shown here represent 48–72 h incubation.
[d]Concentrations > 5.805 mM PABA or 5.85 mM PA (> 1,000 μg/ml) were not used.
[e]Concentrations < 0.585 mM PA (< 100 μg/ml) were not used.
[f]Concentrations < 0.059 mM PA (< 10 μg/ml) were not used.

PRESENTATIONS/PUBLICATIONS

None.

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TABLE 2. Effect of PA on Dihydropteroate Synthase of *Pseudomonas aeruginosa* and *Escherichia coli*

	Pseudomonas aeruginosa		*Escherichia coli*	
	Enzyme Activity[a]	Inhibition (%)	Enzyme Activity [a]	Inhibition (%)
0.02 mM PABA	0.216; 0.247[b]		0.136	
0.02 mM PABA + 0.2 mM Sulfadiazine	-	100	-	100
0.02 mM PABA + 0.2 mM Phosphanilate	0.244	1	NOT DONE	
0.02 mM PABA + 2 mM Phosphanilate	0.193	22	0.098	28
0.02 mM PABA + 20 mM Phosphanilate	0.035	84	-	100

[a]Enzyme activity = nmol dihydropteroate produced min^{-1} mg $protein^{-1}$.
[b]The two enzyme activity base values cited were derived from separate experiments. The percent inhibition values were calculated using the base value which was appropriate for that particular experiment.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
				DAOG1842	1 Oct 89	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
1 Oct 88	D. Chg	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS14	CI	304
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP	FY90-01		

11. TITLE *(Precede with Security Classification Code)*

(U) Role of Thyroid Hormones in Burn Pathophysiology

12. SUBJECT AREAS

0601 Biochemistry 0605 Medicine and Medical Research

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
7908	9909	DA	C. In-House

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

a. DATE EFFECTIVE		18. RESOURCES ESTIMATE		
b. CONTRACT/GRANT NUMBER		FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS *(In thousands)*
c. TYPE	d. AMOUNT	89	1.2	86
e. KIND OF AWARD	f. CUM/TOTAL	90	1.2	95

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME: US Army Institute of Surgical Research	a. NAME: US Army Institute of Surgical Research
b. ADDRESS *(Include zip code)*: Fort Sam Houston, San Antonio, Texas 78234-5012	b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-5012
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR: VAUGHAN, G M
d. TELEPHONE NUMBER *(Include area code)*: 512-221-2720	d. TELEPHONE NUMBER *(Include area code)*: 512-221-5416
21. GENERAL USE: FINA 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) L-Triiodothyronine; (U) Therapy; (U) Deiodinase; (U) Hypothyroidism; (U) Thyroxine; (U) Volunteers;

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

22. (Continued) (U) Adults; (U) Lab Animals: (U) Rats; (U) Hamsters; (U) RA II

23. (U) A DTIC literature search was conducted under DTIC request number W6L23A dated 20 October 1989 for the technical report database and request number W6L24A dated 20 October 1989 for the work unit database. The proposed effort will not result in the duplication of effort. The objective of this work is to assess postburn alterations in thyroid function and develop treatment to improve survival in burned patients.

24. (U) To characterize alterations in thyroid axis control in a burned rat model.

25. (U) 8810 - 8909. Serum T_4 and T_3, *in vitro* T_3 uptake, dialyzable fractions of T_4 and T_3, and thyrotropin (TSH) were measured after rats received a 17% total body surface area standard scald burn or sham burn. The initial T_4 and T_3 fall was not TSH-dependent, as TSH was elevated at 1-2 days. Burn suppression of total T_4 and T_3 was greater than that explainable by variations in food intake. Iodothyronine binding was inhibited first (6 h, 1 day) to serum proteins and then later also to the T_3 uptake test matrix, compatible with a circulating inhibitor which changes character with time after burn. In another study, rats received a 25% burn (or sham) and infusions with either T_4 or diluent until sacrificed at 6 days. Thyroidectomized controls received similar infusions. Serum TSH was inversely related to T_4 and free T_4, but in burned rats relatively more depressed than explained by normal feedback. Thus, after a transient rise in TSH, burn injury appears to enhance negative feedback control of the TSH-thyroid axis, possibly contributing to suppression of the axis. For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1989.*

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

ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3M161102BS14-00, BASIC RESEARCH

PROJECT TITLE: ROLE OF THYROID HORMONES IN BURN PATHOPHYSIOLOGY: The Rat Model of Nonthyroidal Illness

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

1 October 1988 - 30 September 1989

INVESTIGATORS

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ABSTRACT

PROJECT NUMBER: 3M161102BS14-00, BASIC RESEARCH

PROJECT TITLE: ROLE OF THYROID HORMONES IN BURN PATHOPHYSIOLOGY: The Rat Burn Model of Nonthyroidal Illness

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

PERIOD COVERED IN THIS REPORT: 1 Oct 88 through 30 Sep 89

INVESTIGATORS: George M. Vaughan, MD, Colonel, MC
Mary K. Vaughan, PhD
J. Paul Waymack, MD, Major, MC
Basil A. Pruitt, Jr., MD, Colonel, MC

Analyses of TSH and serum thyroid hormones and their serum binding indices at 6, 24, and 48 h and 7 days after a 17% total body surface area (TBSA) burn, and of these variables 6 days after a 25% TBSA burn or thyroidectomy with and without T_4 infusion between burn or operation and sampling were made in young adult male rats. The findings suggest the following assertions. The fall in T_4 and T_3 is not initially TSH-dependent. Serum T_4 and T_3 are depressed beyond what might be explained by reduced food intake. Iodothyronine binding is inhibited first to serum proteins, then later also to the T_3U test matrix. Then, the results are consistent with a circulating inhibitor of thyronine binding not only to serum proteins but also to the competitor in the T_3U test. In the rat burn model, by 6 days after a 25% TBSA burn, serum TSH is relatively more depressed than normal by a given level of available circulating T_4. At this time, enhanced negative feedback prevents a rise in TSH despite low thyronines in burn injury.

ROLE OF THYROID HORMONES IN BURN PATHOPHYSIOLOGY: THE RAT BURN MODEL OF NONTHYROIDAL ILLNESS

Thyroid function and its relation to TSH in illness and injury are not fully understood (1,2). The often normal serum thyrotrophin (TSH) level in critical illness and burn injury, despite low iodothyronine levels, has been taken indirectly to suggest altered control of TSH, possibly by increased sensitivity to negative feedback. We investigated the time course of serum TSH after burn injury and assessed changes in feedback sensitivity.

MATERIALS AND METHODS

For Phase I, adult male Sprague-Dawley rats received a sham burn (S) or a 17% total body surface area (TBSA) full-thickness scald burn (B). Sacrifice by guillotine was at 6, 24, or 48 h or 7 days (10-14 animals per group) for assays of serum tetra- and triiodothyronine (T_4, T_3), their binding (T_3 talc uptake, T_3U; equilibrium dialyzable fractions by tracer method, DFT_4, DFT_3), free indices FT_4I and FT_3I (T_4 or T_3 X T_3U), free concentrations FT_4 and FT_3 (T_4 or T_3 X DF), and thyrotropin (TSH). Hormones were measured by RIA. The NIDDK TSH antibody and kit with RP2 standard from the National Hormone and Pituitary Program (Baltimore MD) was used with a sample volume of 0.2 ml. The least detectable value was 0.5-1.0 ng/ml in buffer. Analyses were by t tests or analyses of covariance.

For Phase II, adult male Sprague-Dawley rats were given a standard 3° scald burn (B) of 25% of the TBSA under anesthesia or a sham burn (S). Control rats were left untreated (C) or were thyroidectomized (T). Half of S, B, and T rats were implanted with subcutaneous Alzet® mini-osmotic pumps (ALZA Corporation, Palo Alto, CA) (SP, BP, TP) to deliver T_4 at 11 μg/100 g/day, the other half (S, B, or T) receiving diluent pumps. For 7 groups, n ranged 12-20. Serum was sampled by guillotine sacrifice 6 days later for thyroid hormones and TSH (RIA) as above, binding assessment (immobilized antibody T_3U test; tracer dialyzable fractions (DF)), and free indices (FT_4I or FT_3I as T_4 X or T_3 X T_3U) and concentrations (FT_4 = T_4 X DFT_4; FT_3 = T_3 X DFT_3).

RESULTS

Results are given as comparisons or relationships at $P < 0.05$ or better. For Phase I, T_4, FT_4I, T_3, and FT_3I were low in B (vs. S) at all four time points. FT_4 (6, 24 h) and FT_3 (24 h) were low in B (vs. S). TSH was elevated in B (vs. S) (24, 48 h) (Fig 1). T_3U (6, 24 h), DFT_4 (all time points), and DFT_3 (24, 48 h; day 7) were elevated in B (vs. S) (Fig 2). Only in the 48-h and day 7 data was FT_4I lower in B than in S at any given level of FT_4 (analyses of covariance; similar for FT_3I, FT_3) (Fig 3). Figure 4 shows weight change (initially reflecting the 30 ml resuscitation

fluid, NaCl IP) and food consumption. The latter was estimated by weighing the food container (hung on the inside cage wall) daily. All total and free measures of T_4 and T_3 were depressed in B (vs. S) for a given level of food consumption (FC) in the lower FC range (Fig 5). In each panel of Figure 5, the slopes were different (S vs. B) except for FT_4I, where the position is different ($P < 0.05$ or better). Furthermore, in the food consumption range marked by the vertical lines (between which the number of S and B samples are the same), t tests indicate lower hormone values in B ($P < 0.05$ or better). Food consumption was that recorded in the time period (usually 24 h) just before hormone samples were taken. Samples from the various time points (excluding 6 h) are considered together.

For Phase II, mean T_4, FT_4I, and FT_4 were depressed in B (vs. S) and T (vs. C), normal or elevated in BP, and elevated in SP (vs. S) and TP (vs. C) (Fig 6). T_3 variables were depressed in B (vs. S except for normal FT_3 in B), BP (vs. SP), and T (vs. C), but normal in SP and TP (Fig 6). T_3U, DFT_4, and DFT_3 were elevated in burn groups, in which for any given level of FT_4, FT_4I was lower than in other groups (analysis of covariance; similar results for FT_3 and FT_3I) (Fig 7). As in human burns and nonthyroidal illness (NTI), in the rat burn model the serum thyronine binding defect was less strongly indicated by the T_3U than by the DF (Fig 7). The same finding in rat and human burns was previously obtained by use of T_3U tests with other matrices (charcoal, talc)(2, Phase I, unpublished data).

TSH was depressed in SP (vs. S), BP (vs. B), and TP (vs. C) and elevated in T (vs. C) (Fig 6). Values of T_4, FT_4I, and FT_4 in B and BP were within a range surrounded by respective values in the other groups. Analyses of covariance (Fig 8) revealed that log TSH was lower (all $P \leq 0.01$) in B and BP (than in nonburn groups, whether or not T_4 (the only nonburn group with low T_3) was included) for any given level of T_4, FT_4I, or FT_4. With or without burn, raising serum T_4 variables with subcutaneous pumps produced significant negative rectilinear slopes with log TSH.

DISCUSSION

The elevated TSH 24 and 48 h after burn for Phase I (17% TBSA burn) indicates that the fall in T_4 and T_3 (present by 6 h) is not initially TSH-dependent. Whether accelerated iodothyronine disposal may thus account for this fall, or whether extravasation into the extracellular compartment in the area of the burn, with dilution of the plasma compartment is responsible for the fall in T_4 and T_3 is not yet known. Furthermore, the elevated TSH at 24 and 48 h suggests that, at least at first, the pituitary responds in a qualitatively normal fashion to the low iodothyronines. Elevated TSH was resolved before 6 or 7 days postburn, possibly related to amelioration of low T_4 and T_3 variables, particularly FT_4 and FT_3, after this relatively small burn. However, with a larger

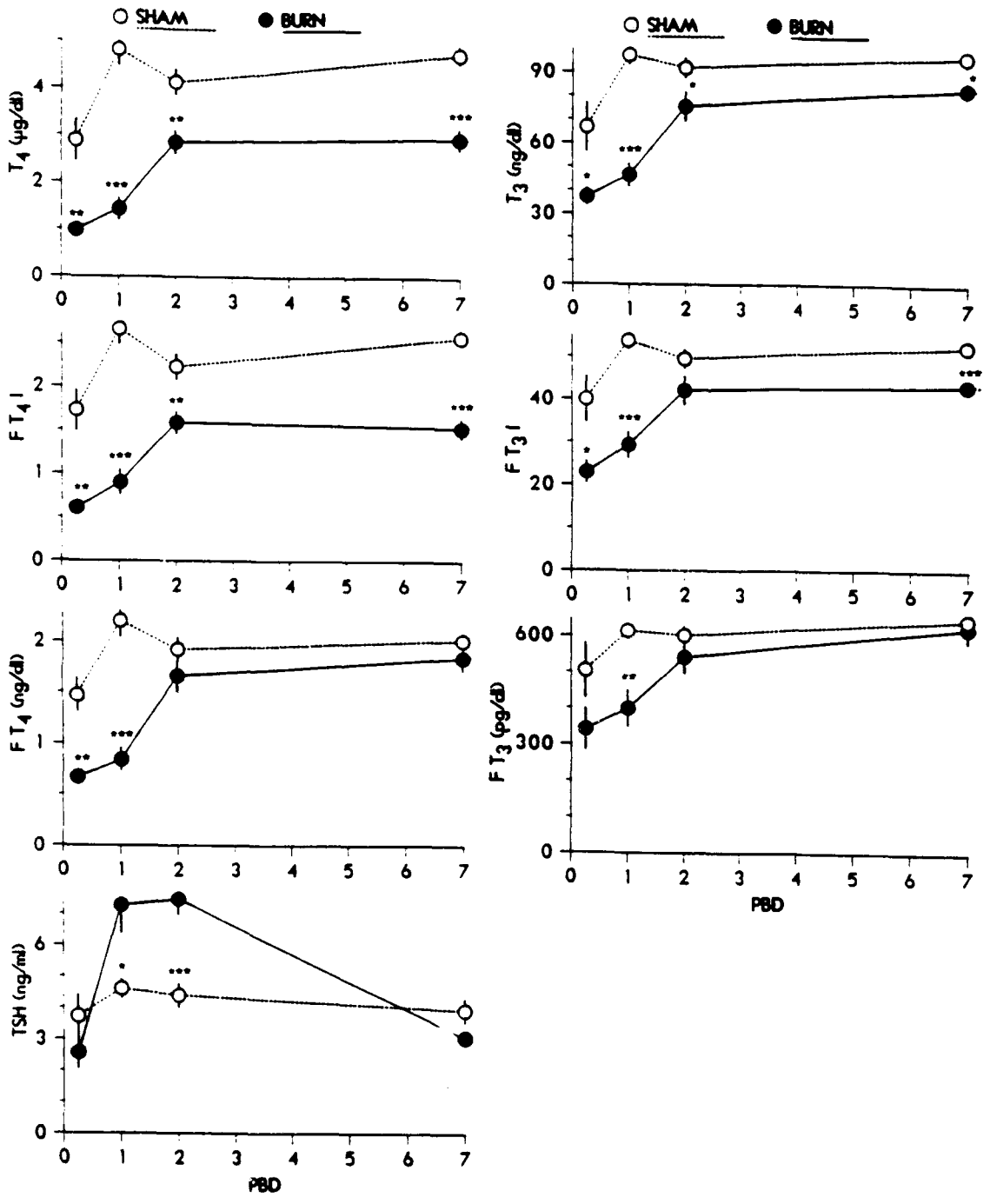

FIGURE 1. Mean ± SE of Phase I serum T_4 and T_3 variables and TSH in rats with a TBSA burn of 17% according to postburn day (PBD). *P < 0.05, **P < 0.01, ***P < 0.001 (burn vs. sham). Figures 2-5 also represent Phase I.

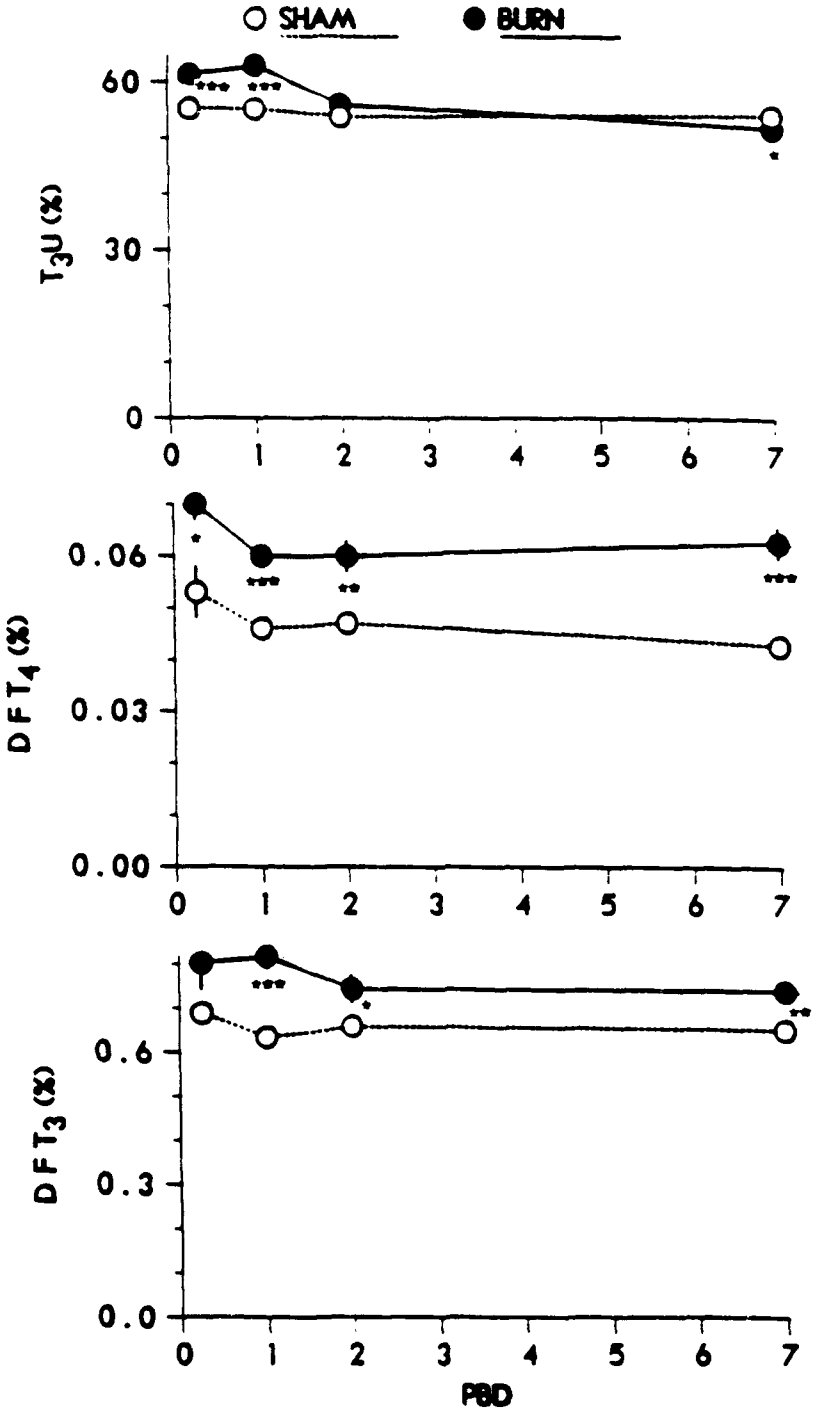

FIGURE 2. Mean ± SE for three assessments (competitive T_3U onto talc, and DF) of serum iodothyronine binding on several postburn days (PBD). *P < 0.05, **P < 0.01, ***P < 0.001 (burn vs. sham).

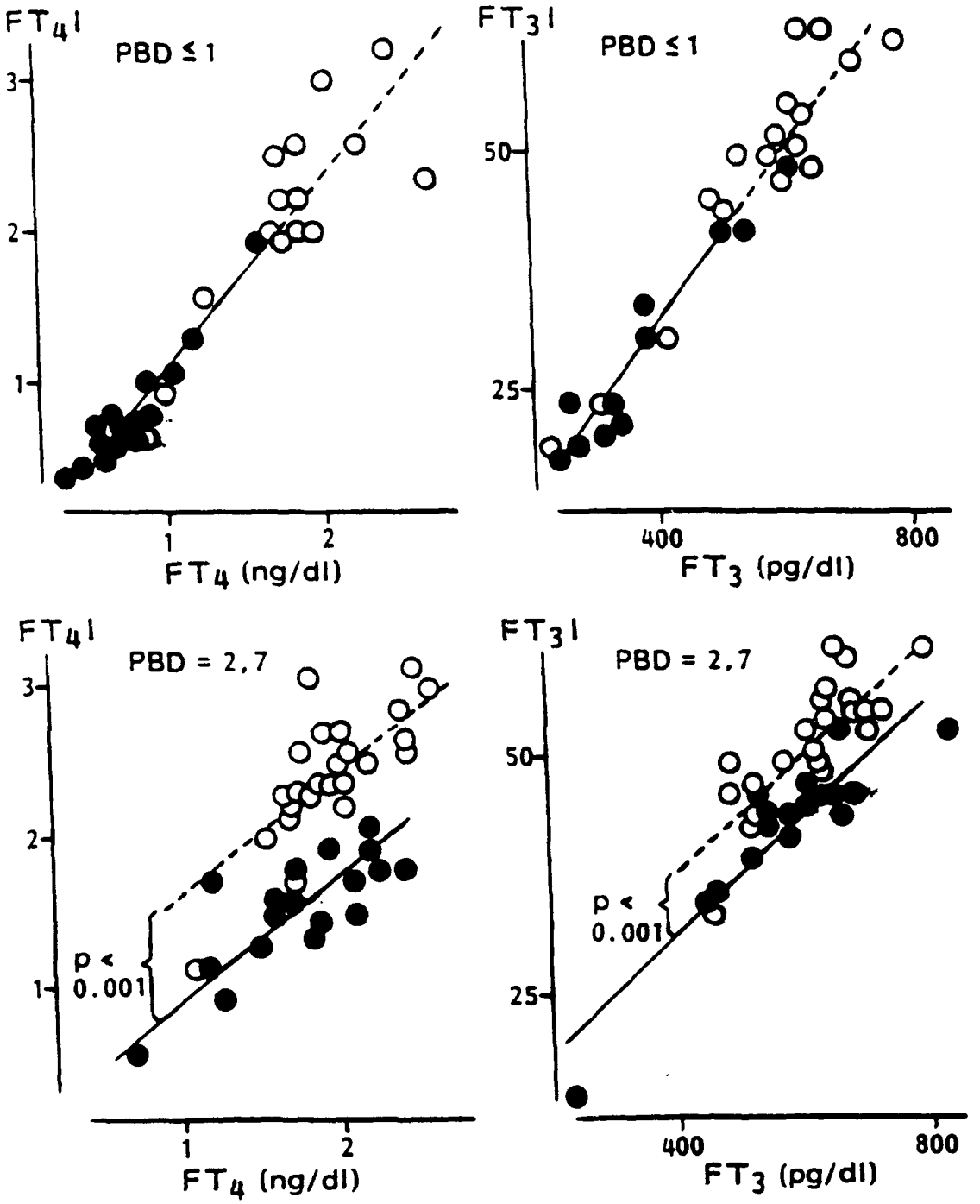

FIGURE 3. Analyses of covariance of FT_4I with FT_4 (and FT_3I with FT_3) between burn (closed circles) and sham (open circles) rats, separately for pooled 6- and 24-h data (top panels) and pooled postburn day (PBD) 2 and 7 data (bottom panels). Burn and sham slopes were not different, and positional differences are indicated.

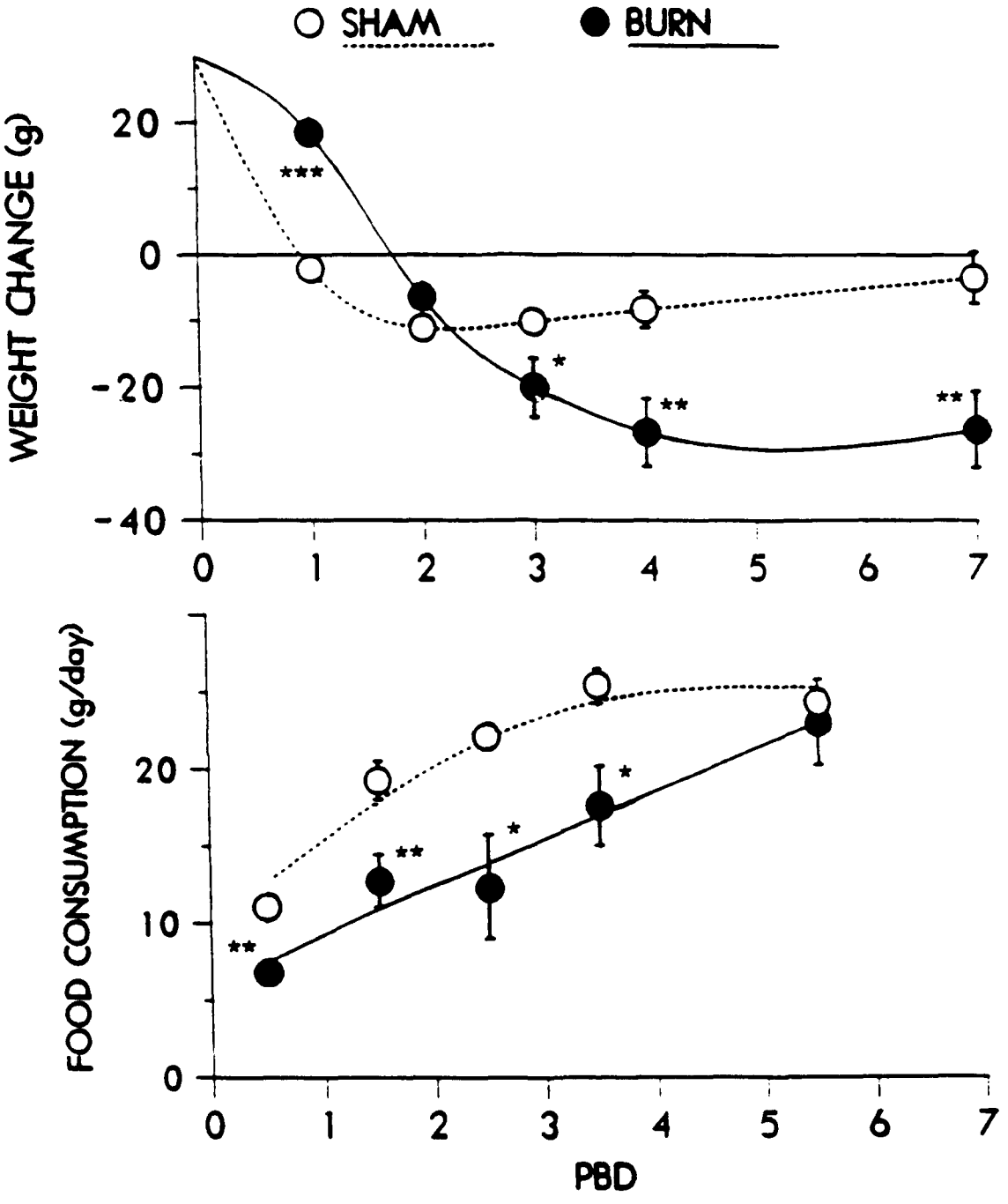

FIGURE 4. Mean ± SE of weight change (difference from weight obtained prior to burning and administration of resuscitation fluid) and food consumption over 1-day periods for postburn day (PBD) < 4 and over a 3-day period for PBD 4-7. *P < 0.05, **P < 0.01, ***P < 0.001 (burn vs. sham).

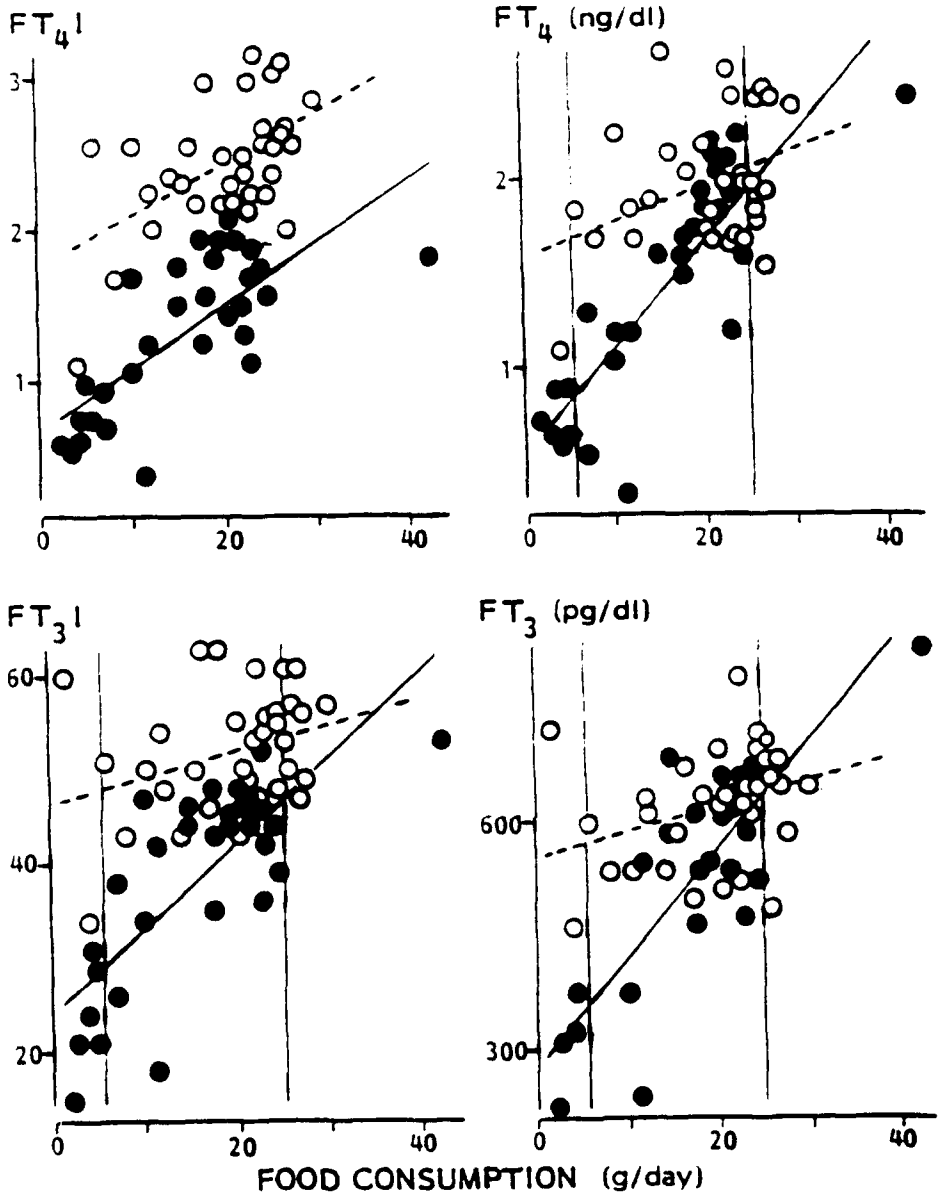

FIGURE 5. Analyses of covariance for iodothyronine variables with food consumption between burn (closed circles) and sham (open circles) groups. Except for FT_4I, slopes differed between groups ($P < 0.05$ or better). In all cases, the positional difference was significant. Vertical lines represent the interval of food consumption (with equal representation for both groups, and samples only to one side of the point of crossing of burn and sham best-fit lines) over which t test comparison showed a difference between burn and sham ($P < 0.05$ or better).

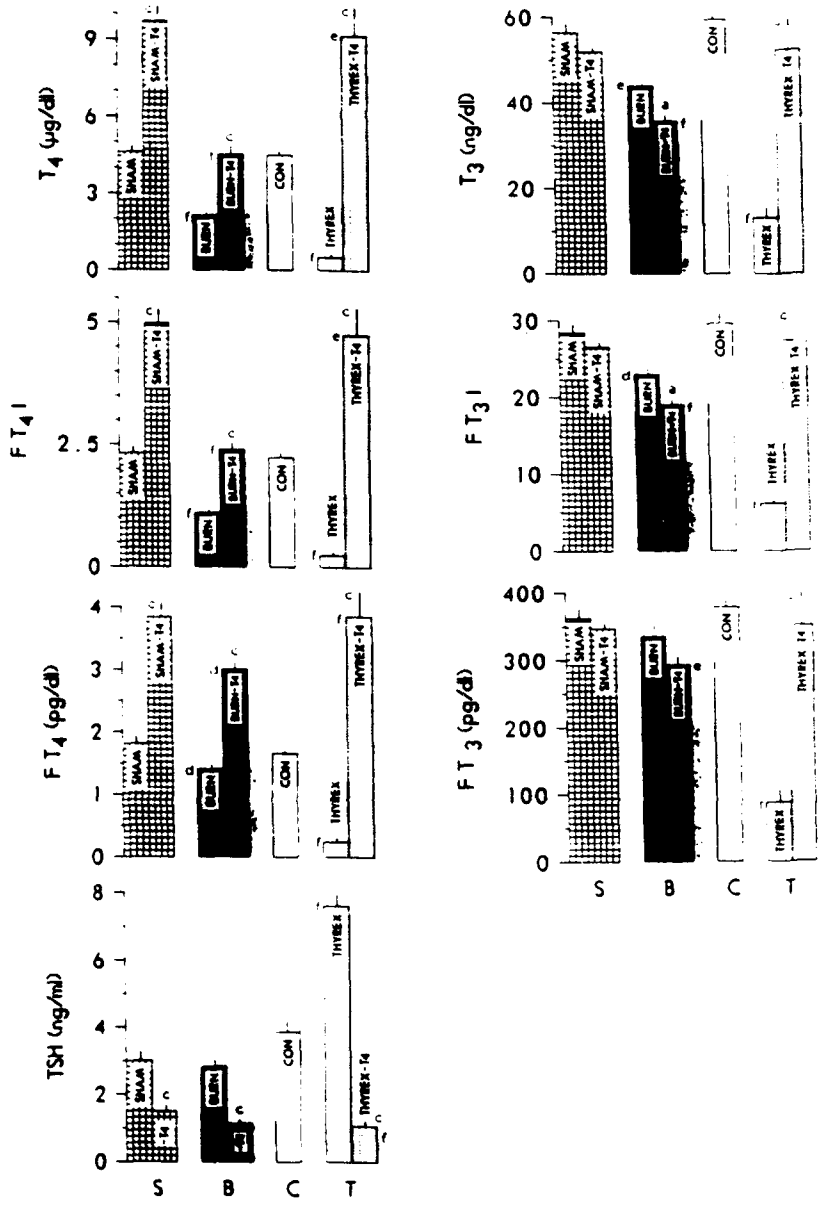

FIGURE 6. Mean ± SE of Phase II serum T_4 and T_3 variables and TSH on postburn or postoperative day 6 in sham (S), 25% TBSA burn (B), control (C), and thyroidectomized (T) rats. In each case, the left of the paired bars (vehicle) is cited in the text as S, B, or T; the right bar in each pair (T_4 by pump) is cited in the text as SP, BP, or TP, respectively. Small case letters near tops of bars indicate significant differences as noted in the legend of Figure 7. Figures 7 and 8 also represent Phase II.

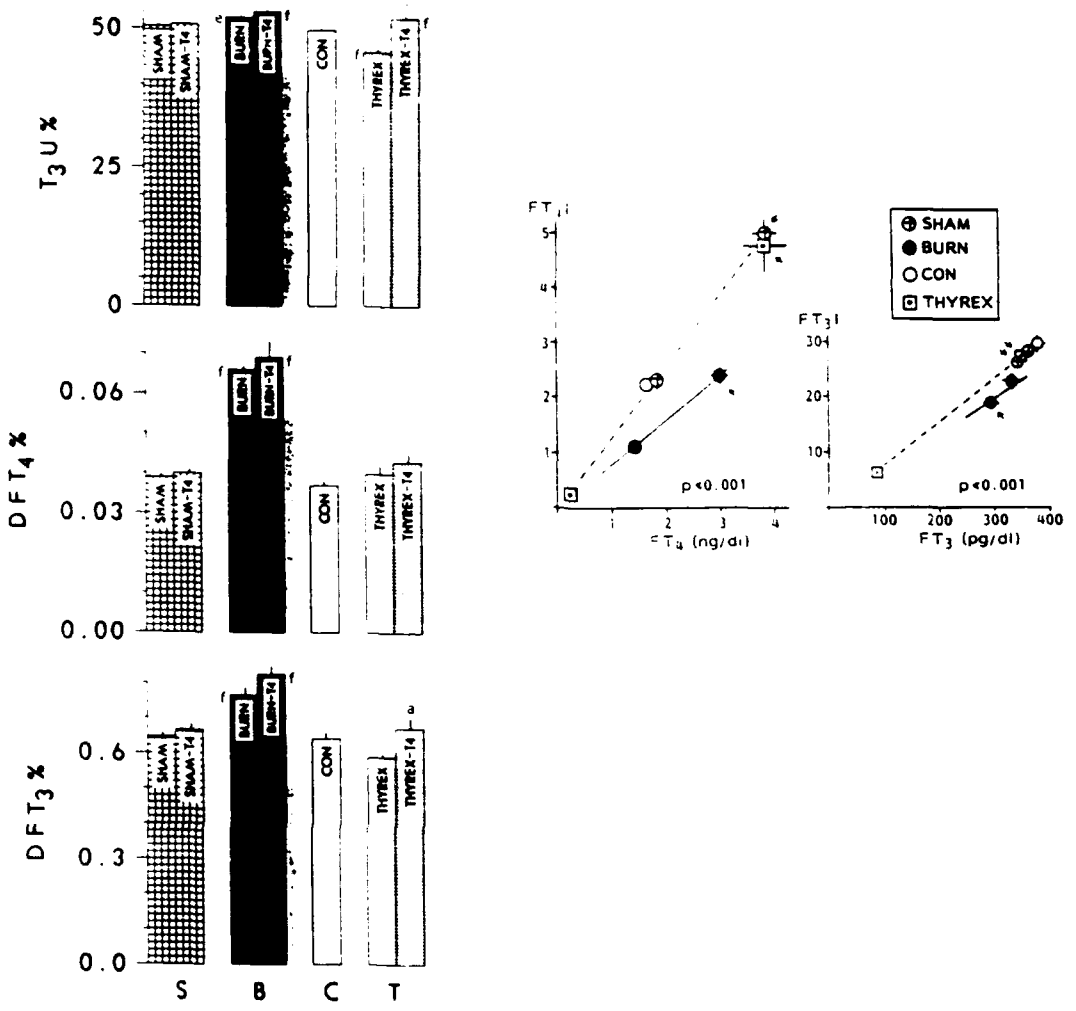

FIGURE 7. Mean ± SE for three assessments (competitive T_3U onto immobilized antibody and DF) of serum iodothyronine binding (bar panels, as described in the legend of Figure 6) and analyses of covariance of FT_4I with FT_4 (and FT_3I with FT_3) between burn and other groups (Cartesian plots). P values for this and Figure 6 are a < 0.05, b < 0.01, c < 0.001 (T_4-treated vs. respective vehicle-treated group) and d < 0.05, e < 0.01, and f < 0.001 (burn vs. respective sham or thyroidectomized vs. control). In the Cartesian plots, small arrows point to groups given T_4. THYREX indicates thyroidectomized and CON, unoperated controls. In both panels, though the slopes differed when all data were included, when only control and T_4-untreated burn and sham groups were included, slopes did not differ but positional differences were significant (P < 0.001).

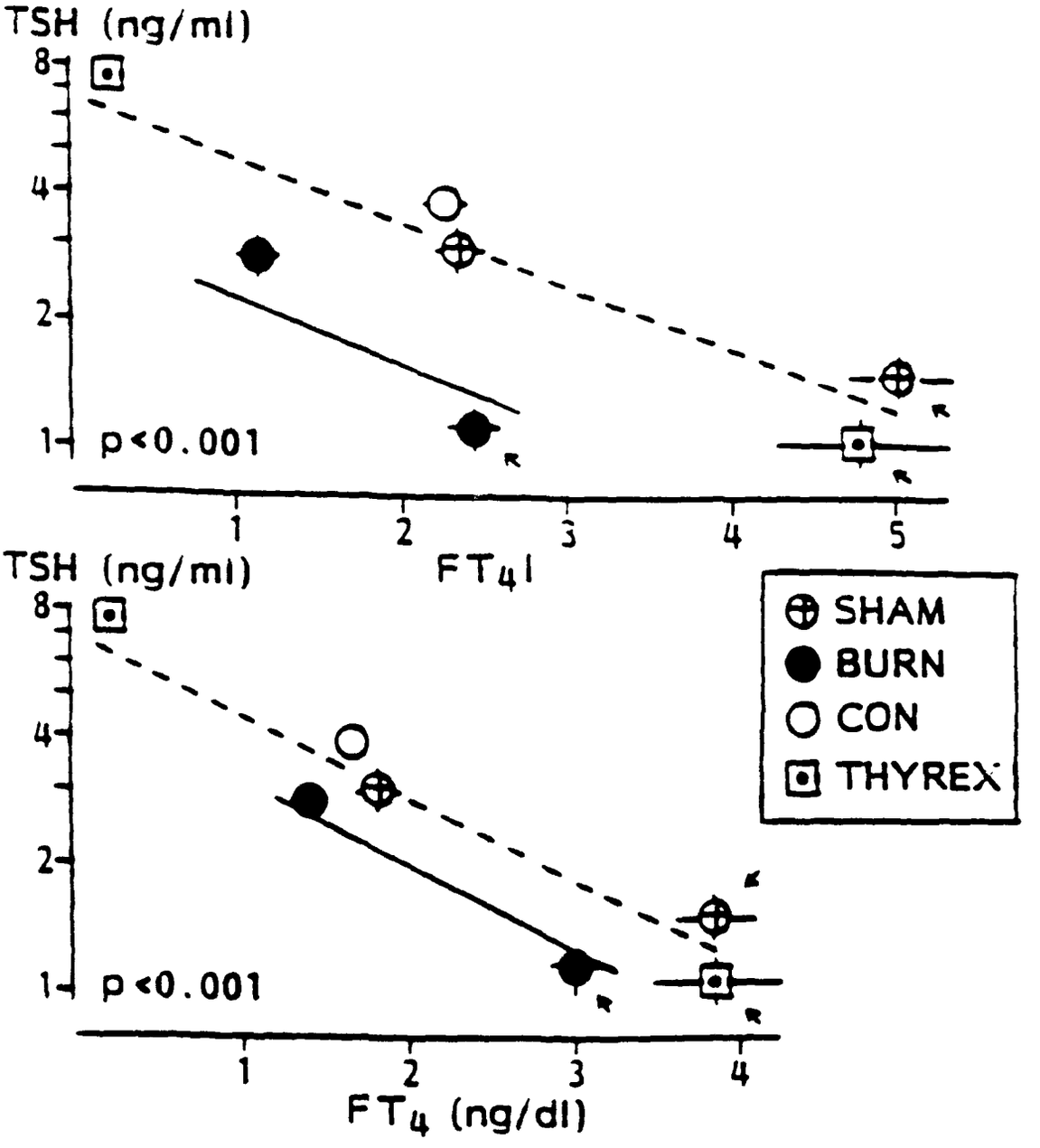

FIGURE 8. Mean ± SE and analyses of covariance of log serum TSH with FT_4I (top) and FT_4 (bottom) between burn and other (combined) groups. Small arrows point to the groups receiving T_4. THYREX indicates thyroidectomized and CON, unoperated controls. Slopes were not significantly different, and significances for positional differences are indicated.

burn (60%) in which depression of FT_4 and FT_3 persists beyond this period (2), TSH nevertheless fell, after the initial elevation, even to low levels (1). With the larger burn (1), TSH was elevated only at 24 (not 48) h. Thus, an initial phase of relatively normal TSH response to low T_4 and/or T_3 can occur in that these low iodothyronine levels are not able to produce excessive negative feedback at this time sufficient to block the rise of TSH.

Following the first 1 or 2 days, however, there occurs relative suppression of TSH to normal levels even in the presence of low iodothyronines (1,2), suggesting later resetting of the pituitary-thyroid axis for enhanced negative feedback. Thus, in Phase II with a somewhat larger burn (25%), even FT_4 was depressed on postburn day 6 and TSH was not elevated. Furthermore, at this time administration of exogenous T_4 disclosed serum TSH relatively more depressed in burns than normal by a given level of available circulating T_4. That negative feedback from T_3 was not involved in this difference is indicated by the lowering of T_3 variables with raising of T_4 in burns by T_4 administration. This suggests that T_4 may be more important than T_3 in the enhanced negative feedback after burn injury. Though the results fit the rubric of enhanced negative feedback post the first couple of days after burn, this may reflect a number of possible mechanisms, including reduced secretion of TSH-releasing hormone (TRH) from the hypothalamus and enhanced production of substances which inhibit TSH secretion at the level of the pituitary, such as dopamine and corticosteroid which are elevated after burn (1).

The results for Phase I suggest that serum T_4 and T_3 are depressed beyond what might be explained by reduced food intake, also known to produce such a response (2). However, this must be tested after larger burn injury in which greater depression of T_4 occurs beyond the first 1 or 2 days and the phase of relative depression of TSH is more secure.

The T_4 and T_3 binding defect in serum (elevated DFT_4 and DFT_3) observed herein confirms that found previously beyond the first week after large burns (2). The previous findings of relatively depressed FT_4I in burns for a given level of FT_4 (2) were also confirmed in these studies. This is thought to result not only from inhibition of iodothyronine binding to serum proteins (affecting dialyzable fractions as well as the T_3U) but also to the competing matrix in the T_3U test (2). The absence of this altered FT_4I-FT_4 relationship at 24 h after burn in Phase I, however, suggests that after burn iodothyronine binding is inhibited first only to serum proteins then later also to the T_3U test matrix. Then, the results are consistent with a circulating inhibitor of thyronine binding to both circulating proteins and the T_3U matrix, suggesting a change of character of the inhibitor with time after injury.

The presence of nonelevated TSH despite low T_4 and T_3 and the occurrence of the serum thyronine binding defect with the altered FT_4I-FT_4 relationship are characteristic of human burn injury and critical NTI (2). Thus, the burned rat is a useful model of human injury and NTI.

PRESENTATIONS/PUBLICATIONS

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

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

REFERENCES

1. Vaughan GM: Neuroendocrine and sympathoadrenal response to thermal trauma. In Dolecek R, Brizio-Molteni L, Brizio-Molteni A, and Traber D (eds), **Endocrinology of Thermal Trauma: Pathophysiologic Mechanisms and Clinical Interpretation**. Philadelphia: Lea & Febiger (in press).

2. Vaughan GM, Pruitt BA Jr, and Mason AD Jr: Burn trauma as a model of severe illness. In Dolecek R, Brizio-Molteni L, Brizio-Molteni A, and Traber D (eds), **Endocrinology of Thermal Trauma: Pathophysiologic Mechanisms and Clinical Interpretation**. Philadelphia: Lea & Febiger (in press).

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
				DA318018	1 Oct 89	DD-DRA&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
16 Feb 89	D. Chg	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS14	F	305
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP,	FY90-01		

11. TITLE *(Precede with Security Classification Code)* (U) Investigation of the Immunologic Sequelae of Blood Transfusions in Rats

12. SUBJECT AREAS
0605 Medicine and Medical Research

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
8909	9009	DA	C. In-House

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

18. RESOURCES ESTIMATE

a. FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS *(In thousands)*
89	0.1	30
90	0.1	35

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME US Army Institute of Surgical Research	a. NAME US Army Institute of Surgical Research
b. ADDRESS *(include zip code)* Fort Sam Houston San Antonio, Texas 78234-5012	b. ADDRESS Fort Sam Houston San Antonio, Texas 78234-5012
c. NAME OF RESPONSIBLE INDIVIDUAL PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR WAYMACK, J P
d. TELEPHONE NUMBER *(include area code)* 512-221-2720	d. TELEPHONE NUMBER *(include area code)* 512-221-2018
21. GENERAL USE FINA MILITARY/CIVILIAN APPLICATION: M	f. NAME OF ASSOCIATE INVESTIGATOR *(If available)* BURLESON, D G g. NAME OF ASSOCIATE INVESTIGATOR *(If available)* OKERBERG, C V

22. KEYWORDS *(Precede EACH with Security Classification Code)* (U) Blood Transfusions; (U) Immunologic Effects; (U) Trauma; (U) Lab Animals; (U) Rats; (U) RA II

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

23. (U) A DTIC literature search was conducted under DTIC request number W6Q35J dated 19 October 1989 for the technical report database and request number W6Q51L dated 19 October 1989 for the work unit database. The proposed effort will not result in the duplication of effort. The objective of this work is to identify the immunological effects of blood transfusions in traumatized patients utilizing a rat transfusion model and determine whether pharmacological manipulation can prevent the adverse immunologic sequelae of the blood transfusions.

24. (U) Healthy and traumatized Lewis rats will be administered blood transfusions. The animals will then be evaluated for blood transfusion effects on their resistance to bacterial infection.

25. (U) 8810 - 8909. Allogeneic blood transfusions were documented to impair NK cell function at both 1 and 2 weeks following blood transfusion. This impairment was further evidenced by an increased rate of metastatic tumor growth. Transfusions were not found to significantly alter the percentage of helper T cells, suppressor T cells, IL-2-bearing lymphocytes or transferrin-bearing lymphocytes. Transfusions were found to enhance survival in endotoxin shock. Investigations of the effect of transfusions on TNF production are currently being performed to determine whether the beneficial effect of transfusions in endotoxin shock are due to alterations in TNF production. For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1989.*

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ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3M161102BS14-00, BASIC RESEARCH

PROJECT TITLE: Investigation of the Immunologic Sequelae of Blood Transfusions in Rats

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

31 January 1989 - 30 September 1989

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

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Transfusions are reported to increase the incidence of tumor metastasis in clinical studies and primary tumor growth in animal studies. We evaluated the effect of transfusions on immunologic response to primary and metastatic tumors in multiple rat models. One-half of the animals were administered lactated Ringer's solution and one-half ACI rat blood at the time of tumor challenge. In 80 animals, a slow-growing colon tumor was implanted subcutaneously. At 4 months, there were no significant differences in tumor size or leukocyte infiltration of the tumor. Similar results were obtained with a rapidly growing colon cancer. Analysis of T-lymphocyte subpopulations in both groups showed no differences. Rats transfused at the time of intravenous challenge with a suspension of 1×10^6 tumor cells had a mean survival time of 38.3 ± 0.8 days and the control group had a mean survival time of 41.1 ± 0.8 days ($P = 0.016$). One week after transfusion, NK cell lysis of tumor cells at a 100:1 effector/target cell ratio was $18.0\% \pm 1.8\%$ for the transfusion group and $23.0\% \pm 1.3\%$ for the control group ($P = 0.034$). In conclusion, transfusion in multiple rat cancer models did not affect primary tumor growth or the host's immunologic response to it but did significantly impair NK cell function and survival with tumor metastases.

INVESTIGATION OF THE IMMUNOLOGIC SEQUELAE OF BLOOD TRANSFUSIONS IN RATS

Before the 1970s, it was generally believed that administration of blood products to patients would result in immunostimulation in the recipient (1). This belief was based on the demonstration by Medawar (2,3) that the administration of blood products resulted in a specific donor immunization in a rabbit skin allograft model. This led transplant surgeons to avoid administering blood transfusions to patients awaiting solid organ transplantation in the belief that such transfusions would increase the immune responsiveness of the recipient and lead to an eventual rejection of the allograft (4). This policy was reversed in the early 1970s when a number of publications reported that transfusions administered before transplantation decreased the rate of allograft rejection (5-8).

With the acceptance of the concept that blood transfusions cause immunosuppression in transplant recipients, surgeons in the 1980s have addressed the question of whether this transfusion-induced immunosuppression is also present in patients not undergoing transplantation. There has been a large number of reports that indicate that perioperative blood transfusions increase the rate of tumor growth and metastasis in patients undergoing oncologic surgery (9). These reports involved retrospective patient analyses. Therefore, the possibility exists that the patients with more advanced tumors received a greater proportion of the blood transfusions. If true, this would indicate that the detrimental effect observed with blood transfusion was not the result of the transfusion itself but rather the more advanced nature of the patient's tumor.

To evaluate this possibility in a controlled prospective study, several investigators have investigated the effect of blood transfusions on primary tumor growth and a diminished long-term survival in animals receiving allogeneic transfusions (9). In clinical practice, control of the primary tumor can usually be achieved through surgical intervention. It is rather the metastasis of the tumor that results in the eventual death of the patient. We have evaluated the effect of blood transfusion on host response to both primary tumor growth and metastatic tumor growth.

MATERIALS AND METHODS

Animals. Two hundred and fifty adult male Wistar-Furth rats weighing ±250 g were used as tumor recipients. Twenty adult male ACI rats were used as blood donors. All animals were housed in individual cages and were allowed food and water ad libitum throughout the study. Animals were observed for 1 week before entry into the study to exclude the possibility of any preexisting diseases.

Transfusion Protocol. Blood was obtained from the donor animals by vena cava puncture and mixed at 4:1 vol ratio with CPDA-1 anticoagulant. Animals in the control group were given 3 ml lactated Ringer's solution and those in the transfusion group were given 1 ml ACI blood intravenously at the time of primary tumor implantation. The increased volume of lactated Ringer's solution was chosen because it was believed that it approximated the intravascular volume changes achieved in the animals receiving the 1 ml whole blood.

Tumor Protocol. Three tumor models were used to evaluate the transfusion effect. In the first model, 80 animals (saline = 40, transfusion = 40) were anesthetized with sodium pentobarbital (35 mg/kg IP) and a 1-cm incision was made in the thigh of the left lower extremity. A 2-mm^3 aliquot of a 1,2-dimethylhydrazine-induced, poorly differentiated syngeneic colon adenocarcinoma (supplied by Drs. Ravikumar and Steele, New England Deaconess Hospital, Boston, MA) was implanted into the thigh. The incision was closed with surgical clips and the tumor was allowed to grow for a period of 4 months. The animals were then sacrificed with a lethal dose of sodium pentobarbital (600 mg/kg IP) and the tumor was excised and weighed. A biopsy specimen of the tumor obtained at its periphery was fixed in formalin and stained with an H&E stain for later assay of leukocyte infiltration.

The second tumor model had the same size aliquot of a more rapidly growing 1,2-dimethylhydrazine-induced, poorly differentiated syngeneic colon carcinoma (also supplied by Drs. Ravikumar and Steele) implanted in the subcutaneous tissue overlying the thoracic vertebrae.

In 20 of these animals (saline = 10, transfusion = 10), the abdomens were painted with 1 ml 0.5% DNFB solution in a 4:1 vol ratio of acetone/olive oil. The tumors were allowed to grow for 1 week. On the day before sacrifice, the ear thickness of each animal was determined using an engineer's caliper. The ear was then painted with 0.1 ml of the same 0.5% DNFB solution. Twenty-four h later, the animals were anesthetized with sodium pentobarbital (35 mg/kg IP). The tumors were excised and weighed and periphery specimens of the tumors were fixed in formalin and stained with H&E for later analysis of leukocyte infiltration. Ear thickness was again measured and the percent ear swelling in comparison with measurements on the preceding day was determined. Ear swelling measured by this technique has previously been shown to correlate with cell-mediated immunity (10). The ears were amputated, fixed in formalin, stained with H&E, and histologically assayed for leukocyte infiltration in response to DFNB. Finally, the spleens were removed and lymphocytes were harvested by Ficoll-Hypaque centrifugation. The lymphocyte preparations so obtained were stained with anti-lymphocyte monoclonal antibody preparations, washed, and reacted with affinity-purified,

fluorescent-labeled, goat anti-mouse IgG as a second-step reagent. Fluorescent-labeled cells were analyzed by flow cytometry. For each sample, 5,000 cells were assayed and the numbers of cells labeled by the monoclonal antibodies specific for pan-T lymphocytes (OX-19), helper/inducer lymphocytes (W3/25), and suppressor/cytotoxic lymphocytes (OX-8) cell surface markers were determined. For each sample, a negative control with a monoclonal antibody of the same isotype (IgG_1) to human T cell (anti-Leu-2) was run to determine cutoffs. The positive cutoff was set at a point determining the upper 2% or less of background control and the number of background control cells were subtracted from each count. Nonlymphoid cell contamination was assayed by analysis of forward and 90° light scatter. Cells with light-scattering intensities outside limits established for normal lymphocytes were removed from analysis. The percentage of lymphocytes bearing IL 2 and transferrin receptors was also determined.

An additional 40 Wistar-Furth rats (saline = 20, transfusion = 20) with the same tumor were followed until death to determine mean survival times. Weekly tumor size determinations were made in these animals according to the technique of Chance et al (11).

For the final tumor model, a tumor-cell suspension was prepared from the rapidly growing 1,2-dimethylhydrazine-induced solid tumor. Briefly, viable tumor was obtained by excision of the rapidly growing tumor from a Wistar-Furth rat. The tumor was mechanically disaggregated by first slicing the tumor into approximately 1-mm^3 pieces and then vigorously shaking the suspension in complete RPMI-1640 medium with penicillin, streptomycin, and 10% fetal calf serum. The cells were washed 3X in the same medium. A sample of this suspension was stained with trypan blue and the number of viable tumor cells was determined. The cells were then resuspended in sufficient RPMI medium to achieve a final concentration of 1×10^6 cells/ml. A 1-ml aliquot of this suspension was injected intravenously into 80 Wistar-Furth rats immediately after the administration of either lactated Ringer's solution (n=40) or blood (n=40). The animals were followed until death and mean survival times were determined. Necropsy was performed in all animals and biopsy specimens of the pulmonary metastases were fixed in formalin and stained with H&E for later analysis of leukocyte infiltration.

Leukocyte Infiltration. Leukocyte infiltrates were quantified by enumeration of WBCs in the tumor periphery or the dermis of the DNFB-treated ear. Fifteen high-power fields (hpf) were counted in each specimen with an image-analysis system (Optomax 40-10, Optomax, Inc., Hollis, NH) as described previously (12). An average was obtained for these 15 hpf.

NK Cell Function. The effect of blood transfusions on NK cell function was assayed at two points in relation to the time of blood administration. Forty animals (control = 20, transfusion = 20)

were sacrificed by decapitation 1 week after the administration of blood or lactated Ringer's solution, and 20 animals (control = 10, transfusion = 10) were sacrificed 2 weeks after the administration of blood or lactated Ringer's solution. After decapitation, the spleens were rapidly removed and homogenized in RPMI-1640 medium supplemented with 10% fetal calf serum, penicillin, and streptomycin. The number of viable nucleated cells in each homogenate was determined in dilutions appropriate to the desired concentration of mononuclear cells. Dilutions were done to achieve effector/target cell ratios of 100:1, 50:1, 25:1, and 12.5:1. Then 1×10^4 YAC target cells that had previously been labeled with ^{51}Cr were added to each well, with a final volume of 0.2 ml/well in V-bottom microfilter plates. After centrifugation at 40 g for 2 min, the plates were incubated at 37°C in 5% CO_2 for 4 h. The plates were then centrifuged at 500 g for 5 min and a 100-μl aliquot of the supernatant was collected and assayed for ^{51}Cr on a γ-counter. The percentage of cell lysis was calculated as the mean counts/min (cpm) related in the presence of effector cells minus the mean cpm spontaneously released by target cells incubated with medium alone divided by the cpm released after treating target cells with Triton X-100 (1:100 dilution) minus the cpm spontaneously released with medium alone; the quotient was multiplied by 100.

All data are expressed as mean ± SE. Comparisons among groups were made with the Savage (Mantel-Cox) test for survival time and ANOVA for all other data. Significance was assumed at $P < 0.05$.

RESULTS

Blood transfusion was found to have no effect on the size of the primary tumor in the slow-growing colon carcinoma model. The mean tumor weight for saline-treated animals was 26.0 ± 1.1 g and for transfused animals, 28.1 ± 1.4 g ($P = 0.098$). Assays of leukocyte infiltration into the tumors failed to demonstrate any effect of blood transfusion. With the slow-growing tumor, there were 171.0 ± 12.6 cells/hpf in the control group and 181.6 ± 16.6 cells/hpf in the animals that had undergone transfusion ($P = 0.6$).

The tumor weights over time in the rapidly growing tumor model are shown in Table 1. None of these differences were statistically significant. The average tumor weights at the time of death for the two groups were 69.4 ± 4.1 g for the control group and 62.2 ± 2.1 g for the transfusion group ($P = 0.135$). The lower tumor weight recorded at the time of death compared with that measured 22 days after tumor implantation is the result of two factors. First, a significant percentage of the animals had died of the tumor before this time, and thus, there was a difference in the populations. Second, the tumor weight at 8, 15, and 22 days after transplantation was a calculated value whereas the value at the time of death was a direct measurement.

TABLE 1. Tumor Weights at 8, 15, and 22 Days after Tumor Implantation

Post-Tumor Implantation Day	Tumor Weights (g) Control Group	Transfusion Group	P Value
8	18.1 ± 0.9	21.2 ± 2.1	0.130
15	65.1 ± 1.9	64.4 ± 3.5	0.865
22	73.4 ± 2.1	73.2 ± 2.0	0.946

Blood transfusions also did not affect ear swelling in this model. The control group had a mean of 33.6% ± 5.8% ear swelling and the animals that had undergone transfusion had a mean of 42.2% ± 7.1% ear swelling ($P = 0.358$). There were 98.3 ± 6.3 cells/hpf in the rapidly growing tumor from the control group and 87.1 ± 6.1 cells/hpf in the tumor from animals that had undergone transfusion ($P = 0.21$). Analysis of leukocyte infiltration in the DNFB-treated ears revealed 97.5 ± 6.9 cells/hpf for the control group and 113.3 ± 5.6 cells/hpf for the animals that had undergone transfusion ($P = 0.087$). The mean survival times in the rapidly growing, subcutaneously implanted tumor model were 23.6 ± 0.5 days for the control group and 22.0 ± 0.7 days for the group that had undergone transfusion ($P = 0.127$).

Survival curves of the tumor metastasis model are shown in Figure 1. The mean survival time for the control group was 41.1 ± 0.8 days and for the transfusion group, 38.3 ± 0.8 days. This difference was statistically significant ($P = 0.016$). Two animals in the transfusion group were noted to have tumor metastases to supraclavicular lymph nodes at the time of death. In the control group, tumor metastases were confined to the lungs. Analysis of leukocyte infiltration of the metastatic pulmonary tumor nodules revealed an average of 281.2 ± 13.0 cells/hpf for the control group. The transfusion group was found to have an average of 269.0 ± 8.2 cells/hpf ($P = 0.42$ vs. control group).

Blood transfusions depressed NK cell function as measured at the 100 effector/target cell ratio both 1 and 2 weeks after transfusion (Table 2). One week after transfusion, the percent cell lysis at a 100:1 ratio with cells obtained from the control group was 23.0% ± 1.3% and for the group that underwent transfusion, 18.0% ± 1.8% ($P = 0.034$). Two weeks after transfusion, the cell lysis for the control group was 25.9% ± 1.7% and for the transfused group, 17.9% ± 2.7% ($P = 0.025$).

The percentages of pan-T lymphocytes (OX-19), helper/inducer T lymphocytes (W3/25), and suppressor/cytotoxic T lymphocytes (OX-8) among splenocytes obtained from control and transfused animals are

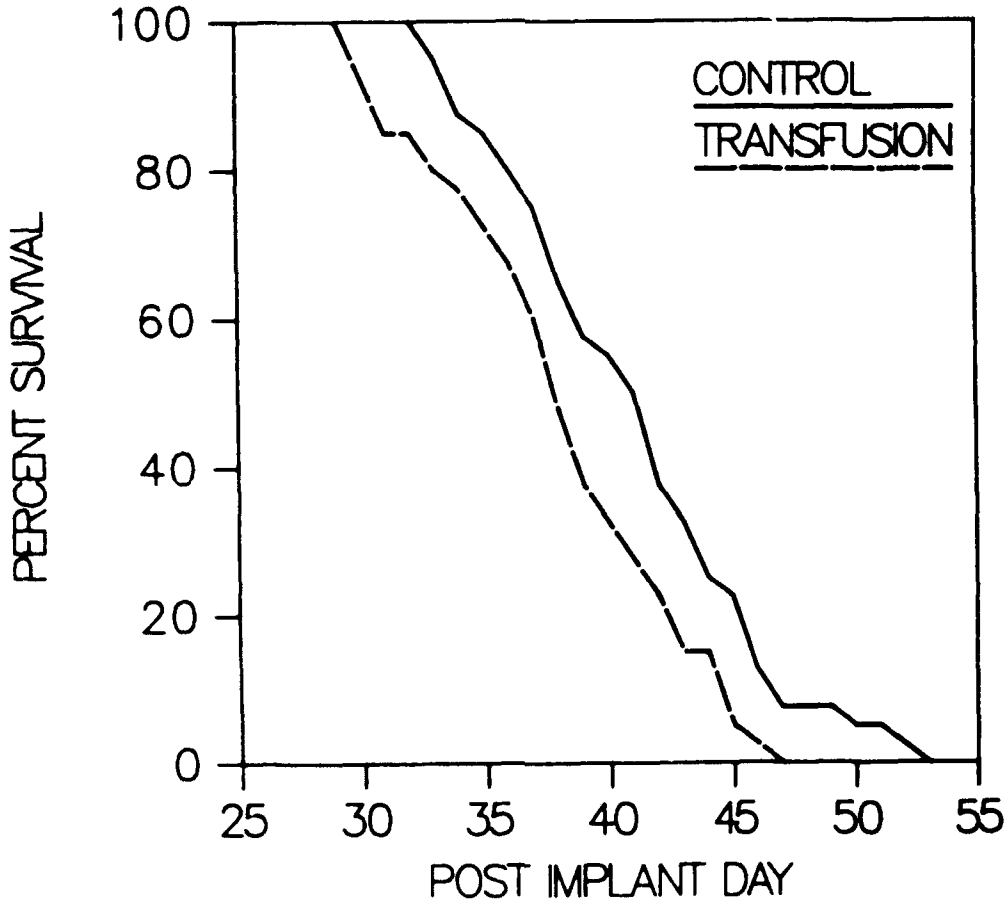

FIGURE 1. Survival curves for rats administered blood transfusions or lactated Ringer's solution at the time of challenge with 1 X 10^6 tumor cells administered intravenously.

shown in Table 3. The percentage of lymphocytes bearing IL 2 and transferrin receptors are also shown in Table 3. Transfusions did not significantly alter these percentages.

DISCUSSION

Surgical intervention is often able to control the primary site of common solid malignant neoplasms. It is commonly the distant metastasis that eventually leads to a fatal outcome. Such metastases can take place by three primary methods, i.e, migration through coelomic cavities, spread through lymphatic vessels, and spread through blood vessels. Tumor spread by these methods does not always result in metastasis. For such tumor spread to eventually become a successful metastasis, the tumor cell, or group of tumor cells, must implant in a distant site and then escape control by the body's immune system.

TABLE 2. NK Cell Cytotoxicity as Measured by Percentage YAC Cell Lysis

Effector/Target Ratio	Control Group	Transfusion Group	P Value
	1 Week Posttransfusion		
100:1	23.0 ± 1.3	18.0 ± 1.8	0.034
50:1	15.2 ± 0.9	12.4 ± 1.8	0.176
25:1	10.1 ± 1.3	8.8 ± 1.6	0.552
12.5:1	5.5 ± 0.8	4.6 ± 1.0	0.487
	2 Weeks Posttransfusion		
100:1	25.9 ± 1.7	17.9 ± 2.7	0.025
50:1	20.9 ± 2.3	15.1 ± 3.4	0.175
25:1	11.9 ± 2.5	6.6 ± 3.0	0.184
12.5:1	4.1 ± 1.2	2.1 ± 1.2	0.255

The tumor host generates a complex immunologic response to both the primary and metastatic tumor cells. Among the more important components of this response are cytotoxic macrophages, helper/inducer T lymphocytes, cytotoxic T lymphocytes, and NK cells. The NK cell appears to be particularly important in controlling tumor cells that are disseminated from the primary tumor source (13,14). The NK cell is able to attack tumor cells without any major histocompatibility complex restrictions and is also able to initiate tumor lysis on initial exposure to the tumor cells. In contrast, cytotoxic T lymphocytes require a period of 7–10 days after exposure to tumor cells before the onset of tumor cell lysis.

This study attempts to delineate the effect of blood transfusions on host response to tumor, with specific emphasis on the immunologic response to metastases. We were unable to demonstrate any effect of blood transfusions on primary tumor growth with two different colon cancer models. This finding differs from our earlier report (15). However, there have been other reports of studies demonstrating no effect of blood transfusions on primary tumor growth (9). These contradictory findings indicate the complex nature of the host response to blood

TABLE 3. T-Lymphocyte Subset Populations Plus Lymphocytes Bearing IL 2 and Transferrin Receptors in Rat Splenocytes

	Control Group (%)	Transfusion Group (%)	P Value
Pan-T cells	74.5 ± 3.0	80.2 ± 2.6	0.1634
Helper-inducer T cells	51.8 ± 1.6	52.1 ± 1.0	0.8703
Suppressor/cytotoxic T cells	22.0 ± 1.0	25.0 ± 1.3	0.0807
IL 2 receptors	2.01 ± 0.34	2.72 ± 0.32	0.1400
Transferrin receptors	0.94 ± 0.12	1.09 ± 0.15	0.4599

transfusions, the host response to tumor, and the variety of animal tumors with regard to antigenicity and growth characteristics.

Our study failed to demonstrate any effect of blood transfusions on T-lymphocyte- and macrophage-mediated immunologic parameters, including tumor infiltration by WBCs. Such WBCs are usually predominantly T lymphocytes (16). Because immunologic control of primary tumor growth is principally dependent on macrophage and T-lymphocyte activity, our failure to demonstrate any effect of transfusion on primary tumor growth and T-lymphocyte or macrophage functions is entirely consistent.

Our study did demonstrate a significant effect of blood transfusions on NK cell function, both 1 and 2 weeks after transfusion. It has previously been reported that blood transfusions impair NK cell function in humans (17), but this study was flawed in that the transfusions were administered to patients with blood dyscrasias to correct anemia. The possibility therefore exists that the sicker patients received a greater proportion of the transfusions and the diminished NK cell function observed in the patients that had undergone transfusion was caused by more severe preexisting disease. This study indicates that this was probably not the sole reason for the diminished NK cell function in the patients that had undergone transfusion.

The finding of diminished survival time with tumor metastases in transfused rats is consistent with NK cell findings. Because NK cells are of primary importance in controlling metastases, impairment of NK cell function should enhance tumor metastasis and growth. These findings thus support the retrospective clinical studies in patients with tumors that report increased rates of tumor metastases in patients receiving perioperative blood transfusions.

The mechanism by which blood transfusion impairs NK cell function has not yet been determined. One possibility is the previous demonstration that blood transfusions decrease IL 2 production (18). IL 2 has been shown to be necessary for optimal NK cell activity (19). If this is found to be the case, concomitant administration of IL 2 with blood transfusions might block or correct the immunosuppressive transfusion effect. Other immunologic interventions that might be used at the time of transfusion to prevent posttransfusion immunosuppression include the use of the H_2 blocker, ranitidine (20), or cyclooxygenase inhibitors (21).

PRESENTATIONS/PUBLICATIONS

None.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION: DA311499	2. DATE OF SUMMARY: 1 Oct 89	REPORT CONTROL SYMBOL: DD-DR&E(AR) 636
3. DATE PREV SUM'RY: 1 Oct 88	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

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a. PRIMARY	61102A	3M161102BS14	IA	306
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP,	FY90-01		

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	9009	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)*
89	1.8	61
90	1.8	65

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 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) Thermal Injury; (U) Zinc Homeostasis; (U) Immunocompetence; (U) Lab Animals; (U) Rats; (U) RA II

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

23. (U) A DTIC literature search was conducted under DTIC request number W6L28F dated 20 October 1989 for the technical report database and request number W6L29E dated 20 October 1989 for the work unit database. The proposed effort will not result in the duplication of effort. The objective of this work is to 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.

24. (U) Studies will use the T cell and monocyte isolation procedure that has been developed in our laboratory over the past year. An *in vitro* bioassay will be used to determine the involvement of interleukin-1 in the model. The effect of burn injury and zinc nutriture on the secondary humoral response will be investigated. Vascular perfusion studies will be performed to determine the effect of burn injury on zinc absorption.

25. (U) 8810 - 8909. The effect of burn injury and zinc nutriture on the anamnestic response was investigated. The kinetics of the anamnestic response to sheep RBCs showed that burn injury did not affect the peak day response; both burned and nonburned rats gave a peak response on the third day following immunization. The response for burn-injured rats was significantly lower than for nonburned control rats. Preliminary data indicated that this response was further suppressed by placing burn-injured rats on a zinc-restricted regimen. Initial data using isolated lymphocytes and monocytes in crossover-type studies indicated that burn injury suppresses monocyte function and that this phenomena was aggravated by zinc restriction. For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1989.*

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1 October 1988 - 30 September 1989

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ABSTRACT

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

PERIOD COVERED IN THIS REPORT: 1 Oct 88 through 30 Sep 89

INVESTIGATORS: Ronald L. Shippee, PhD, Captain, MS
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Previous studies have shown that rats maintained on a zinc-restricted diet for 10 days postburn had a less plaque-forming cell (PFC) response to a single injection of sheep RBCs (SRBC) than burned rats maintained on an adequate zinc regimen. The present report shows that burn injury caused a decrease in PFCs to a booster injection of SRBC and that this response was further depressed when the rats were maintained on a zinc-restricted regimen. The data are consistent with the hypothesis that burn injury places an increased burden on zinc body stores as assessed by immunocompetence.

PRELIMINARY STUDIES ON ZINC HOMEOSTATIC CONTROL AND IMMUNOCOMPETENCE IN A BURNED ANIMAL MODEL

Past studies at this Institute have used a functional test of the humoral response, the Jerne plaque assay, to define interactions between zinc nutriture, burn injury, and immunological response. Our initial studies investigated the effect of a 30% total body surface area burn injury on the primary humoral response to sheep RBCs (SRBC) in the rat. The kinetics of the response revealed that both burned and sham-burned rats had a peak response on day 4 following SRBC injection (postburn day 10) and that the response of the burned animals was significantly higher than that of sham-burned animals ($P < 0.05$). The effect of zinc nutriture was determined by feeding a zinc-free diet (< 0.5 ppm) to all the rats and injecting either zinc (1 mg/kg/day SC) or saline for 10 days following burn injury. The results showed that zinc restriction caused a lower plaque-forming cell (PFC) response in burned animals, but that this response was still higher than for sham-burned, zinc-sufficient animals. Zinc restriction of sham-burned animals had no significant effect on the humoral response.

This report describes methods and results of studies concerning the effect of burn injury and zinc nutriture on the secondary response using the rat model. In addition to the studies concerned with the humoral response, we have worked on methods and techniques to study the effect of zinc nutriture and burn injury on monocyte function.

MATERIALS AND METHODS

Male Sprague-Dawley rats weighing 300-350 g were housed and fed as described previously (1). All animals used for these studies were given a primary immunization by injecting intravenously a 20% suspension of SRBC. One month after the primary injection, the animals were anesthetized and administered a 30% total body surface area (TBSA) scald burn. Seven days postburn, the animals were given a second intravenous injection of SRBC.

Thirty-six animals were used to determine the kinetics of the secondary response. Twelve received a 30% TBSA scald burn injury and 12 received a 30% TBSA sham burn. All animals were maintained on a 1 mg Zn/kg/day regimen. Animals were serially sacrificed on 1, 2, 3, and 4 days postimmunization (8, 9, 10, and 11 days postburn).

Twenty-four animals were used to determine the effect of zinc restriction on the secondary response. After administering the burn, the animals were randomly assigned to one of the following treatment groups:

CZS = No Burn, Zinc Supplementation (1 mg Zn/kg/day)

CZD = No Burn, No Zinc Supplementation

BZS = 30% TBSA Burn, Zinc Supplementation (1 mg Zn/kg/day)

BZD = 30% TBSA Burn, No Zinc Supplementation

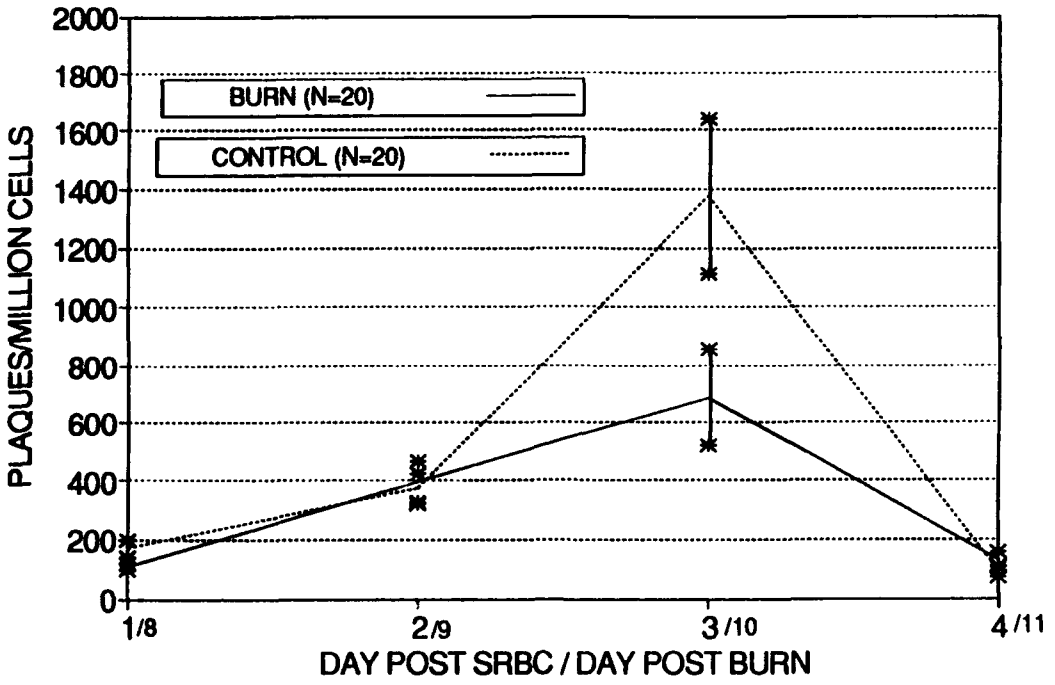
Seven days postburn, the animals were given a booster injection of SRBC. Three days after the injection, the animals were sacrificed. The spleens were excised and lymphocytes isolated by Ficoll-Paque centrifugation. Details concerning the Jerne plaque assay have been described previously (1). IgM class-secreting cells were determined by incubating agar plates containing SRBC and lymphocytes with guinea pig complement. Total PFCs were determined by incubating a duplicated set of plates with goat anti-rat antibody (Accurate Chemical and Scientific Corp, Westbury, NY) for 1 h prior to the complement step. IgG-secreting cells were determined by the difference. Plasma obtained at sacrifice was analyzed for zinc and copper concentration as described previously.

RESULTS

The kinetics of the secondary response are shown in Figure 1A. The kinetics of the primary response determined in earlier studies are presented in Figure 1B for comparison. As expected for a secondary response, the peak response was earlier than the primary response. Contrasted to the primary response, the peak response (day 3 postimmunization) of the burn group (62 ± 39 SEM) was less than the sham-burn group (169 ± 82 SEM). This difference was significant ($P < 0.05$, student's t test).

Table 1 shows the response of both groups for each class of Ig-secreting PFCs. On day 3 postimmunization, 47% of the mean total Ig-secreting PFCs of the sham-burn group were IgG-secreting cells. Statistical difference for IgM- and IgG-secreting PFCs between burn and sham-burn groups was analyzed using a t test. While the difference in IgM-secreting PFCs between burn (46 ± 34 SEM) and sham-burn groups (89 ± 50 SEM) was significant ($P = 0.039$), the difference in IgG-secreting PFCs was highly significant ($P < 0.01$), 19 ± 7 SEM and 80 ± 40 SEM for burn and control groups, respectively.

The results of the studies to determine the effect of zinc nutriture on the secondary response to SRBC are shown in Table 2 and Figure 2. Two rats had to be dropped from the study due to reasons not related to the experimental treatments. Burn injury and zinc deficiency caused a decrease in PFCs and, when both existed together, the depression of the humoral response was compounded.



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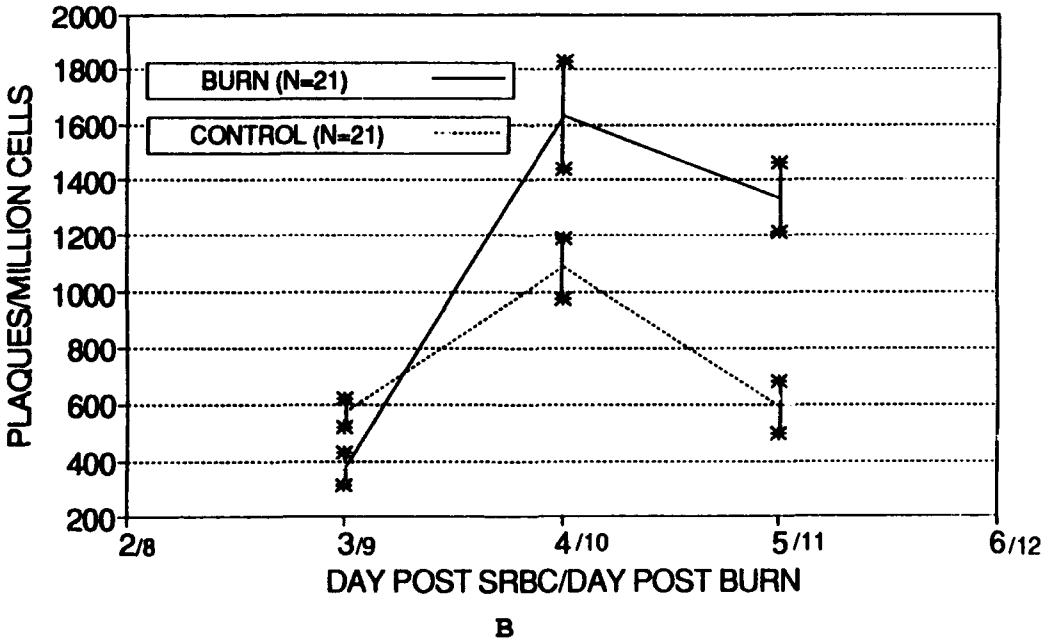

B

FIGURE 1. Kinetic response of SRBC for Groups BZS (burn) and CZS (control) animals. **A**, booster injection; **B**, single injection. Data are expressed as PFCs/1,000,000 lymphocytes.

TABLE 1. Kinetic Response to SRBC for IgM, IgG, and Total Plaques (PFCs/1,000,000 Lymphocytes)

Animal Number	Postimmunization Day 1						Postimmunization Day 2						Postimmunization Day 3						Postimmunization Day 4					
	Sham-Burn Group			Control Group			Sham-Burn Group			Control Group			Sham-Burn Group			Control Group			Sham-Burn Group			Control Group		
	IgM	IgG	Total	IgM	IgG	Total	IgM	IgG	Total	IgM	IgG	Total	IgM	IgG	Total	IgM	IgG	Total	IgM	IgG	Total	IgM	IgG	Total
1	-	25	25	4	2	6	18	2	20	41	13	54	162	161	323	7	22	15	5	8	13	12	2	14
2	-	21	21	-	10	10	8	14	22	38	37	75	142	50	192	12	20	8	1	-	2	5	11	16
3	-	24	24	-	11	11	30	18	48	19	1	20	97	70	167	36	13	49	5	-	5	7	15	22
4	-	17	17	-	-	7	18	26	44	25	18	43	87	122	209	31	30	61	7	-	7	5	-	5
5	2	9	11	2	10	12	6	39	45	29	4	25	50	40	90	81	29	110	9	4	13	1	-	2
6	1	14	15	2	6	8	19	6	25	20	17	37	34	54	88	88	12	100	10	5	15	3	8	11
7	2	9	11	7	5	12	13	8	21	40	25	65	152	106	258	10	11	21						
8	1	6	7	4	10	14	24	22	46	23	8	31	92	96	188	34	21	55						
9	-	25	25	2	6	8	13	22	35	25	6	31	42	47	89	85	20	105						
10	-	21	21	-	9	9	30	17	47	30	2	32	28	53	81	80	15	95						
Mean	1	17	18	2	7	10	18	17	35	29	13	41	89	80	169	46	19	62	6	3	9	6	6	12
SEM	1	7	6	2	4	3	8	11	12	8	11	18	50	40	82	34	7	39	3	3	9	4	6	7

TABLE 2. PFC Response for Sham-Burned and Control Group Animals With and Without Zinc Supplementation (PFCs/1,000,000 Lymphocytes)

Animal Number	Zinc-Sufficient						Zinc-Deficient					
	Sham-Burn Group			Control Group			Sham-Burn Group			Control Group		
	IgM	IgG	Total	IgM	IgG	Total	IgM	IgG	Total	IgM	IgG	Total
1	72	7	79	47	2	49	84	30	114	21	1	22
2	81	27	108	150	55	205	26	3	29	59	4	63
3	89	16	105	69	21	90	75	9	84	4	4	8
4	68	13	81	14	2	16	26	14	40	18	2	20
5	54	26	80				29	1	30			
Mean	71	18	89	72	20	91	46	11	57	22	3	25
SEM	5	3	6	22	10	32	11	4	14	10	1	10

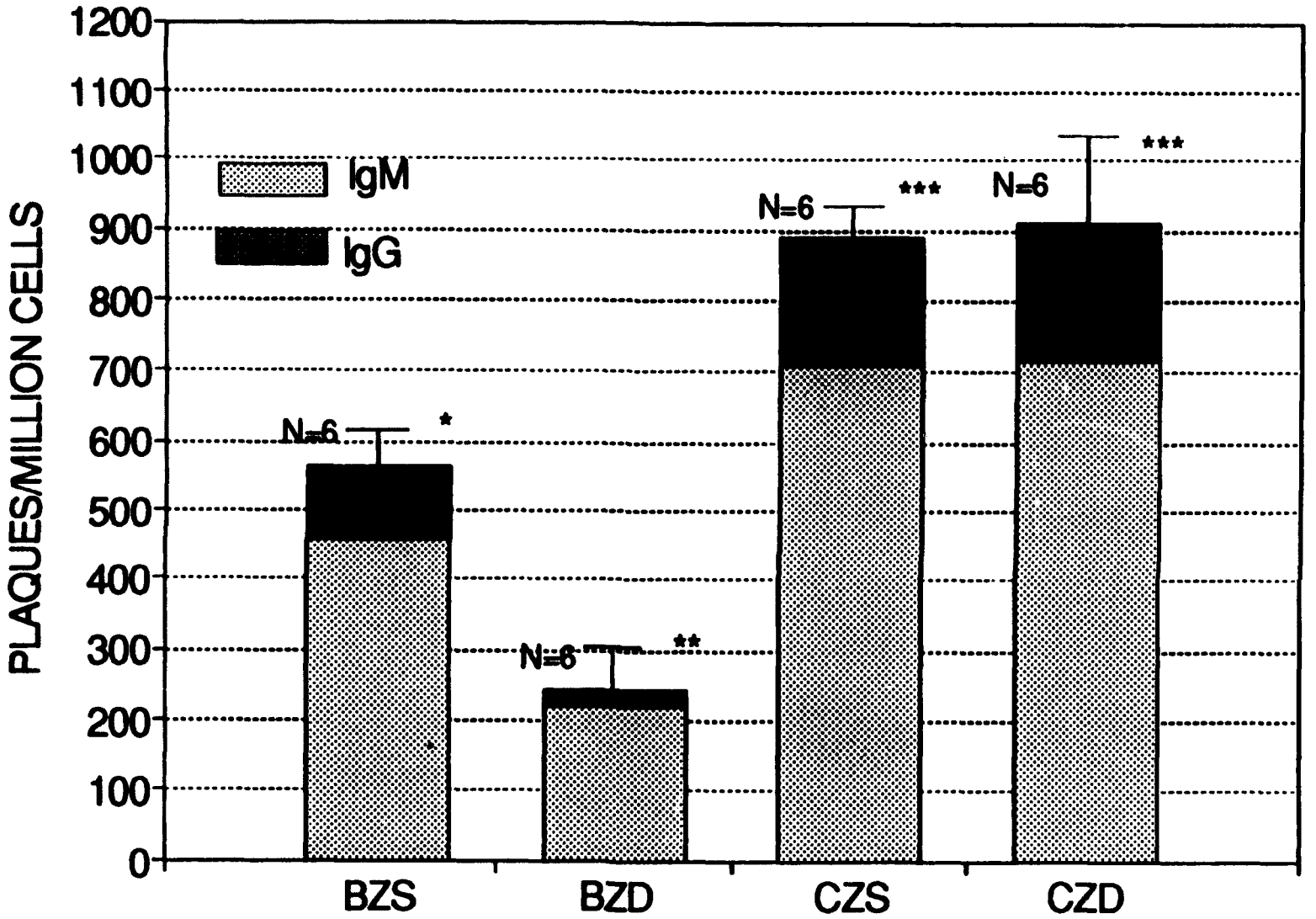

FIGURE 2. PFC response to a booster injection of SRBC on postimmunization day 4/postburn day 10.

Plasma zinc and copper concentrations are shown in Table 3. The zinc restriction regimen lowered the plasma zinc concentration in both sham burned and burned rats. Copper concentration was elevated in both burn groups compared to sham-burn animals, a finding consistent in all studies to date. This is presumably due to elevated levels of ceruloplasmin. No statistical analysis was attempted due to the low sample size. Two additional repetitions are planned.

DISCUSSION

Very little information exists in the literature concerning the use of animal models to study humoral responses during recovery from burn injury. The most referenced studies are those reported by Alexander and Moncrief (2-3) which measured antibody titers to primary and secondary immunization with human RBCs (HRBC) in a burned rat model similar to our model. The salient results from their studies are presented in Figures 3 through 9. Alexander and Moncrief concluded from their data that the degree of inhibition of the primary humoral response parallels the degree of thermal injury. Animals receiving the 20% TBSA burn showed only a slight inhibition of the response compared to the striking inhibition seen in the 30% TBSA burned animals. However, there was no explanation for the smaller dose of HRBC given to the animals receiving the 30% TBSA burn. The authors concluded that the primary response is somewhat dependent upon the temporal relationship at which the antigen is given following thermal injury. A progressive inhibition of the response was seen as the interval between burn injury and antigen administration increased. Contrasting the primary response to *Pseudomonas aeruginosa* to the response to HRBC in the burned rat model, Alexander and Moncrief determined that the nature of the antigen is important to humoral response.

The reduced anamnestic response to SRBC shown in this study and HRBC shown by Alexander and Moncrief (2) in rats administered a 30% TBSA burn is in contrast to the greater than normal response in burned humans given a booster injection of tetanus toxoid antigen (2). In contrast to the Alexander and Moncrief study (2), Wood et al (4) found persistent depression of the humoral responses to tetanus toxoid in the population of burned patients they studied.

Although our findings of a depressed anamnestic response to SRBC in the rat model is in agreement with Alexander and Moncrief (2), our results concerning the primary response differ dramatically. When comparing different studies investigating in vivo immunological responses, there are a number of variables that need to be considered. The particular lymphoid compartment assessed, type, dose, and route of antigen, and type of assay used to assess the response are important variables. Alexander and Moncrief tested peripheral blood with an agglutination assay while we tested splenic tissue with a functional test of lymphocyte activity. Calvano et al (5) reported marked differences in

TABLE 3. Plasma Zinc and Copper Concentrations in Burned and Sham-Burned Rats With and Without Zinc Supplementation (μg/ml)

Animal Number	Zinc				Copper			
	Group BZS	Group BZD	Group CZS	Group CZD	Group BZS	Group BZD	Group CZS	Group CZD
1	1.66	1.88	1.34	0.96	0.88	0.78	1.06	1.62
2	1.74	1.16	1.08	1.06	1.06	0.88	1.34	1.00
3	1.88	0.68	1.64	0.62	0.88	0.80	1.02	1.08
4	1.64	0.52	1.34	0.68	0.94	0.76	1.30	0.98
5	1.38	0.84	1.20	0.46	0.90	0.70	1.06	1.10
6	1.36		1.20		0.88		1.76	
Mean	1.61	1.02	1.34	0.76	0.92	0.78	1.34	1.18
SEM	0.08	0.24	0.08	0.11	0.03	0.03	0.08	0.12

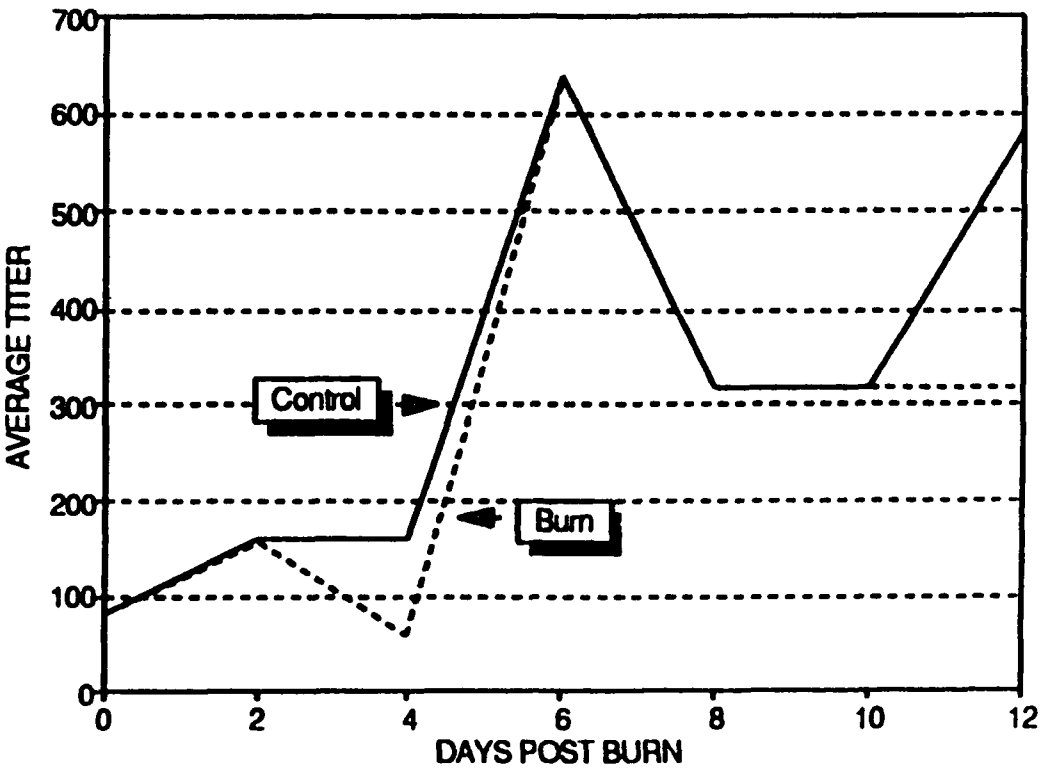

FIGURE 3. Average plasma agglutination titers for rats receiving a 20% TBSA burn and an intraperitoneal injection of 2% (in 0.5 ml vol) O+ HRBC 6 days prior to burn injury (2).

proliferation of lymphocytes to ConA stimulation when cells from splenic and draining lymph nodes were compared from rats receiving a 50% TBSA burn. While the burn injury caused an enhanced response in lymph node cells, splenic lymph nodes gave a depressed response.

We gave a large dose of SRBC (20% in 0.2 ml) as recommended (6). Alexander and Moncrief (2) used a dose of 2% in 5 ml for 20% TBSA burned rats and switched to a relatively small dose of 0.02% in the 32% burn model. We chose to use the intravenous route of injection as opposed to intraperitoneal because the intravenous route has produced less variability in plaque response. We chose to use SRBC as the antigen since this has been the standard used in the mouse model. The mouse response to SRBC shows the typical hallmarks of a primary and secondary response. The primary response is characterized by a relatively long period before the peak response (4 days) and the response is mainly in the form of IgM-secreting cells. The secondary response is characterized by a shorter peak response time (2-3 days), a greater magnitude of response, and a greater proportion of the response is in the form of IgG. Although the response in our model to a booster injection of SRBC is characterized by a higher and earlier peak response when compared to the primary, the response is predominately from the IgM

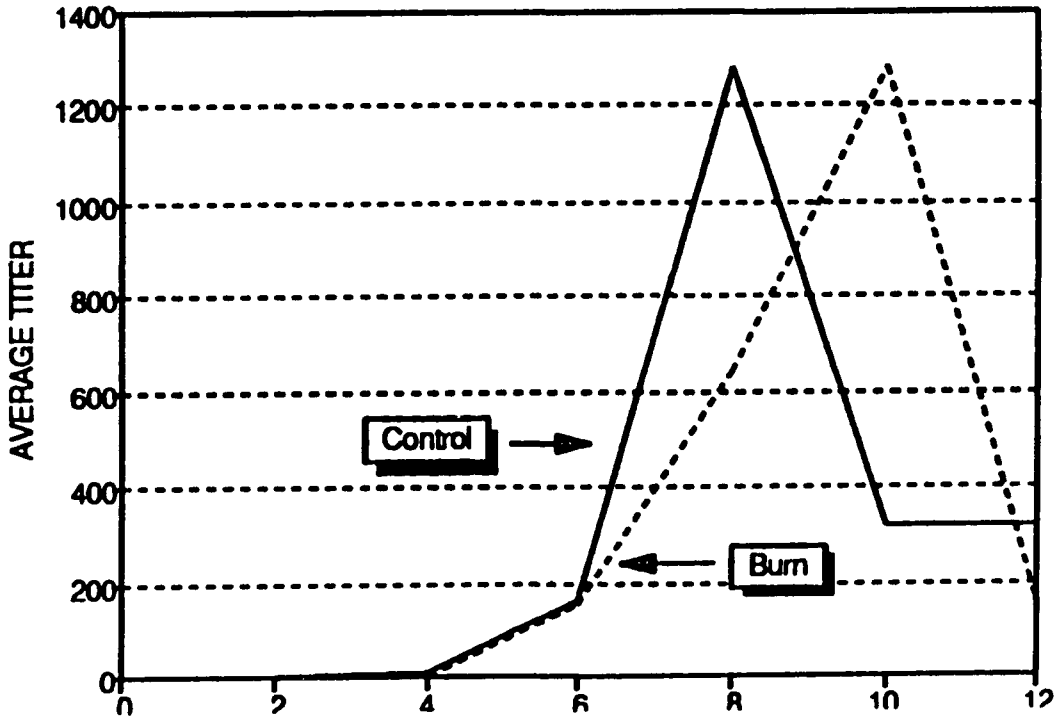

FIGURE 4. Average plasma agglutination titers for rats receiving a 20% TBSA burn and an intraperitoneal injection of 2% (in 0.5 ml vol) O+ HRBC 1 day postburn (2).

class of antibody. A possible explanation is that a single dose of SRBC causes a secondary response in the rat. If this were the case, then our increased response of the burned animals to a single injection would be in agreement with the results reported by Soderberg (7) which demonstrated an elevated secondary response to SRBC in a burned mouse model.

Additional studies will be necessary to fully characterize the humoral response in the burned rat model and to explain the discrepancies between reported studies. The burned rat model has been used by this Institute and other burn centers conducting basic research for a number of years. Although the mouse model would be the obvious model of choice based on immunological considerations, the rat model is the burn model of choice for a number of metabolic and physiological reasons.

An additional variable, one that is overlooked in most studies involving the effect of burn injury on immunocompetence, is the nutritional status of the subjects. In the last 15 yr, there has been a plethora of information relating host defense to all the individual essential nutrients. It is well documented that zinc nutriture can have profound effects on immunocompetence.

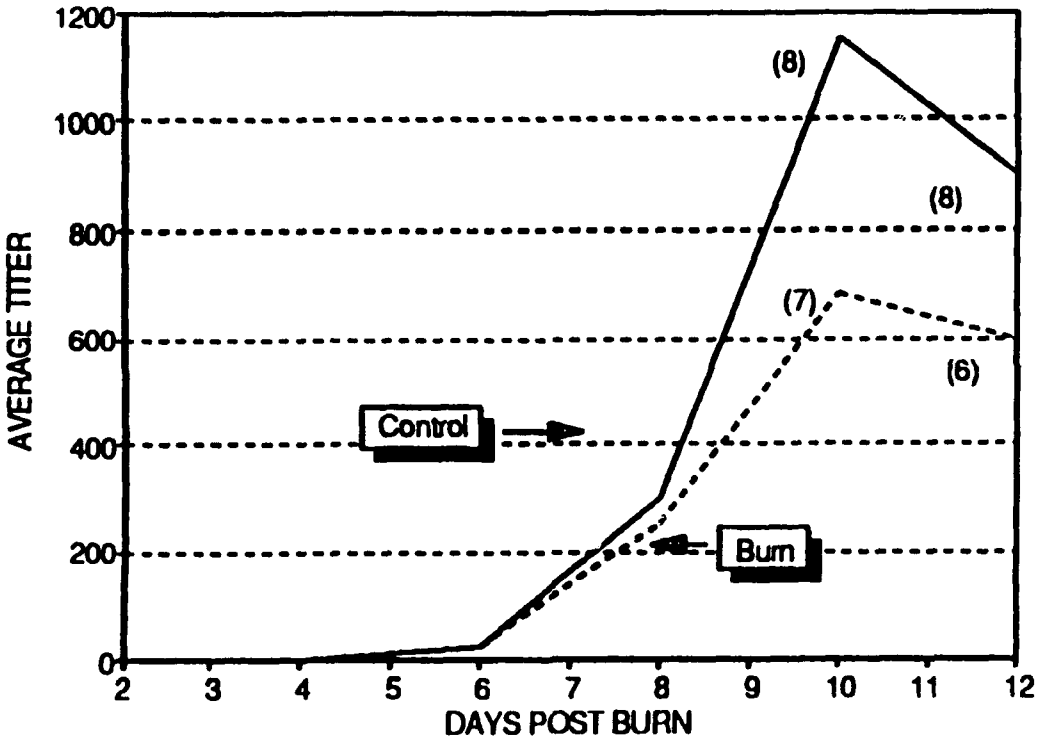

FIGURE 5. Average plasma agglutination titers for rats receiving a 32% TBSA burn and an intraperitoneal injection of 0.2% (in 0.5 ml vol) O+ HRBC 4 days postburn (2).

Zinc nutriture is of particular interest in relation to burn injury. Our earlier work (1) as well as work by Powanda (8) at this Institute have shown that dramatic alterations in zinc metabolism occur after a major burn injury in a rat model. Clinically, the issue of zinc nutriture is important based on the reliance on total parenteral nutrition in burn patients. This modality of nutritional supplementation has been shown to have a pervasive effect on zinc deficiency if supplementation and status are not monitored carefully.

The results of the present study as well as our previous reports support the hypothesis that an interrelationship between zinc nutriture and burn injury exists. We are careful in our rat model to insure that all animals have sufficient zinc body stores prior to administering the burn injury and starting the zinc restriction regimen. When evaluating studies reporting immunocompetence in burn patients, it is likely that a burn patient population used in a particular study are heterogenous with respect to nutritional status. We feel that our studies involving zinc nutriture indicate that some of the variably, both within and between studies, may be due to the nutritional status of the patients.

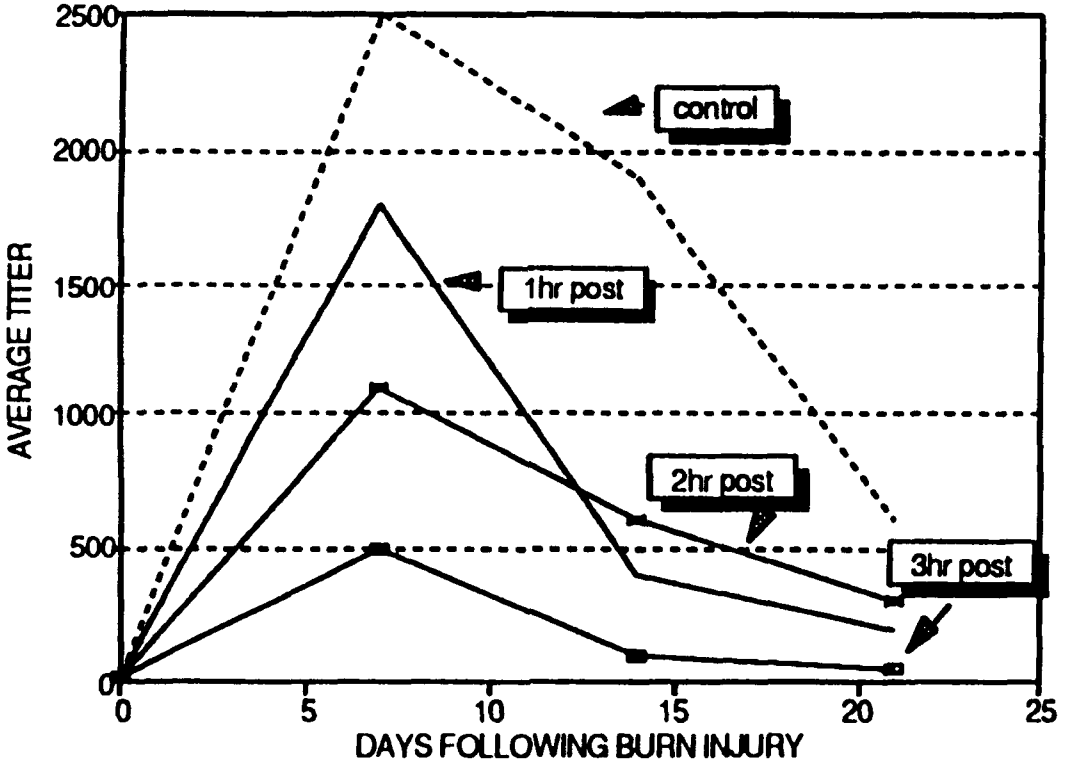

FIGURE 6. Average plasma agglutination titers for rats receiving a 30% TBSA burn and an intraperitoneal injection of 0.2% (in 0.5 ml vol) O+ HRBC 1, 2, and 3 h postburn (2).

PRESENTATIONS/PUBLICATIONS

None.

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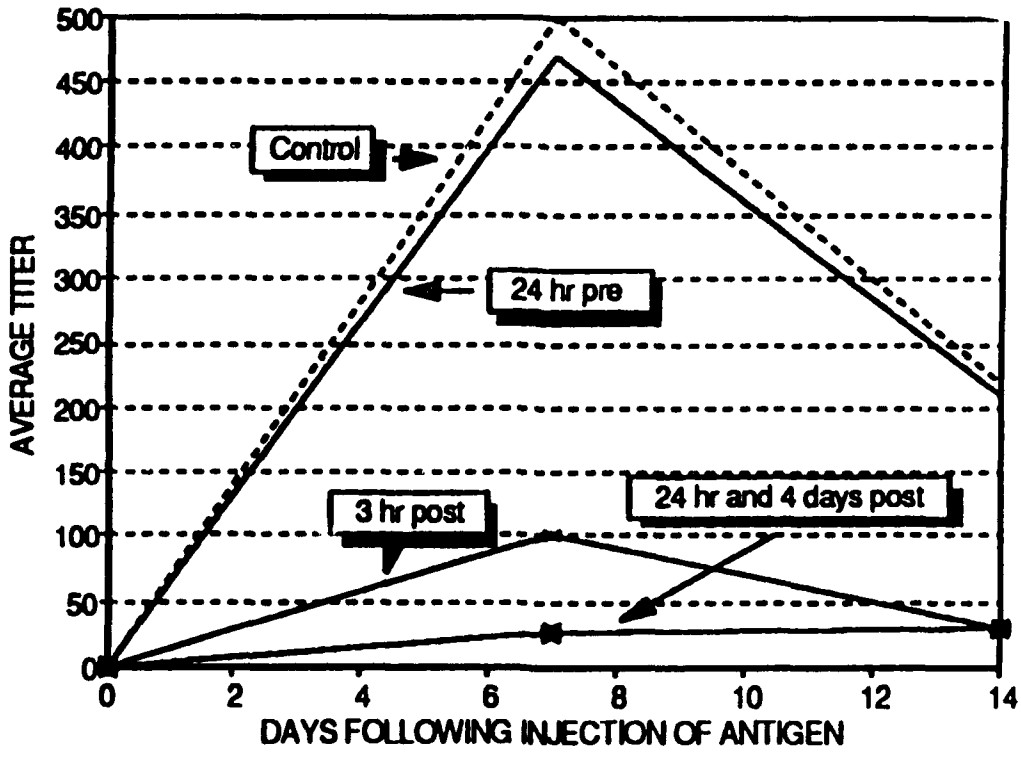

FIGURE 7. Average plasma agglutination titers for rats receiving a 30% TBSA burn and an intraperitoneal injection of 0.02% (in 0.5 ml vol) O+ HRBC 24 h prior to and 3 h, 24 h, and 4 days postburn (2).

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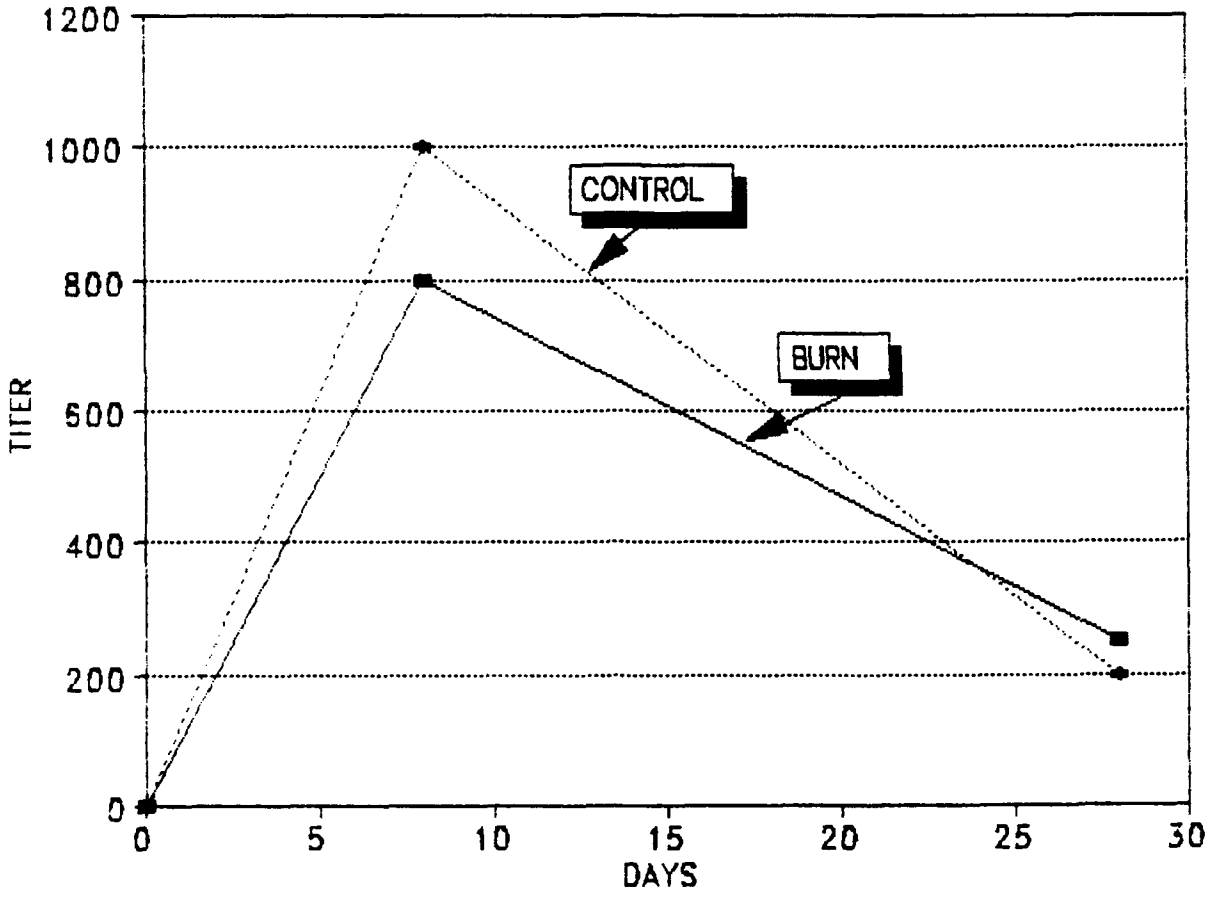

FIGURE 8. Average plasma agglutination titers for rats receiving a 30% TBSA burn and an intraperitoneal injection of partially purified antigen from *Pseudomonas aeruginosa* 48 h postburn (3).

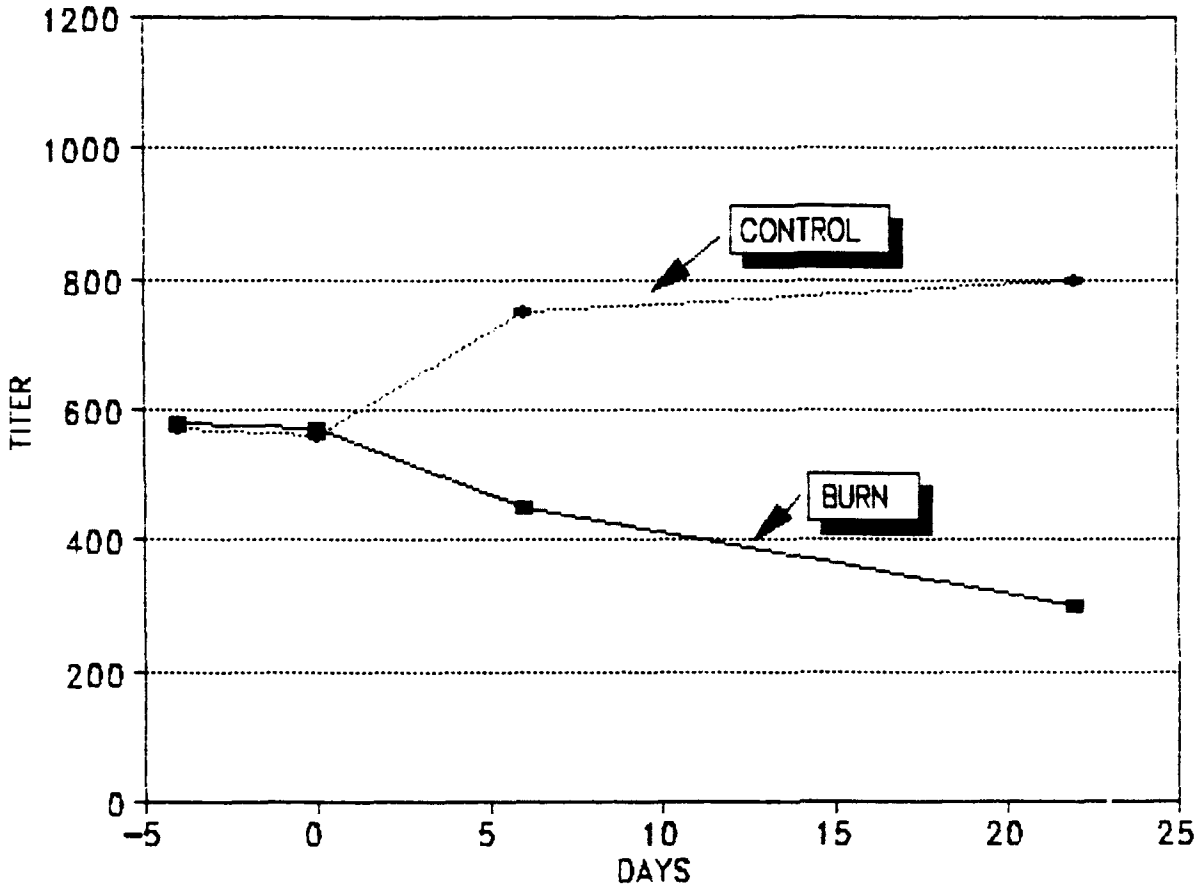

FIGURE 9. Average plasma agglutination titers for rats receiving a 30% TBSA burn and an intraperitoneal injection of 0.02% HRBC given 23 days prior to burn and booster injection given 48 h postburn (3).

ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3M161102BS14-00, BASIC RESEARCH

PROJECT TITLE: Preliminary Studies on Zinc Homeostatic Control and Immunocompetence in a Burned Animal Model: Zinc Nutriture, Burn Injury, and Monocyte Function

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

1 October 1988 - 30 September 1989

INVESTIGATORS

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

ABSTRACT

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We have been developing techniques to isolate and purify lymphocytes and monocytes from rat spleen tissue. Assays are being developed to use the purified cell types to determine which cell types are affected by the zinc restriction in our burned rat model.

PRELIMINARY STUDIES ON ZINC HOMEOSTATIC CONTROL AND IMMUNOCOMPETENCE IN A BURNED ANIMAL MODEL: ZINC NUTRITURE, BURN INJURY, AND MONOCYTE FUNCTION

Severe burns adversely affected both specific and nonspecific host defense mechanisms. A large amount of evidence exists demonstrating that thermal injury causes depressed neutrophil activity, aberrant changes in both the complement and coagulation-fibrinolytic systems, and alterations in specific immunocompetence. A common link between specific and nonspecific host defense mechanisms is their regulation and interaction with monocytes.

Miller et al (1) have performed extensive studies concerning the effect of burn injury on monocyte function in both severely burned patients and in a murine model. Using decreased plasminogen activator (PA) as an indicator of monocyte dysfunction, it was shown that decreased monocyte PA response was consistently seen in patients who developed excessive suppressor-T cell activity and who eventually succumbed to fatal sepsis.

A primary in vitro antibody-forming cell (AFC) assay was used to assess the effect of a 20% full-thickness burn in Balb/c mice. The AFC response was totally abrogated in burn-injured mice at approximately 5-7 days postinjury. A temporal relationship between early loss of PA function by murine monocytes and a later increase in suppressor-T cell activity was observed.

Given this relationship between burn injury and monocyte function and our interest in zinc metabolism, we found the results of James et al (10) of interest. Splenic T cells and monocytes (adherent cells) from zinc-deficient and pair-fed control mice were isolated, purified, and recombined in vitro to evaluate the contribution of each cell type to effect PHA-stimulated mitogenesis. The results revealed that the observed depression in T-cell proliferation in the zinc-deficient mice was indirect and due to a primary defect in the monocyte population.

We have been developing techniques to isolate and purify lymphocytes and monocytes from rat spleen tissue. Assays are being developed to use the purified cell types to determine which cell types are affected by the zinc restriction in our burned rat model.

MATERIALS AND METHODS

Male Sprague-Dawley rats weighing approximately 250-300 g were maintained on the purified diet and given a daily subcutaneous injection of zinc (1 mg/kg) for at least 2 weeks prior to being sacrificed. Spleens were excised under aseptic conditions, minced with stainless steel spatulas in HBSS, centrifuged at 800 g, and suspended in HBSS.

After trying a number of cell isolation and purification techniques, we settled on the scheme presented in Figure 1. Briefly, suspended splenocytes are pipetted onto a gradient containing 40%, 55%, and 60% Percoll™ and centrifuged at 500 g for 20 min. A staining procedure (#91, Sigma Diagnostics, St. Louis, MO) that indicates alpha-naphthyl acetate esterase activity was used to differentiate monocytes and lymphocytes using a light microscope. This enzyme is primarily in monocytes, macrophages, and histiocytes and is virtually absent in lymphocytes. Based on this staining technique, we determined that the cell layer at the 40/55% interface (Ma) contained more monocytes than unfractionated spleen cells while the 55/60% interface contained mostly lymphocytes (Ta). Viability of each fraction was determined to be > 89% based on trypan-blue exclusion.

An aliquot containing 2 X 10^7 viable cells from the Ma fraction was incubated with mouse anti-rat T cell monoclonal antibody (OX-19, 1:100 dilution) for 1 h at 37°C in 5% CO_2. After washing off the unbound monoclonal antibodies, the cell fraction was incubated with goat anti-mouse ferric-containing monoclonal antibody for 20 min at room temperature. The suspension was exposed to a magnetic field for 15 min. The enriched monocytes (Mb) suspension was pipetted from the tube and placed in HBSS and washed 2X by centrifugation. The cells from the 55/60% interface were eluted through a T-cell recovery column (Biotex Laboratories, Inc., Edmonton, Alberta, Canada) to obtain an enriched T-cell fraction (Tb).

In each study, an aliquot of splenocytes was placed on a 40%/60% Percoll™ gradient. The cells (unfractionated) at the 40/60% interface (MT) were pipetted and washed in HBSS. Slides were prepared from each enriched fraction and stained with the alpha-naphthyl acetate stain procedure. Aliquots from each fraction were labeled with florescent conjugated anti-T cell monoclonal antibodies (OX-19, W3/13). The percent positive cells were determined by flow cytometry.

Aliquots of the MT, Ma, Mb, Ta, and Tb fractions were pipetted into 96-well microtiter plates in all possible combinations. T cells and monocytes were plated at 2.5 X 10^5 and 2.5 X 10^4 cells/well, respectively. Each combination was run in 6 wells, 3 unstimulated with mitogen and 3 stimulated with 2 μg/ml ConA. Plates were incubated at 37°C in 5% CO_2. Plates were pulsed with 1 μCi of ^{3}H-thymidine after 48 h. Cells were harvested at 65 h. Radioactivity was determined by scintillation counting.

One study has been completed using the techniques above to assess the effect of burn injury on lymphocyte and monocyte function. Four Sprague-Dawley rats were maintained for a least 2 weeks on the zinc-adequate regimen. Two of the animals were administered a 30% total body surface (TBSA) burn and 2 were administered a 30% TBSA sham burn. All animals were continued on

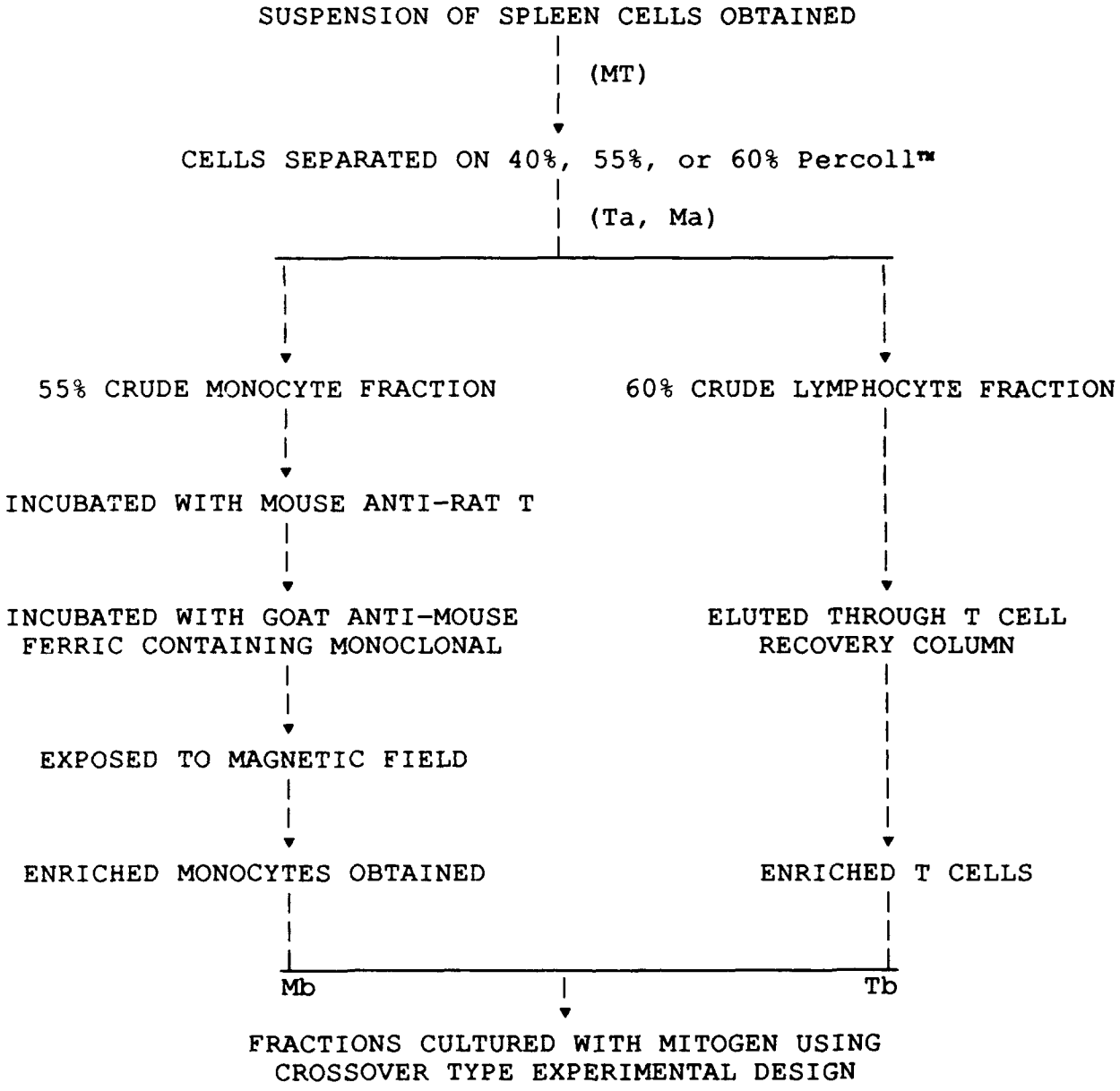

FIGURE 1. Spleen cell separation technique. MT indicates unfractionated mononuclear cells at the 40/60% interface; Ta, crude preparation of T cells at the 55/60% interface; Ma, crude preparation of Mo cells at the 40/55% interface; Tb, enriched T cells after eluting Ta fraction through a rat T-cell recovery column; Mb, enriched Mo cells after incubation of Ma fraction with anti-T-cell monoclonal and followed by incubation with anti-mouse ferric containing monoclonal and subsequent removal of contaminating T cells by exposure to a magnetic field.

the zinc-adequate regimen. Five days postburn, the animals were sacrificed and the spleen lymphocytes were processed as described above. Cells were pooled within treatments after the Percoll™ separation step. The enriched monocytes (Mb) and lymphocytes (Tb) from each treatment were plated in 96-well microtiter plates in all possible combinations. Cells were pulsed and harvested as described above.

RESULTS

The results of the proliferation assay for three separate repetitions of the separation technique are shown in Table 1 and graphically in Figure 2. The proliferative response of the Tb fraction was about half that of the unfractionated (MT) cells. The addition of the purified monocytes increased the proliferative response in the range of the MT cells. This cannot be attributed to just an increase in cell quantity because the Mb fraction allowance gave proliferative responses barely above background.

Results of the flow cytometry analysis of cells from the first repetition are shown in Figure 3. The percent positive for both T-cell marking monoclonals decreased in the monocyte enrichment fraction and increased in the T-cell enrichment fraction.

The effect of burn injury on proliferation is shown in Figure 4. Proliferation of both burn and control lymphocytes was depressed when co-cultured with monocytes from burned animals. However, the addition of control monocytes to burn lymphocytes supported proliferation almost equal to the control lymphocyte/monocyte combination.

DISCUSSION

This work is still in the preliminary stages. One persistent problem has been the large between-repetition variability. We feel that part of the problem has to do with the fact that the Sprague-Dawley rats are an outbred strain. We have started using in-bred, Lewis strain rats.

An additional problem has been the alpha-naphthyl acetate esterase-staining procedure. The differentiation between a lymphocyte and a monocyte is not always clear cut and considerable subjectivity arises. Although the staining procedure has allowed us to make a gross determination about which fractions from the Percoll™ separation contain more or less monocytes, we have not felt comfortable with the staining procedure. We have discussed the problem with the technical representatives at Sigma Corporation who have suggested that part of the problem may be that the procedure was developed for human samples. The suggestion was made that a procedure that stains for alpha-naphthyl butyrate esterase may work better on murine cells. We are in the process of evaluating this possibility.

TABLE 1. Stimulation Index Values from in vitro Culture of Various Fractionated Enriched Cells (cpm)

Repetition	MT	Ta	TaMa	TaMb	TaMc	Tb	TbMa	TbMb	TbMc	Ma	Mb	Mc
1	78411	36053	23993	42044	56499	60248	58980	79893	71659	149	350	550
2	124913	9344	12953	39862	23514	60856	109469	162909	155822	81	124	-
3	117487	26734	60783	69996	57613	41139	93155	107452	100469	2422	2165	391
Average	106937	24044	32576	50634	45875	54081	87201	116751	109317	884	880	314
SEM	20397	11069	20448	13720	15818	9155	21038	34523	34924	1088	914	231

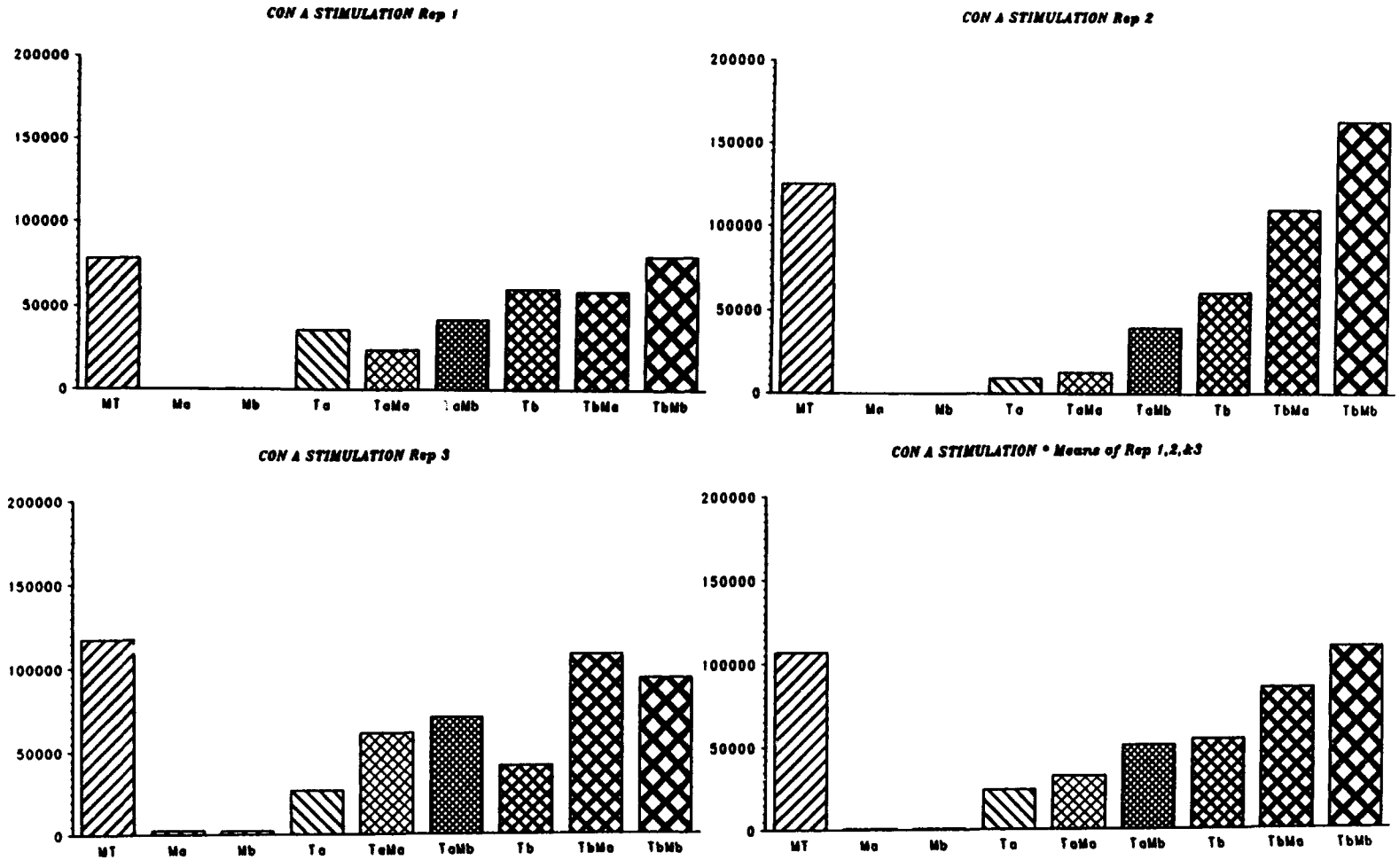

FIGURE 2. Results from a crossover study expressed as percent stimulation index (cpm of unstimulated cells ÷ cpm unstimulated cells) of in vivo co-culture of control Tb (CTb) with control Mb (CMb) stimulated with ConA.

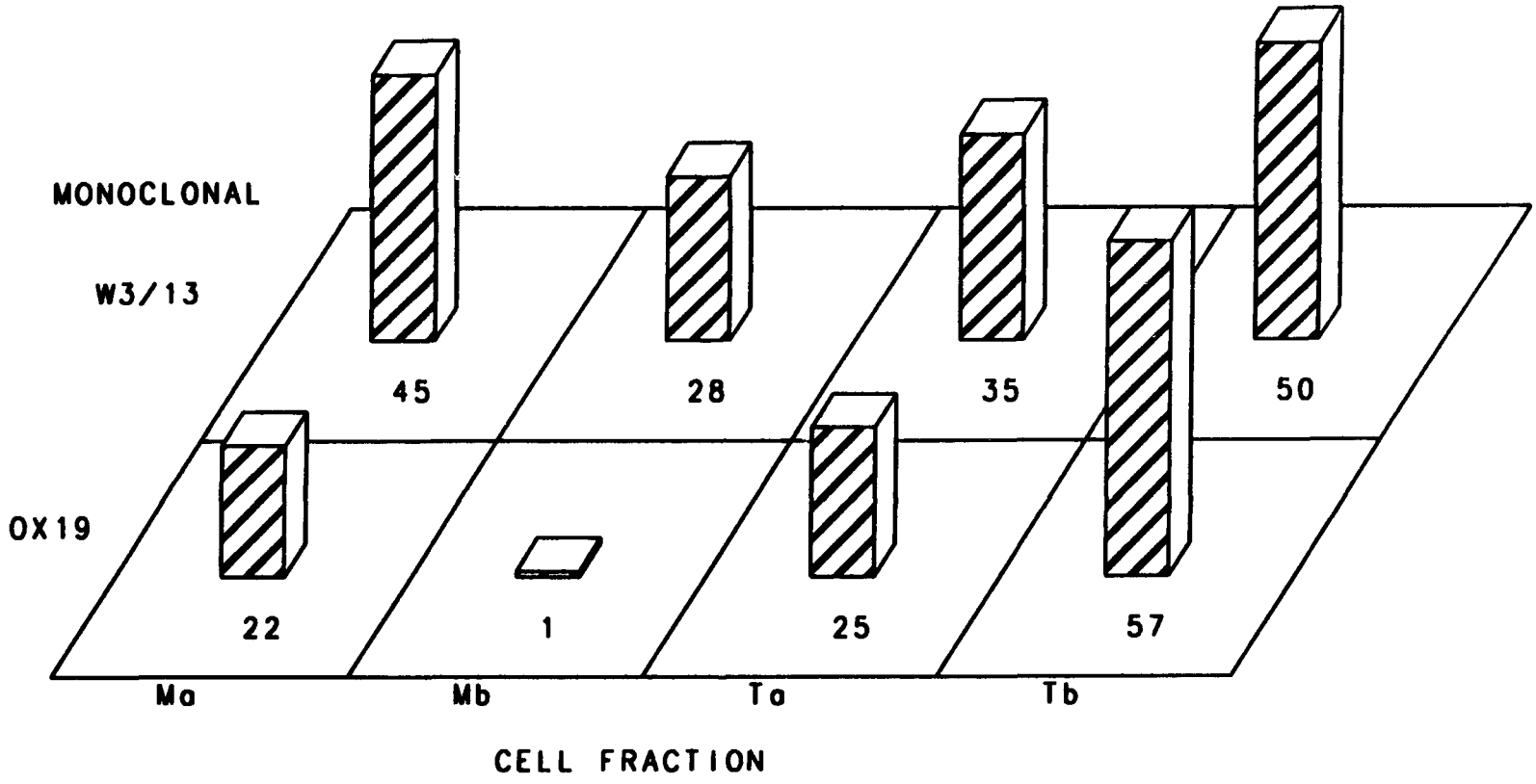

FIGURE 3. Results using monoclonal antibodies to determine enrichment. Data are presented as percent positives.

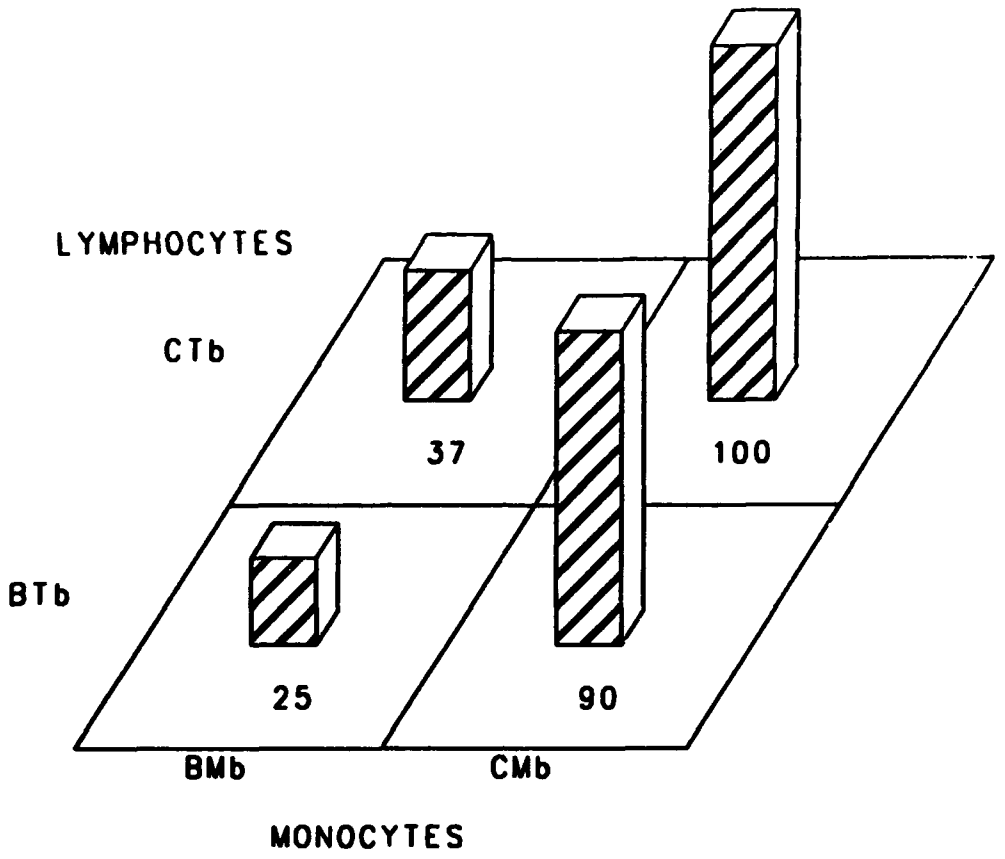

FIGURE 4. Results from a crossover study between cells separated from burned and control animals expressed as a percent of the control monocyte/T cell combination. Data are presented as percent of CTb/CMb combination.

One principal disadvantage of using a rat model as opposed to a mouse model is the lack of monoclonal antibodies available. Although the two T-cell monoclonals that we have been using (OX-19, W3/13), appear adequate for determining T-cell enrichment, we have been unable to find a reliable monoclonal for monocytes.

We feel that these problems can be solved and that this research direction will add valuable information concerning the interaction between nutritional supplementation, thermal injury, and host defense mechanisms.

PRESENTATIONS/PUBLICATIONS

Shippee RL, Burleson DG, and Mason AD Jr: Primary and secondary humoral response to sheep red blood cells in a burn rat model. Presented at the Annual Meeting of the Society for Leukocyte Biology, Marco Island, Florida, 15 October 1989.

Shippee RL and Wilson J: 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, 23 March 1989.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
				DA313320	88 11 07	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
88 10 01	K	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS14	IA	307
b. CONTRIBUTING			BD	
c. CONTRIBUTING	DA LRRDAP,	FY89-01		

11. TITLE (Precede with Security Classification Code) (U) Evaluation of Serum Visceral Protein Levels as Indicators of Nitrogen Balance in Thermally Injured Patients

12. SUBJECT AREAS
06 04 Anatomy and Physiology 06 05 Medicine and Medical Research

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
87 10	88 11	DA	C

17. CONTRACT/GRANT MILITARY RELEVANCY CERTIFIED APPROVED BY: David Trunkey

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)
88	0.5	30
89	0.1	1

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 78234-6200	b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-6200
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR: CARLSON, D
d. TELEPHONE NUMBER (Include area code): 512-221-2720	d. TELEPHONE NUMBER (Include area code): 512-221-6532
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):

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Nitrogen Balance; (U) Serum Visceral Protein; (U) Albumin; (U) Transferrin; (U) Retinol-Binding Protein;

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

22. (Continued) (U) Volunteers: (U) Adult; (U) RA II

23. (U) The purpose of this study was to assess whether circulating levels of albumin, prealbumin, transferrin, or retinol-binding protein could be used as indicators of nitrogen balance in burned patients. A literature was performed and indicated no duplication of effort.

24. (U) Baseline serum visceral protein levels were measured on postburn day 5 following stabilization of the patient's fluid status. Serum levels were repeated every 3 days until postburn day 30. Changes from baseline level were correlated with nitrogen balance.

25. (U) 8809 - 8811. Data collection was completed on 10 patients enrolled in the study. Results indicated that serum protein concentrations were not useful in predicting nitrogen balance in patients with burns.

ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3M161102BS14-00, BASIC RESEARCH

PROJECT TITLE: Evaluation of Serum Visceral Protein Levels as Indicators of Nitrogen Balance in Thermally Injured Patients

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

1 October 1988 - 7 November 1988

INVESTIGATORS

Dawn E. Carlson, RD, Major, SP
William G. Cioffi, Jr., MD, Major, MC
William F. McManus, MD, Colonel, MC
Basil A. Pruitt, Jr., MD, Colonel, MC

ABSTRACT

PROJECT NUMBER: 3M161102BS14-00, BASIC RESEARCH

PROJECT TITLE: Evaluation of Serum Visceral Protein Levels as Indicators of Nitrogen Balance in Thermally Injured Patients

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

PERIOD COVERED IN THIS REPORT: 1 Oct 88 through 7 Nov 88

INVESTIGATORS: Dawn E. Carlson, RD, Major, SP
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Basil A. Pruitt, Jr., MD, Colonel, MC

The use of serum visceral protein concentrations as predictors of nitrogen balance was assessed during the first 4 weeks following thermal injury. The correlation between nitrogen balance and serum albumin was not significant. Statistically significant correlations were found between nitrogen balance and serum prealbumin, retinol-binding protein, and transferrin. However, even the best correlation (retinol-binding protein, $r = 0.388$) was too weak to permit prediction of nitrogen balance on the basis of the visceral protein concentration. The correlation between change in direction of nitrogen balance and change in direction of protein concentration over time showed all four visceral proteins to be poor predictors of change in nitrogen balance. The efficiency was $< 50\%$ for each visceral protein. Stepwise multiple regression analysis performed to determine which indices were most closely correlated with nitrogen balance showed that a calculation using readily available information (nitrogen intake, postburn day, percent total body surface area burned, and age) provided better prediction of nitrogen balance ($4 = 0.765$) than any of the visceral protein concentrations. In view of these findings, measurement of serum visceral protein concentrations to monitor adequacy of nutritional support seems an unwarranted expense in patients with thermal injury.

EVALUATION OF SERUM VISCERAL PROTEIN LEVELS AS INDICATORS OF NITROGEN BALANCE IN THERMALLY INJURED PATIENTS

The importance of providing adequate calories and protein for healing of serious burn injury is well recognized. An optimal method to monitor the adequacy of intake, however, remains to be defined. Measurement of nitrogen balance is an accepted method of determining the efficacy of a nutritional regimen, but accurate determination of nitrogen intake and output of nitrogen is labor-intensive and impractical for general clinical use.

Several researchers have suggested that measures of serum visceral protein levels can be used to predict nitrogen balance and that this method is useful in assessing the efficacy of nutritional support programs. Starker et al (1) demonstrated an association between decreased serum albumin levels and negative nitrogen balance. Comparing changes in measures of serum albumin, prealbumin, transferrin, and retinol-binding protein with changes in nitrogen balance, Church and Hill (2) that increases and decreases in prealbumin levels were correlated with positive and negative changes in nitrogen balance in general surgical patients. Tuten et al (3) reported positive correlations between change in nitrogen balance and change in both prealbumin and transferrin levels in patients receiving parenteral or enteral nutrition. Both Fletcher et al (4) and Smale et al (5) reported that changes in serum transferrin concentrations correlated with changes in nitrogen balance.

In view of these reports, we felt serial measurements of these serum visceral proteins might also provide a method of predicting nitrogen balance in thermally injured patients.

The purpose of this study was to determine whether changes in serum levels of albumin, prealbumin, transferrin, or retinol-binding protein are correlated with changes in nitrogen balance in patients with thermal injury.

MATERIALS AND METHODS

Number of Patients. Nitrogen balance and serum protein levels were measured in 10 adult patients with thermal injuries involving at least 20% of the total body surface area, admitted to the US Army Institute of Surgical Research between November 1987 and March 1988.

Inclusion Criteria. Patients meeting all of the following criteria were eligible for enrollment in this study:

1. Patients hospitalized for burn injuries comprising > 20% of the total body surface area.

2. Male or female patients > 18 yr of age. Female patients were previously surgically sterilized, were postmenopausal (> 45 yr of age and the lack of menstrual periods for > 1 yr), or had a negative pregnancy test.

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

1. Outpatients.

2. Patients with contact electrical or chemical burns.

3. Patients who were pregnant.

Study Design. An assessment of nutritional status was performed for each patient upon entry into the study. This assessment included preburn height/weight, history of any recent weight changes, history of any drug/alcohol abuse, evaluation of the patient's preburn nutrition, and history of any medical diseases. Data collection was initiated on postburn day 5, after stabilization of fluid status, and then repeated every 3 days until postburn day 30.

Dietary Regimens. Daily energy requirements were estimated for studied patients by indirect calorimetry or by using a formula derived at this Institute based on earlier indirect calorimetry data. Protein requirements were calculated to provide a kilocalorie to nitrogen ratio of 150:1. Patients were provided regimens designed to meet these goals using oral diets, enteral feedings via nasoenteric tube, total parenteral nutrition, or combinations of these feedings modes. Daily calorie counts were calculated to determine the percentage of the requirement met each day.

Serum Visceral Proteins. Serum samples were analyzed for albumin, transferrin, prealbumin, and retinol-binding protein. Serum albumin assays were performed on an SMA autoanalyzer (Chemistry Autoanalyzer Instrumental Laboratory, Lexington, MA). Serum transferrin, prealbumin, and retinol-binding protein were measured using radioimmunodiffusion techniques.

Nitrogen Balance. Nitrogen intakes for each 24-h period were calculated from records of the parenteral and enteral nitrogen administered. Oral diet data were obtained by weighing quantities of foods served to and rejected by patients. Food composition data were obtained from the United States Department of Agriculture data or from manufacturers' information for commercial products.

Twenty-four-hour urine samples were collected and aliquots were analyzed for total nitrogen using a digital nitrogen detector (Antek Instruments, Inc., Houston, TX). Nitrogen loss across the burn wound was estimated using the formula of Waxman et al (6) with

a correction factor for the effect of silver sulfadiazine. The percent of remaining open wound was calculated daily. The formula used was as follows:

$$g\ \text{nitrogen} = 0.1 \times \%\ \text{TBSA} \times \%\ \text{TBSA Burn} \times 0.8$$

where TBSA = total body surface area. An estimate of 2 g/day was used for fecal loss of nitrogen. Nitrogen balances were calculated by subtracting nitrogen output (urine + wound + 2) from nitrogen intake.

Statistical Analysis. Regression analysis was used to evaluate the relationship between serum visceral protein concentrations and nitrogen balance.

Changes in nitrogen balance across time were determined for each subject. Changes in each serum visceral protein concentration were calculated for each subject at corresponding time points. The directions and magnitudes of these changes were determined. A change of at least 10% was considered a significant difference and was recorded as either a positive or negative change. Changes of < 10% were recorded as no change. A change of nitrogen balance and a serum visceral protein concentration in the same direction at the same time point was considered a correct prediction. The number of correctly predicted positive and negative nitrogen balance changes were determined for each subject for each serum visceral protein. An overall efficiency for each serum protein was determined as the number of correctly predicted nitrogen balance changes divided by total nitrogen balance changes multiplied by 100.

RESULTS

Ten patients (9 males, 1 female) were enrolled in this study (Table 1). The age of these patients ranged from 20–62 yr and burn size from 20–69.5% of the total body surface area. Eight of the 10 patients had at least one operative procedure performed during the study period and half of the patients had more than one such procedure. Nine of the 10 patients were transfused at least 1 unit of blood during the study period and 7 of these patients received multiple transfusions.

The study population was purposed varied to assess the applicability of this method of predicting nitrogen balance in the usual clinical situation. Detailed information regarding the patient population is presented in Table 2.

Nitrogen intake and balance for postburn days (PBD) 5, 11, 20, and 29 are tabulated in Table 3. Nitrogen balance was initially negative in all but one subject. As a general trend, nitrogen balance became more positive with time, but individual variation was seen. For example, patient 9 had a more negative balance on PBD 20 than on PBD 11 because his nutritional regimen was

TABLE 1. Patient Characteristics and Clinical Data

Patient Number	Age (Yr)	Sex	Body Weight (kg)	Burn Size Total	Burn Size 3°	Surgical Procedures	Transfusions	Dietary Regimen	Estimated Requirement (kcal/d)	% of Energy Requirements Met PBD 11	PBD 20	PBD29
1	54	M	118	27	-	-	5	PO	2575	55	57	57
2	22	M	65	26	1	1	1	PO	3349	135	99	104
3	59	M	56	36	30	1	6	TPN/TF	3257	85	89	-
4	31	F	60	57	41	4	5	TF/TF + PO	2850	92	102	97
5	57	M	66	34	18	1	1	PO	3623	79	69	76
6	62	M	61	34	24	2	6	TF	3397	76	92	73
7	29	M	102	70	11	2	7	PO/TF/TPN	5640	72	73	76
8	25	M	119	28	5	-	-	PO	4099	89	103	97
9	20	M	80	49	49	5	15	TF/TPN/TF	4378	67	75	72
10	25	M	68	29	9	2	5	TF/PO	3856	103	100	92

PBD indicates postburn day; PO, oral diet; TPN, total parenteral nutrition; and TF, tube feeding.

TABLE 2. Patient Population Characteristics

Age	38.4 ± 17.2 yr (range 20–60 yr)
Burn size	38.8% ± 14.7% (range 20–69.5%)
Assessment of medical conditions:	
Healthy prior to injury - Patients 2, 4, 5, 7, 8, 9, 10	
Complicating conditions - Patients 1, 3, 6	
Adequacy of dietary intake:	
Met > 80% of estimated requirement - 6 patients	
Met > 70% of estimated requirement - 3 patients	
Met < 70% of estimated requirement - 1 patient	

TABLE 3. Nitrogen Balance Data

Patient Number	Intake/Balance (g)*			
	PBD 5	PBD 11	PBD 20	PBD 29
1	9.1/-13.0	12.3/- 4.3	7.5/- 3.5	8.2/+ 0.8
2	13.9/+ 1.8	26.4/+ 3.6	20.2/- 2.1	18.6/- 9.6
3	3.4/-18.9	17.4/+ 0.7	15.8/- 3.5	-
4	3.0/-29.5	19.2/- 7.6	15.0/- 6.4	13.0/- 3.0
5	0.0/-16.0	17.4/- 8.1	12.3/+ 1.2	15.4/+ 3.8
6	0.0/-17.1	3.0/- 7.8	15.8/-11.3	14.2/ -
7	18.7/-29.9	20.0/-18.9	25.9/+ 7.9	32.6/+16.1
8	13.6/-15.3	15.5/-10.5	24.8/+ 5.3	21.9/+ 9.8
9	4.5/-22.6	19.8/-17.2	0.0/-20.3	20.0/+ 9.4
10	6.1/-13.5	23.8/+ 4.9	23.4/-10.1	18.7/+ 2.2

interrupted for surgery. Seven of the patients demonstrated neutral or positive balance by the end of the study period.

Regression data for each serum visceral protein versus nitrogen balance are presented in Figure 1. Serum transferrin, retinol-binding protein, and prealbumin showed statistically significant positive correlations with nitrogen balance, whereas serum albumin did not. Retinol-binding protein showed the best correlation of the four proteins measured ($r = 0.388$), but even retinol-binding protein would account for only 15% of the observed variation in nitrogen balance and did not correlate at a level that could be used for clinical prediction of nitrogen balance.

Stepwise multiple regression analysis was performed to determine what parameters could best predict nitrogen balance. Variables tested included age, percentage of the total body surface area burned, postburn day, nitrogen intake, and serum albumin, transferrin, retinol-binding protein, and prealbumin concentration. Nitrogen intake, postburn day, percent total body surface area burned, and age were identified as the variables that most closely correlated with nitrogen balance. None of the serum proteins entered this regression equation. The data plot (fig 2) showing the regression analysis of observed nitrogen balance on a predictor developed using nitrogen intake, postburn day, percent total body surface area burned, and age demonstrated a better correlation ($r = 0.765$) for this predictor than for the best serum protein correlation ($r = 0.388$). Even so, the calculated predictor explains less than 60% of the observed variation in nitrogen balance and is too insensitive for clinical use.

The analyses of the relationship between change in nitrogen balance and change in serum albumin, prealbumin, retinol-binding protein, and transferrin showed all four serum visceral proteins to be poor indicators of changes in nitrogen balance. Nitrogen balance changes in the positive direction were more accurately predicted than those in the negative direction (Table 4). Changes in serum retinol-binding protein, prealbumin, and transferrin had a better correlation than serum albumin. However, even retinol-binding protein, which had the highest number of correct predictions, corresponded to positive nitrogen balance changes in only 57% of these cases. The percentages of correct positive balance change predictions for the other proteins were 55% for prealbumin, 51% for transferrin, and 23% for albumin. Prediction of nitrogen balance changes in the negative direction was worse. Percentages of correct positive balance changes were 30% for prealbumin, 29% for transferrin, 24% for retinol-binding protein, and 5% for albumin. Thus, the overall efficiencies were less than 50% for each of the four visceral proteins.

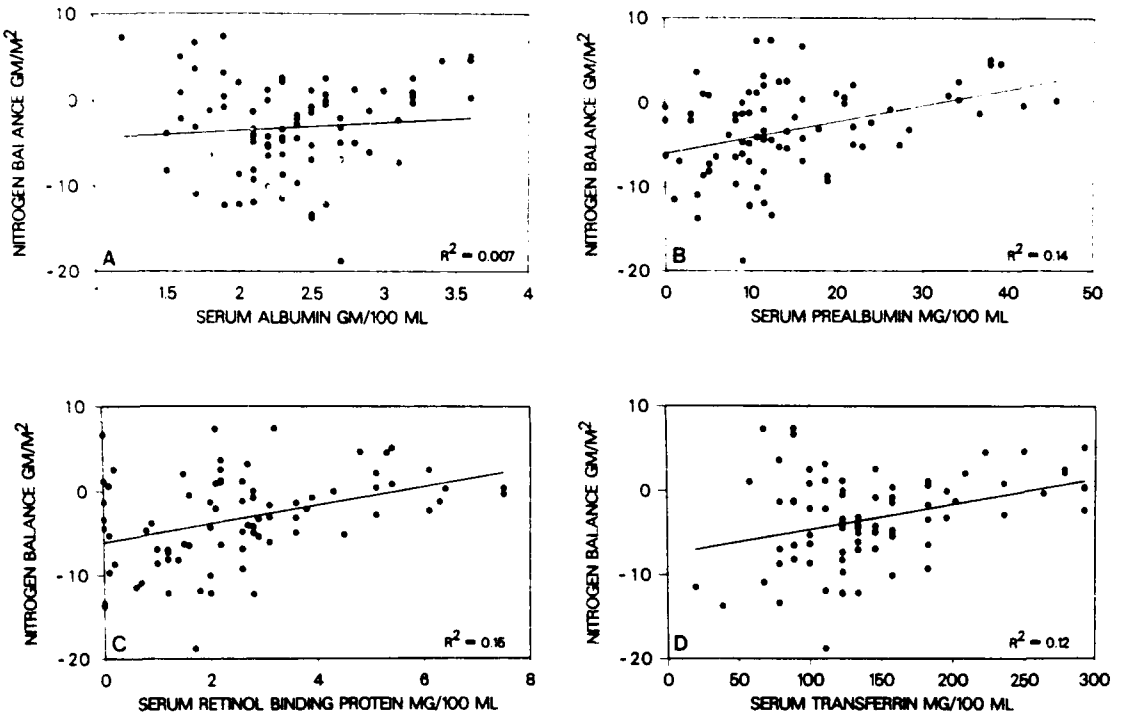

FIGURE 1. Nitrogen balance versus serum visceral protein concentrations. Correlations between nitrogen balance and serum prealbumin, retinol-binding protein, and transferrin were statistically significant, but the large statistical variances demonstrated in these scatter plots preclude prediction of nitrogen balance from measures of any of these proteins.

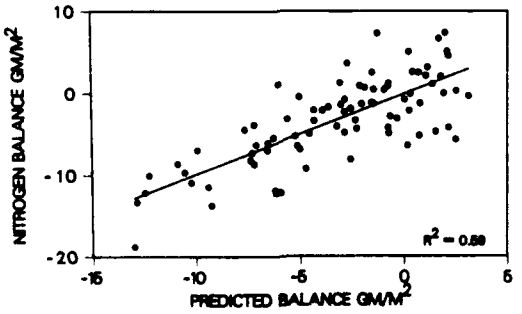

FIGURE 2. Nitrogen balance versus nitrogen balance predictor based on nitrogen intake, postburn day, percent total body surface area burned, and age. A factor using readily available variables selected in stepwise multiple regression analysis as most closely correlated with nitrogen balance was used to predict nitrogen balance. The correlation of this predictor with measured nitrogen balance was better than that for any of the serum visceral proteins.

TABLE 4. Prediction of Nitrogen Balance Using Correlation of Changes in Nitrogen Balance with Changes in Serum Visceral Protein Concentrations

Serum Visceral Protein	Correct Predictions*		Efficiency (%)**
	+ Balance	- Balance	
Albumin	10/44	1/21	17
Prealbumin	23/42	6/20	47
Retinol-Binding Protein	24/42	5/21	46
Transferrin	22/43	6/21	44

*Correct prediction = change of serum visceral protein in same direction as nitrogen balance/total number of nitrogen balance changes. **Efficiency = correct prediction/total nitrogen balance changes X 100.

DISCUSSION

To use serum protein levels as a basis for nutritional monitoring in patients with thermal injury, one must recognize deviations which occur in these measurements secondary to thermal injury. Serum albumin levels are decreased following burn injury due to increased loss of albumin into the burn wound, although total albumin synthesis rates are increased (7). Additionally, serum albumin levels may be affected by treatment measures or by complications which may occur. For example, exogenous infusion of albumin may artificially raise the serum albumin concentration. Serum transferrin is decreased following burn injury and blood loss or blood transfusion may further alter this serum level (8). Decreases in prealbumin and retinol-binding protein have been described following injury (9). Other proteins such as serum IgG are lowest within 48 h following thermal injury and subsequently rise to normal and supranormal levels over a 2- to 4-week period.

Our results demonstrate that none of the serum visceral proteins that we measured can be used to predict nitrogen balance in patients with thermal injury. This agrees with Starker et al (1) who found that serum albumin did not predict nitrogen balance changes during depletion and repletion with total parenteral nutrition, and with McCauley and Brennan (12) who found no change in serum albumin levels in cancer patients receiving total parenteral nutrition. In view of the long half-life of serum albumin, these changes are not surprising, We had expected that the shorter half-lives of prealbumin, transferrin, and retinol-binding protein might make them better indicators of nitrogen balance in this population. Although we did find

statistically significant correlations between nitrogen balance and each of these protein concentrations, their large statistical variances preclude using these measures as proxies for nitrogen balance. The wide scatter of individual points did not even allow reliable prediction of whether an individual patient's nitrogen balance was positive or negative. These results are consistent with those of Shenkin et al (9) who found no difference in serum visceral protein levels in trauma patients receiving supplemental amino acids compared to those not receiving amino acids.

The attempt to correlate change in direction of nitrogen balance with change in direction of the serum visceral protein concentrations was not successful. Fewer than one-half of positive and negative changes in nitrogen balance were accurately predicted by corresponding changes in any of the serum visceral protein concentrations.

Our results show that the serum visceral proteins, which are expensive to measure, are less effective than other readily available predictors of nitrogen balance. Prices quoted by commercial laboratories for performing serum visceral protein assays ranged from $26 to $60 per test for albumin, $26 to $67 for retinol-binding protein, and $17.50 to $69 for transferrin. Monitoring serum visceral protein levels on a weekly basis would be expensive For example, monitoring prealbumin and transferrin once a week would cost approximately $80/week/patient, using the average cost based upon quotes from five laboratories. In view of the poor predictive value of these measures, this expense does not seem warranted. Moreover, a calculated "predictor" based on use of nitrogen intake, percent total body surface area burned, postburn day, and age predicted nitrogen balance between than did serum albumin, prealbumin, transferrin, or retinol-binding protein concentrations.

Based upon the results of this study as well as a review of the current literature, the use of serum visceral protein concentrations as indicators or predictors of nitrogen balance in thermally injured patients is unwarranted and expensive.

PRESENTATIONS/PUBLICATIONS

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

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

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION DA318368	2. DATE OF SUMMARY 1 Oct 89	REPORT CONTROL SYMBOL DD-DR&E(AR) 636
3. DATE PREV SUM'RY 13 Jun 89	4. KIND OF SUMMARY D. Chg	5. SUMMARY SCTY U	6. WORK SECURITY U	7. REGRADING	8. DISB'N INSTR'N CX	9. LEVEL OF SUM A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS14	DA	307
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP	FY90-01		

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	9009	DA	C. In-House

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

a. DATE EFFECTIVE	d. RESOURCES ESTIMATE		
b. CONTRACT/GRANT NUMBER	a. FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (*In thousands*)
c. TYPE / d. AMOUNT	89	0.5	20
e. KIND OF AWARD / f. CUM/TOTAL	90	0.5	25

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	f. 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) Interleukin-1; (U) Burns; (U) Assaying; (U) Immunosuppression; (U) Cytokine; (U) Lab Animals: (U) Rats;

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

22. (Continued) (U) Volunteers: (U) Adults; (U) RA II

23. (U) A DTIC literature search was conducted under DTIC request number W6L37A dated 20 October 1989 for the technical report database and request number W6L37E dated 20 October 1989 for the work unit database. The proposed effort will not result in the duplication of effort. The objective of this work is to delineate the role of IL-1, IL-6, and TNF in burn patients, with emphasis on establishing a correlation between serum IL-1 levels, IL-6, and TNF and the degree of burn injury or infection.

24. (U) The first part of this study includes duplication of the methodology used to detect serum IL-1, IL-6, and TNF activity in a burned rat model. The second part of the study will involve detection of serum IL-1, IL-6, and TNF activity in burned patients. If serum IL-1, IL-6, and TNF activity is significantly increased in burn patients above that of healthy controls, serum IL-1, IL-6, and TNF activity will be correlated with time postburn, burn size, infection, or other burn-associated manifestations.

25. (U) 8810 - 8909. This project was approved by the USAISR Research Council, US Army Institute of Surgical Research Human Use Committee, and US Army Institute of Surgical Research Animal Care and Use Committee during the second and third quarters of fiscal year 1989. Nine patients were enrolled in the study during this reporting period. Analysis will be carried out when sufficient samples are available. For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1989.*

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ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3M161102BS14-00, BASIC RESEARCH

PROJECT TITLE: Interleukin 1 (IL-1) Activity in the Serum of Burned Rats and Thermally Injured Patients

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

1 October 1988 - 30 September 1989

INVESTIGATORS

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

ABSTRACT

PROJECT NUMBER: 3M161102BS14-00, BASIC RESEARCH

PROJECT TITLE: Interleukin 1 (IL-1) 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

INVESTIGATORS: David G. Burleson, PhD, Lieutenant Colonel, MS
Adriana C. Drost, MS
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IL-1 is involved in many inflammatory and infectious responses and may be elevated in the blood of burn patients. We examined different methods to measure IL-1 in the blood of burn patients and rats. Burn sera were fractionated, concentrated, dialyzed, and tested for IL-1 activity in a biological assay. Rat serum prepared in this manner contained IL-1 activity above unburned controls and human samples were negative. In addition, IL-1 activity was not detected in human samples tested in an ELISA assay specific for human recombinant IL-1ß.

Several attempts were made to insure that substances in plasma were not interfering with the assays. A recently developed chloroform extraction procedure was compared to the fractionation/dialysis method. Chloroform extraction alone interfered with the ELISA assay in some cases and did not increase the ability to detect plasma IL-1 beyond that seen with the fractionation procedure. IL-1 was consistently below detection limits in untreated plasma or extracted plasma, yet IL-1 added to the plasma samples could be fully recovered. This suggested that nothing in the plasma interfered with the assay.

A total of 196 plasma samples from 15 burn patients with a total body surface area burn ranging from 20-80% were tested for IL-1 with the ELISA. IL-1 levels above the sensitivity level of the ELISA were found in only 9 plasma samples from 2 patients. Thus, IL-1 could not consistently be detected in the plasma of burn patients at concentrations above the sensitivity limit of the ELISA.

INTERLEUKIN 1 (IL-1) ACTIVITY IN THE SERUM OF BURNED RATS AND THERMALLY INJURED PATIENTS

The monokine IL-1 is a hormone-like polypeptide which is produced during inflammation, infection, and antigenic challenge (1). Its activities include the induction of acute-phase proteins, fever, and alterations of endocrine responses, i.e., glucocorticoid levels (2). These manifestations are also seen following severe thermal injury, indicating that IL-1 may be involved in eliciting some burn-associated pathophysiological alterations.

Recently, a methodology has been developed to measure serum IL-1 activity in a burned rat model (3). It was found that IL-1 activity is significantly increased in the serum of burned rats compared to unburned control rats. In the present study, we extended this technique to measure IL-1 in the plasma of burned patients. We also compared the column fractionation technique to other IL-1 extraction and detection techniques and chose the optimal technique to measure IL-1 in patients.

MATERIAL AND METHODS

Animal Model. Fourteen male Sprague-Dawley rats were anesthetized with sodium pentobarbital (35 mg/kg IP). After shaving the dorsal area, the animals were exposed to 100°C water for 10 sec which inflicted a 30% total body surface area full-thickness burn injury. Ten animals served as unburned controls. On postburn day 7, all animals were sacrificed by exsanguination and the sera were stored at -70°C until assayed.

Patient Selection. Fifteen patients with > 20% total body surface area burns were enrolled in the study. All patients were normotensive and hemodynamically stable after uneventful resuscitation. The presence of inhalation injury was not an exclusionary. Blood was drawn into sterile EDTA blood collection tubes 3X weekly for 6 weeks. The tubes were centrifuged at 3,000 rpm for 20 min and a 1-ml aliquot of the plasma was placed into a tube and stored at -70°C until assayed.

Fractionation and Dialysis. Serum samples were fractionated on a reverse-phase Sephacryl-S-200 superfine column. The buffer was RPMI supplemented with 50,000 U penicillin, 50 mg streptomycin, and 50 mM mercaptoethanol. Molecular weight standards in a range from 400-6.5 kD served to determine the fraction in the molecular weight range of IL-1 (~15 kD). Eight-milliliter factions were collected, concentrated 8-fold, and concomitantly dialyzed against a semipermeable membrane with a molecular weight cutoff at 10 kD. The liquid remaining in the membranes was stored at -20°C until tested for IL-1 activity.

Chloroform Extraction. Lipids have been noted to interfere in some immunoassays. To assure that this potential interference was removed, a lipid extraction procedure was performed as previously described by Cannon et al (1). Briefly, 2 vol of chloroform were mixed with 1 vol of plasma and vortexed until emulsified. The samples were centrifuged at 10,000 g for 10 min and the aqueous phase tested for IL-1 activity.

LBRM Assay. This biological assay, which measures rat and human IL-1 activity, was performed as previously described (4). Briefly, 5 X 10^4 LBRM-33-A5 cells were incubated with 100 µl fractionated and dialyzed rat serum and 0.25 µg PHA. After 24 h, 100 µl of cell supernatants were added to 2 X 10^4 CTLL-2 cells and incubated for an additional 24 h. Proliferation was measured by ^{3}H-thymidine uptake (1 µg/well) during the last 4 h of incubation.

IL-1ß ELISA. This double sandwich ELISA detects human IL-1ß in the natural or recombinant form. Its sensitivity range is 20-1,000 pg/ml. A standard curve containing different concentrations of human recombinant IL-1 (hrIL-1) diluted in either BSA or pooled plasma from 3 healthy laboratory personnel was used to calculate IL-1 concentrations of unknown plasma samples. The absorbance at 490 nm was measured with a MR600 microplate reader.

Statistical Analysis. The IL-1 concentrations of patient samples measured in the ELISA were calculated from a regression of the standard curve by ANOVA. The same procedure was used for all calculations.

RESULTS

In order to apply the previously developed assay to human samples, we repeated the IL-1 measurements in the serum of burned rats. Sera from burned and unburned rats were fractionated, concentrated, and dialyzed as described previously. The IL-1 levels in all burned animals were above background as well as in 3 out of 4 control rats (Table 1). When human sera from burn patients and healthy laboratory personnel were prepared in the same fashion and tested in the LBRM assay, IL-1 activity was at or below the PHA background stimulation levels in all samples (Table 2).

To examine whether the serum preparation technique or the IL-1 assay might be inhibiting the detection of IL-1 in humans, we first retested B4, B6, C1, and C3 (from Table 2) in an ELISA specific for human IL-1ß. The column on the far right in Part A of Table 3 shows that all samples were below the detection limit of the assay.

When we spiked the same samples with 200 pg/ml hrIL-1ß before testing in the ELISA, we could fully recover the spike (Table 3, Part A). This finding indicated that the assay was working properly and that the serum fractions did not contain

TABLE 1. Detection of IL-1 Activity from Concentrated/Dialyzed Rat Serum Fractions by the LBRM Assay[a]

Group	LBRM Cells PHA (μg/ml)	Stimulated With IL-1 (U/ml)	^{3}H-Thymidine Uptake of CTLL Cells (cpm)
		1 March 1989	
Unburned	–	–	10,243
	5	–	12,961
	–	13.3	17,427
	5	13.3	23,523
Burned	5	B12[b]	23,642
	5	B13[b]	26,677
	5	C4[b]	23,248
	5	C5[b]	13,971
		8 March 1989	
Unburned	–	–	1,455
	5	–	4,068
	–	13.3	927
	5	13.3	12,728
Burned	5	B6[b]	9,151
	5	B7[b]	13,221
	5	C2[b]	13,368
	5	C3[b]	16,195

[a]5×10^4 LBRM cells were incubated with 100 μl rat serum fractions in the presence of PHA. IL-2 release was measured by ^{3}H-thymidine uptake of CTLL cells.

[b]B12, B13, B6, and B7 were serum samples from burned animals and C4, C5, C2, and C3 were serum samples from unburned control animals. All serum samples were fractionated and the fractions in the MW range corresponding to IL-1 were concentrated and dialyzed before incubation with LBRM cells.

TABLE 2. Detection of IL-1 Activity from Concentrated/Dialyzed Human Serum Fractions by the LBRM Assay[a]

Group	LBRM Cells PHA (μg/ml)	Stimulated With IL-1 (U/ml)	^{3}H-Thymidine Uptake of CTLL Cells (cpm)
		14 March 1989	
Unburned	–	–	2,734
	5	–	59,820
	–	13.3	3,097
	5	13.3	99,768
Burned	5	P1[b]	46,465
	5	P2[b]	18,428
	5	P3[b]	12,734
		26 March 1989	
Unburned	–	–	5,877
	5	–	8,282
	–	13.3	5,309
	5	13.3	30,747
Burned	5	P4[b]	4,826
	5	P5[b]	5,956
	5	P6[b]	4,076
	5	C1[b]	6,730
	5	C2[b]	4,958
	5	C3[b]	5,530

[a] 5×10^4 LBRM cells were incubated with 100 μl rat serum fractions in the presence of PHA. IL-2 release was measured by ^{3}H-thymidine uptake of CTLL cells.

[b] P1, P2, P3, P4, P5, and P6 were serum samples from burned patients and C1, C2, and C3 were serum samples from unburned control subjects. All serum samples were fractionated and the fractions in the MW range corresponding to IL-1 were concentrated and dialyzed before incubation with LBRM cells.

TABLE 3. Comparison of Two Serum Extraction Methodologies - Fractionation-Dialysis Concentration vs. Chloroform Extraction[a]

Serum Sample	Spiked with hrIL-1 (pg/ml)	Recovery (pg/ml)	% Recovery	Unspiked (pg/ml)
		Fractionation-Dialysis/Concentration[b]		
C1	200	169.1	84.5	< 20
C3	200	144.1	72.0	< 20
B4	200	238.3	119.2	< 20
B6	200	199.8	99.9	< 20
		Chloroform Extraction[c]		
B8[d]	50	69.0	88.0	28
B9	20	34.0	54.0	43
B9	100	163.0	114.0	43
B9	200	343.0	144.0	43
		Chloroform Extraction and Dialysis[e]		
B10	20	38.0	71.0	26
B10	50	100.0	131.0	26
B7	100	107.0	107.0	< 20

[a]Serum samples from burn patients and healthy laboratory personnel were prepared in different ways and tested for IL-1 activity in an ELISA.

[b]All samples were fractionated on a G-50 Sephadex column, which separated IL-1 activity from a higher MW inhibitory activity. The IL-1 containing fractions were concentrated 8-fold and concomitantly dialyzed to remove molecules with a MW below 10 kD. C1-B6 were spiked with 200 pg/ml hrIL-1β before fractionation.

[c]Serum samples were mixed with 2 vol chloroform, vortexed for 5 min, and centrifuged at 10,000 g and the aqueous phase was tested for IL-1 activity.

[d]Serum samples were spiked with different amounts of rhIL-1β chloroform extracted, and measured for IL-1 activity.

[e]Pooled serum was spiked with different amounts of rhIL-1β chloroform extracted, and dialyzed against PA-10 membranes before tested for IL-1 activity.

components which bound to free IL-1, thereby masking its active site.

To compare the fractionation and concentration/dialysis technique to another serum preparation technique, we extracted patient sera with chloroform as described in the **MATERIALS AND METHODS** section before measuring IL-1 in the ELISA. The right column of Part B in Table 3 displays the IL-1 concentration measured. They were not significantly higher than the NSB wells, indicating that the error in this particular assay was such that 28 or 43 pg/ml of IL-1 were not different from background. When we spiked the same sera with different amounts of hrIL-1ß before chloroform extraction, the recovery ranged from 54-144%, indicating that the chloroform extraction introduced a significant amount of error into the assay. Visually, it was noted that residual chloroform in the samples caused detachment of the primary monoclonal antibody in some wells of the ELISA plates, altering the results of those wells. In an attempt to remove the residual chloroform, we dialyzed the aqueous phase of some chloroform-extracted patient sera. Part C of Table 3 displays results of three samples prepared in this manner. The recovered amount of IL-1 in this series ranged from 71-131%. Since the recovery was not very consistent and the residual chloroform was destructive to the plastic dialysis membranes, we abandoned this technique as well.

A recent report in the literature (1) noted the risk of in vitro release of IL-1 by leukocytes during the serum generation procedure. We compared IL-1 levels in patient and control sera to IL-1 levels in plasma. Plasma was prepared using heparin, EDTA, or EDTA and aprotinin (aprotinin is a serine protease inhibitor). We could not find a significant difference between the sample generation techniques (data not shown); but as a precaution against the induction of monocyte IL-1, we collected plasma samples with EDTA as anticoagulant for all subsequent patient and control samples.

The major reason for extracting, fractionating, or dialyzing serum before measuring its IL-1 activity is to remove interference of serum or plasma components with the IL-1 assay. To examine whether the ELISA was susceptible to nonspecific activation or inhibition by other serum factors, we measured IL-1 in plasma which had not been fractionated or chloroform-extracted. Table 4 displays results of such an assay. IL-1 could not be detected in 3 control plasma samples and 1 burn patient plasma sample. When the samples were spiked with different amounts of hrIL-1ß before testing, the recovery ranged from 90-126%, indicating no interference with hrIL-1ß detection. From these results, we concluded that free IL-1, if present in the plasma of burn patients, could be measured in the ELISA without prior extraction or fractionation.

TABLE 4. Recovery of Exogenous rhIL-1 from Human Plasma Tested by ELISA[a]

Sample[b]	Unspiked (pg/ml)	Spiked with hrIL-1ß (pg/ml)	IL-1 Recovery (pg/ml)	% Recovery
C1	< 20	50	45.4	90
C1	< 20	200	260.0	130
C1	< 20	1,000	1,129.4	113
C2	< 20	50	59.8	120
C3	< 20	50	63.1	126
C3	< 20	500	525.9	105
B16	< 20	200	245.0	123

[a]Human plasma generated in EDTA was spiked with hrIL-1ß and tested for IL-1 activity by ELISA.
[b]C indicates control subjects; B, burn patients.

We have tested 196 plasma samples from a total of 15 burn patients with total body surface burn sizes ranging from 20-80% (Table 5). IL-1 was found in 9 plasma samples, 8 of which were from the same patient. Figure 1 shows the plasma IL-1 concentrations of this patient over a 42-day period. During the first 21 days postburn, the plasma IL-1 levels measured below the detection limit of the assay. After that time, the IL-1 concentrations fluctuated above the detection limit. This was late in the recovery period when the patient was apparently infection-free.

DISCUSSION

The preponderance of evidence suggests that IL-1 is a central mediator in inflammatory and immune responses. Its inflammatory nature when injected in vivo and its central role in the in vitro stimulation of immune cells are well known. Yet, to date, few investigators have reported the detection of IL-1 in the circulation of patients undergoing inflammatory or immune responses.

One explanation for the difficulty in measuring circulating IL-1 is that it is present in minute quantities. Cannon et al (1) have reported IL-1 levels of 70 pg/ml in serum from normal individuals using RIA. In contrast, we have been able to measure endogenous IL-1ß above background in plasma from only 2 of 15

TABLE 5. Plasma IL-1 Levels in Burn Patients*

Patient Number	Burn Size (%)	Postburn Days of Samples	Number of Samples	IL-1 Detection (pg/ml)
4	62	8–48	11	< 20
5	89	10–12	2	< 20
6	24	4–20	8	< 20
7	60	5–47	18	< 20–34
8	59	1–43	18	< 20
9	39	3–45	19	< 20
10	36	2–44	18	< 20
11	62	7–49	19	< 20–22
12	30	1–40	17	< 20
13	44	1–17	8	< 20
14	30	5–40	15	< 20
15	54	2–31	14	< 20
16	32	2–30	13	< 20
18	65	2–23	10	< 20
19	43	2–14	6	< 20

TOTAL NUMBER OF PATIENTS = 15 **TOTAL NUMBER OF SAMPLES = 196**

*All plasma samples were generated with EDTA and stored at −70°C until tested for IL-1 activity.

patients using an ELISA assay sensitive to 20 pg/ml. Problems with false-positives in the RIA (1) may be the cause of the discrepancy between our results and those in the cited report. We were puzzled by our failure to measure IL-1 with this more sensitive assay in patients who had clinical indications of inflammation. The clinical symptoms of the IL-1 positive patients were not those which we would have predicted for patients with systemic inflammation. They were not unusually febrile, had no evidence of

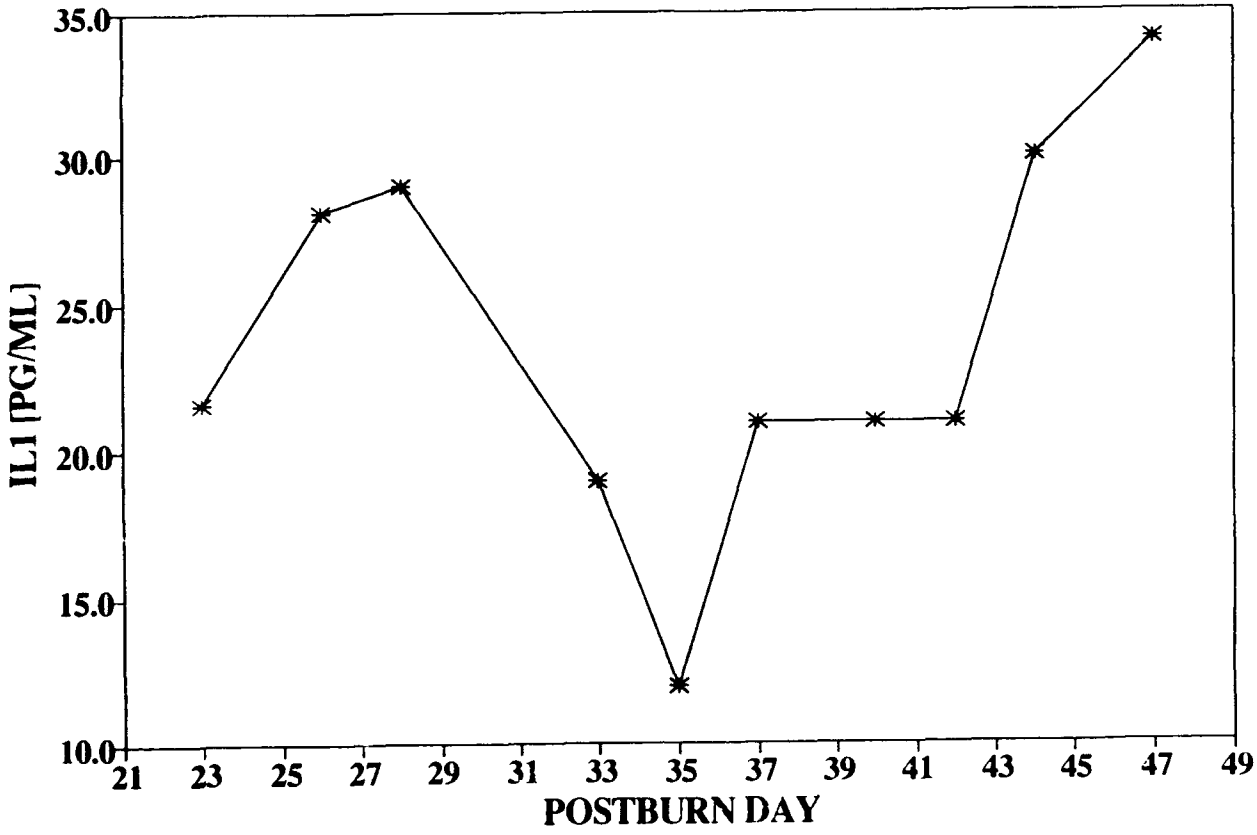

FIGURE 1. Temporal plasma IL-1 profile of a burned patient. Plasma IL-1 was measured 3X weekly by ELISA and plotted over time.

infection, and were well on their way to recovery from their injury.

Another explanation for our difficulty in detecting circulating IL-1 is interference with the assay by serum or plasma factors. Two groups of investigators have claimed to have circumvented this barrier. IL-1 activity in serum from burned rats was found to be increased above that from unburned controls (4). The IL-1 activity was detectable only after the rat serum had been fractionated and dialyzed to remove interfering substances. No quantitative measurements were possible with this technique. In the other report, a chloroform extraction procedure was applied to allow IL-1 detection in normal human plasma by RIA (1).

Our attempts to measure IL-1 in plasma from burn patients using either of these preparation procedures has failed to reveal IL-1 in amounts greater than background. We were able to repeat the measurement of IL-1 activity in burned rat serum by fractionation, dialysis, and bioassay. We could not detect IL-1 in human burn

patient serum with this procedure even though we could recover hrIL-1 which had been added to the serum before fractionation. If 2-endogenous human IL-1 was masked by other serum substances, they differed from interfering substances in the rat. A purification procedure appropriate for human serum or plasma may have to be devised before we can measure human IL-1 by bioassay.

In our hands, the chloroform extraction resulted in inconsistent recovery of hrIL-1, which was attributed to disruption of the protein-coated ELISA plates by residual chloroform in the plasma. The purpose of chloroform extraction is to remove lipid from the aqueous phase. Chloroform-extracted plasma samples from burn patients did not reveal IL-1ß levels above those from unextracted patient plasma. This suggested that lipids were not interfering with the ELISA.

Our results lead us to conclude that IL-1 is either not normally present in human plasma or serum at levels above 20 pg/ml or that substances present in the circulation are masking its presence. A recent relevant report disclosed that plasma α_2-macroglobulin is capable of binding IL-1ß through sulfhydryl linkage and suggested a carrier role for α_2-macroglobulin in the circulation (5). An anti-IL-1ß monoclonal antibody only partially recognized IL-1ß when bound to α_2-macroglobulin. This suggested that only a portion of the IL-1ß molecule was accessible to anti-IL-1ß. Since the ELISA assay is a "sandwich" assay, partial blockage of binding sites could have profound effects on its detection by that assay. α_2-macroglobulin is found in increased levels in burn patient serum (6). If it is a principal carrier of IL-1ß in the circulation, our inability to measure it by the techniques we have employed so far could be explained.

PRESENTATIONS/PUBLICATIONS

None.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION DA313374	2. DATE OF SUMMARY 1 Oct 89	REPORT CONTROL SYMBOL DD-DR&E(AR) 636
3. DATE PREV SUM'RY 1 Oct 88	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	IA	308
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP,	FY90-01		

11. TITLE *(Precede with Security Classification Code)* (U) Medium-Chain Triglyceride Utilization in the Thermally Injured Patient

12. SUBJECT AREAS
0605 Medicine and Medical Research

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
8710	9009	DA	C. In-House

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18. RESOURCES ESTIMATE

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89	0.3	5
90	0.3	5

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
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22. KEYWORDS *(Precede EACH with Security Classification Code)* (U) Thermal Injury; (U) Nutrition; (U) Medium-Chain Triglycerides; (U) Long-Chain Triglycerides;

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

23. (U) A DTIC literature search was conducted under DTIC request number W6L42E dated 20 October 1989 for the technical report database and request number W6L43E dated 20 October 1989 for the work unit database. The proposed effort will not result in the duplication of effort. The objective of this work is to examine the ability of thermally injured patients to use medium-chain triglycerides when administered in significant quantity as an effective energy source to maintain nitrogen balance.

24. (U) REE will be measured on postburn day 10 and the patient started on enteral nutrition consisting of a carbohydrate load of 5 mg/kg/min with the residual caloric requirements administered as long-chain triglycerides. After a 3-day stabilization period, metabolic measurements will made. Finally, the patient will be changed to a nutrition regimen consisting of a carbohydrate load of 3.8 mg/kg/min and the remaining necessary calories will be administered as 90% medium-chain triglycerides and 10% long-chain triglycerides.

25. (U) 8810 - 8909. Data from 2 study patients are being analyzed. Intestinal intolerance prompted a search for an alternate medium-chain triglyceride preparation. For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1989.*

DD FORM 1498 83 MAR — EDITION OF MAR 86 IS OBSOLETE. — * USGPO. 1988 -491-003/50329

ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3M161102BS14-00, BASIC RESEARCH

PROJECT TITLE: Medium-Chain Triglyceride Utilization in the Thermally Injured Patient

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

1 October 1988 - 30 September 1989

INVESTIGATORS

William G. Cioffi, Jr., MD, Major, MC
Theresa A. Graves, MD, Captain, MC
Dawn E. Carlson, RD, Major, SP
William F. McManus, MD, Colonel, MC
Basil A. Pruitt, Jr., MD, Colonel, MC

ABSTRACT

PROJECT NUMBER: 3M161102BS14-00, BASIC RESEARCH

PROJECT TITLE: Medium-Chain Triglyceride Utilization in the Thermally Injured Patient

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

PERIOD COVERED IN THIS REPORT: 1 Oct 88 through 30 Sep 89

INVESTIGATORS: William G. Cioffi, Jr., MD, Major, MC
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The optimal nutritional support program for the thermally injured patient has yet to be defined. The ability of burn patients to use fat, normally administered as long-chain triglycerides, as an effective energy source has been questioned. Few studies comparing the efficacy of medium-chain triglycerides to standard carbohydrate and long-chain triglycerides regimens have been performed. This study was designed to use medium-chain triglycerides, when administered in significant quantity, as an effective energy source to maintain nitrogen balance. Two patients out of a projected 10 have been enrolled and these patients have completed the study.

MEDIUM-CHAIN TRIGLYCERIDE UTILIZATION IN THE THERMALLY INJURED PATIENT

The optimal nutritional support program for the thermally injured patient has yet to be defined. Various studies have questioned the ability of burn patients to use fat, usually administered as long-chain triglycerides (LCT), as an effective energy source (1-3). Few studies comparing the efficacy of medium-chain triglycerides (MCT) to standard carbohydrate and LCT regimens have been performed. The purpose of this study is to examine the ability of thermally injured patients to use MCT when administered in significant quantity as an effective energy source to maintain nitrogen balance.

Multiple studies have documented that carbohydrate is used preferentially over fat as an energy source when both are administered to injured animals and humans (4-7). However, there is a physiological limit to the amount of carbohydrate which can be effectively utilized by man. Burke et al (8) has suggested that 5 mg/kg/min is the amount of carbohydrate which can be administered before excessive CO production occurs. Excess carbohydrate administered is first transformed to fat, a process which has a RQ of 8, meaning for every molecule of oxygen utilized, 8 molecules of CO are produced. The fat may then be used as an energy source after this conversion. In thermally injured patients, it is usually impossible to meet the increased kilocalorie requirements using carbohydrate alone if one adheres to this limit. Fat administered as LCT can be given to supplement the carbohydrate load and meet energy requirements even though the LCT may be utilized ineffectively.

Recent studies have suggested that MCT may be utilized more effectively than LCT by injured animals (2-3,9). In comparison to LCT, MCT are not stored in liver or fat deposits and are quickly oxidized when administered (10). Maiz et al (3) has demonstrated in a rat burn model that MCT in combination with carbohydrate are just as effective as carbohydrate alone in maintaining nitrogen balance. However, Stein et al (4) has demonstrated that when MCT are administered as the sole energy source, there is a negative nitrogen balance, decreased protein synthesis, and decreased peripheral fat stores as compared to carbohydrate alone.

On a cellular level, Goodwin et al (1) has investigated the ability of hepatocyte mitochondria from burned rats to oxidize both MCT and LCT. Oxidation of LCT was decreased when compared to normals while beta oxidation of MCT was increased.

These studies suggest that MCT administered in combination with carbohydrate could serve as an effective energy source in thermally injured patients. The safety of administration of MCT to humans has been documented (10). No untoward effects of these compounds

have been reported to date (11-12). Excessive ketone production does not occur and ketoacidosis has not been a problem. The RQ has been noted to decrease after administration, suggesting utilization as an energy source.

The objective of this study is to determine the effects of MCT on the RQ and nitrogen balance as compared to LCT.

MATERIALS AND METHODS

Number of Patients. Ten consecutive patients will be enrolled in the study. Two patients have been enrolled to date.

Criteria for Admission into the Study. Patients admitted to the US Army Institute of Surgical Research who require the use of enteral nutrition to meet all nutritional requirements are eligible for enrollment in this study.

Patient Inclusion. Ten patients meeting the following criteria will be considered for enrollment in the study:

1. Male or female patients ≥ 18 yr old. Female patients must be previously surgically sterilized or postmenopausal (> 45 yr old and lack of menstrual periods > 1 yr) or have a negative pregnancy test.

2. Patients with burns > 30% of the total body surface area.

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

1. Patients < 18 yr old.

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

3. Any pregnant patient.

4. Patients who have clinical and/or laboratory indications of sepsis. Any patient who develops sepsis during participation in the study will also be excluded from the study at that time.

Procedures During the Study Period. On postburn day 10, each patient is transported to the Metabolic Room prior to the morning dressing changes. VO_2 and VCO_2 are measured utilizing the Horizon MMC Metabolic Cart™. The resting energy expenditure (REE) is calculated and baseline triglyceride, cholesterol, ketone, and insulin levels are obtained.

Each patient is started on enteral nutrition with energy requirements calculated as 1.2 X REE (13). Daily nitrogen requirements are calculated as 1 g/150 kcal. Carbohydrates are administered at a dose of 5 mg/kg/min. The remainder of the kilocalories requirement is administered as LCT. Electrolyte composition is tailored according to each individual patient's needs. Each patient receives standard vitamins and trace minerals. Once the patient's intake has reached his/her projected requirement for three continuous days, the VO_2, VCO_2, REE, nitrogen balance calculated from a 24-h UUN, and RQ are measured. Triglyceride, cholesterol, insulin, and ketone levels are obtained from a blood sample.

The following calculation is then made: ME (energy metabolism in kcal/min) = (5.083 X VO_2) + (0.138 X VCO_2) - (0.125 X NM). NM equals nitrogen metabolized in grams per minute calculated from the 24-h UUN.

Grams per minute of carbohydrate, fat, and protein metabolized are then calculated. The formulae used as previously described by Weir depends upon the RQ of the patient at each time.

The enteral formula is then changed. Protein and carbohydrate concentrations remain the same. Fat is administered as 90% MCT and 10% LCT. After a 3-day stabilization period, the same measurements are repeated.

The enteral formula is again altered. Protein remains the same, carbohydrates are reduced to 3.8 mg/kg/min, and fats consisting of 90% MCT and 10% LCT are then administered in sufficient quantity to meet the REE. The total amount of fat does not exceed 3 g/kg/day. After a 3-day stabilization period, all measurements are again repeated.

Upon completion of the study, the patient's enteral formula is changed back to a conventional formulation with 5 mg/kg/min carbohydrate and the residual caloric needs as 50% LCT and 50% MCT.

Statistical Analysis. The RQ, nitrogen balance, and percentage of calories from carbohydrate, fat, and protein will be compared by ANOVA for each patient.

RESULTS

Two patients have been enrolled in the study to date and both have completed the study. Intestinal intolerance has prompted a search for an alternate MCT preparation.

DISCUSSION

When the projected total of 10 patients have completed the study, the data will be analyzed as to the ability of medium-chain

triglycerides to serve as an effective energy source in thermally injured patients.

PRESENTATIONS/PUBLICATIONS

None.

REFERENCES

1. Goodwin CW, Whitson JD, Mason AD Jr, et al: The study of metabolism and nutritional effects of burn injury in soldiers - metabolic effect of injury: mitochondrial studies. In **US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1983**. San Antonio: US Government Printing Office, 1984, pp 230-43.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION DA313583	2. DATE OF SUMMARY 1 Oct 89	REPORT CONTROL SYMBOL DD-DR&E(AR) 636
3. DATE PREV SUM'RY 1 Oct 88	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	IA	309
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP,	FY90-01		

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	9009	DA	C. In-House

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

18. RESOURCES ESTIMATE

FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS *(In thousands)*
89	0.3	5
90	0.3	5

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: BUESCHER, T M
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)*: CARLSON, D g. NAME OF ASSOCIATE INVESTIGATOR *(if available)*: MC MANUS, W F

22. KEYWORDS *(Precede EACH with Security Classification Code)* (U) Thermal Injury; (U) Nutrition; (U) Metabolism; (U) Volunteers; (U) Children; (U) RA II

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

23. (U) A DTIC literature search was conducted under DTIC request number W6L57B dated 20 October 1989 for the technical report database and request number W6M03C dated 20 October 1989 for the work unit database. The proposed effort will not result in the duplication of effort. The objective of this work is to determine optimal nutritional support for the burned child.

24. (U) On the fifth postburn day, the REE will be calculated as will the RQ. Baseline laboratory data will be collected and liver function and partial thromboplastin time tests will be performed. The patient will then be begun on alimentation that will be adjusted every 3 days until the caloric need is determined and met for two successive, 3-day cycle measurements as determined by a positive nitrogen balance, a RQ between 0.85 and 1.0, and a caloric intake equal to 1.25 X REE.

25. (U) 8810 - 8909. No suitable pediatric patients were admitted to the Institute during this reporting period. Patients will be asked to enroll into the study as they become available. For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1989*.

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

ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3M161102BS14-00, BASIC RESEARCH

PROJECT TITLE: Caloric Requirements of Thermally Injured Children

US ARMY INSTITUTE OF SURGICAL RESEARCH
FORT SAM HOUSTON
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1 October 1988 - 30 September 1989

INVESTIGATORS

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ABSTRACT

PROJECT NUMBER: 3M161102BS14-00, BASIC RESEARCH

PROJECT TITLE: Caloric Requirements of Thermally Injured Children

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

PERIOD COVERED IN THIS REPORT: 1 Oct 88 through 30 Sep 89

INVESTIGATORS: Teresa M. Buescher, MD, Captain, MC
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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 signed and witnessed volunteer agreement affidavits will be obtained for each patient prior to enrollment in the study.

Inclusion Criteria. Patients meeting the following criteria will be eligible for enrollment in the study:

1. Patients admitted to the US Army Institute of Surgical Research with burn injury.

2. Male or female patients < 13 yr old.

3. Patients with burn wounds > 30% of the total body surface area.

Exclusion Criteria. Patients meeting the following criteria will be excluded from enrollment in the study:

1. Patients ≥ 13 yr 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.

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 wil 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 adjustd 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	1 Oct 89	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
1 Oct 89	D. Chg	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS14	IA	310
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP	FY90-01		

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	9009	DA	C. In-House

MILITARY RELEVANCY CERTIFIED
APPROVED BY

17. CONTRACT/GRANT	18. RESOURCES ESTIMATE		
a. DATE EFFECTIVE	FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS *(In thousands)*
b. CONTRACT/GRANT NUMBER			
c. TYPE / d. AMOUNT	89	0.1	7
e. KIND OF AWARD / f. CUM/TOTAL	90	0.1	7

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	f. NAME OF ASSOCIATE INVESTIGATOR *(If available)*
MILITARY/CIVILIAN APPLICATION: M	g. NAME OF ASSOCIATE INVESTIGATOR *(If available)*

22. KEYWORDS *(Precede EACH with Security Classification Code)* (U) Burns; (U) Antidiuretic Hormone; (U) Atrial Natriuretic Peptide; (U) Renin-Angiotensin; (U) Aldosterone Axis;

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

22. (Continued) (U) Resuscitation; (U) Blood Volume; (U) Renal Plasma Flow; (U) Volunteers: (U) Adults; (U) RA II

23. (U) A DTIC literature search was conducted under DTIC request number W6M09B dated 20 October 1989 for the technical report database and request number W6M10A dated 20 October 1989 for the work unit database. The proposed effort will not result in the duplication of effort. The objective of this work is to 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.

24. (U) Twenty consecutive patients will be enrolled in 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) 8810 - 8909. An addendum to the study has recently been approved by the USAISR Research Council, the Brooke Army Medical Center Radiation Control Committee, the US Army Institute of Surgical Research Human Use Committee, and The Surgeon General's Human Subjects Research Review Board and patient studies will be initiated during fiscal year 1990. For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1989*.

DD FORM 83 MAR 1498 EDITION OF MAR 68 IS OBSOLETE. * U.S.G.P.O. 1988 -491-083/30329

ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3M161102BS14-00, BASIC RESEARCH

PROJECT TITLE: Salt and Water Balance in the Thermally Injured Patient

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

1 October 1988 - 30 September 1989

INVESTIGATORS

William G. Cioffi, Jr., MD, Major, MC
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ABSTRACT

PROJECT NUMBER: 3M161102BS14-00, BASIC RESEARCH

PROJECT TITLE: Salt and Water Balance in the Thermally Injured Patient

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

PERIOD COVERED IN THIS REPORT: 1 Oct 88 through 30 Sep 89

INVESTIGATORS: William G. Cioffi, Jr., MD, Major, MC
Cheryl M. Crowley, MD, Captain, MC
Theresa A. Graves, MD, Captain, MC
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Factors responsible for sodium and volume regulation following injury are not clearly understood. This study was designed to describe the alterations of plasma levels of antidiuretic hormone, atrial natriuretic peptide, and the renin-angiotensin-aldosterone axis following thermal injury as related to plasma volume, osmolality, and tonicity. The information generated will permit refinement of fluid resuscitation regimens for severely burned and critically ill patients. One patient has been enrolled in the study to date.

SALT AND WATER BALANCE IN THE THERMALLY INJURED PATIENT

Factors responsible for sodium and volume regulation following injury are not clearly understood. Several authors have interpreted their data to imply that resetting of hormonal control mechanisms occurs following thermal injury and that this is a stress response and not sodium and volume-dependent (1-2). Although various studies have examined one or two factors responsible for sodium and volume regulation following thermal injury, no one has studied the system as a whole. In normal man, antidiuretic hormone (ADH), atrial natriuretic peptide (ANP), and the renin-angiotensin-aldosterone loop are responsible for salt and water balance. How these systems interact following thermal injury is unknown.

The ADH response following thermal injury has been recently examined (2-4). Morgan et al have concluded that ADH levels are elevated postburn and remain so for 7-10 days. In addition, the increased ADH levels appear to have little relation to the serum osmolality and do not affect urine output. There is no satisfactory explanation for this at present. None of these studies have measured blood or plasma volume simultaneously with the measurements of ADH.

The renin-angiotensin-aldosterone axis has been examined in thermal injury (1). Shirani et al suggested that the elevated plasma levels of renin, angiotensin I, angiotensin II, and aldosterone following thermal injury reflect a resetting of hormonal control and are not dependent upon an effective plasma volume deficit. No volume measurements were made in this study. In this group of patients, combinations of these hormones did remain volume-responsive as verified by saline-loading tests.

ANP, a potent natriuretic and diuretic as well as a vasorelaxant agent, is present in mammalian cardiac atria (5). Central hypervolemia and increased blood pressure have been postulated as factors promoting ANP secretion (6). The vasorelaxant properties of ANP appear to be mediated by an increased level of intracellular cyclic guanosine monophosphate (GMP) which antagonizes the vasopressor effects of angiotensin II and norepinephrine (6). Infusion of ANP in humans effects a profound natriuresis with an accompanying diuresis (7). The mechanism by which ANP causes these changes in unclear. In animal models, infusion of ANP causes an increase in the glomerular filtration rate (GFR) as well as sodium excretion (8). It appears, at least in part, that the mechanism responsible for natriuresis is the increase in the GFR. No proximal tubular effect of ANP has been documented. The renal effects of ANP can be blocked by calcium channel blockers, suggesting that its effects are calcium-dependent (8).

ANP also has an effect on ADH release and the renin-angiotensin-aldosterone axis. In isolated rat posterior pituitary lobes, ANP causes a massive release of ADH (9). Other investigators have indicated that in isolated hypothalamic hypophyseal preparations, ANP induces a decrease in ADH. ANP induction of ADH release may serve as a negative feedback loop regulating the actions of ANP. The effects of ANP on the renin-angiotensin-aldosterone axis appear to be more constant. Infusion of ANP causes a decrease of plasma renin activity and renin excretion (10,12) and at the same time blunts aldosterone release stimulated by angiotensin II (11-12).

A syndrome of inappropriately low plasma aldosterone levels in the presence of elevated plasma renin activity has been identified in a subset of critically ill patients and was associated with a higher mortality during critical illness (13). The nature of this abnormality has not been elucidated. Elevated ANP could explain this dissociation during critical illness, with its ability to decrease aldosterone levels in the face of an activated renin system (14).

The effects of thermal injury on plasma ANP level and how it in turn affects salt and water balance have not been described. The purpose of this study will be to describe the alterations of plasma levels of ADH, ANP, and the renin-angiotensin-aldosterone axis following thermal injury as related to plasma volume, osmolality, and tonicity. The information generated will permit refinement of fluid resuscitation regimens for severely burned and critically ill patients.

MATERIALS AND METHODS

Number of Patients. Twenty consecutive patients admitted to the US Army Institute of Surgical Research will be eligible for enrollment in this study.

Inclusion Criteria. Patients meeting the following criteria will be eligible for enrollment in the study:

1. Male or female patients ≥ 18 yr old. Female patients be previously surgically sterilized or postmenopausal (> 45 yr old and lack of menstrual periods for > 1 yr) or have a negative pregnancy test.

2. Patients with burns between 30% and 80% of the total body surface area.

3. Patients admitted to the US Army Institute of Surgical Research within 24 h of the time of injury.

Exclusion Criteria. Patients meeting the following criteria will be excluded from the study:

1. Patients < 18 yr old.

2. Any pregnant patient.

3. Patients with burns < 30% or > 80% of the total body surface area.

4. Patients admitted to the US Army Institute of Surgical Research > 24 h postburn.

5. Patients with a history of diabetes mellitus or congestive heart failure.

6. Patients with a history of treatment for hypertension within the previous month.

7. Patients with concomitant CNS injury.

8. Patients with sepsis or who develop sepsis during the study period.

9. Patients with acute renal failure or who develop acute renal failure during the study period (defined as an acute rise in serum creatinine to a level > 1.5).

Patient Procedures During the Study Period.

Part I. Upon enrollment into the study, the following data will be collected each day for each patient on postburn days 2-10:

1. Percentage of the total body surface area burned.

2. Medications administered.

3. Body weight.

4. Total intake of water and salt.

5. Urine and nasogastric output, to include volume as well as sodium and potassium content.

6. Serum concentrations of sodium, potassium, chloride, glucose, phosphate, uric acid, urea nitrogen, creatinine, and β_2-microglobulin.

7. Serum and urine osmolality.

8. Urine concentrations of creatinine, urea nitrogen, phosphate, total protein, β_2-microglobulin, and aldosterone from a 24-h urine sample.

From this data, the endogenous GFR, osmolality clearance, $H_2O(CH_2O)$ clearance, and fractional excretion of sodium will be calculated.

At 0700 h each morning at the time of the routine blood drawings, blood will be obtained for ADH, ANP, plasma renin activity, and aldosterone assays.

Part II. On postburn days 2, 5, and 10, intravascular volume measurements will be made utilizing chromium-labeled RBCs to measure RBC volume. Total blood volume will then be calculated after measuring a central hematocrit.

On postburn day 5, a Swan-Ganz catheter, if not already in place, will be inserted through the central line which the patient will already have for clinical care and readings of cardiac output and pulmonary artery occlusion pressures will be recorded. Systemic vascular resistance will be calculated from the appropriate variables.

Also on postburn day 5, GFR will be measured utilizing the inulin technique. Effective renal plasma flow will be measured using a colorimetric hippurate study. Inulin clearance will be repeated for any patient who demonstrates a subsequent decrease in renal function during the hospital course.

RESULTS

One patient has been enrolled in the study to date.

DISCUSSION

When 20 patients have completed the study, the data will be analyzed as to the alterations of plasma levels of ADH, ANP, and the renin-angiotensin-aldosterone axis following thermal injury as related to plasma volume, osmolality, and tonicity.

PRESENTATIONS/PUBLICATIONS

None.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
				DA315349	1 Oct 89	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
1 Oct 88	D. Chg	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS14	DA	311
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP,	FY90-01		

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	9009	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	89	0.2	8
e. KIND OF AWARD	f. CUM/TOTAL	90	0.5	10

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	f. NAME OF ASSOCIATE INVESTIGATOR *(If available)* g. NAME OF ASSOCIATE INVESTIGATOR *(If available)*

22. KEYWORDS *(Precede EACH with Security Classification Code)* (U) Epidermal Growth Factor; (U) Fibroblast Growth Factor; (U) Platelet-Derived Growth Factor; (U) Epithelization;

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

22. (Continued) (U) Thermal Injury; (U) Lab Animals: (U) Guinea Pigs; (U) RA II

23. (U) A DTIC literature search was conducted under DTIC request number W6M16F dated 20 October 1989 for the technical report database and request number W6M17E dated 20 October 1989 for the work unit database. The proposed effort will not result in the duplication of effort. The objective of this work is to determine whether infusion of a recently described copolymer will enhance burn epithelization in a deep partial-thickness burn wound in the guinea pig.

24. (U) 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) 8810 - 8909. An addendum was approved by the USAISR Research Council and US Army Institute of Surgical Research Animal Care and Use Committee during this reporting period. Initial studies revalidating the deep partial-thickness burn wound model in guinea pigs was initiated. For technical reports refer to the *US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1989*.

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

ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3M161102BS14-00, BASIC RESEARCH

PROJECT TITLE: Effect of Growth Factors on the Healing of Partial-Thickness Scald Wounds in the Guinea Pig

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

1 October 1988 - 30 September 1989

INVESTIGATORS

William G. Cioffi, Jr., MD, Major, MC
Chi-Sing Chu, MD
Carlin V. Okerberg, DVM, PhD, Major, VC
Arthur D. Mason, Jr., MD
Basil A. Pruitt, Jr., MD, Colonel, MC

ABSTRACT

PROJECT NUMBER: 3M161102BS14-00, BASIC RESEARCH

PROJECT TITLE: Effect of Growth Factors on the Healing of Partial-Thickness Scald Wounds in the Guinea Pig

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

PERIOD COVERED IN THIS REPORT: 1 Oct 88 through 30 Sep 89

INVESTIGATORS: William G. Cioffi, Jr., MD, Major, MC
Chi-Sing Chu, MD
Carlin V. Okerberg, DVM, PhD, Major, 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.

REFERENCES

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
				DA315350	1 Oct 89	DD-DR&E(AR) 636
3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
1 Oct 88	D. Chg	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS14	DA	312
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP,	FY90-01		

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	9009	DA	C. In-House

17. CONTRACT/GRANT — MILITARY RELEVANCY CERTIFIED — APPROVED BY: Lea C. 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)*
89	2.0	60
90	2.0	60

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME: US Army Institute of Surgical Research	a. NAME: US Army Institute of Surgical Research
b. ADDRESS *(include zip code)*: Fort Sam Houston, San Antonio, Texas 78234-5012	b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-5012
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR: 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	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) Flow Cytometry; (U) Lymphocyte Subpopulations; (U) Burn Injury; (U) Infection; (U) Immunocompetence;

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

22. (Continued) (U) Volunteers: (U) Adults; (U) RA II

23. (U) A DTIC literature search was conducted under DTIC request number W6M25D dated 20 October 1989 for the technical report database and request number W6M27E dated 20 October 1989 for the work unit database. The proposed effort will not result in the duplication of effort. The objective of this work is to 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.

24. (U) The immune status of burn patients will be assessed in terms of lymphocyte subpopulation composition and function using flow cytometry to differentiate the subpopulations. Data will be correlated with patient morbidity and compared to data from unburned control subjects.

25. (U) 8810 - 8909. Data was collected for 17 burned patients and compared to data collected from 15 unburned control subjects. The function of T lymphocyte subsets was measured as the expression of IL-2R after mitogen stimulation. The expression of IL-2R by helper and suppressor T lymphocyte subsets after mitogen stimulation was decreased in burned patients as compared to controls. The ability of B lymphocytes and NK cells to express the IL-2R appeared to be unimpaired by thermal injury. For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1989.*

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

ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3M161102BS14-00, BASIC RESEARCH

PROJECT TITLE: Cellular Host Defense Function after Thermal Injury: Assessment by Flow Cytometry of Peripheral Blood Cells

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

1 October 1988 - 30 September 1989

INVESTIGATORS

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

ABSTRACT

PROJECT NUMBER: 3M161102BS14-00, BASIC RESEARCH

PROJECT TITLE: Cellular Host Defense Function after Thermal Injury: Assessment by Flow Cytometry of Peripheral Blood Cells

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

PERIOD COVERED IN THIS REPORT: 1 Oct 88 through 30 Sep 89

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

The susceptibility of burned patients to infection correlates with a reduced functional responsiveness of their peripheral blood T lymphocytes. In order to establish which of the lymphocyte subsets are less functional, we determined the proportion of each lymphocyte subset from burned patients that bear the IL-2R after mitogenic stimulation. Twenty-six burned patients were enrolled into the study within 5 days of their injury. Lymphocytes isolated from peripheral blood were stimulated for 24 h with ConA, removed from culture, washed, and stained with monoclonal antibodies to the IL-2R and one of several lymphocyte subset markers. The number of lymphocytes positive for both the IL-2R and a subpopulation marker were compared to the total number of lymphocytes positive for the subpopulation marker to determine the proportion of that subpopulation responding to mitogen. The results show that a lower percentage of T lymphocytes from burned patients was able to respond to mitogen than for 27 unburned control subjects. The proportion of IL-2 positive lymphocytes from patients was decreased as compared to control subjects for both the helper and the suppressor subsets (CD4 and CD8 positive). In contrast, the proportion of IL-2 positive B lymphocytes (CD19 positive) and NK cells (CD16 positive) was similar for both patients and control subjects. We have previously reported that burned patients have decreased numbers of circulating helper and suppressor T lymphocytes as compared with unburned control subjects. Though the cause of the susceptibility of burned patients to infection remains unknown, the combination of the decreased numbers of circulating T lymphocytes and the further decreased proportion of these T lymphocytes able to respond to mitogen are suggestive of their importance in the host's defense against infection.

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

The number of immunosuppressive events described for burned patients compels us to inquire in what way these changes are related to the increased susceptibility to infection seen in these patients. One of the many changes in immune competence following thermal injury is a decreased ability of lymphocytes to respond to mitogenic stimulation. Since the lymphocyte population is a complex mixture of effector and regulator cells, it is not clear which of the subpopulations express the decrease in mitogenic capacity. Are suppressor cells hyperactive as has been suggested by some (1-4) or are the effector cells incapable of responding to stimuli? The expression of IL-2R is correlated with the ability of lymphocytes to respond to mitogenic stimuli (5). The expression of IL-2R on the lymphocyte surface and the expression of phenotypic surface markers can be monitored by flow cytometry methods. If multiple chromophores are used on the appropriate monoclonal antibodies, these two parameters can be determined simultaneously. By using these methods, we have determined the mitogenic capacity of lymphocyte subpopulations from burned patients as determined by expression of IL-2R.

MATERIALS AND METHODS

Patient Data. Data from 26 burned patients were compared with that from 27 control subjects. All patients were entered into the study within 5 days of their injury. Admission to the study required that the patient's expected mortality (determined from burn size and age statistics from previous experience at this Institute) be between 20% and 90% (average 50.1%). The average burn size was 41.7% and the average age was 46.1 yr. The control subjects were healthy individuals made up of laboratory and hospital staff. The average age of the control subjects was 33.8 yr.

Cell Preparations. Heparinized blood samples were obtained from patients twice weekly for up to 8 weeks postburn. Blood samples were taken at random from the 27 control subjects. Lymphocytes were separated from RBCs and nonlymphoid cells by Ficoll-Hypaque gradients. The cells isolated from the gradients were washed 3X in HBSS and resuspended for counting with a Coulter™ counter (Model ZM, Coulter Electronics, Inc., Hialeah, FL). The lymphocyte preparations were monitored for nonlymphoid contamination by preparing a slide on a cytocentrifuge using a portion of each sample.

Cell Culture. Cells were cultured for 24 h using RPMI-1640 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 per

milliliter 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 2X in RPMI culture medium without ConA and once with HBSS. The cell suspension was counted and the cell concentration adjusted for staining.

Cell Staining. Cells were stained with the appropriate monoclonal antibodies chemically bound to phycoerythrin, fluorescein isothiocyanate, or biotin. Allophycocyanin conjugated to streptavidin was used to bind allophycocyanin to the biotinylated monoclonal antibodies for the third chromophore. Monoclonal antibodies were purchased from Becton Dickinson (Mountain View CA). The antibodies used were anti-Leu-2 (CD8 T suppressor/cytotoxic subpopulation), anti-Leu-3 (CD4 T helper/inducer subpopulation), anti-Leu-12 (CD19 B lymphocyte), anti-Leu-7 (large granular lymphocyte), anti-Leu-8 (a monoclonal binding to the surface of a subset of helper and suppressor lymphocytes as well as some B cells, monocytes, and neutrophils), anti-Leu-11 (CD16 NK cell or IgG Fc receptor), and anti-Leu-M3 (CD14 monocyte) and anti-IL-2R (CD25). IgG_1 or IgG_2 conjugated with the appropriate dye marker was employed as an isotypic control. The staining procedure followed that specified by the manufacturer of the monoclonal antibody. Cells were fixed immediately after staining in 1% paraformaldehyde.

Flow Cytometry Analysis. Subpopulations were analyzed by flow cytometry using a FACSTAR Plus™ (Becton Dickinson). Electronic gates were set on forward angle and side scatter intensity using normal human peripheral blood lymphocytes. This gate was used to exclude as many nonlymphoid cells as practical. 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 samples as positive.

Data Analysis. Group means for data that are normally distributed were compared by a t test (Program 7D, BMDP Statistical Software, San Francisco, CA). Data that were not normally distributed were compared by nonparametric analysis (Program 3S, BMDP Statistical Software).

RESULTS

The proportions of several lymphocyte subpopulations in the circulation were determined. Figure 1 depicts the decrease in the proportion of the T lymphocyte subpopulations that was found in the circulation of burned patients as compared to control subjects. CD3, CD4, and CD8 subpopulations were all decreased in the burned patients. The proportion of large granular lymphocytes and NK cells in the blood of burned patients was also decreased when

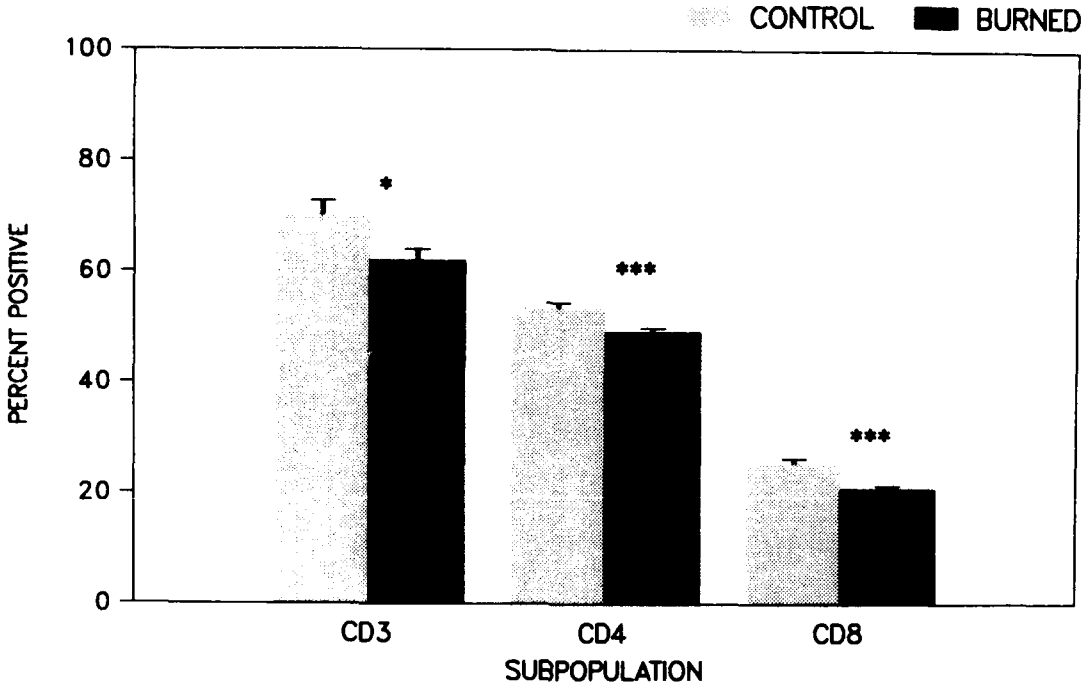

FIGURE 1. Peripheral blood T-lymphocyte subpopulations from burned patients and control subjects. The percentage that each subpopulation represents of the total blood lymphoid population is displayed as mean percent ± 1SEM for both burned patients and control subjects. CD3 indicates pan-T lymphocytes; CD4, helper/inducer; and CD8, suppressor/cytotoxic. *P < 0.05, **P < 0.01, ***P < 0.001.

compared to control subjects (Fig 2). However, the proportion of B lymphocytes in burned patients was not different from control subjects.

IL-2R expression has been reported to be increased in circulating lymphocytes in burned patients (6). We measured the IL-2R expression of freshly isolated lymphocytes to determine the endogenous level of receptor expression in CD4 and CD8 subpopulations. These subpopulations from control subjects had relatively low percentages of IL-2R-expressing cells of approximately 10% and 5%, respectively. A significantly higher proportion of burned patient cells expressed the IL-2R (Fig 3).

IL-2R expression was monitored for each lymphocyte subpopulation after 24 h of culture with and without mitogen. After 24 h of culture in the absence of mitogen, T- and B-lymphocyte subpopulations from both burned patients and control subjects had similar low levels of IL-2R expression (Fig 4).

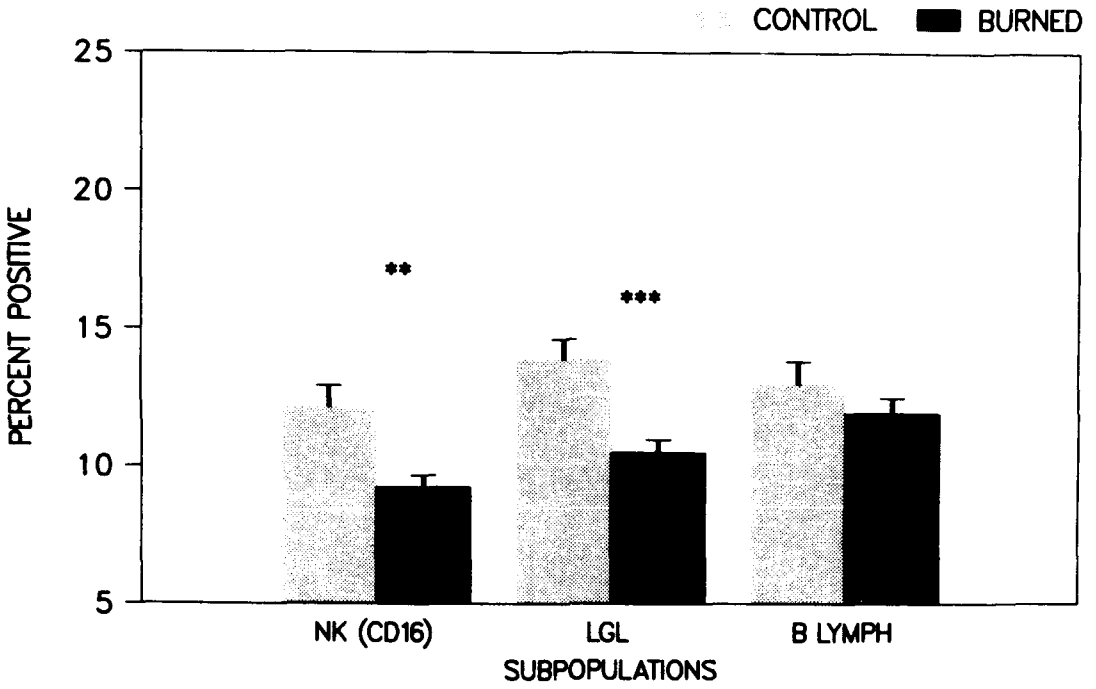

FIGURE 2. Peripheral blood non-T-lymphocyte subpopulations from burned patients and control subjects. The percentage that each subpopulation represents of the total blood lymphoid population is displayed as mean percent ± 1SEM for both burned patients and control subjects. NK (CD16) indicates natural killer cells; LGL, large granular lymphocytes and B LYMPH, B lymphocytes. *P < 0.05, **P < 0.01, ***P < 0.001.

Exposure of the cells to culture conditions for 24 h increased the level of receptor expression in control subject cells up to and slightly above the level of IL-2R expression seen in freshly isolated burned patient cells. IL-2R expression in the large granular lymphocytes and B cell subpopulations were also similar after 24 h of culture without ConA (Fig 5), but the proportion of IL-2R positive unstimulated NK cells from patients was higher than that of controls.

The increase in IL-2R expression after 24-h exposure to ConA in culture above that obtained in the absence of ConA was used as a measure of the mitogenic capacity of the cells. After 24 h ConA stimulation, the proportion of the cultured lymphocytes expressing IL-2R from burned patients was about half that of control subjects (Fig 6). The depressed response seemed evenly distributed between the CD4, CD8, and large granular lymphocyte subpopulations (Figs 6 and 7). In contrast, the proportions of IL-2R positive NK cells and B cells from burned patients and control subjects were similar after the 24-h stimulation period (Fig 7). Therefore, the

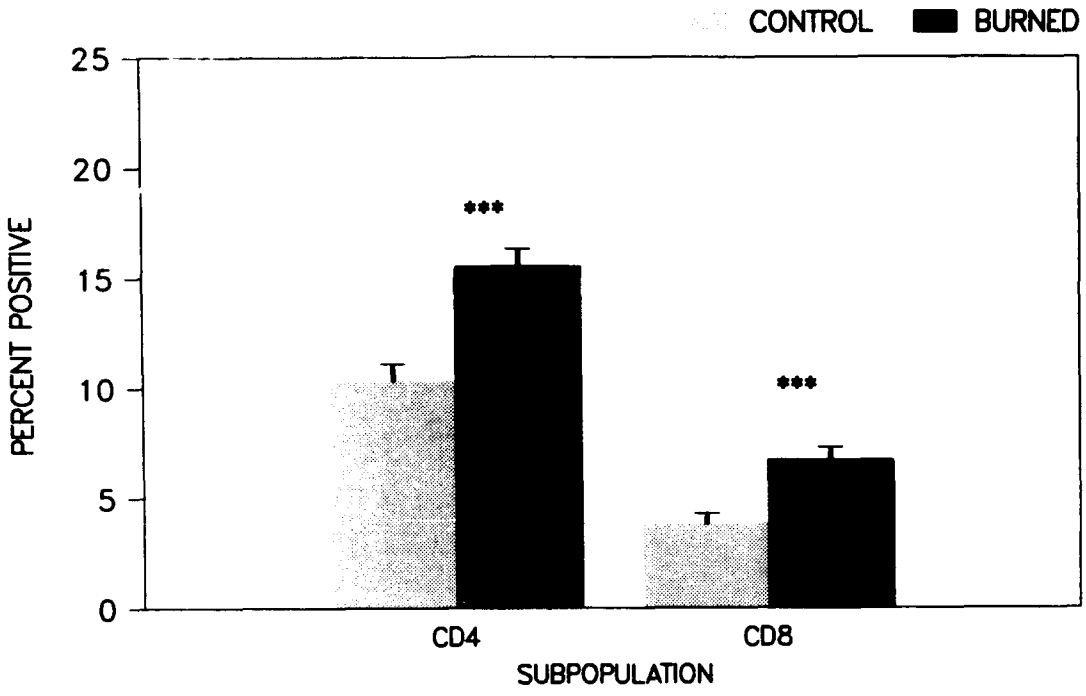

FIGURE 3. The proportion of IL-2 positive peripheral blood T-lymphocyte subpopulations from burned patients and control subjects. The percentage of subpopulation positive lymphocytes that are also IL-2 positive are expressed as mean percent ± 1SEM of that subpopulation. CD4 indicates helper/inducer and CD8, suppressor/cytotoxic. *P < 0.05, **P < 0.01, ***P < 0.001.

mitogenic capacity of the T-cell subpopulations, both the helper/inducer and the suppressor/cytotoxic subpopulations, was greatly reduced in burned patients. In contrast, the NK cell and B cell mitogenic capacity seem unaffected by thermal injury.

DISCUSSION

Burned patients are normally considered immunosuppressed because they have decreased cellular immune responses. They are unable to reject skin grafts and respond to delayed hypersensitivity antigens in vivo (7-13), and lymphocytes from burned patients have a corresponding decrease in response to mitogen and other T-lymphocyte function assays in vitro (14,15). It is generally assumed that these depressed responses are related to the high susceptibility of burned patients to infection. The decreased responsiveness could be due to a passive defect in functionality of the effector cells of the immune system, active suppression by regulator cells, or a combination of both. Some

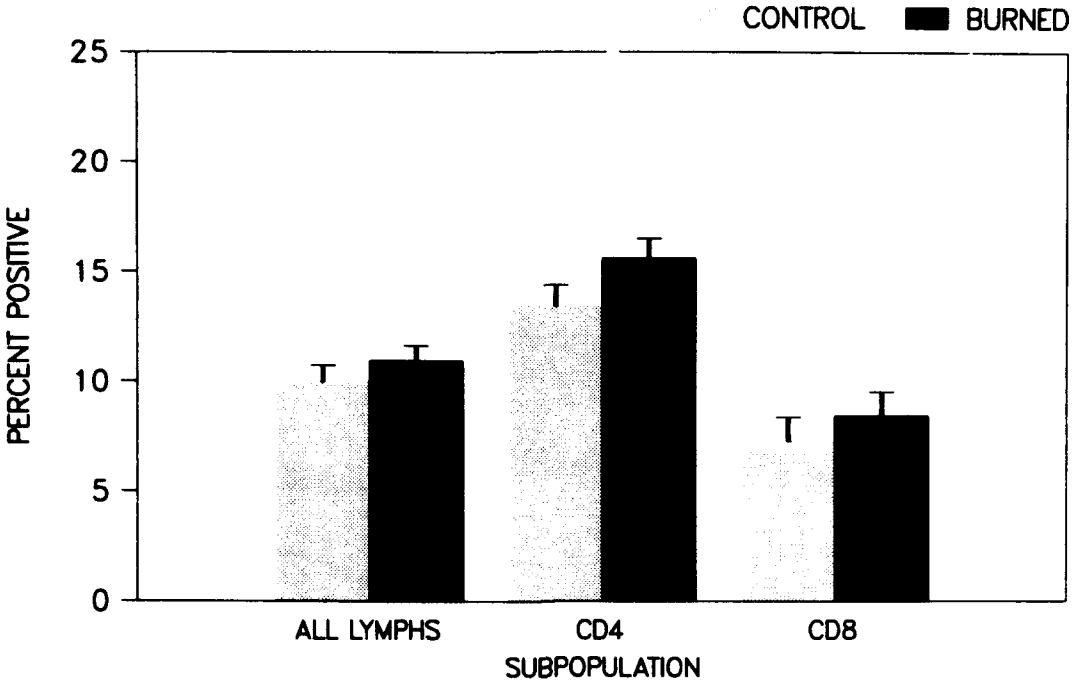

FIGURE 4. The proportion of IL 2 positive T lymphocytes after 24 h culture without ConA. The percentage of IL-2 positive lymphocytes determined for each subpopulation is expressed as mean percent ± 1SEM of that subpopulation. ALL LYMPHS indicates all lymphocytes; CD4, helper/inducer; and CD8, suppressor/cytotoxic.

authors have suggested that suppressor cells in the CD8 subpopulation may be activated in burned patients (1-4). If there was active suppression by cells in this subpopulation, then it was reasonable to assume that they might express IL-2R.

We have previously reported that there is a decrease in the number and the proportion of circulating T lymphocytes (both CD4 and CD8) and NK cells but not B lymphocytes (16,17). The data in the present report suggests that there is an increased proportion of activated cells in both the CD 4 (10% vs. 15%) and CD8 (5% vs. 10%) subpopulations as well as NK cells (8.8% vs. 14.5%) in the circulation of burned patients than in unburned control subjects. Yet after stimulation by ConA for 24 h, burned patient T-lymphocyte subpopulation expression of IL-2R is greatly decreased. This limited functional capacity is evident for both T lymphocyte subpopulations but not for NK cells.

The increased IL-2R expression in unstimulated circulating lymphocytes and the decreased capacity to express IL-2R after stimulation would represent evidence for the active suppression mechanism if the activated IL-2 expressing CD4 lymphocytes are

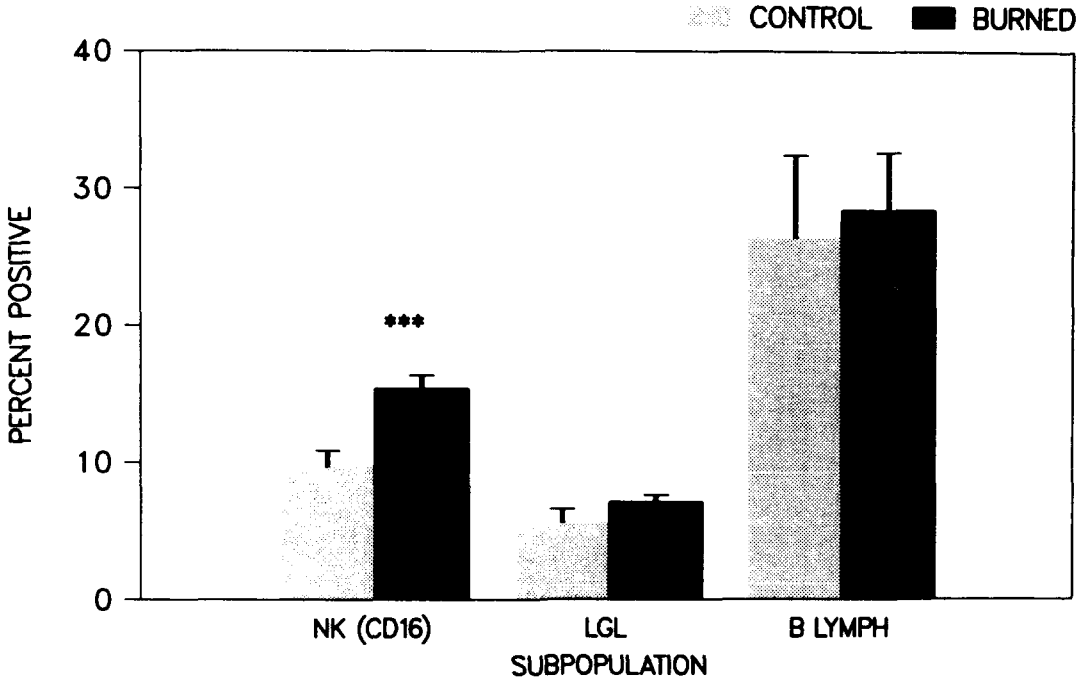

FIGURE 5. The proportion of IL-2-positive non-T lymphocytes after 24 h culture without ConA. The percentage of IL-2-positive lymphocytes determined for each subpopulation is expressed as mean percent ± 1SEM of that subpopulation. NK (CD16) indicates natural killer cells; LGL, large granular lymphocytes; and B LYMPH, B lymphocytes.

"suppressor-inducer" T lymphocytes and if the activated IL-2 expressing CD8 lymphocytes are "suppressor-effector" cells. Conversely, the large number of lymphocytes that do not express IL-2R in comparison to control lymphocytes may represent evidence for either mechanism. That is, the activated cells isolated from the circulation could exert a suppressive influence on the cells while in culture or the cells simply could not respond because they lacked an inherent capacity to respond. More definitive work is needed to distinguish between these possibilities.

PRESENTATIONS/PUBLICATIONS

None.

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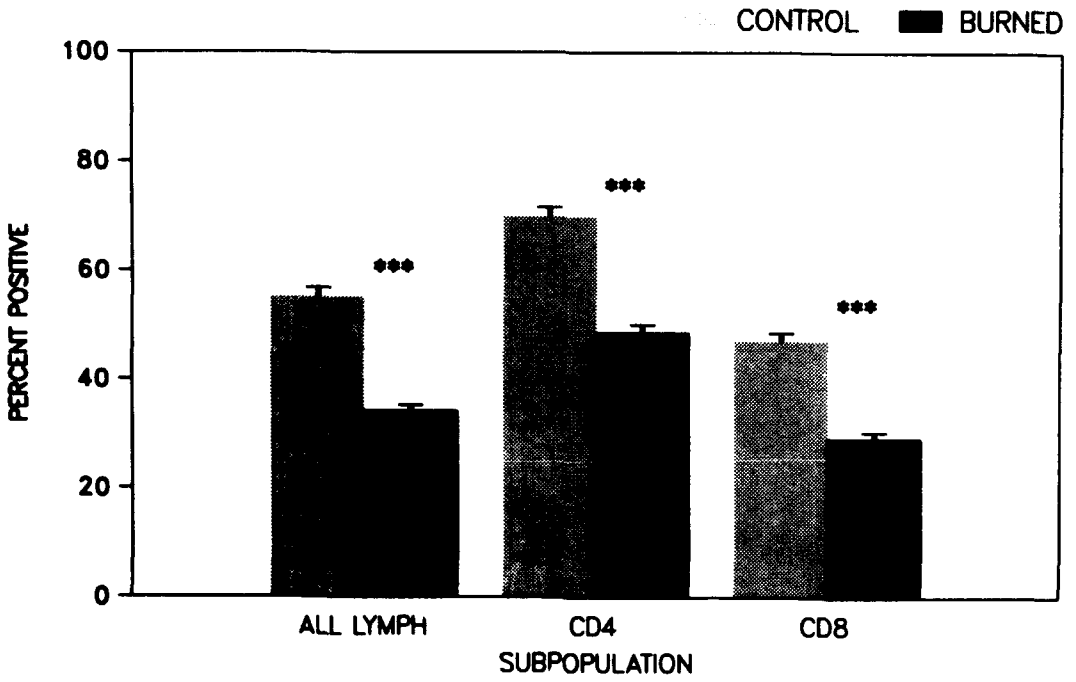

FIGURE 6. The proportion of IL-2-positive T lymphocytes after 24 h culture with ConA as mitogen. The percentage of IL-2-positive lymphocytes *determined for each* subpopulation is expressed as mean percent ± 1SEM of that subpopulation. ALL LYMPHS indicates all lymphocytes; CD4, helper/inducer; and CD8, suppressor/cytotoxic.

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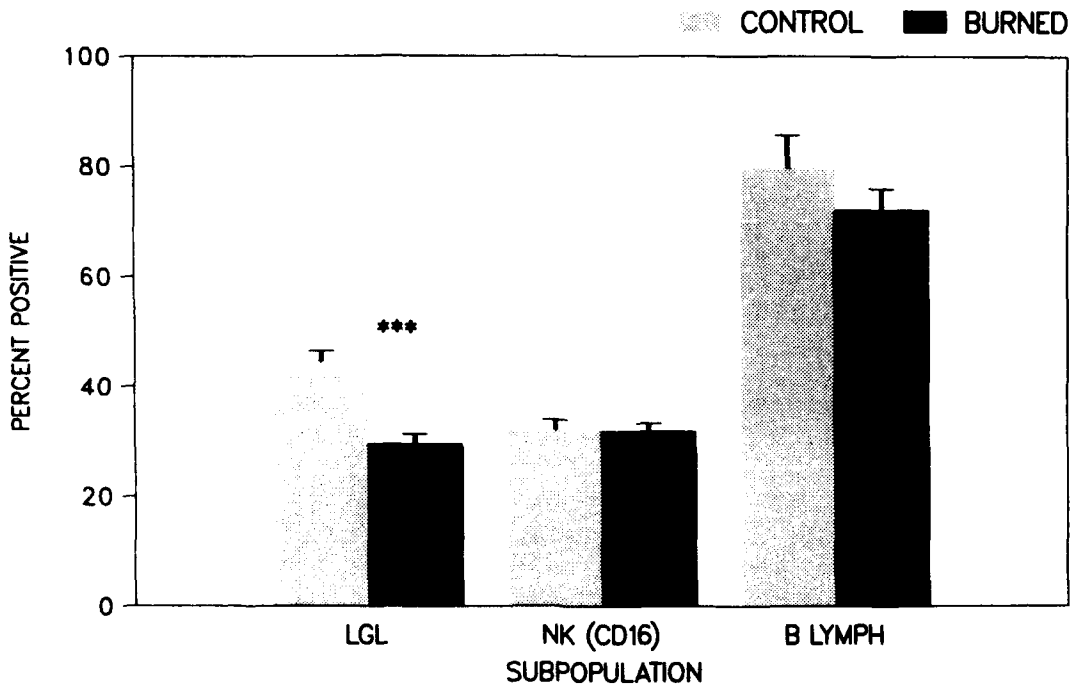

FIGURE 7. The proportion of IL-2-positive non-T lymphocytes after 24 h culture with ConA as mitogen. The percentage of IL-2-positive lymphocytes determined for each subpopulation is expressed as mean percent ± 1SEM of that subpopulation. LGL indicates large granular lymphocytes; NK (CD16), natural killer cells; and B LYMPH, B lymphocytes.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
				DA315351	1 Oct 89	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
1 Oct 88	D. Chg	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS14	DA	313
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP,	FY90-01		

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	9009	DA	C. In-House

MILITARY RELEVANCY CERTIFIED APPROVED BY [illegible]

17. CONTRACT/GRANT
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 WORKYEARS	b. FUNDS *(In thousands)*
89	1.1	55
90	1.1	55

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME: US Army Institute of Surgical Research	a. NAME: US Army Institute of Surgical Research
b. ADDRESS *(include zip code)*: Fort Sam Houston, San Antonio, Texas 78234-5012	b. ADDRESS: Fort Sam Houston, San Antonio, Texas 78234-5012
c. NAME OF RESPONSIBLE INDIVIDUAL: PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR: BROWN, W L
d. TELEPHONE NUMBER *(include area code)*: 512-221-2720	d. TELEPHONE NUMBER *(include area code)*: 512-221-4652
21. GENERAL USE: FINA	f. NAME OF ASSOCIATE INVESTIGATOR *(if available)*: MASON, A D
MILITARY/CIVILIAN APPLICATION: M	g. NAME OF ASSOCIATE INVESTIGATOR *(if available)*

22. KEYWORDS *(Precede EACH with Security Classification Code)* (U) Burn Wound Metabolism; (U) Edema; (U) Burn Injury; (U) Lab Animals: (U) Rats; (U) RA II

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

23. (U) A DTIC literature search was conducted under DTIC request number W6M37B dated 20 October 1989 for the technical report database and request number W6N22D dated 20 October 1989 for the work unit database. The proposed effort will not result in the duplication of effort. The objective of this work is to determine the biochemical and metabolic changes that occur in the *in vivo* partial-thickness rat burn wound during the early postburn period and identify criteria of reversibility. The data generated may identify means to block or reverse the metabolic changes and limit progression in the extent and severity of injury in wounds of burned soldiers.

24. (U) Microelectrodes will be used to measure changes in extracellular potassium ion content and in pH and/or carbon dioxide partial pressure at various sites in the *in vivo* burn wound. Samples from sites adjacent to the microelectrodes will be taken to measure selected metabolites using enzymatic methods. Cells and subcellular organelles will be isolated for measurement of changes in function with time postburn.

25. (U) 8810 - 8909. A variety of preparation procedures for cell suspensions from rat skin that would be suitable for measurement of cellular function in partial-thickness burn wounds were tested. The best yield of viable cells (trypan-blue impermeable) was obtained from minced tissue incubated first in trypsin solution followed by washing and subsequent incubation in a solution of collagenase and hyaluronidase. Biopsy samples of wound tissue were obtained from areas adjacent to the area in which microelectrode measurements were made and blood samples were obtained for determination of changes in the levels of selected metabolites with time postburn. For technical reports, refer to they *US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1989.*

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ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3M161102BS14-00, BASIC RESEARCH

PROJECT TITLE: A Study of Biochemical Changes in the Cellular Environment of the in vivo Partial-Thickness Rat Burn Wound

US ARMY INSTITUTE OF SURGICAL RESEARCH
FORT SAM HOUSTON
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1 October 1988 - 30 September 1989

INVESTIGATORS

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

ABSTRACT

PROJECT NUMBER: 3M161102BS14-00, BASIC RESEARCH

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

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

A variety of methods for preparing cell suspensions from different types of skin was tested to find an adaptation suitable for isolating cells from sham and partial-thickness burn wound tissue of adult rats for measurement of cellular function. The best yield of viable cells (trypan-blue impermeable) was obtained from minced tissue incubated first in trypsin solution followed by washing and subsequent incubation in a solution of collagenase and hyaluronidase.

Procedures were also adapted to quick-freeze tissue and extract metabolites from blood and wound tissue of rats with sham and partial-thickness burns for measurement of ATP and lactate using enzymatic methods. Preliminary results showed that the content of ATP decreased and of lactate increased in burn wound tissue during the first 24 h postburn. The content of ATP in blood of burned rats showed little change whereas lactate content varied around normal values during that time.

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

In a previous report, we described procedures that we had used in an attempt to isolate viable cells from sham and partial-thickness burned skin of adult rats for use in studies of cellular metabolic activity (1). Technics reported in the literature describing isolation of cells from human, pig, newborn and adult mice, and neonatal rat skin indicated that epidermal-dermal layers could be selectively separated following incubation of skin with the proper enzymes (2). Treatment of skin with trypsin results in separation of skin above or through the basal cell layer, leaving most of the basal cells attached to the dermis. Following treatment of skin with collagenase, separation occurs at the epidermal-dermal junction. The layers can then be teased apart using tweezers or can be scraped off using a sharp scalpel.

The surface layer of normal adult rat skin could not be separated following incubation with trypsin alone. Following subsequent incubation in collagenase/hyaluronidase solution, the epidermal layer of normal skin could be completely removed by scraping the surface with a scalpel.

Following incubation of burned adult rat skin with trypsin or with trypsin followed by collagenase/hyaluronidase, the epidermal layer could be easily removed by scraping with a scalpel. It should be noted, however, that histological examination of untreated burned skin revealed multifocal separation of the epidermal and dermal layers as early as 5 h postburn (PB). Scraping did cause some disruption in the architecture of the burned skin, especially the hair follicles and the sebaceous glands. Such changes were minimal in the scraped sham-burn skin.

Once the cell preparations had been separated from the debris and washed well in calcium-free Hepes balanced salt buffer solution, they showed a tendency to aggregate and could not readily be redispersed, making them difficult to count or to use in metabolic studies. Klein-Szanto showed that the use of ice-cold, calcium-free medium supplemented with 10% fetal bovine serum was essential to prevent aggregation of isolated skin cells (3). We have now modified our previously used procedure to include 10% fetal bovine serum in the solutions used to wash and to store the isolated skin cells. In addition, Percoll® solutions (Pharmacia, Piscatawy, NJ) used for density gradient centrifugation of the cells will now be supplemented with 10% 10X calcium-free balanced salt solution and 10% fetal bovine serum. A much sharper separation of the different types of cells was achieved using this Percoll® solution than in the one diluted with saline which we had originally used for the density gradient centrifugation. The cell fractions can be washed free of the fetal bovine serum, if

necessary, before they are transferred to the appropriate substrate for metabolic studies.

We also have had to adapt procedures to be used to quick-freeze and extract metabolites from small samples of rat sham and burn wound tissue, which is difficult to homogenize, from methods developed for use with large samples of liver which are easily frozen in situ and are easy to disrupt. Several variations of the liver procedures are described in *Bergmeyer's Methods of Enzymatic Analysis* (4).

Rather than using "quick-freeze" tongs to freeze the wound sample, we removed the samples with a 4-mm stainless steel, hollow-bore drill bit driven by a high-speed pneumatic drill (Model 950, Alko Diagnostics Corp, Holliston, MA) through which a continuous vacuum drew the tissue sample into a container of 2-methyl butane (-150°C) chilled with liquid nitrogen, where it was instantly frozen. The frozen samples were transferred to chilled tubes, tightly capped, and placed in liquid nitrogen until they were stored in a freezer at -80°C.

Our attempts to homogenize the frozen wound tissue PCA using glass homogenizers, ultrasonic cell disruptors, and a Polytron™ homogenizer with smooth-tipped generators were unsuccessful. Powdering the frozen tissue in liquid nitrogen in a mortar was very time-consuming and inconvenient for handling the large number of small samples we were planning to analyze. We obtained the best disintegration of the wound tissue samples using a Polytron™ PT10/35 homogenizer with PT 20s or PT 10s saw-toothed generators (Brinkmann Instruments, Inc., Westbury, NY) with the samples contained in a close-fitting tube.

For the typical sample which weighed 150-250 mg, the frozen sample was added to a 16 X 176-mm thick-walled polyallomer tube that contained 1 ml ice-cold 6% (w/v) PCA containing 1 mM/l EDTA. The samples were kept ice-cold throughout the extraction procedure. The PT 10s generator, which had been chilled in crushed ice, was placed in the tube and run at setting 5 for 15 sec. A second milliliter of PCA was added to the tube, allowed to cool in ice for 1 min, and the homogenizer was again run at setting 5 for 15 sec. Typically this was sufficient to totally disrupt the tissue. If not, the homogenization was repeated for another 15 sec. The tubes were then held in an ice-salt bath for 30 min to allow denaturation and aggregation of the proteins before they were centrifuged in the cold at 5000 g for 20 min. The supernates were decanted into 12 X 75-mm polypropylene tubes, tightly capped, and placed in liquid nitrogen until they were transferred to a freezer at -80°C for storage.

Blood samples, obtained by cardiac puncture, were immediately mixed with an equal volume of 12% TCA, capped, held in an ice bath for 5 min, frozen in liquid nitrogen, and stored in the freezer.

They were not centrifuged until just before they were to be analyzed.

Enzymatic methods using commercially prepared reagent kits (Sigma Chemical Company, St. Louis, MO) were used to measure ATP (5) and lactate (6) in the blood and tissue extracts.

The extracts to be used for ATP measurements must be thawed in cold water and kept in an ice-salt bath until analyzed. No more tubes should be thawed at one time than one can analyze in an hour. Lactate content in the extracts remained stable for several hours in the cold.

Preliminary results show that blood ATP in burned rats varies slightly around normal values during the first 48 h PB but is not significantly decreased. Burn wound ATP content decreased slightly at 1 h PB before decreasing to 20-30% of sham values during the later postburn period. Lactate content of blood of burned rats decreased during the first 24 h PB but was equal to that of sham rats at 48 h PB. Lactate content of burn wound was approximately one and one-half times that of sham wound from 3 to 18 h PB but decreased to 60% of sham values at 24 and 48 h PB. We are currently collecting and processing more samples for ATP and lactate measurements and extending the time period of the study to 7 days PB.

PRESENTATIONS/PUBLICATIONS

None.

REFERENCES

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL DD-DR&E(AR) 636
				DA315353	1 Oct 89	
3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
1 Oct 88	D. Chg	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS14	F	314
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP	FY90-01		

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	9009	DA	C. In-House

17. CONTRACT/GRANT MILITARY RELEVANCY CERTIFIED APPROVED BY

a. DATE EFFECTIVE		RESOURCES ESTIMATE		
b. CONTRACT/GRANT NUMBER		FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS *(In thousands)*
c. TYPE	d. AMOUNT	89	0.4	27
e. KIND OF AWARD	f. CUM/TOTAL	90	0.4	30

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	f. NAME OF ASSOCIATE INVESTIGATOR *(If available)*
MILITARY/CIVILIAN APPLICATION: M	g. NAME OF ASSOCIATE INVESTIGATOR *(If available)*

22. KEYWORDS *(Precede EACH with Security Classification Code)* (U) Interleukin-2; (U) Pseudomonas; (U) Sepsis; (U) Burns; (U) Lab Animals; (U) Rats; (U) RA II

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

22. (Continued) (U) Pseudomonas; (U) Sepsis; (U) Burns; (U) Lab Animals: (U) Rats; (U) RA II

23. (U) A DTIC literature search was conducted under DTIC request number W6N47A dated 20 October 1989 for the technical report database and request number W6N48A dated 20 October 1989 for the work unit database. The proposed effort will not result in the duplication of effort. The objective of this work is to study the effects of exogenous IL-2 administration following burn wound infection in a rodent model.

24. (U) Due to the failure of IL-2 to improve survival in this infection model, the study has been extended to evaluate the effects of indomethacin, gamma interferon, and other cytokines on IL-2R expression. The ability to enhance IL-2R expression may allow one to reverse T cell abnormalities with the exogenous administration of IL-2.

25. (U) 8810 - 8909. The protocol for measuring IL-2R expression in rodent splenocytes and peripheral blood mononuclear cells was developed. The study will now be continued to assess the ability of indomethacin, PGE_2, and gamma interferon to improve IL-2R expression in thermally injured rats. For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1989*.

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

ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3M161102BS14-00, BASIC RESEARCH

PROJECT TITLE: The Effect of Interleukin 2 (IL-2) Administration on Mortality to Rats with Pseudomonas Burn Wound Sepsis

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

1 October 1988 - 30 September 1989

INVESTIGATORS

William G. Cioffi, Jr., MD, Major, MC
Bryan S. Jordan, RN, MS
David G. Burleson, PhD, Lieutenant Colonel, MS
Brian C. Rakestraw, Staff Sergeant
Basil A. Pruitt, Jr., MD, Colonel, MC

ABSTRACT

PROJECT NUMBER: 3M161102BS14-00, BASIC RESEARCH

PROJECT TITLE: The Effect of Interleukin 2 (IL-2) Administration on Mortality to Rats with Pseudomonas Burn Wound Sepsis

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

PERIOD COVERED IN THIS REPORT: 1 Oct 88 through 30 Sep 89

INVESTIGATORS: William G. Cioffi, Jr., MD, Major, MC
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Decreases IL-2 production, activity, and receptor (IL-2R) expression have been described following injury. Previous investigators have reported increased survival following pretreatment with recombinant IL-2 in secondary infection, thermal injury models. Attempts to improve survival in a rat invasive burn wound infection model have been unsuccessful. Not only is IL-2 production decreased following injury, but the presentation of IL-2R is also altered. PGE_2 production from macrophages is increased following thermal injury and these elevated levels have been shown to decrease IL-2 production and possibly decrease IL-2R expression on T cells. The current studies were designed to investigate the role of PGE_2 on T-cell subpopulations and IL-2R expression following thermal injury. Preliminary data indicate 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 (IL-2) 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 IL-2 (T cell growth factor) (4) is produced by T helper cells. The stimulus for this IL-2 production comes from circulating IL-1, which is itself released from macrophages when they are exposed to foreign antigens (5). The IL-2 then binds to specific IL-2 receptors (IL-2R) to promote the proliferation of T lymphocytes, regardless of their antigenic specificity (6). The number of IL-2R, the amount of IL-2 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. IL-2 production has been shown to be significantly reduced postburn and returns to normal only in those patients who eventually survive (5). IL-2R are also decreased on T cells following thermal injury (7). This has been related to the ability of IL-2 to modulate the expression of its own receptors.

Previous investigators have reported increased survival following pretreatment with recombinant IL-2 in secondary infection, thermal injury models. Gough et al (8) treated mice with recombinant IL-2 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 IL-2 had a 55% mortality. The splenocytes harvested from IL-2-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 IL-2 administration in a rat model of invasive burn wound sepsis (9). The failure of exogenous IL-2 to increase survival in this model may result from the fact that exogenous IL-2 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 IL-2 levels. Hancock et al (11) have shown that macrophages fail to express IL-2R in response to gamma interferon when PGE_2 is added in vitro. LaLa et al (12) have suggested that PGE can down-regulate IL-2R expression on activated T cells.

The suggestion that PGE may modulate IL-2R 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 IL-2R 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 IL-2R density following culture and stimulation as previously described (14).

RESULTS/DISCUSSION

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 IL-2R 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 IL-2R expression on splenic T cells from normal rats (Table 6).

PRESENTATIONS/PUBLICATIONS

None.

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. IL-2R Antibody Concentration (µl/ml) vs. ConA Concentration (µg/ml) for Maximal IL-2R Expression in Normal Rats

	IL-2R 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

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
				DA315354	1 Oct 89	DD-DRAE(AR) 636
3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISB'N INSTR'N	9. LEVEL OF SUM A. WORK UNIT
1 Oct 88	D. Chg	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS14	IA	315
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP	FY90-01		

11. TITLE *(Precede with Security Classification Code)* (U) Development of Thermal Ionization Mass Spectrometry Methodology for the Study of Calcium Metabolism

12. SUBJECT AREAS
0601 Biochemistry 0604 Anatomy and Physiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
8805	9009	DA	C. In-House

17. CONTRACT/GRANT MILITARY RELEVANCY CERTIFIED APPROVED BY Samuel A. Truitt

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)*
89	0.3	21
90	0.3	22

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 MILITARY/CIVILIAN APPLICATION: M	f. NAME OF ASSOCIATE INVESTIGATOR *(if available)*: VAUGHAN, G M g. NAME OF ASSOCIATE INVESTIGATOR *(if available)*: OKERBERG, C V

22. KEYWORDS *(Precede EACH with Security Classification Code)* (U) Burns; (U) Mass Spectrometry; (U) Metabolism; (U) Homeostasis; (U) Lab Animals; (U) Rats; (U) RA II

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

23. (U) A DTIC literature search was conducted under DTIC request number W6N55C dated 20 October 1989 for the technical report database and request number W6N56B dated 20 October 1989 for the work unit database. The proposed effort will not result in the duplication of effort. The objective of this work is to develop thermal ionization mass spectrometry techniques to investigate the homeostatic mechanisms of calcium regulation after thermal injury. Methodology developed from this study will be applied to clinical studies of calcium metabolism in thermally injured soldiers.

24. (U) Male Sprague-Dawley rats will be used as a burn model to study the effect of burn injury on calcium homeostasis. Stable isotopes of calcium will be used to perform calcium kinetic analyses after burn injury. Total fecal and urine excretion will be collected to perform calcium balance calculations. The combined data from the kinetic analyses and the balance calculations will be analyzed using a mathematical modeling computer program to determine the effect of burn injury on the various aspects of calcium metabolism.

25. (U) 8810 - 8909. Modifications of the mass spectrometer have been completed. Methods have been developed to determine natural abundance of calcium stable isotopes in standard solutions. Work will proceed upon delivery of enriched standards of stable calcium isotopes that will enable us to spike standards and biological samples. For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1989.*

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ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3M161102BS14-00, BASIC RESEARCH

PROJECT TITLE: Development of Thermal Ionization Mass Spectrometry (TIMS) Methodology for the Study of Calcium Metabolism in a Burned Rat Model

US ARMY INSTITUTE OF SURGICAL RESEARCH
FORT SAM HOUSTON
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1 October 1988 - 30 September 1989

INVESTIGATORS

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Avery A. Johnson, BS
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ABSTRACT

PROJECT NUMBER: 3M161102BS14-00, BASIC RESEARCH

PROJECT TITLE: Development of Thermal Ionization Mass Spectrometry (TIMS) Methodology for the Study of Calcium Metabolism in a Burned Rat Model

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

PERIOD COVERED IN THIS REPORT: 1 October 1988 - 30 September 1989

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DEVELOPMENT OF THERMAL IONIZATION MASS SPECTROMETRY (TIMS) METHODOLOGY FOR THE STUDY OF CALCIUM METABOLISM IN A BURNED RAT MODEL

Hypocalcemia (6-7 mg/100 ml) is a consistent observation in patients with burns > 30% of the total body surface area (1). This sequel may be due to a number of reasons. Lennquist et al (2) reported low serum calcium in burn patients which correlated with serum albumin; serum calcium/albumin ratios were always within normal range. Szyfelbein et al (3) found good correlation of total serum calcium to total protein. Ionized calcium was depressed and showed a poor correlation to total protein. Ionized calcium remained depressed even when serum calcium and total protein were restored to normal levels. Intercellular calcium sequestering in burned muscle tissue has been reported by a number of investigators (4-6). Transeschar calcium leaching, which may occur in patients treated with silver nitrate soaks, has been reported to lower the serum calcium level to a point where symptoms occur (7). Abnormalities may exist in the secretion and metabolic function of calcium-regulating hormones, calcitonin and parathyroid hormone, in the postburn period. Loven et al (8) and Lennquist et al (2) have reported that low serum ionized calcium levels persist in burn patients, even though serum concentrations of calcitonin are elevated.

Despite numerous reports concerning abnormalities in calcium metabolism, the mechanisms involved are ill-defined. Recent technological advances in TIMS have made it practical to use stable isotope methodology to study the dynamics of calcium metabolism in biological systems (9-10).

MATERIALS AND METHODS

Study Design. Thirty-six male Sprague-Dawley rats weighing 250-300 g will be individual housed in stainless steel hanging cages and observed for 1 week prior to entry into the study to exclude the presence of any preexisting diseases. Twelve animals will be used to develop techniques. The remaining 24 animals will be divided into two groups (n=12), control and burn. A semipurified diet designed to meet all known nutrient requirements of the adult rat and distilled deionized water will be fed ad libitum. After a 2-week equilibration period on this diet, the animals will be anesthetized with sodium pentobarbital (35 mg/kg IP), the dorsal surface will be shaved, and a 30% total body surface area scald or sham burn will be administered. For animals in the burn group, the dorsal area will be exposed to 100°C water for 10 sec. Animals in the control group will be handled in the same manner as those in the burn group, but they will exposed to water at room temperature. All animals will be placed back in their cages and allowed to recover from anesthesia without resuscitation. A pair-feeding regimen will be used from the day of

burn injury until final disposition of the animals. Control animals will be fed the amount consumed by a weight-matched burned rat during the previous 24-h period. Blood samples will be taken daily for 3 days from the tail vein with no anesthesia while the animals are restrained in a tube-type restraint device. Ten days postburn, the animals will be administered two isotopes of calcium, one given orally (^{44}Ca, 0.5 mg/kg) and the other intravenously (^{42}Ca, 0.1 mg/kg). Blood samples (300 μl) will be collected at 0.5, 1, 2, 4, 8, 12, 24, and 48 h following isotope administration. At 72 h, the animals will be reanesthetized with sodium pentobarbital (35 mg/kg IP). A ventral laparotomy will be performed and the animal will be exsanguinated via the caudal vena cava. Calcium will be extracted from the serum using ammonium oxalate precipitation, and the ratios of the calcium isotopes will be measured using TIMS (10). Feces and urine will be collected over the total 72-h study period. Serum samples collected at 72 h will be analyzed for total calcium, albumin, total protein, calcitonin, and parathyroid hormone. Aliquots of the feces and urine collections will be analyzed for total calcium and for the ratio of the stable calcium isotopes. Mathematical modeling procedures will be accomplished as outlined by Moore et al (9). The experimental design allows for determination of net endogenous calcium fecal excretion, net dietary calcium absorption, calcium balance, and bone calcium deposition and resorption.

Determination of Number of Animals Required. Twelve animals will be necessary for refinement of a number of techniques associated with the TIMS. Twenty-four animals will be needed for the study, 12 for the control group and 12 for the burn group.

Data Analysis Plan. Significant differences between the control and burn groups for the various parameters will be accomplished using the student's t test ($P < 0.05$).

RESULTS

Modifications of the MS have been completed and methods have been developed to determine natural abundance of calcium stable isotopes in standard solutions.

DISCUSSION

Work will proceed upon delivery of enriched standards of stable calcium isotopes that will enable us to spike standards and biological samples.

PRESENTATIONS/PUBLICATIONS

None.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION DA315355	2. DATE OF SUMMARY 1 Oct 89	REPORT CONTROL SYMBOL DD-DR&E(AR) 636
3. DATE PREV SUM'RY 1 Oct 88	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	CA	316
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP,	FY90-01		

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	9009	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)*
89	0.2	7
90	0.2	8

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	f. NAME OF ASSOCIATE INVESTIGATOR *(If available)* g. NAME OF ASSOCIATE INVESTIGATOR *(If available)*

22. KEYWORDS *(Precede EACH with Security Classification Code)* (U) Inhalation Injury; (U) High Frequency Ventilation; (U) Ventilation-Perfusion Ratio; (U) Cardiac Output; (U) Lab

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

22. (Continued) (U) Animals: (U) Sheep; (U) RA II

23. (U) A DTIC literature search was conducted under DTIC request number W6003E dated 20 October 1989 for the technical report database and request number W6006A dated 20 October 1989 for the work unit database. The proposed effort will not result in the duplication of effort. The objective of this work is to compare the effects of volumetric diffusive ventilation and conventional ventilation on pulmonary and hemodynamic indices which are altered in an ovine inhalation injury model.

24. (U) Inhalation injury will be induced using the standard ovine smoke inhalation model developed at this Institute. Animals will be randomized to treatment with either conventional or high frequency ventilation. Changes in V_A/Q as well as other pulmonary and physiologic measurements will be compared between groups.

25. (U) 8810 - 8909. The multiple inert gas elimination technique was revalidated and 8 animals with inhalation injury and 6 normal control animals were studied during this reporting period. For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1989.*

DD FORM 83 MAR 1498 — EDITION OF MAR 68 IS OBSOLETE. — * U.S.G.P.O.: 1988 -491-083/50329

ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3M161102BS14-00, BASIC RESEARCH

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

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

1 October 1988 - 30 September 1989

INVESTIGATORS

William G. Cioffi, Jr., MD, Major, MC
Bryan S. Jordan, RN
Avery A. Johnson, MS
Basil A. Pruitt, Jr., MD, Colonel, MC
Arthur D. Mason, Jr., MD

ABSTRACT

PROJECT NUMBER: 3M161102BS14-00, BASIC RESEARCH

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

PERIOD COVERED IN THIS REPORT: 1 Oct 88 through 30 Sep 89

INVESTIGATORS: William G. Cioffi, Jr., MD, Major, MC
Bryan S. Jordan RN
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Arthur D. Mason, Jr., MD

Severe inhalation injury has been shown to cause hypoxia, hypercarbia, and a shift of V_A/Q to the left, i.e., increase in segments with $V_A/Q > 0$ but < 1. Attempts to alter these derangements with conventional ventilation 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.

Initial studies were performed on normal sheep. High frequency ventilation (HFV) had no apparent effect on PO_2, hemodynamic parameters, to include heart rate, mean blood pressure, cardiac index, and LVSW index or calculated shunt (Qs/Qt). Proximal airway peak inspiratory pressure was significantly lower in HFV animals. Analysis of V_A/Q utilizing the multiple inert gas elimination technique (MIGET) in 2 animals revealed a trend towards increased blood flow to high V_A/Q lung segments, with V_A/Q between 1 and 10. HFV was then compared to conventional ventilation in a series of smoke-injured sheep. Two sheep with mild injury, 2 with moderate injury, and 1 with severe injury were studied. HFV resulted in a lower arterial PO_2 level, a lower heart rate, and a lower Qs/Qt in most animals, despite maintaining the same ventilation as indexed by arterial PCO_2. MIGET analysis of the V_A/Q data showed that HFV resulted in increased true shunt in 3 out of 4 animals studied, decreased blood flow to normal V_A/Q areas, and increased blood flow to low V_A/Q areas. The fact that HFV failed to maintain oxygenation in this model when compared to conventional ventilation may be a reflection of the model and physiologic differences between sheep and man, as clinical experience with this ventilator does not support these data.

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 (2).

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, Wagner (3) 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 (4). 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 positioned prone and conventional mechanical ventilation was continued with a volume-limited ventilator (Bear II™, Bear Medical Systems, Inc., Riverside, CA). Ventilator settings were altered to maintain a pH between 7.35 and 7.40 and a PO_2 between 80 and 100 mmHg. Lactated Ringer's was constantly infused at a rate of 1 ml/kg/h. CVP and pulmonary artery pressure (PAP) were monitored with Statham P23Db transducers (Statham Instruments, Oxnard, CA) and systemic artery pressures with a Hewlett-Packard 1290A quartz transducer (Hewlett-Packard Company, Waltham, MA). Transpulmonary pressure was monitored by a differential transducer (MP-451, Valadine Engineering Corporation, Northridge, CA). Inspiratory and expiratory gas concentration (N, O_2, and CO_2) were monitored by a medical gas analyzer (MGA-1100, Perkin Elmer). Percutaneous O_2 saturation and PO_2 were continuously monitored.

Heart rate, blood pressure, CVP, PAP, cardiac output, arterial blood gases, tidal volume, flow rates, transpulmonary pressures, and O_2 saturation were measured every 30 min. Once the ventilator settings were maximized yielding a PO_2 between 80 and 100 mmHg and a pH between 7.35 and 7.40, the animal was allowed to stabilize for 2 h. V_A/Q distributions were then measured 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 volumetric-diffusive ventilator. Ventilator settings were made to maintain a pH between 7.35 and 7.40 and a PO_2 between 80 and 100 mmHg. Cardiopulmonary parameters were then measured every 30 min. 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 was compared utilizing the student's t test.

RESULTS

Technical problems with the MIGET have been resolved since the last reporting period. Because earlier experience with this ventilator in smoke-injured sheep indicated the inability of this form of HFV to adequately oxygenate severely injured sheep, 7 normal sheep were instrumented and studied comparing conventional ventilation with room air and 0 PEEP to HFV with room air and 0 PEEP. Conventional ventilation tidal volumes were set at 18 ml/kg and the ventilatory rate was adjusted in an attempt to maintain a PCO2 between 30 and 35 torr. After the study period was completed, the sheep were switched to HFV at a high frequency rate of 10 Hz with a 2-sec inspiratory time and a flow interruption rate set at two less than the conventional ventilator rate. Proximal PAP was then adjusted in an attempt to maintain the PCO_2 the same as that obtained using conventional ventilation. Table 1 contains arterial blood gas and hemodynamic data for 7 sheep for both conventional ventilation and HFV. Ventilation, oxygenation, and hemodynamic variables, to include heart rate, mean systemic blood pressure, cardiac index, LVSW index, and Os/Qt were not different between the two forms of ventilation. The proximal peak inspiratory pressure was statistically lower for HFV. Data were analyzed using the paired t test. Table 2 contains V_A/Q data obtained utilizing the MIGET for 2 normal sheep. No distinct differences could be seen between the conventional ventilation or HFV, although a trend towards increased blood flow to higher low V_A/Q segments (V_A/Q = 1-10) was noted. Table 3 shows a summary of V_A/Q distribution for 2 normal sheep. With n=2, no statistical conclusions could be drawn. However, in both animals, the mean blood flow on a log scale was similar, irrespective of type of ventilation, while the SD of blood flow on a log scale was increased by the application of HFV. The mean ventilation on a log scale as well as the SD of ventilation on a log scale were increased when HFV was applied.

Arterial blood gas and hemodynamic data for 5 smoke-injured sheep are presented in Table 4. Conventional ventilation was first established at a tidal volume of 18 ml/kg, an FIO_2 of 0.21, a PEEP of 0, and a rate to maintain the PCO_2 at approximately 30 torr.

TABLE 1. Arterial Blood Gas and Hemodynamic Data in Normal Sheep (Conventional Ventilation/High Frequency Ventilation)

Animal Number	pH	PCO^2	PO^2	PIP*	HR	$\bar{x}$BP	CI	LVSW Index	Qs/Qt
1	7.51/7.45	27.1/24.0	108/104	22/15	167/170	113/110	4.80/4.30	39.1/39.0	18.6/17.8
2	7.41/7.43	23.5/24.0	102/103	22/24	140/129	122/120	2.99/3.06	33.9/36.1	7.1/4.3
3	7.45/7.43	31.5/34.0	89/93	20/18	195/177	125/128	2.95/2.55	23.9/23.7	18.6/17.8
4	7.50/7.52	35.3/33.5	89/88	24/18	168/165	141/132	3.22/3.19	36.1/35.0	10.0/15.0
5	7.58/7.58	32.2/30.0	84/88	20/18	165/181	112/115	5.90/7.00	49.0/47.0	14.0/17.0
6	7.48/7.40	36.1/34.1	91/105	20/18	185/165	120/114	2.09/2.04	20.0/20.1	7.0/0.0
7	7.45/7.42	32.6/36.7	91/87	22/20	110/115	118/112	2.98/2.84	40.8/35.3	11.0/13.0

*$P < 0.05$. HR indicates heart rate; $\bar{x}$BP, mean blood pressure; CI, cardiac index; and Qs/Qt, calculated shunt.

TABLE 2. V_A/Q Data Utilizing MIGET in Normal Sheep (Conventional Ventilation/High Frequency Ventilation)

Animal Number	% Blood Flow to VA/Q Compartment					
	0	0-0.01	0.01-0.1	0.1-1	1-10	> 10
1	0.000/0.000	0.135/0.019	0.015/0.053	0.617/0.546	0.353/0.420	0.000/0.000
2	0.000/0.015	0.000/0.010	0.375/0.000	0.606/0.497	0.356/0.478	0.000/0.000

TABLE 3. V_A/Q Distribution in Normal Sheep (Conventional Ventilation/High Frequency Ventilation)

Animal Number	Mean Q	SD of Q	Mean V	SD of V
1	0.780/0.760	0.85/1.13	1.08/1.50	0.53/0.69
2	0.770/0.930	0.80/1.26	1.00/1.26	0.37/0.80

Mean Q indicates mean blood flow (l/min) on log scale; SD of Q, standard deviation of blood flow on log scale (log SDq); mean V, ventilation (l/min) on log scale; and SD of V, standard deviation of ventilation on log scale. The log SDq is calculated as the square root of the second moment about the mean on a natural log scale for compartmental blood flow in 48 V_A/Q compartments other than shunt ($V_A/Q = 0$) and dead space (V_A/Q = infinity). The log SDq is an index of dispersion of blood distribution pattern.

Upon completion of analysis on the conventional ventilator, HFV was instituted at a high frequency rate of 10 Hz, an FIO_2 of 0.21, a PEEP of 0, a flow interruption rate two less than the conventional ventilator rate, an inspiratory time of 2 sec, and a PAP equal to that of the conventional ventilator. PAP and the flow interruption rate were then altered in an attempt to maintain the PCO_2 similar to that obtained by conventional ventilation. If oxygenation was inadequate following this maneuver, inspiratory time and PAP were then increased in an attempt to improve oxygenation. This had the added effect of increasing CO_2 clearance and respiratory alkalosis. These are the standard maneuvers for increasing oxygenation for this type of ventilator once high frequency rates have reached 10 Hz.

Animals 1 and 2 were subjected to a mild smoke exposure, animals 3 and 4 to a moderate exposure, and animal 5 to a severe injury. Hemodynamic and arterial blood gas data are depicted in Table 4 and V_A/Q distribution data in Table 5 for these animals. No discernible differences in hemodynamic or arterial blood gas data were noted for animals 1 and 2. V_A/Q data available from one animal indicated an increase in blood flow to low ($V_A/Q = 0.01-0.1$) and high ($V_A/Q = 1-10$) lung segments. Animals 3 and 4, which received moderate injuries, had different results when HFV was applied. Animal 3 had a marked decrease in arterial oxygenation, despite increases in peak inspiratory pressure, and inspiratory time. Calculated shunt rose from 40.3 to 74.5 upon institution of HFV. Other hemodynamic variables showed a marked increase in true shunt ($V_A/Q = 0$), indicating a marked increase in numbers of lung segments which were unventilated. True shunt increased from 4% to 27% of cardiac output. Blood flow to high V_A/Q segments was also markedly increased with the institution of HFV (17% vs. 55% of

TABLE 4. Arterial Blood Gas and Hemodynamic Data in Smoke-Injured Sheep (Conventional Ventilation/High Frequency Ventilation)

Animal Number	pH	PCO_2*	PO_2	PIP	HR*	$\bar{x}$BP	CI	LVSWI	Qs/Qt*
1	7.55/7.57	27.7/26.7	90/87	20/15	183/188	127/124	4.37/4.58	40.5/39.9	18.4/23.6
2	7.63/7.65	26.8/24.6	91/83	20/15	113/91	112/109	3.11/2.95	48.8/46.3	17.8/28.8
3	7.45/7.58	28.6/21.2	72/48	20/25	148/117	124/108	4.74/5.02	48.0/55.1	40.3/74.5
4	7.51/7.46	29.7/30.4	71/76	20/15	159/149	109/117	3.84/2.93	34.8/30.5	49.1/51.8
5	7.45/7.73	38.6/14.6	41/26	30/60	175/163	124/87	5.95/2.73	53.1/17.1	80.4/88.5

*$0.05 < P > 0.1$. HR indicates heart rate; $\bar{x}$BP, mean blood pressure; CI, cardiac index; and Qs/Qt, calculated shunt.

TABLE 5. V_A/Q Distribution in Smoke-Injured Sheep (Conventional Ventilation/High Frequency Ventilation)

Animal Number	% Blood Flow to V_A/Q Compartment					
	0	0–0.01	0.01–0.1	0.1–1	1–10	> 10
2	0.000/0.000	0.032/0.028	0.064/0.276	0.669/0.165	0.232/0.510	0.002/0.022
3	0.040/0.272	0.032/0.000	0.000/0.000	0.746/0.160	0.173/0.550	0.008/0.018
4	0.000/0.052	0.141/0.144	0.065/0.160	0.634/0.452	0.154/0.190	0.004/0.000
5	0.393/0.550	0.238/0.045	0.044/0.000	0.126/0.000	0.160/0.214	0.000/0.189

cardiac output). Animal 4 demonstrated maintenance of arterial oxygenation upon the institution of HFV, without changes in other hemodynamic parameters or calculated shunt. Analysis of V_A/Q data, however, showed mild changes in the distribution of cardiac output in various segments of the lung. Increased blood flow was noted for both low and high V_A/Q segments when compared to conventional ventilation. Animal 5, which received a severe injury, demonstrated the inability of either type of ventilator to adequately oxygenate the animal on room air. However, the HFV was markedly inferior compared to the conventional ventilator (PO_2 = 40.89 vs. 25.9 torr), despite marked increases in inspiratory pressure resulting in a severe respiratory alkalosis. Calculated shunt increased from 80.4% to 88.5% while cardiac index and LVSW index and mean systemic blood pressure were all reduced secondary to the high inspiratory pressures. Analysis of V_A/Q data indicates a significant increase in true shunt (39% vs. 55%) when the animal was switched to HFV, as well as an increase in blood flow to high V_A/Q areas (16% vs. 40% of the cardiac output).

Because the order in which ventilatory support was instituted was not randomized, animals were placed back on conventional ventilation at the conclusion of the study in an attempt to ascertain whether the injury had progressed over the time course of the study. In each instance, reinstitution of support with conventional ventilation at the settings previously used resulted in arterial blood gas levels similar to those prior to institution of HFV.

DISCUSSION

Hemodynamic and arterial blood gas data from normal sheep were remarkably similar, regardless of the type of ventilatory support utilized. However, the V_A/Q data obtained by the MIGET showed that HFV appears to increase ventilation and blood flow to high V_A/Q areas, disturbing the normal V_A/Q pattern. These alterations, however, do not result in changes in ventilation and oxygenation as indexed by arterial blood gases. When this form of HFV was utilized in smoke-injured sheep, this same trend was noted, with additional increases either in true shunt or blood flow to low V_A/Q areas, further disrupting V_A/Q distribution. Arterial PO_2 was affected only in those animals in which significant increases in shunt was noted following the institution of HFV. Attempts to improve oxygenation in the two animals which showed significantly lower arterial PO_2 were unsuccessful and resulted in marked hemodynamic compromise in the most severely injured animal.

Although the number of animals studied is small and thus no statistical conclusions can be reached, the worsening of V_A/Q distribution following institution of HFV is disturbing. Clinical experience from this Institute involving patients with severe ARDS secondary to inhalation injury and pneumonia demonstrated a marked improvement in oxygenation and ventilation in patients who were

switched to HFV from standard conventional ventilation. One possible explanation for the failure of HFV in this model may be that the strategy used in these animals for manipulation of HFV variables was inappropriate, resulting in a failure to recruit collapsed lung segments and overventilation of already expanded lung segments. This is unlikely, however, because varying strategies were used in these animals as well as previously studied animals in an attempt to improve oxygenation in smoke-injured animals without apparent benefit and often significant hemodynamic compromise. The one variable which was not manipulated in these animals which is routinely used in the clinical application of this ventilator is PEEP. It may be that application of significant levels of PEEP may have altered the V_A/Q distribution in a more favorable manner. However, previous data from this Institute utilizing PEEP with conventional ventilation in this model failed to show any benefit from application of PEEP and often demonstrated a decline in oxygenation variables and a worsening of V_A/Q distribution.

Failure of this form of HFV to support smoke-injured sheep in combination with the previously reported data of application of PEEP often worsening V_A/Q distribution may indicate a flaw in this sheep model. Clinical experience with this ventilator as well as the use of PEEP and conventional ventilation normally results in an improvement in oxygenation variables. Future plans for continued study will involve the use of high frequency percussive ventilation in other animal models and the use of other forms of HFV (high frequency oscillation) in the sheep model in an attempt to ascertain both the utility of the currently used ventilator as well as the sheep injury model.

PRESENTATIONS/PUBLICATIONS

None.

REFERENCES

1. Shimazu T, Yukioka T, Hubbard GB, et al: Inequality of V_A/Q ratios following smoke inhalation injury and the effect of angiotensin analogues. In **US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1985**. Davis CC (ed). San Antonio: Fort Sam Houston, pp 425-42 (in press).

2. Shimazu T: Acute effects of positive end-expiratory pressure (PEEP) on cardiopulmonary indices including V_A/Q ratios (unpublished data).

3. Kaisor HG, Davies NJH, Rodriguez RR, et al: Efficacy of high-frequency ventilation in presence of extensive ventilation-perfusion mismatch. **J Appl Physiol** 58:996-1004, 1985.

4. Shinozaki T, Deane RS, Perkins FM, et al: Comparison of high-frequency lung ventilation with conventional mechanical lung ventilation. Prospective trial in patients who have undergone cardiac operations. **J Thorac Cardiovasc Surg** 89:269-74, 1985.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION: DA315356	2. DATE OF SUMMARY: 1 Oct 89	REPORT CONTROL SYMBOL: DD-DR&E(AR) 636
3. DATE PREV SUM'RY: 1 Oct 88	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	DA LRRDAP	FY90-01		

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	9009	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)
89	0.6	40
90		

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	f. NAME OF ASSOCIATE INVESTIGATOR (If available): MASON, A D g. NAME OF ASSOCIATE INVESTIGATOR (If available): OKERBERG, C V

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Shock; (U) Resuscitation; (U) Hemodynamics; (U) Plasmaphoresis; (U) Fluid Replacement; (U) Albumin;

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

22. (Continued) (U) Crystalloids; (U) Lab Animals: (U) Sheep; (U) RA II

23. (U) A DTIC literature search was conducted under DTIC request number W6021F dated 20 October 1989 for the technical report database and request number W6023D dated 20 October 1989 for the work unit database. The proposed effort will not result in the duplication of effort. The objective of this work is to determine the hemodynamic consequences of controlled pure plasma loss in sheep using a method to simulate the acute burn.

24. (U) 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) 8810 - 8909. Eight animals were studied during this reporting period. Data from these 8 animals are currently being analyzed. 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. For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1989.*

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ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3M161102BS14-00, BASIC RESEARCH

PROJECT TITLE: Effects of Replacement Therapy on Hemodynamic Parameters in an Ovine Model of Controlled Pure Plasma Loss

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

1 October 1988 - 30 September 1989

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A controlled plasma loss ovine model designed to simulate the rate of intravascular plasma loss in the acute burn has been developed. Fluid replacement therapy will be correlated with the amount of plasma loss while measuring hemodynamic changes in this animal model. Preliminary studies have been completed which have demonstrated the necessity for splenectomy in this model. This standardized model will now be used to evaluate the effects of varying resuscitation formulae on a pure plasma loss model. In the near future, this model will be compared to pure plasma loss seen in an ovine model of thermal injury.

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 (3), 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 (Hewlett-Packard Model 7754A) using calibrated pressure transducers (Hewlett-Packard Model 1290A). Arterial blood gas and cardiac output by the thermodilution method (Edwards Model 9520) 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) 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 (4).

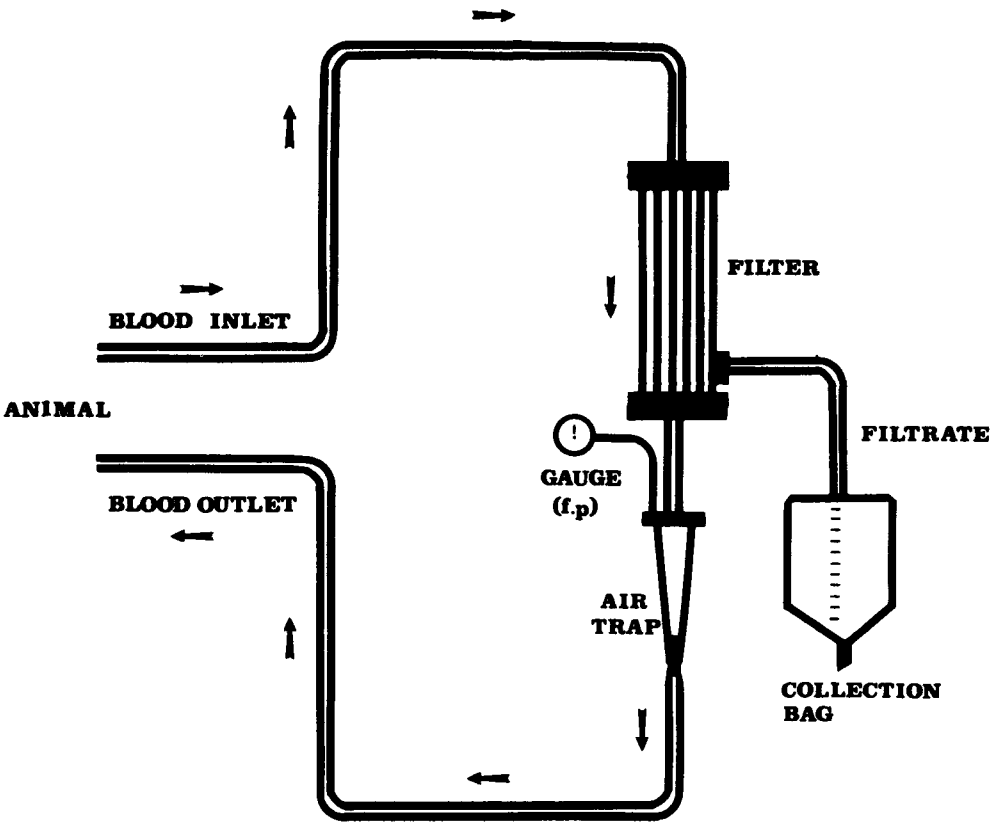

FIGURE 1. Graphic representation of the filtration circuit.

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.

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4. Pruitt BA Jr, Mason AD Jr, and 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	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
				DA315357	1 Oct 89	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
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10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
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c. CONTRIBUTING	DA LRRDAP	FY90-01		

11. TITLE *(Precede with Security Classification Code)* (U) Antibacterial and Wound Healing Effects of Silver-Nylon Electrodes with Weak Direct Current

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17. CONTRACT/GRANT MILITARY RELEVANCY CERTIFIED APPROVED BY Paul A. Trunkey

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b. CONTRACT/GRANT NUMBER		a. FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS *(In thousands)*
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19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
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c. NAME OF RESPONSIBLE INDIVIDUAL PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR CHU, C S
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22. KEYWORDS *(Precede EACH with Security Classification Code)* (U) Silver; (U) Silver-Nylon Cloth; (U) Skin Healing; (U) Skin Grafts; (U) Direct Current; (U) Burns; (U) Lab Animals;

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

22. (Continued) (U) Guinea Pigs; (U) Rats; (U) RA II

23. (U) A DTIC literature search was conducted under DTIC request number W6035C dated 20 October 1989 for the technical report database and request number W6036B dated 20 October 1989 for the work unit database. The proposed effort will not result in the duplication of effort. The objective of this work is to 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.

24. (U) 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) 8810 - 8909. In addition to effective topical antimicrobial action, direct current-conducting silver-nylon dressings were found to improve wound healing. Using a guinea pig model of scald injury, application of low amperage current reduced scar formation, reduced wound contraction, and improved hair follicle survival. Similar treatment following excision of partial-thickness scalds and autografting improved graft healing time and reduced scarring. Studies are in progress to test the effects of electrical dressing treatment on survival after excision and grafting of infected burn wounds. For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1989.*

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The time required for wound healing, contraction, and hypertrophic scarring often limit the use of deep partial-thickness burn wounds as donor sites for split-thickness grafts. We have examined the effects of weak direct current and silver-nylon dressings on the healing of partial-thickness scald burns, split-thickness grafts taken from these wounds when healed, and the resulting donor sites in a guinea pig model. Dorsal scald wounds treated with weak direct current reepithelized by 12 days postinjury. Split-thickness grafts taken from healed scald wounds showed more rapid revascularization with direct current treatment than did control grafts. Grafts and donor sites treated with direct current showed more rapid reepithelization, decreased contraction, improved hair survival, and decreased dermal fibrosis when compared to controls not treated with direct current. Only donor wounds treated with weak direct current were reusable as donor sites.

ANTIBACTERIAL AND WOUND HEALING EFFECTS OF SILVER-NYLON ELECTRODES WITH WEAK DIRECT CURRENT

The closure of skin defects by autografting was an historic advance in the treatment of burns. In patients with major burns, however, the burned area is commonly larger than the unburned surface and closure of the wounds with autograft necessitates a staged series of operations in which available donor sites must be repeatedly harvested. The healing time of these donor sites is a major factor in prolonging hospitalization. We have examined the effects of direct current applied through silver-nylon dressings (1) on the healing of experimental partial-thickness scald wounds, split-thickness grafts taken from these wounds after healing, and the resulting donor sites.

MATERIALS AND METHODS

Silver-Nylon Cloth. Silver nylon cloth (SN) (Swift Textile Metalizing Corporation, Hartford, CT) is a knit nylon fabric, which can be varied from a light weave to a heavy mesh fabric, that is coated with metallic silver to achieve a very conductive, yet flexible material (2). Based on preliminary in vitro studies, Style A-2589-5, a heavy rip-stop fabric, was selected for in vivo studies. The material weighed 84.8 g/m^2 and contained 22.6 g/m^2 of silver. All SN dressings measured 6.5 X 10.5 cm.

Animals. Two hundred and twenty male Hartley guinea pigs weighing 400 ± 25 g were anesthetized with sodium pentobarbital (35 mg/kg IP). The dorsal trunk hair was clipped and a depilatory cream (Nair™, Carter Products, New York, NY) was applied for 15 min. Partial-thickness scald wounds were inflicted by a 10-sec exposure of the depilated areas to 78°C water using a Walker-Mason burn template with a window measuring 5.5 X 10.5 cm (3). Following scald injury, animals were divided into treatment (n=180) and control (n=40) groups as described below. All animals were individually caged in plastic cages insulated from contact with the metal cage stands.

Scald Burn Healing Model. Following scald injury, SN was secured to the wounds with surgical sutures and three layers of gauze and a layer of sponge with a small polyethylene tube attached were placed over the SN dressing. A second contact point was established by adhering a 4 X 5 cm piece of SN to the shaved ventral abdominal skin with electrical-conducting gel. The dorsal SN was connected as the anode in the circuit. The gauze was then fixed in place with a flexible tubular bandage. To prevent the animals from disturbing the electrode wires and irrigation tube, the wires and tube were passed through a hole cut in a wooden tongue blade and a 4-in length of meshed wire insulator. The blade was then sutured to the back over the dressings (Fig 1). The gauze was moistened daily with 3 to 5 ml of saline through the

FIGURE 1. Locations of SN anode and cathode on scalded animal. **(A)** The dorsal dressing is sutured over the wound and connected to DC as an anode. **(B)** SN cloth coated on the underside with conductive gel is stapled to the abdomen and connected to DC as a cathode.

tube. A constant 40 μA direct current (DC) was applied for 2 days, followed by 20 μA for 3 days, using a previously described constant DC generator (4). Scald wounds in the control group were dressed as above, but no DC was applied. No dressings were applied after the 5th postburn day. Gross and microscopic comparisons of wound healing in 10 treatment animals and 2 control animals were made at 2, 3, 4, 7, and 14 days and 3 months postburn. The depth of microcirculation in the wounds of animals sacrificed at 2, 3, 4, and 7 days postburn was estimated by perfusion via a cannula placed in the superior mesenteric artery with black ink (Drawing Ink A, Pelikan AG, D-3000, Hanover, West Germany). All biopsy specimens were taken from the central area of each site, and estimation of the extent of microcirculation in the wounds was made by microscopic examination for the presence of carbon particles in the vessels.

Wound Excision and Grafting Model. When the scald wounds reepithelialized (see below) in 120 animals randomized to the treatment group and 20 animals randomized to the control group, 0.015-in split-thickness grafts were taken using a Padgett™ electrodermatome. Each graft was divided in half and the anterior portion of the graft bed was covered with the posterior half of the graft. The anterior portion of the graft was discarded. Each animal thus had an anterior autografted wound segment and an open posterior donor site segment of equal size. A diagram of the model is presented in Figure 2. Animals were then dressed as above with those in the treatment group receiving 40 μA for 2 days followed by 20 μA for 3 days. Graft and donor sites were either biopsied at the time of sacrifice for microscopic examination or visually examined daily for 3 weeks and then weekly. The extents of reepithelialization, hair growth, and gross wound contraction were recorded. Samples of graft and donor site tissues were obtained and examined microscopically in 10 treatment animals and 2 control animals for anatomic evidence of scarring and depth of microcirculation on the day of harvesting and 2, 3, 4, 7, and 12 days after excision and grafting.

Multiple Harvesting Model. The remaining 60 animals in the excised, grafted, and electrically treated group reepithelialized both donor sites and grafted areas by 14 days after the harvesting. The entire area (0.015 in) of the original scald was again harvested and the graft from the healed donor site was placed on the anterior portion of the bed. The posterior area was again not grafted and left as an open donor site. Animals were again and treated as had been previously done. Second grafts and donor sites were examined as above.

Estimation of Hair Follicle Survival. Fourteen days after the last treatment in each group, hair follicles were counted in the histologic sections. Sections from 10 individual animals in each group were examined at a magnification of 100X. Three random fields of the upper half and the lower half of the dermis were

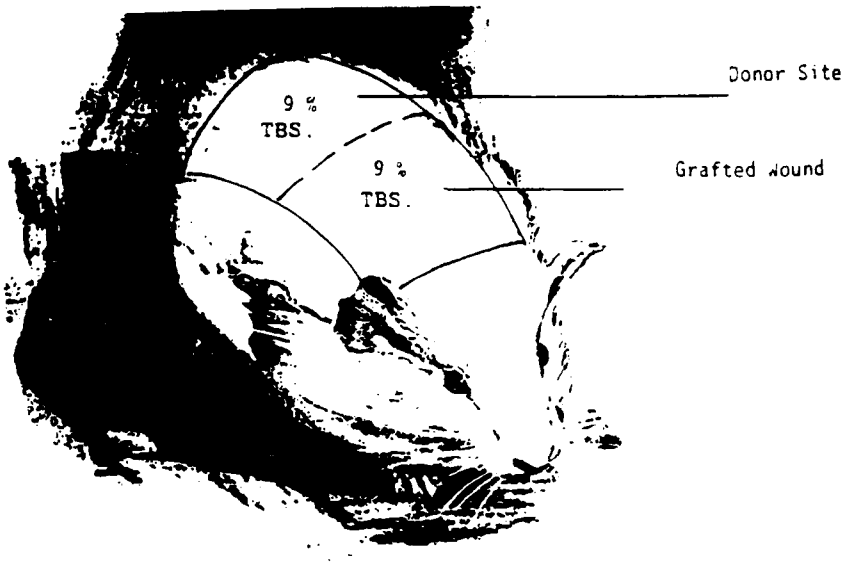

FIGURE 2. Diagram of scalded wound area and location of donor site and grafted wound.

counted. Comparison of follicle counts were made by t testing of treatment and control results.

RESULTS

Scald Wound Healing Model. By 12 days postburn, all animals in the treatment group had completely resurfaced wounds. In contrast, only 50% of the animals in the control group had reepithelialized wounds by 16 days postburn. Examples of treated and control wound healing are shown in Figure 3. Figure 3A shows the wound appearance of treated animals at 12 days postburn. Microscopic examination (Fig 3B) showed that these treated animals had minimal dermal loss and little subepidermal granulation tissue. Most of the hair follicles had survived. Reepithelialized control wounds remained partially covered with a thick layer of dried eschar at 16 days (Fig 3C). Microscopic examination showed that approximately 30% of the original dermis has been replaced by a layer of granulation tissue (Fig 3D) and surviving hair follicles were rare. When present, these follicles originated in the deeper dermis.

The animals in the control group that had reepithelized wounds by 16 days postburn were used as donors of split-thickness autografts. The control scald wounds not healed at 16 days required several additional weeks for complete healing and could not be used for subsequent excision and grafting studies.

Graft Healing Model. Autografts taken from the reepithelialized scald wounds of treated animals were firmly adherent 4 days after grafting. Microscopic examination at this time showed the presence of ink carbon in the grafted tissue (Fig

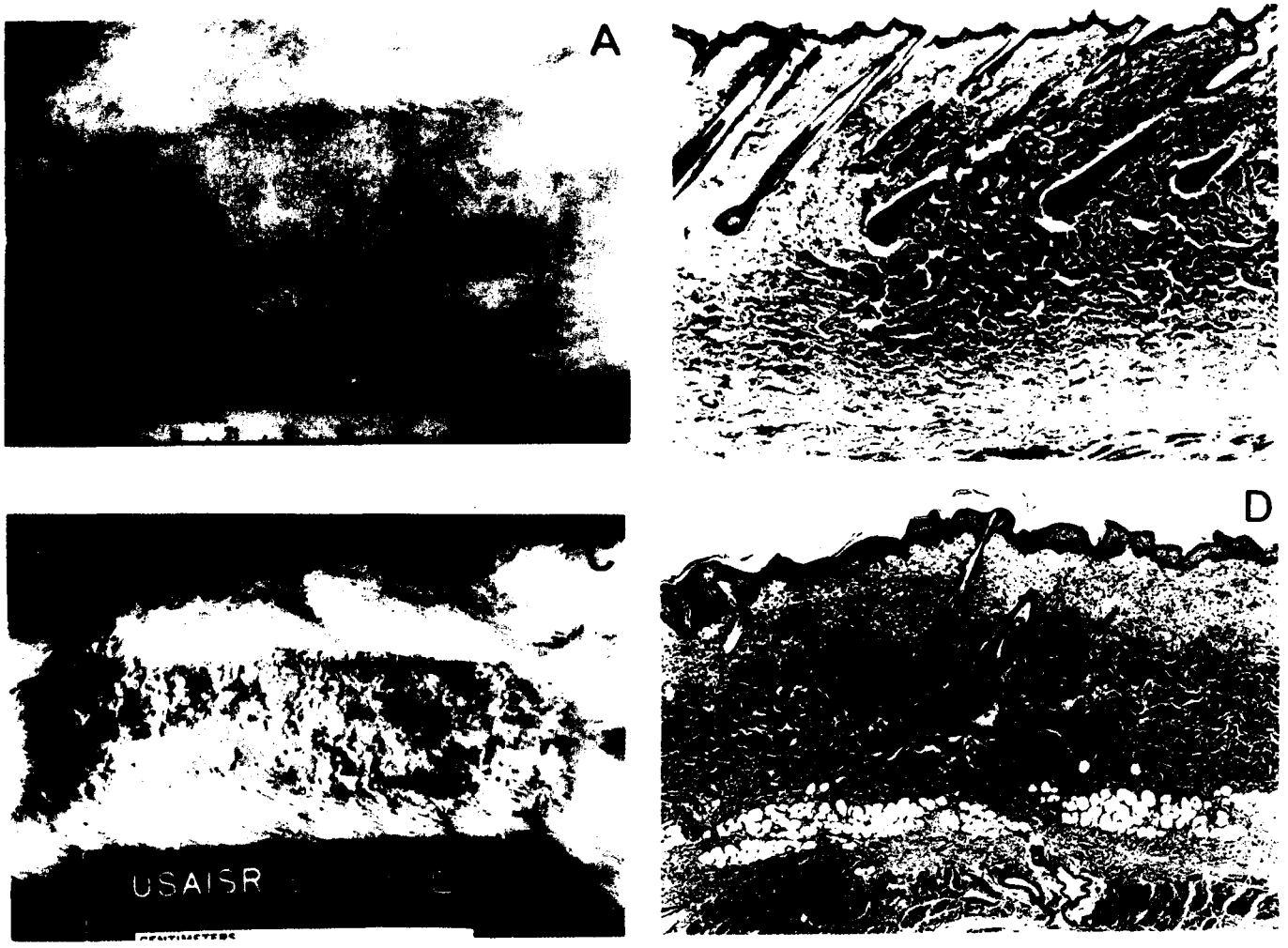

FIGURE 3. Comparison of treatment and control animals. **(A)** Gross appearance of a treated scald wound 12 days after wounding. The wound is completely reepithelialized. **(B)** Microscopic appearance of the wound in Panel A (trichrome, 10X). The wound is reepithelized and contains numerous hair follicles. **(C)** Gross appearance of a control group scald wound that reepithelialized 16 days after wounding. There is a dried crust containing epithelial debris on the wound surface. **(D)** Microscopic appearance of the wound in Panel C (trichrome, 10X). Even in an area of healing, there is subepidermal inflammatory infiltrate and loss of hair follicles in the healed wound (arrow).

4), indicating that union between graft and wound microcirculation had been established. In addition, there was an unusual hyperplasia of hair follicle epithelium at the graft-wound interface. This hyperplasia was most obvious at 4 days after grafting when it gave the appearance of an epithelial layer joining the graft with the wound bed. Figure 5 shows that by 7 days after grafting, hyperplasia of hair follicle epithelium at the graft-wound interface was less prominent and nearly normal hair follicles had reformed. At 12 days after grafting, a stratum corneum was evident and the architecture was that of normal guinea pig skin. Second grafts taken 14 days after primary harvesting in treated animals and again treated with DC showed gross and microscopic findings essentially similar to those described for primary grafts.

FIGURE 4. Grafted wound from a treated animal 4 days after harvesting from a healed scald wound (H&E, 10X). Carbon from black ink is evident in grafted tissue (top arrow). Hyperplasia in hair follicle epithelium is present at graft-wound interface and gives the appearance of an epithelial layer joining the graft with the wound bed (bottom arrow).

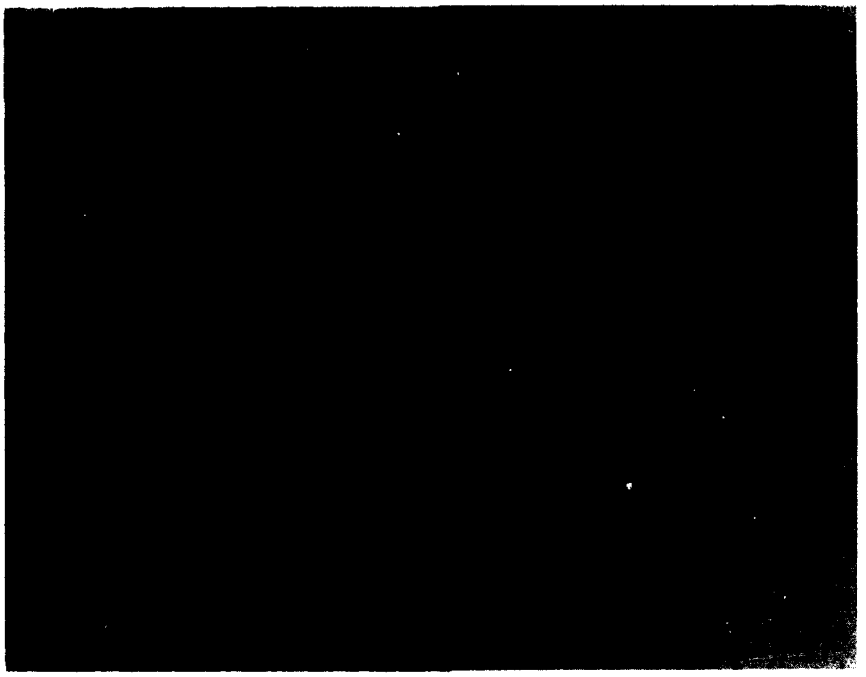

FIGURE 5. Grafted wound from a treated animal 7 days after harvesting from a healed scald wound (H&E, 10X). In this longitudinal section, the hyperplasia of hair follicle epithelium at the graft-wound interface is less prominent and nearly normal hair follicles have reformed (arrow).

Autografts from reepithelialized control scald wounds showed first evidence of revascularization and weak adherence 7 days after grafting. This was 5 full days later than in treated animals. As shown in Figure 6, further degeneration of the hair follicles had occurred by 1 week after grafting. Cords of hyperplasic epithelium extended down from the epidermis. By 3 months after harvesting, graft contraction and hair loss were marked in these animals.

Donor Site Healing Model. By 48 h after the first harvest and DC treatment, epithelial cells in the donor site hair follicles showed evidence of initial surface migration and no significant inflammatory reaction was seen in 10 of 10 examined animals. Samples taken at 3 days showed donor wounds covered with a neoepidermis of 3- to 8-cell thickness in 10 of 10 treated animals examined, but stratum corneum was not obvious. As shown in Figure 7A, treated animals at 14 days after harvesting had healed grafted wound and donor sites. Wound contraction was not obvious. Figure 7B shows that these donor sites had an established stratum corneum and an essentially normal distribution of the hair follicles. There was moderate acanthosis of the epidermis and minimal fibroblast growth in the subepidermis in some areas. No gross

FIGURE 6. Revascularization of a grafted wound from a control animal 7 days after harvesting (H&E, 10X). Black ink carbon is present in the graft tissue. Most of the hair follicles have degenerated. The inset of the superficial dermis and epithelium shows ink particles in the vessels (arrow) and cords of the hyperplastic epithelium (arrowhead) (H&E 40X).

contraction was evident 2 weeks or 3 months after harvesting and both graft and donor sites expanded with growth of the animal. Hair density was only slightly reduced.

Donor site healing after the second harvesting in treated animals required several days longer for reepithelialization and stratum corneum formation than did the primary donor sites. Donor sites had an established stratum corneum and survival of most hair follicles at 16 days after the second harvesting. Figure 8A shows the appearance of treated animals at 16 days after the second harvesting of a donor site. As shown in Figure 8B, minimal subepidermal fibroblast proliferation was present in the donor site. At 3 months, the graft and donor sites showed minimal contraction and hair loss (Fig 9). Although further graft harvesting from healed, twice-harvested donor sites was not

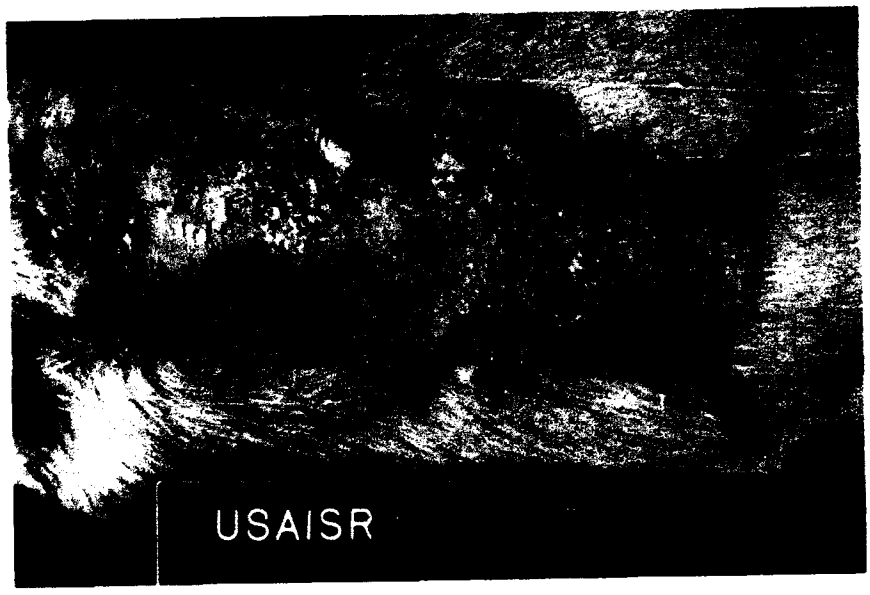

FIGURE 7. Previously healed scald wound of a treated animal 14 days after harvesting and grafting. **(A)** Gross appearance shows healed graft wound and donor site. **(B)** Microscopic appearance shows survival of many hair follicles and essentially normal epidermis with moderate acanthosis (H&E, 10X).

FIGURE 8. Scald wound of a treated animal 16 days after the second split-thickness skin graft harvesting. **(A)** Gross appearance shows no obvious contraction. **(B)** Microscopic examination of the donor site reveals only minimal evidence of subepidermal fibrosis and little inflammatory cell infiltration (H&E, 10X).

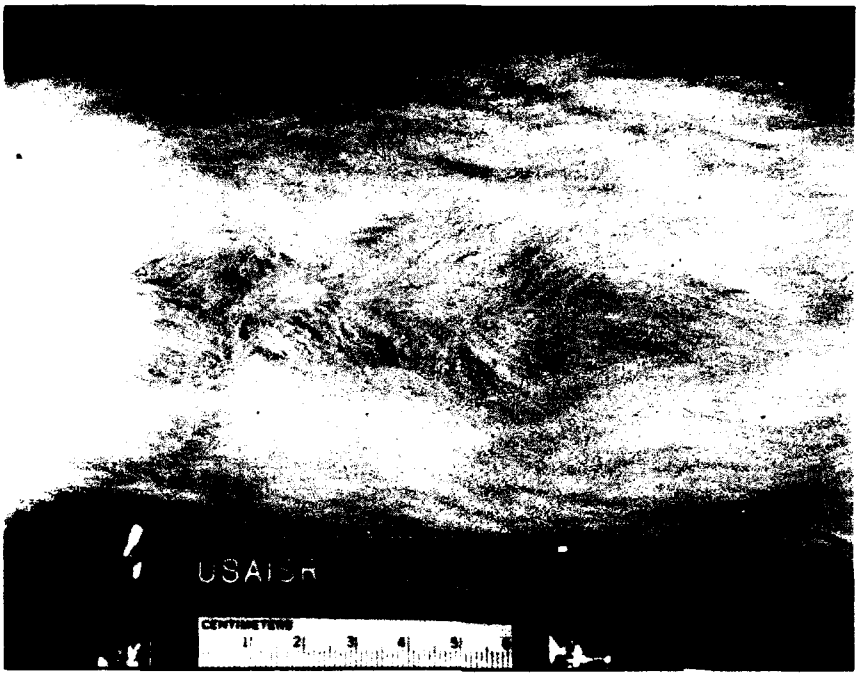

FIGURE 9. Scald wound of a treated animal at 3 months after the second harvesting. There is minimal contraction and hair loss.

attempted, there was no gross or microscopic evidence to suggest that such grafts could not be successfully taken.

Donor sites of control animals showed neutrophil accumulation at the wound surface 48 h after an initial graft harvest. At 3 days, epithelial cell migration from the remaining hair follicles had initially advanced under a thin layer of inflammatory cells. Reepithelialization of donor wounds after harvesting in control animals required more than 3 weeks. As shown in Figure 10, donor site wounds had marked fibrosis in the upper dermis and most hair follicles had degenerated in the reepithelized areas at 3 weeks after harvesting. Contraction was marked and no further harvesting of grafts from control animals was attempted.

DISCUSSION

The original hypothesis that DC would improve burn wound healing was based on observations of healed wounds of animals surviving after treatment of experimental burn wound sepsis with silver-nylon anodal dressings (1). The results of the present study, designed to examine this possibility using partial-thickness scalds, clearly show that DC markedly reduces the time required for healing and improves the quality of the healed wounds. Although the anatomic evidence is obvious, the strongest argument is that

FIGURE 10. Microscopic appearance of the donor site from a control animal 21 days after harvesting and grafting from a healed scald wound (trichrome, 10X). The donor site shows subepidermal fibrosis (arrow) and loss of hair follicles.

treated scald wounds could be repeatedly harvested as donor sites for highly successful split-thickness autografts.

Although the mechanisms of the improvement in healing were not addressed in this study, the results lead us to hypothesize that the observed effects may be due to the prevention of deleterious events that occur in the wound following injury and cause progression from cell injury to cell death. DC treatment may not cause improvement in wound healing but may instead limit the extent of tissue destruction. In all treated groups, the wounds showed less inflammation, granulation tissue, and fibrosis than in the control groups. The observed shortening in reepithelialization time may simply be a consequence of a much greater number of surviving active hair follicles. As shown in Table 1, the improved survival of hair follicles with DC treatment was readily demonstrated by microscopic counts of hair follicles in the upper

TABLE 1. Hair Follicle Survival in Healed Wounds (Mean Counts/Field)

	Scald Wound		1st Use Donor Site		
	Treatment Group	Control Group*	Treatment Group	Control Group*	Treatment Group
Upper dermis	8.8 ± 0.8	3.8 ± 0.7**	6.6 ± 1.0	3.8 ± 0.4**	3.8 ± 0.6
Lower dermis	6.4 ± 0.4	2.0 ± 0.7**	5.6 ± 0.9	1.2 ± 0.3**	3.7 ± 0.4

Surviving hair follicles were counted at 14 days after scalding and after first and second harvestings. Groups were compared by t test and data are presented as means of 30 random fields examined in 10 animals per group. *Tissue specimens obtained from the epithelized portions of wounds and donor sites. **$P < 0.02$.

and lower halves of the dermis of all groups. The active hair follicles of treated animals allowed healing to occur predominantly from within the partial-thickness wounds and donor sites themselves, rather than from the wound edges as occurred in control animals with few residual hair follicles within the wound. The mechanism for this proposed prevention of progression of injury could be relief of circulatory stasis and ischemia in the wound margins (5). If limitation of blood flow and depletion of required metabolic factors causing cell death are secondary effects of injury which evoke inflammation and influence other stages of wound healing, any reduction in such tissue loss would be expected to result in reduced inflammation and, in turn, less scarring and wound contraction. The microscopic findings in this study, i.e., less inflammation and less fibrosis in treated animals, are fully consistent with this speculation. Such a mechanism might also explain improved graft and donor site healing with DC treatment, since such wounds also experience at least transient periods of ischemia. The more rapid reestablishment of the microcirculation in the wounds and grafts of the animals treated with direct current may account for the reported observations.

PRESENTATIONS

Chu C-S: Multiple graft harvestings from deep partial-thickness scald wounds healed under the influence of weak direct current. Presented at the 48th Annual Session of the American Association for the Surgery of Trauma, Newport Beach, California, 6 October 1988.

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

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

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

PUBLICATIONS

Chu C-S, McManus AT, Pruitt BA Jr, and Mason AD Jr: Therapeutic effects of silver nylon dressings with weak direct current on *Pseudomonas aeruginosa*-infected burn wounds. **J Trauma** 28(10):1488-92, October 1988.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
				DA317765	1 Oct 89	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
1 Oct 88	D. Chg	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS14	CA	319
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP	FY90-01		

11. TITLE *(Precede with Security Classification Code)* (U) The Effect of Platelet-Activating Factor (PAF) and PAF Antagonist (CV-3988) on Smoke Inhalation Injury Using an Ovine Model

12. SUBJECT AREAS
0604 Anatomy and Physiology 0615 Pharmacology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
8812	8909	DA	C. In-House

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

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)*
89	1.0	38
90	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: IKEUCHI, H
d. TELEPHONE NUMBER *(include area code)*: 512-221-2720	d. TELEPHONE NUMBER *(include area code)*: 512-221-7832
21. GENERAL USE: FINA MILITARY/CIVILIAN APPLICATION: M	f. NAME OF ASSOCIATE INVESTIGATOR *(if available)*: SAKANO, T g. NAME OF ASSOCIATE INVESTIGATOR *(if available)*: MASON, A D

22. KEYWORDS *(Precede EACH with Security Classification Code)* (U) Platelet-Activating Factor; (U) Platelet-Activating Factor Antagonist; (U) Inhalation Injury; (U) Burns; (U) Arachidonic Acid; (U) Lab Animals: (U) Sheep; (U) RA II

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

23. (U) A DTIC literature search was conducted under DTIC request number W6044B dated 20 October 1989 for the technical report database and request number W6045A dated 20 October 1989 for the work unit database. The proposed effort will not result in the duplication of effort. The objective of this work is to determine the role of PAF and the pharmacologic effects of CV-3988 on smoke inhalation injury in an ovine model and to study the relationship between PAF and oxygen radical production and arachidonic acid metabolism.

24. (U) Physiologic measurements of cardiovascular and pulmonary function will be performed. Ventilation-perfusion ratios will be measured utilizing the six-inert gas elimination technique. These variables will be correlated with smoke inhalation injury severity and with treatment using a PAF antagonist. The thiobarbituric acid assay will be used to measure malondialdehyde as a product of membrane lipid peroxidation and prostaglandin derivatives will be assayed. Bronchoalveolar lavage fluid will be collected to evaluate the relationship of these metabolites to cytological changes after smoke inhalation injury.

25. (U) 8810 - 8909. Analysis of data revealed that treatment with a PAF antagonist reduces intrapulmonary neutrophil infiltration and bronchiolar edema. Both pre- and postinjury treatment with the PAF antagonist maintained arterial oxygen pressure at higher levels than in controls. For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1989.*

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

ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3M161102BS14-00, BASIC RESEARCH

PROJECT TITLE: The Effect of Platelet-Activating Factor (PAF) and a PAF Antagonist (CV-3988) on Smoke Inhalation Injury Using an Ovine Model

US ARMY INSTITUTE OF SURGICAL RESEARCH
Fort Sam Houston
San Antonio, Texas 78234-5012

1 October 1988 - 30 September 1989

INVESTIGATORS

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Jose E. Sanchez, Staff Sergeant
Basil A. Pruitt, Jr., MD, Colonel, MC

ABSTRACT

PROJECT NUMBER: 3M161102BS14-00, BASIC RESEARCH

PROJECT TITLE: The Effect of Platelet-Activating Factor (PAF) and a PAF Antagonist (CV-3988) on Smoke Inhalation Injury Using an Ovine Model

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

PERIOD COVERED IN THIS REPORT: 1 Oct 88 through 30 Sep 89

INVESTIGATORS: Hisashi Ikeuchi, MD
Tsutomu Sakano, MD
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Smoke inhalation injury is one of the determinants of mortality following major burn injuries. The physiologic changes after smoke inhalation injury have been observed in a sheep model developed at this Institute. The influence of chemical mediators in this model, however, has not been thoroughly investigated. Recently, PAF, one of the most potent mediators, has been studied in systemic inflammation and shock models in relation to the production of arachidonic acid derivatives and superoxide. The effects of PAF on smoke inhalation injury and/or major burn have been investigated. If PAF modulates the inflammatory process after smoke inhalation injury, analysis of its relationship to the production of arachidonic acid derivatives or superoxide should enhance our understanding of the pathophysiologic consequences of such injury. A PAF antagonist might improve the treatment of inhalation injury by limiting or preventing inflammatory response.

The objective of this study is to determine the role of PAF and the pharmacologic effects of CV-3988 on smoke inhalation in an ovine model, including the relationship with arachidonic acid derivatives.

THE EFFECT OF PLATELET-ACTIVATING FACTOR (PAF) AND A PAF ANTAGONIST (CV-3988) ON SMOKE INHALATION INJURY USING AN OVINE MODEL

Smoke inhalation injury is one of the major complications of severe burns (1). The roles of chemical mediators have been studied to clarify the mechanism of pathophysiologic changes in smoke inhalation injury (2,3). PAF (1-O-hesadecyl/octadecyl-2-acetyl-sn-glyceryl-3-phosphorylcholine) has been studied as one of the chemical mediators causing gamma E immunoglobulin systemic anaphylaxis and many inflammatory responses. In vitro, PAF activates platelets and neutrophils, resulting in platelet aggregation and chemotaxis (4-8). PAF also may modulate neutrophil and monocyte-mediated reactions, since the production of lipoxygenase pathway-derived products of arachidonic acid and generation of oxygen-derived free radicals are evoked in human neutrophils by PAF injection (4,9,10). In vivo, systemic injection of PAF results in acute pulmonary hypertension with systemic hypotension, increased airway resistance, and decreased dynamic lung compliance resulting from bronchoconstriction. It produces pulmonary edema in rats, dogs, and baboons and increased pulmonary lymph flow in sheep (6,11-17). These are the alterations observed after smoke inhalation injury. In our sheep model, the level of PAF from bronchoalveolar lavage fluid increased 6 h after smoke inhalation, although PAF was not found in blood or pulmonary lymph samples (18). PAF receptor antagonists (CV-3988, BN52021) have been studied in endotoxin shock, hemorrhagic shock, and PAF injection models. Improvement in survival rate, prevention of systemic hypotension, and decreased production of arachidonic acid derivatives, i.e., PGE_2 and thromboxane B_2, by PAF antagonists as pretreatment agents have been observed in endotoxin shock models (19-24). CV-3988 (rac-3-(N-n-octadecylcarbamoyloxy)-2-methoxypropyl-2-thiazoethyl phosphate) also reversed endotoxin-induced hypotension in an endotoxin shock model and improved the survival rate in an anaphylactic shock model, even though it was injected after the endotoxin and antigen injection (19,25). CV-3988 decreased thromboxane B_2 levels in bronchoalveolar lavage fluid after ozone exposure in a dog model (26). These findings strongly suggest a significant relationship between PAF and the pathophysiologic changes after inhalation injury. PAF antagonists may be beneficial in treating smoke inhalation injury and decreasing the production of prostaglandin derivatives and superoxide.

MATERIALS AND METHODS

Study Design. Fifteen 1- to 2-yr-old male sheep weighing 25-30 kg will be equally divided into three groups. Group I will receive smoke inhalation injury without treatment, Group II will receive smoke inhalation injury with a continuous treatment infusion both pre- and postinjury, and Group III will receive smoke inhalation

injury with a continuous treatment infusion postsmoke only. All sheep will be maintained in outdoor covered runs and fed commercial chow and water ad libitum. Baseline hematologic data will be established 3 weeks prior to entry into the study.

The day before smoke exposure, all sheep will be anesthetized with methohexital sodium (1 mg/kg IV) and orally intubated. The sheep will then be ventilated, using halothane/oxygen anesthesia, and placed in the supine position. One Silastic® medical-grade tube (30 cm) will be placed in a carotid artery and another in a femoral vein using sterile technique. A radiopaque sheath introducer (8.5F, Arrow-Flex™) will be placed in an external jugular vein using sterile technique. The arterial line will be used to draw blood samples for gas analysis and metabolites. The venous line will be used for infusion of anesthetic agents and the PAF antagonist.

On the day of smoke exposure, a Swan-Ganz catheter will be inserted through the sheath in the jugular vein prior to presmoke measurements. After induction of anesthesia with methohexital sodium (1 mg/kg IV) and oral intubation, smoke insufflation at a dose that produces a 50-60% carboxyhemoglobin level will be produced by the method developed at this Institute (27). Group I will receive only lactated Ringer's solution (70 $ml/m^2/h$) postsmoke. Group II will be pretreated with CV-3988 (10 mg/kg) 5 min presmoke and will receive a continuous infection of CV-3988 (2 mg/kg/h) for the first 24 h postsmoke. Group III will be treated with CV-3988 (10 mg/kg) for the first 23 h postsmoke prior to multiple inert gas elimination measurement.

Cardiopulmonary variables and blood gases will be measured presmoke, prior to CV-3988 infusion, and at 1, 3, 6, 12, 18, and 24 h postsmoke. Blood samples for PGE_2, thromboxane B_2, and malondialdehyde measurement will be drawn prior to infusion of CV-3988, at 1, 3, 6, 12, 18, and 24 h postsmoke, and just prior to reinfusion of CV-3988. Physiologic measurements of cardiopulmonary changes and V_A/Q measurement by the multiple inert gas elimination technique (28) will be performed 24 h postsmoke.

At the end of 24 h, the animals will again be anesthetized with methohexital sodium (1 mg/kg IV) and orally intubated. The animals will be maintained on anesthesia with alpha-chloralose (0.05 g/kg IV), paralyzed with pancuronium bromide (1 cc), and placed in the prone position. Mechanical ventilation will be given with a volume-limited ventilator (tidal volume = 15 ml/kg, respiratory rate = 12/min, and fractional percent of oxygen in inspired gas = 0.21). Measurements will include systemic blood pressure, heart rate, pulmonary artery pressure, pulmonary capillary wedge pressure, cardiac output, static compliance, pulmonary resistance, and blood gas analysis of arterial and mixed venous blood. After an 1-h stabilization period, lactated Ringer's solution containing 6 inert gases (sulfur hexafluoride, ethane, cyclopropane,

halothane, 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 ml each) will be drawn simultaneously and anaerobically into preweighed, heparinized syringes (30 ml, matched, glass, Becton Dickinson). Mixed-expired gas will be collected from a temperature-controlled copper coil (OD = 3.49 cm, L = 640 cm) about 1 min after blood sampling, thus compensating for the delay in the mixing chamber. Sulfur hexafluoride from blood and expired gas samples will be analyzed immediately by GC-MS (Hewlett-Packard, Models 5890 and 5970). The other five gases will be analyzed using GC (Hewlett-Packard, Model 5840A) with a flame ionization detector. Cardiopulmonary indexes measured at this time will be taken as the representative values.

Blood samples for PGE_2, thromboxane B_2, and malondialdehyde measurements will be drawn from the carotid artery catheter into heparinized syringes containing 0.1 ml of a 100 mg/l sodium meclofenamate solution to prevent further platelet synthesis of metabolites. Samples will be performed prior to infusion of CV-3988 and at 1, 3, 6, 12, 18, and 24 h postsmoke. After centrifugation, the plasma of these samples will be stored at -70°C for possible future testing. After blood sample collections, bronchoalveolar lavage will be performed with a bronchofiberscope (Olympus 1T10) to obtain samples for PGE_2 and thromboxane B_2 measurement from the lower lung lobes. Due to the expense of such measurements, the assays for PGE_2 and thromboxane B_2 will be performed only if expected pathophysiologic changes are seen.

Platelet-Activating Factor (CV-3988). A pure sample of CV-3988 will be provided by Takeda Chemical Industries, Ltd. (Osaka, Japan). A solution of CV-3988 (5 mg/ml) will be prepared in saline at 50°C 2 h prior to injection. The effective dose of CV-3988 in a sheep model is unknown. In other species, doses from 3-10 mg/kg as bolus injections were effective (19-26). The ED_{50} for mice is reported to be from 0.5-1.2 mg/kg (19). We therefore feel that 10 mg/kg as a bolus injection and 2 mg/kg/h for continuous infusion will be effective without hemolysis in the sheep model.

Estimation of Superoxide Production with Thiobarbituric Acid (TBA) Assay for Malondialdehyde. Malondialdehyde, formed from the breakdown of polyunsaturated fatty acids, serves as a convenient index for determining the extent of the peroxidation reaction. Malondialdehyde has been identified as the product of lipid peroxidation that reacts with TBA, which is measured by absorbance at 535 nm (29-33). Details are as follows:

Preparation of Chemical Agents for TBA Assay (Two Mixtures).

First Mixture (TCA-TBA-HCl). 150 g TCA and 3.75 g TBA are added to 0.25N HCl (1 l). The mixture is then heated

gently in a water bath until the TBA is dissolved. The mixture is then filtered through Whatman™ paper and stored capped in the dark.

Second Mixture (TCA-TBA-HCl + BHT). To TCA-TBA-HCl, enough BHT is added to give a 0.01% (w/v) final concentration.

Specimen Collection and Storage. Three milliliters of venous blood is drawn into a heparinized syringe at each sampling. The specimens are then stored at -70°C until measurement.

Procedure. 0.5 ml of well-mixed blood is added to 4 ml of TCA-TBA-HCl and 4 ml of TCA-TBA-HCl + BHT. After mixing well, the mixture is heated at 80°C for 30 min on a heating block. After cooling to room temperature, flocculent precipitate is removed by centrifugation at 1,000 g/10 min. The absorbance of the sample is measured at 535 nm to blank, e.g., TCA-TBA-HCl + 0.1 ml water.

Definition of Extinction Coefficient for MDA. The definition of the extinction coefficient for MDA is 1.56×10^5 $M^{-1} cm^{-1}$ (or 156 $mM^{-1} cm^{-1}$).

Calculation of MDA. The amount of MDA in 1 ml of blood is calculated by the formula as follows:

$$\text{MDA (mM/1 ml blood)} = A_{535\ nm} \times 1/156 \times 2$$

cf A_{535} nm: relative absorbance of sample to blank

The amount of MDA in each sample is calculated by subtracting the TCA-TBA-HCl reading from the TCA-TBA-HCl + BHT reading to reject the extent of MDA production after collection.

RESULTS

Preliminary analysis of data has revealed that treatment with a PAF antagonist reduces intrapulmonary neutrophil infiltration and bronchial edema. Both pre- and postinjury treatment with the PAF antagonist maintained arterial oxygen pressure at higher levels than in control animals.

DISCUSSION

Upon completion of data collection from all animals, the data will be analyzed as to the role of PAF and the pharmacologic effects of CV-3988 on smoke inhalation injury, including the relationship with arachidonic derivatives.

PRESENTATIONS/PUBLICATIONS

None.

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mitochondrial membranes. **Arch Biochem Biophys** 168:463-72, 1975.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
				DA318558	1 Oct 89	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
1 Dec 88	D. Chg	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS14	CA	320
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP,	FY90-01		

11. TITLE *(Precede with Security Classification Code)* (U) The Effect of Cutaneous Burn and Inhalation Injury on Pulmonary Function in Rabbits

12. SUBJECT AREAS
0604 Anatomy and Physiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING ORGANIZATION	16. PERFORMANCE METHOD
8812	9009	DA	C. In-House

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

a. DATE EFFECTIVE		18. RESOURCES ESTIMATE		
b. CONTRACT/GRANT NUMBER		a. FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS *(In thousands)*
c. TYPE	d. AMOUNT	89	1.0	33
e. KIND OF AWARD	f. CUM/TOTAL	90	1.0	34

19. RESPONSIBLE DOD ORGANIZATION	20. PERFORMING ORGANIZATION
a. NAME US Army Institute of Surgical Research	a. NAME US Army Institute of Surgical Research
b. ADDRESS *(Include zip code)* Fort Sam Houston San Antonio, Texas 78234-5012	b. ADDRESS Fort Sam Houston San Antonio, Texas 78234-5012
c. NAME OF RESPONSIBLE INDIVIDUAL PRUITT, B A	c. NAME OF PRINCIPAL INVESTIGATOR SAKANO, T
d. TELEPHONE NUMBER *(Include area code)* 512-221-2720	d. TELEPHONE NUMBER *(Include area code)* 512-221-7832
21. GENERAL USE FINA 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) Burn Injury; (U) Inhalation Injury; (U) Pulmonary Function; (U) Prostanoid; (U) Lipid Peroxide; (U) Lab Animals:

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

22. (Continued) (U) Rabbits; (U) RA II

23. (U) A DTIC literature search was conducted under DTIC request number W6048D dated 20 October 1989 for the technical report database and request number W6049C dated 20 October 1989 for the work unit database. The proposed effort will not result in the duplication of effort. The objective of this work was to identify the pulmonary changes in a rabbit model following smoke inhalation, cutaneous burns, and a combination of both.

24. (U) Twenty-percent full-thickness scald burns and/or various severities of smoke inhalation were produced in a rabbit model. Histologic evaluation was performed and carboxyhemoglobin, arterial oxygen pressure, extravascular lung water, etc., were be used to estimate an interaction between burn and inhalation injury.

25. (U) 8812 - 8909. A rabbit model with smoke inhalation injury and/or cutaneous burn injury was developed and observed for 2 days. However, lung injury, as measured by decreased arterial oxygen pressure and lung water accumulation, was less in the group with both injuries than in the group with inhalation injury only. Long-term changes of lung function will be studied to determine whether more deterioration occurs in the group with both injuries due to late shifts of plasma from the burn wound into the systemic circulation. For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1989.*

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

ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3M161102BS14-00, BASIC RESEARCH

PROJECT TITLE: The Effect of Cutaneous Burn and Inhalation Injury on Pulmonary Function in Rabbits in Relation to Prostanoid and Lipid Peroxide Production

US ARMY INSTITUTE OF SURGICAL RESEARCH
Fort Sam Houston
San Antonio, Texas 78234-5012

1 October 1988 - 30 September 1989

INVESTIGATORS

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

ABSTRACT

PROJECT NUMBER: 3M161102BS14-00, BASIC RESEARCH

PROJECT TITLE: The Effect of Cutaneous Burn and Inhalation Injury on Pulmonary Function in Rabbits in Relation to Prostanoid and Lipid Peroxide Production

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

PERIOD COVERED IN THIS REPORT: 1 Oct 88 through 30 Sep 89

INVESTIGATORS: Tsutomu Sakano, MD
Hisashi Ikeuchi, MD
Carlin V. Okerberg, DVM, PhD, Major, VC
Basil A. Pruitt, Jr., MD, Colonel, MC

Recent clinical and experimental evidence suggests that arachidonic acid metabolites and oxygen-free radicals play important roles in the pathogenesis of acute lung injuries such as ARDS, endotoxin-induced lung dysfunction, and hyperoxia-induced pulmonary edema. Similar evidence has been reported concerning the mechanisms of pathophysiologic pulmonary response after skin burns and smoke inhalation. The objectives of this study are to identify the pulmonary changes following smoke inhalation, cutaneous burns, and a combination of both and to clarify the role of prostanoid and lipid peroxide production in the pathogenesis of lung dysfunction if pulmonary injury is most severe after a combination of burn and inhalation injury.

A rabbit model with smoke inhalation injury and/or cutaneous burn injury was developed and observed for 2 days. However, lung injury, as measured by decreased arterial oxygen pressure and lung water accumulation, was less in the group with both injuries than in the group with inhalation injury only. Long-term changes of lung function will be studied to determine whether more deterioration occurs in the group with both injuries due to late shifts of plasma from the burn wound into the systemic circulation.

THE EFFECT OF CUTANEOUS BURN AND INHALATION INJURY ON PULMONARY FUNCTION IN RABBITS IN RELATION TO PROSTANOID AND LIPID PEROXIDE PRODUCTION

Progressive pulmonary failure remains a major cause of death, especially in patients with massive cutaneous burns associated with smoke inhalation (1). The presence of inhalation injury markedly increases the mortality rate after cutaneous thermal injury (2). Since inhalation injury alone rarely produces progressive pulmonary failure, this suggests an interaction between burn and inhalation injury with respect to pulmonary injury. The effects of a combination of both injuries on prostanoid and lipid peroxide production, and of the relationship of these effects to pulmonary dysfunction, have not been studied. Such studies may be pertinent to the care of burned patients.

Recent clinical and experimental evidence suggests that arachidonic acid metabolites and oxygen-free radicals play important roles in the pathogenesis of acute lung injuries such as ARDS, endotoxin-induced lung dysfunction, and hyperoxia-induced pulmonary edema. Similar evidence has been reported concerning the mechanisms of pathophysiologic pulmonary response after skin burns and smoke inhalation. In the early period after thermal injury, pulmonary dysfunctions include a decrease in lung compliance and arterial oxygen pressure (3) and an increase in extravascular lung water (4). These changes sometimes occur in the absence of inhalation injury; one suggested mechanism involves neutrophil sequestration in the lung after complement activation (5). Till et al (6), using a rat model, have reported increased pulmonary vascular permeability due to oxygen radicals generated by complement-activated neutrophils after burn injury. Oxygen radicals can cause not only increased pulmonary vascular permeability and vasoconstriction by themselves (7), but also the formation of lipid peroxides, which are suspected to be another mechanism of oxygen radical-induced cellular damage (8). In burned rats (9) and human patients (10), increased serum levels of lipid peroxide have been demonstrated in association with damage to various organs.

To measure lipid peroxidation, degradation products such as malondialdehyde (MDA), ethane production, pentane production, the presence of fluorescent materials, and chemiluminescence have been used (11). Although thiobarbituric acid assay of MDA is somewhat insensitive to low levels of lipid peroxides, the measurement of MDA is commonly used and correlates well with measurements of other products (11).

Protection from acute lung injury can be achieved by depletion of neutrophils (12) or by systemic treatment with oxygen radical scavengers. Hydroxyl radical ($OH^{\cdot}$) scavengers or catalase significantly diminish the increase in permeability and

morphological alterations after thermal burns in rats (13), suggesting hydrogen peroxide and OH· as key mediators. Several agents such as ethane, mannitol, dimethyl sulfoxide, and dimethylthiourea are known OH· radical scavengers. Of these, dimethylthiourea is the most potent and administration of 600 mg/kg remarkably increased the survival of mice exposed to ionizing radiation, in which OH· is generated as the principal oxidative radical (14). Infusion of dimethylthiourea is also reported to decrease lung edema caused by phorbol myristate acetate in the rabbit (15).

Plasma thromboxane begins to increase within an hour after injury in burned sheep and is linked to pulmonary hypertension and decreased arterial oxygen pressure and lung compliance; the animals can be protected by ibuprofen (3,16). An absence of concomitant increase in prostacyclin is also thought to be responsible for systemic changes after burns (17). Modulation of arachidonic acid metabolism by oxygen radicals or their intermediates has also been reported (8,18).

In a sheep model of inhalation injury, decreased permeability, characterized by increased lung lymph flow with increased lymph-to-plasma protein concentration ratios, occur within several hours after smoke inhalation (19). These pathologic responses are attenuated by leukocyte depletion (20). In rabbits, ibuprofen blocks the increase of extravascular lung water seen after smoke inhalation (21). A significant inverse relationship is reported between plasma vitamin E levels and inhalation injury in thermally injured patients (22). These data suggest the participation of arachidonic acid metabolites and free radicals as the mechanism responsible for pulmonary damage in inhalation injury.

Little is known about the differences between the pulmonary changes due to smoke inhalation and inhalation injuries. Herndon et al (23), recognized no difference in extravascular lung water and a significantly inspired oxygen in patients with burns and smoke inhalation compared to patients with smoke inhalation alone. No decisive conclusion can be obtained from this study because of the wide range in severity of injury, but more severe bronchoconstriction due to thromboxane might have caused the observed decrease in arterial oxygen pressure/fractional inspired oxygen in the patients with a combination of both injuries. Zawacki et al (24), using a murine model, reported a striking increase in mortality with the addition of 10% total body surface area scald burns to inhalation injury. Gasping and labored respiration were seen in a majority of the mice that died, but histological examination showed no remarkable change. These studies suggest that more severe pulmonary damage may have occurred when inhalation injury was added to the burn; interaction between the mediators released by inhalation injury and skin burns is a possible explanation.

The objectives of this study were to identify pulmonary changes following smoke inhalation, cutaneous burns, and a combination of both and to clarify the role of prostanoid and lipid peroxide production in the pathogenesis of lung dysfunction if pulmonary injury was most severe after a combination of burn and inhalation injury.

MATERIALS AND METHODS

Study Design. New Zealand white rabbits weighing 2.5–3.5 kg were housed in individual stainless steel cages and fed food and water ad libitum throughout the study. A catheter was placed in the aorta via the carotid artery under ketamine anesthesia. The animals were then randomized to one of four groups. Group 1 (n=3) was administered 20% total body surface area full-thickness scald burns, Group 2 (n=12) was administered various severities of smoke inhalation injury, Group 3 (n=12) received both 20% total body surface area full-thickness scald burns and various severities of smoke inhalation injury, and Group 4 (n=3) served as uninjured controls. Three days after cannulation, the animals were anesthetized with ketamine and the dorsal area was shaved. Animals in Groups 1 and 3 were administered 20% full-thickness scald burns by immersion in 80°C water for 30 sec (35). Animals in Groups 2 and 3 were administered various severities of smoke inhalation injury. Smoke was produced by burning disposable underpads in a smoke generator. The smoke was led by a copper pipe into a volume-adjustable metal syringe and then into an intratracheal tube. Carboxyhemoglobin concentrations after smoke exposure were used to grade severity in the animals with smoke exposure; exposure was adjusted to yield approximately 15%, 30%, 45%, and 60% carboxyhemoglobin concentrations.

All animals were studied in an unanesthetized state for 2 days with food and water provided ad libitum. Blood samples were taken from the catheter before injury and 4, 8, 24, and 48 h after injury. Arterial oxygen pressure, arterial carbon dioxide pressure, etc., were measured before injury and 4, 8, 24, and 48 h after injury. At the end of the 48-h study period, all animals were sacrificed with sodium pentobarbital (600 mg/kg) and extravascular lung water was determined by a gravimetric method (33). A modified method of Pierce by Drake et al (32) was used to estimate the lung extravascular fluid weight and blood-free dry weight.

Determination of the Number of Animals Required. Thirty animals were used for this study in a 5 X 2 factorial design with 3 replicates in each cell.

Data Analysis Plan. Changes with time in arterial oxygen pressure was examined as functions of severity of inhalation and burn injury using ANOVA to assess the factorial design. Multiple regression technique and analysis of covariance were also used.

The same procedures were applied to extravascular lung water and arterial carbon dioxide pressure.

RESULTS

A rabbit model with smoke inhalation injury and/or cutaneous burn injury was developed and observed for 2 days. However, lung injury, as measured by decreased arterial oxygen pressure and lung water accumulation, was less in the group with both injuries than in the group with inhalation injury only.

DISCUSSION

Long-term changes of lung function will be studied to determine whether more deterioration occurs in the group with both injuries due to late shifts of plasma from the burn wound into the systemic circulation.

PRESENTATIONS AND PUBLICATIONS

None.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION: DA318371	2. DATE OF SUMMARY: 1 Oct 89	REPORT CONTROL SYMBOL: DD-DR&E(AR) 636
3. DATE PREV SUM'RY: 13 Jun 89	4. KIND OF SUMMARY: D. Chg	5. SUMMARY SCTY: U	6. WORK SECURITY: U	7. REGRADING	8. DISP'N INSTR'N: CX	9. LEVEL OF SUM A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS14	AZ	321
b. CONTRIBUTING				
c. CONTRIBUTING	DA LRRDAP,	FY90-01		

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	9009	DA	C. In-House

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

a. DATE EFFECTIVE	d. AMOUNT	18. RESOURCES ESTIMATE: a. FISCAL YEARS	a. PROFESSIONAL WORKYEARS	b. FUNDS (In thousands)
b. CONTRACT/GRANT NUMBER		89	0.3	20
c. TYPE		90	0.3	21
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: BECKER, W K
d. TELEPHONE NUMBER (include area code): 512-221-2720	d. TELEPHONE NUMBER (include area code): 512-221-4652
21. GENERAL USE: FINA MILITARY/CIVILIAN APPLICATION: M	f. NAME OF ASSOCIATE INVESTIGATOR (if available): BUESCHER, T M g. NAME OF ASSOCIATE INVESTIGATOR (if available): MASON, A D

22. KEYWORDS (Precede EACH with Security Classification Code) (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)

22. (Continued) (U) Crystalloids; (U) Hypertonic Saline; (U) Lab Animals: (U) Swine; (U) RA II

23. (U) A DTIC literature search was conducted under DTIC request number W6055C dated 20 October 1989 for the technical report database and request number W6056F dated 20 October 1989 for the work unit database. The proposed effort will not result in the duplication of effort. The objective of this work is to 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.

24. (U) 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) 8810 - 8909. This project was approved by the USAISR Research Council and US Army Institute of Surgical Research Animal Care and Use Committee during the third quarter of fiscal year 1989. For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1989*.

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

ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3M161102BS14-00, BASIC RESEARCH

PROJECT TITLE: Effect of Resuscitation Fluid on Hepatic Blood Flow and Hepatic High Energy Phosphate Production in a Swine Model of Hemorrhagic Shock

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

13 June 1989 - 30 September 1989

INVESTIGATORS

William K. Becker, MD, Lieutenant Colonel, MC
Teresa M. Buescher, MD, Captain, MC
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Carlin V. Okerberg, DVM, PhD, Major, VC
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ABSTRACT

PROJECT NUMBER: 3M161102BS14-00, BASIC 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: 13 Jun 89 through 30 Sep 89

INVESTIGATORS: William K. Becker, MD, Lieutenant Colonel, MC
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Hemorrhagic shock following rapid exsanguination from blunt and penetrating trauma is a frequent cause of death. 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 possible that the adequacy of volume resuscitation immediately following hemorrhage may determine the subsequent development of sequential progression of multisystem organ failure.

The purpose of this study is to evaluate the effects of various resuscitation formulae on hepatic blood flow and hepatic high energy phosphate production following hemorrhagic shock.

This project was approved by the USAISR Research Council and US Army Institute of Surgical Research Animal Care and Use Committee during this reporting period. Equipment and supplies have been ordered and work will be initiated upon their arrival.

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. In addition, there has been recent interest in the use of hypertonic salt solutions in the early resuscitation from hemorrhagic shock. 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.

Hypertonic saline has been used successfully for acute resuscitation in swine hemorrhage models and seems to be superior (as measured by survival and decreased lactate levels) to other salt-containing resuscitative fluids. This is work that has largely been performed at Letterman Army Institute of Research. However, little has been described concerning the effects of different resuscitative fluids on hepatic blood flow and function. This study will measure liver high energy phosphate, hepatic blood flow, and oxygen delivery in swine at baseline, after 25% hemorrhage, after 50% hemorrhage, and after administration of various resuscitative fluids.

The purpose of this study is to evaluate the effects of various resuscitation formulae on hepatic blood flow and hepatic high energy phosphate production following hemorrhagic shock.

MATERIALS AND METHODS

Study Design: Immature swine of either sex weighing 20–25 kg will be anesthetized, intubated, and placed on mechanical ventilation. A thermodilution pulmonary artery catheter will be placed in the right jugular vein and a line for volume infusion will be placed in the left jugular vein. An arterial cannula for blood pressure measurement and hemorrhage will be placed in the right femoral artery. EKG leads will be placed for heart monitoring. The portal vein and hepatic artery will be completely dissected through an upper abdominal flap incision with specific care taken with the hepatic artery to isolate and ligate any branches. All ligamentous attachments of the liver will be divided. This dissection and preparation is similar to that of harvesting the liver for hepatic transplantation. The goal is to insure that no collateral circulation to the liver is maintained and that all blood flow to the liver enters through the hepatic artery or portal vein. Ultrasonic flow probes will be placed around the hepatic artery and portal vein in the porta hepatis. A catheter for blood sampling will be placed through a side branch of the portal vein.

At the conclusion of all surgical procedures, a 30-min stabilization period will commence. Prior to the onset of hemorrhage, baseline studies, including cardiac output, heart rate, arterial blood pressure, total hepatic blood flow, and total hepatic oxygen delivery will be measured. In addition, a small needle biopsy of the liver will be obtained and immediately frozen in liquid nitrogen for later processing for high energy phosphate. Following this 30-min period, the animals will be hemorrhaged over a 10-min period. There will be two hemorrhage groups. Group 1 will undergo hemorrhage of 25% of the blood volume and Group 2 will undergo hemorrhage of 50% of the blood volume. The blood volume of the swine will be assumed to be 71 ml/kg.

Following hemorrhage, the animals will be observed for a 15-min period prior to initiation of resuscitation. There will also be two control groups (25% and 50% hemorrhage) which will not be resuscitated. In the treatment groups, resuscitation fluid (blood, lactated Ringer's, Hespan®, or hypertonic saline) will be given over a 30-min period (see Table 1). After resuscitation, the animals will be observed for a period of 3 h, after which all catheters will be removed and all surgical incisions will be closed. Animals will then be returned to their cages and observed for a period of 24 h. At this point, any surviving animals will be sacrificed with T61 to effect or with sodium pentobarbital (40 mg/kg) with exsanguination.

Laboratory samples and physiologic data will be obtained at baseline (prior to hemorrhage), 15-min posthemorrhage, and 15, 30, 60, 120, and 180 min postresuscitation. Laboratory samples will include arterial, mixed venous, and portal blood gases,

TABLE 1. Resuscitation Fluid Compositions

Resuscitation Fluid	Volume (ml)	Na+ (mEq)
50% Hemorrhage Group:		
Hespan®	710	109
Lactated Ringer's	2130	276
Hypertonic Saline	178	228
Blood	710	-
25% Hemorrhage Group:		
Hespan®	355	54
Lactated Ringer's	1050	138
Hypertonic Saline	89	114
Blood	355	-

hematocrit, serum osmolality, and liver function tests, including SGOT, alkaline phosphatase, and bilirubin. Hepatic tissue samples will be obtained at each time period for analysis of AMP, ADP, and ATP. Hemodynamic indices measured at each time period will include heart rate, blood pressure, cardiac output, pulmonary artery pressure, pulmonary artery wedge pressure, hepatic artery flow, and portal vein flow. Based on these measurements, the total body oxygen delivery and consumption and hepatic oxygen delivery will be calculated.

Selection of Appropriate Animal Model: Swine have been widely used in hemorrhagic shock models at various institutions. In addition, swine do not require a splenectomy prior to the initiation of hemorrhagic shock. In addition, the anatomic characteristics of the hepatic artery and portal vein in the swine are very similar to that in the human.

Methods for Appropriate Alleviation of Pain and Distress: The anesthetic agent for surgery will be methohexital sodium (1 cc/3 kg) followed by halothane gas anesthesia. All procedures will be performed after the induction of anesthesia. Laboratory specimens will also be removed while the animal is under anesthesia. Animals surviving hemorrhage will euthanized after 24 h. Buprenorphine

(0.005–0.01 mg/kg IM) will be administered every 12 h for postoperative pain.

Description of Procedures: Under anesthesia, a thermodilution pulmonary catheter will be placed percutaneously in the right jugular vein and an intravenous catheter for volume infusion will be placed in the left jugular vein. A right femoral arterial line will be placed percutaneously. EKG leads will be applied to shaven skin. An upper abdominal flap incision will be performed through the which the hepatic artery and portal vein will be mobilized. Hepatic artery branches outside the liver will be ligated and the liver will be freed of its ligamentous attachments. A catheter for blood drawing will be placed in the portal vein and ultrasonic flow probes will be placed on the hepatic artery and portal vein. After obtaining baseline cardiac output, heart rate, blood pressure, total hepatic blood flow, and oxygen delivery, a liver Truecut™ needle biopsy will then be done. One set of animals will undergo hemorrhage of 25% of the blood volume and the second group will undergo hemorrhage of 50% of the blood volume over 10 min. There will also be a control group that will not undergo hemorrhage. Two other control groups will be hemorrhaged (25% and 50%) but will not be resuscitated. After 15 min, resuscitation fluids will be given to the treatment groups, i.e., hypertonic saline, blood, Hespan®, or lactated Ringer's solution. Hemodynamic data and blood samples will then be collected 15 min after hemorrhage and at 15, 30, 60, 120, and 180 min after resuscitation. When this is completed, the catheter will be removed, the incisions will be closed, and the animals will be kept in their cages for 24 h, receiving Buprenorphine for postoperative pain relief. All surviving animals will be sacrificed at the end of the 24-h period.

Postoperative care plan: All animals will be allowed food and water ad libitum following the completion of all surgical procedures. They will be allowed unrestricted activity inside of their cage. Incisions used to place intravascular catheters or other incisions will have been closed under anesthesia. They will receive Buprenorphine for postoperative pain relief and can be managed easily in open runs. There should be no special restraints necessary and no special monitoring necessary other than insuring that the animals awaken safely from the general anesthetic. The wounds will be treated with dry gauze dressings.

Determination of Number of Animals Required: Previous studies using this swine model of hemorrhage have used 5 animals in each study group. Since this study is looking at two types of hemorrhage (25% and 50%) with four different types of resuscitation fluid and three control groups (no hemorrhage, 25% hemorrhage, and 50% hemorrhage without resuscitation), there is a need for 8 study groups and 3 control group of 5 animals each for a total of 55 animals.

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

This project was approved by the USAISR Research Council and US Army Institute of Surgical Research Animal Care and Use Committee during this reporting period.

DISCUSSION

Equipment and supplies have been ordered and work will be initiated upon their arrival.

PRESENTATIONS/PUBLICATIONS

None.

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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
					1 Oct 89	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
12 Jun 89	D. Chg	U	U		CX	

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS14	DA	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 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)*
89	0.1	20
90	0.1	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)*: 512-221-4652
21. GENERAL USE: FINA MILITARY/CIVILIAN APPLICATION: M	f. NAME OF ASSOCIATE INVESTIGATOR *(if available)*: BUESCHER, T M g. NAME OF ASSOCIATE INVESTIGATOR *(if available)*

22. KEYWORDS *(Precede EACH with Security Classification Code)* (U) Burns (Injuries); (U) Trauma; (U) Sepsis; (U) Morbidity; (U) Amino Acids; (U) Enzymes; (U) Metabolism;

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

22. (Continued) (U) Volunteers: (U) Adults; (U) RA II

23. (U) A DTIC literature search was conducted under DTIC request number W6R20M dated 29 May 1990 for the technical report database and request number W6R22N dated 29 May 1990 for the work unit database. The proposed effort will not result in the duplication of effort. The objective of this work is to measure PLP levels in thermally injured patients and correlate those with abnormalities in amino acid metabolism.

24. (U) 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) 8810 - 8909. Nine patients have been enrolled in the study. Data show severe depression of PLP levels starting at 3 days postinjury and continuing until convalescence. For technical reports, refer to the *US Army Institute of Surgical Research Annual Research Progress Report for Fiscal Year 1989*.

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

ANNUAL RESEARCH PROGRESS REPORT

PROJECT NUMBER: 3M161102BS14-00, BASIC RESEARCH

PROJECT TITLE: Correlation of Plasma Amino Acid and Pyridoxal-5'-Phosphate (PLP) Levels in Thermally Injured Patients

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

12 June 1989 - 30 September 1989

INVESTIGATORS

William K. Becker, MD, Lieutenant Colonel, MC
Teresa M. Buescher, MD, Captain, MC
Basil A. Pruitt, Jr., MD, Colonel, MC

ABSTRACT

PROJECT NUMBER: 3M161102BS14-00, BASIC RESEARCH

PROJECT TITLE: Correlation of Plasma Amino Acid and Pyridoxal-5'-Phosphate (PLP) Levels in Thermally Injured Patients

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

PERIOD COVERED IN THIS REPORT: 12 Jun 89 through 30 Sep 89

INVESTIGATORS: William K. Becker, MD, Lieutenant Colonel, MC
Teresa M. Buescher, MD, Captain, MC
Basil A. Pruitt, Jr., MD, Colonel, MC

The purpose of this study is to measure PLP levels in thermally injured patients and correlate this with abnormalities in amino acid metabolism. 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 consequenes of multiorgan failure and therefore enhance survival of critically ill patients.

This project was approved by the USAISR Research Council and the US Army Institute of Surgical Research Human Use Committee during the fourth quarter of fiscal year 1989.

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. The purpose of this study is to measure PLP levels in thermally injured patients and correlate this with abnormalities in amino acid metabolism.

MATERIALS AND METHODS

Study Design: Twenty-five patients will have plasma PLP and amino acid profiles drawn on admission, weekly, and when indicated

by a change in clinical status. The plasma PLP levels will be sent to MAJ Richard C. Keniston at the William Beaumont Army Medical Center for assay and the aminograms will be performed by Aminograms, Inc. (St. Paul, MN). During the initial phase of this study, 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 B-6 supplementation received in tube feedings or hyperalimentation will be recorded. No additional supplementation of vitamin B-6, beyond that normally present in the diet, enteral, or parenteral feedings, will be given to the patients in the first phase of this study. If after study of the first 25 patients a significant depression of PLP is found, a further study group for vitamin B-6 supplementation will be proposed. The second phase of the study will correlate plasma PLP and amino acid levels in response to supplementation with clinical status (presence of sepsis, burn size, etc.).

Patient Inclusion: Male or female patients ≥ 18 yr old with burns > 20% of the total body surface area (the presence of an inhalation injury not being exclusionary) admitted to the US Army Institute of Surgical Research within 72 h postburn are eligible for enrollment in this study.

Patient Exclusion: Patients < 18 yr old with burns < 20% of the total body surface area or toxic epidermal necrolysis syndrome (TENS) or who are not admitted to the US Army Institute of Surgical Research within the first 72 h postburn are ineligible for enrollment in this study.

Description of Patient Procedures: Blood samples for PLP and plasma amino acids will be obtained upon admission, weekly, and when clinically indicated until discharge. Data collection will include burn size, presence of inhalation injury, mortality, morbidity, caloric intake, amount of vitamin B_6 supplementation for patients on hyperalimentation and enteral feeding, use of aminoglycoside antibiotics, theophylline drugs, and/or digoxin, and nitrogen and liver function tests from routine studies.

Amino Acid Analysis: Blood for amino acid analysis will be collected in a 7-ml green-top tube (lithium-heparin) and placed directly on ice. The plasma will be separated by centrifugation and stored in a plastic cryotube at -80°C prior to shipment to Aminograms, Inc., for performance of amino acid analysis (see attached letter of agreement). Samples will be shipped on dry ice for overnight delivery to Aminograms, Inc., for performance of plasma amino acid analysis on a Beckman 6300 amino acid analyzer. This technique involves lithium-based buffering for high performance liquid chromotography on a 20-cc column with ninhydrin analysis.

Plasma PLP Analysis: Blood for plasma PLP analysis will be collected in a 7-ml purple-top (EDTA preservative) tube on ice protected from light. Plasma will be separated by centrifugation and stored in a plastic cryotube at -80°C prior to shipment to the William Beaumont Army Medical Center for performance of plasma PLP analysis (see attached letter of agreement). Samples will shipped on dry ice for overnight delivery to the William Beaumont Army Medical Center where plasma PLP analysis will performed. The technique for determining PLP is the undeproteinized tyrosine apodecarboxylase radioimmunoassay. This technique appears to correlate better with survival in critically ill patients than bioassays or functional assays.

Determination of Number of Subjects Required: Amino acid and PLP levels will be determined in 100 patients, with data evaluation after the first 25 patients. No baseline reports are readily available for burn patients. Therefore, it is difficult to predict the minimum number of patients needed for scientific validity.

Data Collection: Data collection will include 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. Details of nitrogen balance studies are shown in Table 1. Data will be tabulated for each patient on flow sheets kept by CPT Buescher.

Data Analysis Plan: Multiple regression will be used to detect any relationship between the various independent variables from the collected data and dependent variables which are measured.

RESULTS

This project was approved by the USAISR Research Council and the US Army Institute of Surgical Research Human Use Committee during the fourth quarter of fiscal year 1989.

DISCUSSION

Equipment and supplies have been ordered and work will be initiated shortly.

PRESENTATIONS/PUBLICATIONS

None.

TABLE 1. Formula by Waxman et al with Silver Sulfadiazine Modification*

Nitrogen Intake − Nitrogen Output = Nitrogen Balance

$$\text{Nitrogen Intake} = \frac{\text{Grams Protein Intake}}{6.25}$$

Nitrogen Output by UUN Method = Urinary Urea Nitrogen + 4 g + Wound Loss

Nitrogen Output by TUN Method = Total Urea Nitrogen + 2 g + Wound Loss

Wound Loss = 0.1 X TBSA X %TBSA Burn X 0.8

REFERENCES

1. Keniston RC, Reyna T, Becker W, et al: Prognostic value of undeproteinized plasma pyridoxal 5′-phosphate concentrations. In **Clinical and Physiological Applications of Vitamin B-6**. Alan R. Liss, Inc., pp 425-33, 1988.

2. Martin TR, Halpert M, Kenniston RC, and Becker WK: Plasma pyridoxal 5′-phosphate (PLP), a predictor of outcome in surgical intensive care unit patients (abstr).

3. Keniston RC, Becker W, Enriquez J, and Duncan F: Plasma pyridoxal 5′-phosphate levels in health and disease (in press).

4. Herndon DN, Wilmore DW, Mason AD Jr, and Pruitt BA Jr: Abnormalities of phenylalanine and tyrosine kinetics. **Arch Surg** 113:133-5, 1978.

5. Brown WL, Bowler EG, Mason AD Jr, and Pruitt BA Jr: Protein metabolism in burned rats. **Am J Physiol** 231:476-82, 1976.

6. Aulick HL and Wilmore DW: Increased peripheral amino acid release following burn injury. **Surgery** 85:560-5, 1979.

7. Cerra FB, Siegel JH, Border JR, et al: The hepatic failure of sepsis: cellular versus substrate. **Surgery** 86:409-22, 1979.

8. Waxman K, Rebello T, Pinderski L, et al: Protein loss across burn wounds. **J Trauma** 27(2):136-42, 1987.

PRESENTATIONS

Chu C-S: Multiple graft harvestings from deep partial-thickness scald wounds healed under the influence of weak direct current. Presented at the 48th Annual Meeting of the American Association for the Surgery of Trauma, Newport Beach, California, 6 October 1988.

Graves TA: Relationship of transfusion and infection in a burn population. Presented at the 48th Annual Meeting of the American Association for the Surgery of Trauma, Newport Beach, California, 6 October 1988.

DePew CL: Acid base balance. Presented as part of the Critical Care Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 19 October 1988.

Summers TM: Crisis and families. Presented as part of the Critical Care Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 19 October 1988.

DePew CL: Fluid and electrolytes. Presented as part of the Critical Care Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 20 October 1988.

Gutierrez RT: Physical therapy in burns. Presented as part of the Physical Therapy Specialist Course (91L), Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 20 October 1988.

Jordan BS: An intervention outcome assessment of a fixation device for nasoenteral tubes in burn-injured patients. Presented at the Nursing Research: Read It! Use It! Do It! Seminar, 90th Army Command Headquarters, Fort Sam Houston, San Antonio, Texas, 23 October 1988.

Pruitt BA Jr: Present management of severe burns. Presented at the Annual Meeting of the Association of Military Surgeons, San Antonio, Texas, 31 October 1988.

Burgess MT: Introduction to the care of burn patients. Presented to the Northeast Recruiting Command, St. Mary's College, Newburgh, New York, 1 November 1988.

Hayes MR: Beyond stress management, a model for occupational therapy intervention. Presented to the Occupational Therapy Specialty Group, Association of Military Surgeons of the United States, San Antonio, Texas, 3 November 1988.

Carlson DE: Nutrition and burns - application to mobilization. Presented at the Dietitian Section Meeting, Association of Military Surgeons of the United States, San Antonio, Texas, 3 November 1988.

DePew CL: Standards of nursing care for the large burn victim in the initial 48 hours. Presented at the Spotlights in Critical Care Symposium, Alamo Chapter of the American Association of Critical Nurses, San Antonio, Texas, 3 November 1988.

McManus WF: Advances in burn care. Presented to physicians from the National Aeronautics and Space Administration, San Antonio, Texas, 3 November 1988.

Summers TM: Stress and crisis management. Presented as part of the Critical Care Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 4 November 1988.

Stetz CK: The role of the nurse in burn nursing. Presented at the Annual Meeting of the Student Nurses' Association, 6th Recruiting Brigade, Los Angeles, California, 6 November 1988.

Pruitt BA Jr: The Shriners Burns Units from a national perspective. Presented at the 6th Annual Harvey Beffa Conference, Galveston, Texas, 9 November 1988.

Trevino JD: Recovery room care. Presented as part of the Practical Nurse Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 9 November 1988.

Trevino JD: Role of the practical nurse in burn care. Presented as part of the Practical Nurse Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 9 November 1988.

Lambert JL: IV therapy and the burn patient. Presented at the Intravenous Nursing Society Annual Symposium, San Antonio, Texas, 11 November 1988.

McManus WF: Infections in burns. Presented at the Surgery Grand Rounds, Kansas Medical Education Foundation, Topeka, Kansas, 12 November 1988.

McManus WF: What's new in burn care. Presented at the Surgery Grand Rounds, Kansas Medical Education Foundation, Topeka, Kansas, 12 November 1988.

Gutierrez RT: Changing roles of the US Army Physical Therapist. Presented to the Retired Army Nurses of Fort Sam Houston, Fort Sam Houston, San Antonio, Texas, 15 November 1988.

Pruitt BA Jr: Diagnosis and treatment of inhalation injury. Presented to the Department of Surgery, University of British Columbia, Vancouver, British Columbia, Canada, 17 November 1988.

Pruitt BA Jr: Infection in burn patients. Presented to the Department of Surgery, University of British Columbia, Vancouver, British Columbia, Canada, 17 November 1988.

Zelenka JP: A device to protect skin-grafted ears. Presented at the Great Southern Occupational Therapy Conference, Charleston, South Carolina, 20 November 1988.

Anderson SE: Standards of nursing care for the large burn victim in the initial 48 hours. Presented as part of the Critical Care Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 21 November 1988.

Duncan DJ: Initial management of the burn victim. Presented as part of the Critical Care Course, Academy of Health Sciences, Fort Sam Houston, San Antonio, Texas, 21 November 1988.

Pruitt BA Jr: Criteria for burn centers. Presented to the 8th Annual Comprehensive Care of the Burn Patient Course, Kansas City, Missouri, 2 December 1988.

Pruitt BA Jr: Managing pain in the burn patient. Presented to the 8th Annual Comprehensive Care of the Burn Patient Course, Kansas City, Missouri, 2 December 1988.

Pruitt BA Jr: Burn-induced metabolic disease. Presented to the 8th Annual Comprehensive Care of the Burn Patient Course, Kansas City, Missouri, 3 December 1988.

Pruitt BA Jr: Resuscitation: to do or not to do. Presented to the 8th Annual Comprehensive Care of the Burn Patient Course, Kansas City, Missouri, 3 December 1988.

McManus WF: Burn care - the state of the art. Presented as Visiting Professor, Cornell University-New York Hospital Burn Center, New York, New York, 5 December 1988.

Cioffi WG Jr: Outpatient care of pediatric burns. Presented at the Annual Staff Meeting, Santa Rosa Children's Hospital, San Antonio, Texas, 6 December 1988.

Pruitt BA Jr: Early burn wound excision and closure. Presented at the Burn Injuries Seminar, International Society for Burn Injuries, Denver, Colorado, 9 December 1988.

Pruitt BA Jr: Inhalation injury. Presented at the Burn Injuries Seminar, International Society for Burn Injuries, Denver, Colorado, 9 December 1988.

Pruitt BA Jr: Immunologic effects of burn injury. Presented at the Burn Injuries Seminar, International Society for Burn Injuries, Denver, Colorado, 10 December 1988.

Pruitt BA Jr: Unsolved problems in burn care. Presented at the Burn Injuries Seminar, International Society for Burn Injuries, Denver, Colorado, 10 December 1988.

Chapman TH: Initial management and aeromedical evacuation of the burn victim. Presented to the 34th Aeromedical Evacuation Squadron, Kelly Air Force Base, San Antonio, Texas, 11 December 1988.

Selzer RA: Management of burn victims in the theater of operations. Presented as part of the United States Air Force Battlefield Nursing Course, Brooks Air Force Base, San Antonio, Texas, 13 December 1988.

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

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

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

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

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

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

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

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

Jennings JL: Aeromedical transport of the burn victim. Presented to the Nursing Service Branch, United States Army

Institute of Surgical Research, Fort Sam Houston, San Antonio, Texas, 10 January 1989.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Pruitt BA Jr: Scientific activities of the United States Army Institute of Surgical Research - 1988. Presented at the United States Army Medical Research and Development Command Commanders Conference, Denver, Colorado, 6 February 1989.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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