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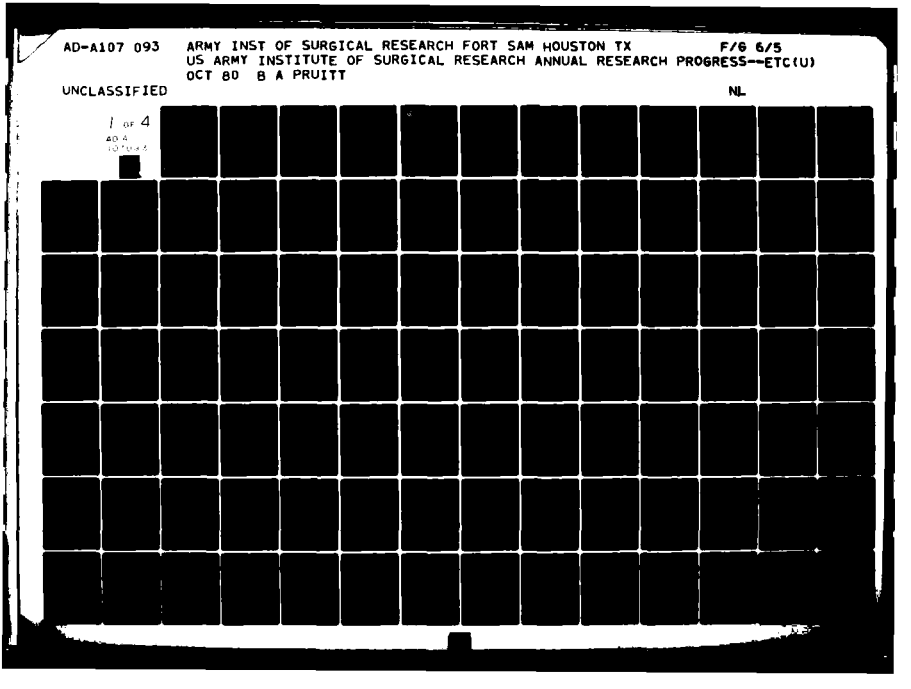

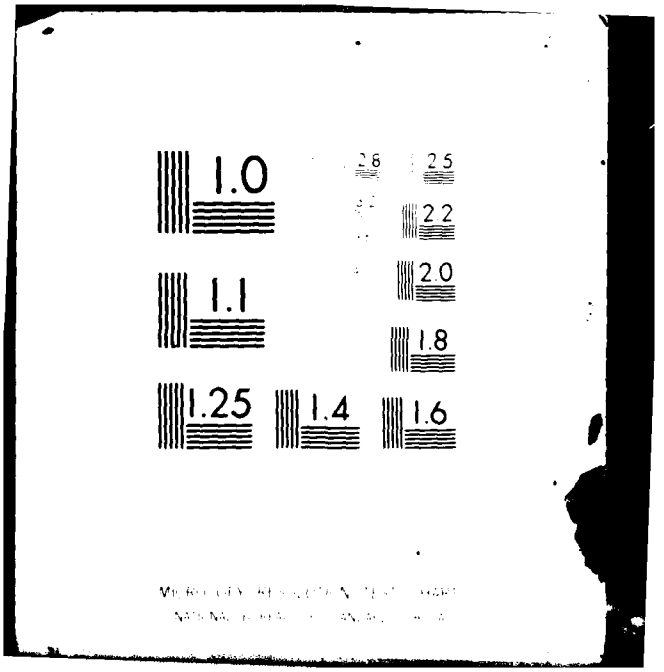
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REPORT MEDDH-288(R1) AD_____

US ARMY INSTITUTE OF SURGICAL RESEARCH ANNUAL RESEARCH PROGRESS REPORT FY 1980

U.S. ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234

(1 October 1979 - 30 September 1980)

1 October 1980

Prepared for:
US ARMY MEDICAL RESEARCH & DEVELOPMENT COMMAND
FORT DETRICK, FREDERICK, MD 21701

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7. AUTHOR(s): Basil A. Pruitt, Jr., COL, MC	8. CONTRACT OR GRANT NUMBER(s)
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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

This report documents the clinical and laboratory activities of the US Army Institute of Surgical Research during the fiscal year 1980. These activities include patient care, clinical investigation and laboratory research in the areas of burn injury and general trauma. Special emphasis is placed on the clinical management of burned patients and on studies related to prevention and treatment of burned wound infection.

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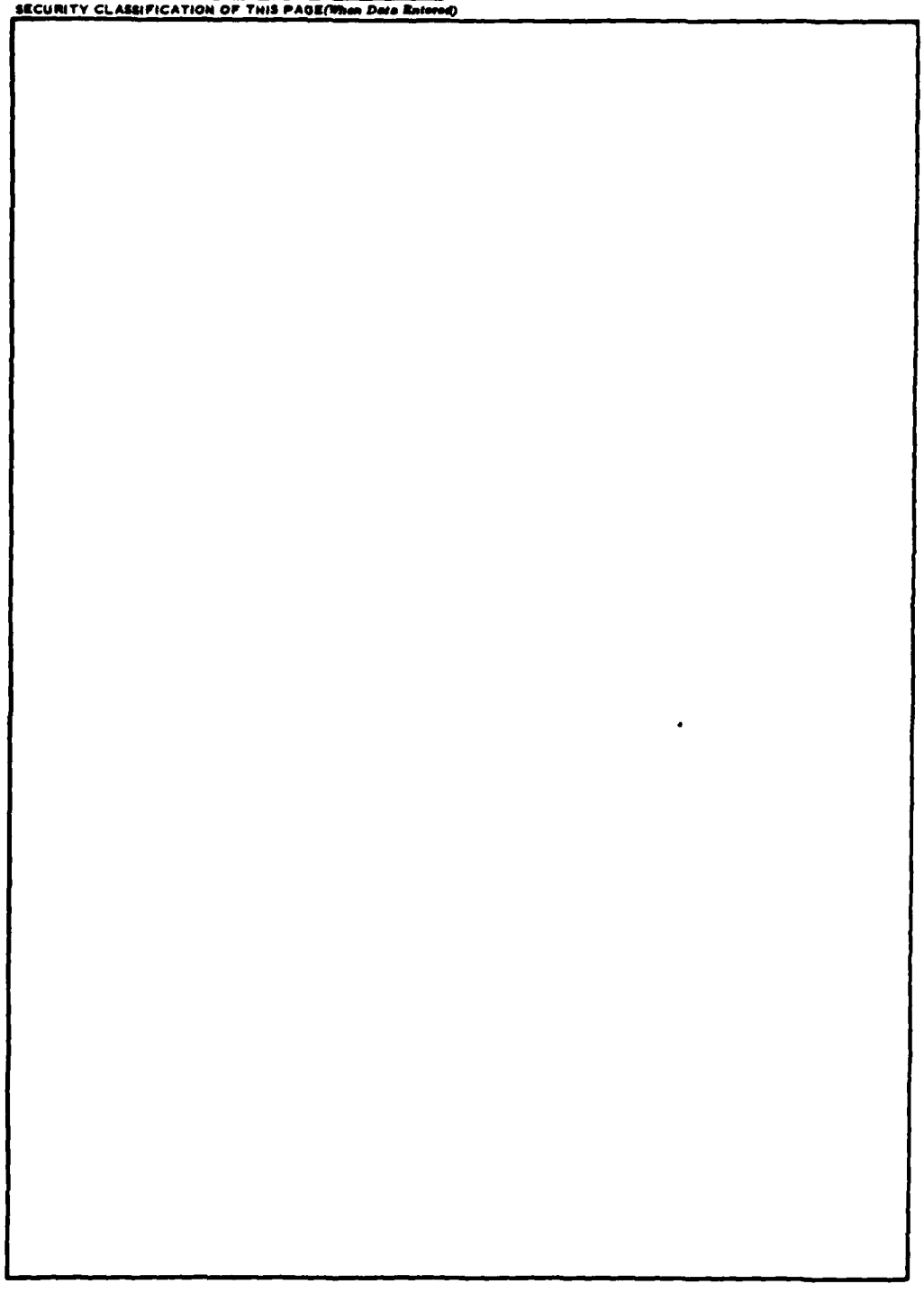

DEPARTMENT OF THE ARMY
US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234

SGRD-USZ

SUBJECT: Annual Research Report FY 1980

TO: SEE DISTRIBUTION

Annual report(s) of the US Army Institute of Surgical Research for FY-80 are forwarded under provisions of the OTSG Regulation 70-31, dated 2 April 1969.

Basil A. Pruitt, Jr.

BASIL A. PRUITT, JR., M.D, FACS
COLONEL, MC
COMMANDER & DIRECTOR

FOREWORD

The work reported in this volume is the result of the effective collaboration of clinicians and basic scientists in the study of the clinically significant physiologic changes and complications which occur in injured man. Regardless of the type of military scenario anticipated or weapons employed, wounded soldiers will experience the same pathophysiologic consequences as do burn patients. If one wishes to provide optimum care for combat casualties, it is imperative that these studies receive continuing support proportional to their military importance.

During the past two fiscal years, this Institute has sustained a steady hemorrhage of clinical personnel until it stands on the verge of irreversible shock. Clinical care is jeopardized and the continued involvement of clinicians in investigative activities is virtually impossible. The assigned clinical personnel who have labored so hard to care for our critically ill patients and advance that care by research activitity have identified the fact that their fatigue is accentuated by a sense of loneliness resulting from both the departure of their fellow clinicians and their perception that the historical commitment to excellence has fallen victim to expediencies employed in dealing with the physician shortage.

The current tendency to equate all physicians is yet another evidence of inflation in American life and by its very nature is self-defeating since it makes no recognition

of professional attainment or productivity. Medical advances do not come about as a result of administrative force, finesse or fiat, but only by virtue of appropriately trained and qualified scientists identifying and then addressing problems of importance. To prevent the previously noted personnel hemorrhage from progressing to disintegration of the corpus, the military physician's scientific productivity must be nurtured, his professional attainment furthered, and his status made comparable to his civilian counterparts. In short, all doctors are not the same and the viability of the Institute of Surgical Research depends upon the recognition of such. On the basis of the past achievements of ISR investigators, both individually and as a group, it is clear that they have been and are a class unto themselves. The material presented within this volume, which was generated during a period of physician shortage, is further testimony to the industry and excellence of the ISR staff.

Basil A. Pruitt Jr.

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

The opinions expressed above are the private views of the author and are not to be construed as official or as reflecting the views of the US Army Medical Research & Development Command, the Department of the Army or the Department of Defense.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
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3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISB'N INSTR'N	8b. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
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a. PRIMARY	62772A	3S162772A874	AF	164
b. [redacted]	62772A	3S162772A814	00	118
c. [redacted]	STOC 80-7.2.5			

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NAME: DA

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(U) Heterograft; (U) Resuscitation; (U) Homograft; (U) Air Evacuation; (U) Thermal Injury; (U) Inhalation Injury; (U) Topical Therapy; (U) Autograft.

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

25. (U) The Clinical Division of the US Army Institute of Surgical Research continues its role as a major specialized clinical treatment center for thermally injured military personnel. Its main objectives are the investigation and modification of new diagnostic and therapeutic methods for optimum care of the burn patient as well as dissemination of the scientific advances to military and civilian medical treatment centers.

24. (U) Thermally injured patients both from the Continental United States and throughout the world are evacuated to the US Army Institute of Surgical Research for intensive inpatient therapy. Carefully controlled evaluation of the efficacy of many treatment modalities is undertaken.

25. (U) 7910 - 8009. Two hundred seventy three seriously burned patients were admitted and treated during 1979. Active clinical research activities include evaluation of laminar flow isolation to delay burn wound colonization; assessment of L-Triiodothyromine therapy following thermal injury; studies of pulmonary function following crystalloid and colloid intravenous fluid resuscitation; investigation of the metabolic response to and nutritional support of acutely burned patients has provided information for the care of burned and injured man.

*Available to contractors upon originator's approval.

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ANNUAL PROGRESS REPORT

PROJECT NO. 3S162774A814-00, APPLIED RESEARCH

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

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

1 January 1979 - 31 December 1979

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ABSTRACT

PROJECT NO. 3S162774A814-00, APPLIED RESEARCH

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

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

Period covered in this report: 1 January - 31 December 1979

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Two hundred and seventy three patients were admitted to the Clinical Division of the United States Army Institute of Surgical Research during the calendar year of 1979. The emphasis of Clinical Division activities over the past year have continued in the areas of excellence of patients care; research in the areas of host response to injury and the improved methods of treatment; and, education of health professionals. Major areas of research included assessment of metabolic and neuroendocrine responses to injury, infection prevention and treatment, and pulmonary response to injury and resuscitation. This report summarizes the activities of the Clinical Division of the U.S. Army Institute of Surgical Research during the calendar year 1979; catalogs the responses to treatment and complications which contributed to morbidity and mortality; summarizes the single largest and most successful management of burn mass casualties; and contains the recommendation of the Chief, Clinical Division for future improvement.

CLINICAL OPERATION, CENTER FOR TREATMENT OF BURNED SOLDIERS

The Clinical Division of the United States Army Institute of Surgical Research continued throughout the calendar year 1979 to provide clinical care for thermally, chemically or electric injured soldiers and other authorized patients.

Two hundred and seventy three patients were admitted during the period of this report. There were 97 aeromedical evacuation flights for 161 patients (60% of admissions) of which 94 were within the Continental United States for a total of 122 patients. The OCONUS flights were to Panama and Alaska for one patient each and Japan for 38 patients. In addition, 67 patients were admitted directly to the Burn Center following pre-hospital care by local Emergency Medical Service agencies. Ninety-four of the 273 patients (34%) were admitted on the day of burn.

The single largest mass casualty operation in the history of the Institute of Surgical Research was initiated on Friday 19 October 1979. A burn team consisting of three surgeons, one microbiologist, three registered nurses, two respiratory therapists and nine hospital corpsmen was dispatched with the supplies and equipment necessary to treat and transport 45 marines injured in an accident on Mount Fuji, Japan that morning. The U.S. Air Force Military Airlift Command transported the team and equipment to Japan in a C-141 Starlifter Medevac plane and pre-positioned a second C-141 in Japan for the return flight. Additional ventilators and supplies were mobilized from Japan, Okinawa, the Philippines and Alaska. Initial triage by the burn team selected 38 of the 45 patients with burn injury to be flown to this Burn Center. Three patients with minor injury were treated locally and four patients died in Japan. The average total body surface injury in the 38 patients was 42% and 11 had clinical evidence of inhalation injury necessitating endotracheal intubation prior to departure from Japan. Five patients had burn injuries greater than 80% of the total body surface, seven were between 61% and 80% TBS, eight were between 41% and 60% TBS, nine were between 21% and 40% TBS and nine patients had burns of less than 20% of the TBS. These 38 patients were then air evacuated to the Institute of Surgical Research in two C-141 MAC Starlifter aircraft (21 patients in one aircraft and 17 patients in the other) within 55 hours of injury. A mortality predictor program based on burn size and patient age predicted 14 deaths in these 38 patients. However, observed mortality was nine, a 36% improvement over predicted mortality. The success of this operation was due to the high quality of professional care combined with the experience of the personnel involved in carrying out the aeromedical transfer of critically ill patients. Both the US Air Force Medical Airlift Command and the U.S. Army Institute of Surgical Research on a daily basis triage, treat and air evacuate severely injured and ill patients and this mass casualty operation was an expansion of daily activity.

CLINICAL MANAGEMENT

The overall management of the thermal, chemical and electrically injured patients as practiced by this Institute has been adequately documented

in previous annual reports and numerous scientific presentations and publications and has remained essentially unchanged during this calendar year.

RESEARCH

Areas of continued investigation include evaluation of methods of infection control, the neuroendocrine responses to injury and infection, pulmonary changes following injury, nutritional support of the compromised host, and injury induced metabolic change.

EDUCATION

Throughout the calendar year 1979, the staff of the Clinical Division of the Institute of Surgical Research conducted extensive educational activities for military and civilian professional and paraprofessional personnel. Sixteen resident physicians (9 Army, 6 Air Force and 1 Navy) from military graduate training programs as well as one physician from Canada and one from Belgium were attached to the Institute of Surgical Research for education and experience in the care of thermally injured patients. Seven medical students, nine civilian physicians and four Army reserve officers on active duty for training were also attached to the Institute of Surgical Research for education and experience. Fifteen scientific publications appeared in refereed medical journals and 155 scientific presentations were conducted for military and civilian medical audiences. Eighteen physicians from various foreign countries visited the Institute of Surgical Research and were briefed on the spectrum of activities at this Institute. Formal educational rounds are conducted by the Institute staff for the Brooke Army Medical Center General Surgical house officers and staff. Numerous presentations at the Academy of Health Sciences and various military installations throughout the Continental United States were also conducted. In addition weekly professional conferences were conducted for and by Institute personnel.

MORBIDITY AND MORTALITY

Seventy four of 267 patients for whom disposition was made during calendar year 1979 died in the hospital for an overall mortality of 27.7%. Sixty eight percent of hospital deaths had autopsies performed. The average total body surface injury of patients who died was 65% and the average third degree burn component was 37%. Six patients who died were under 15 years of age with an average burn size of 55.7% with a full thickness average of 14.2% of the total body surface. Four of these six patients had autopsies. Thirty one of the 74 patients who died (42%) had documented inhalation injury as the contributing or primary cause of death. Unusual causes of mortality included a sudden infant death syndrome with pulmonary edema in one patient, fat emboli in one patient, Aspergillus pneumonitis with Aspergillus septicemia in one patient and gram negative Meningitis with Septicemia in one patient. As previously reported infection continues to be the most common complication following thermal, chemical or electric injury. Ninety seven patients had positive blood cultures reported. Coagulase positive Staphylococcus aureus continues to be the organism most frequently recovered in blood culture and was recovered from 62 patients. Pseudomonas aeruginosa continued to be the second most frequently recovered organism and was recovered in 32 patients, Klebsiella pneumoniae in 17 patients, and Escherichia coli in ten patients. Burn wound sepsis was diagnosed in 38 patients.

Mycotic infection has continued as evidenced by 19 patients with Candida species recovered from blood culture, 11 patients with Aspergillus species invading the burn wound and 7 patients with Phycomycetes invading the burn wound. Unusual infections included Herpes Zoster in one patient and meningitis in four patients. One hundred and eleven patients (41.6%) had an associated injury(s). Seventy three of these 111 patients had inhalation injury; 9 patients had major fractures; 12 patients had head injuries; 1 patient had spinal cord injury; 6 patients had corneal burns, and 1 additional patient had a corneal abrasion; and 1 patient had massive blast injury.

In calendar year 1979 no patient required operation for upper gastrointestinal hemorrhage, however nine patients had minimal upper gastrointestinal hemorrhage, controlled nonoperatively. One patient had a gastrectomy for upper gastrointestinal hemorrhage prior to admission to this Institute, however this patient had not been treated with Cimetidine and/or antacids. One patient required operation for a perforated ulcer; one patient had an appendectomy and one patient had acute pseudo-obstruction of the colon. Two patients were pregnant when injured, one patient delivered a stillborn infant while the other patient carried a successful term pregnancy.

Forty eight patients (18%) had acute renal failure of which 25 patients had acute tubular necrosis, three patients had renal abscesses, four patients had acute pyelonephritis and nine patients required acute hemodialysis. Acute renal failure was diagnosed as a terminal event in the majority.

Cardiac complications during 1979 included five patients with acute myocardial infarctions, two patients with acute myocarditis with abscesses, five patients with acute bacterial endocarditis one of whom required aortic valve replacement for acute bacterial endocarditis with refractory congestive heart failure.

Pulmonary complications included 61 patients (23% of admissions) with bronchopneumonia, seven patients with hematogenous pneumonia and 73 patients with inhalation injury.

STATISTICAL RESUME DURING CALENDAR YEAR 1979

273 thermally injured patients were admitted to the Institute of Surgical Research and there were 267 dispositions during the same period. Subsequent data will be based on dispositions. There were 228 males and 39 females with an average age of 30 years ranging from 9 months to 80 years of age. Thirty eight patients (14.2%) were under 15 years of age and 61 patients (23%) were over 45 years of age. The average total burn size was 35% of the total body surface with an average full thickness burn size of 18%. The average hospital stay for all patients was 43.8 days however excluding convalescent leave for active duty military reduces the average hospital stay to 40.8 days. Ninety four patients were admitted on the day of burn and the average post burn day of admission was 2.6 days.

During 1979, 1,518 operations were performed on 218 patients for an average of six operations per patient. Five hundred and fifty five

anesthetic procedures were administered for an average of two anesthetics per patient. One hundred and forty six patients required cutaneous autografts and 86 patients required biologic dressings for a total of 283 allograft/xenograft applications.

During calendar year 1979 there were 97 rapid section biopsies from 54 patients with suspected wound infection and 288 surgical specimens from 125 patients. Of the 97 biopsies, 47 were read as bacterial or fungal infection four biopsies had multiple organisms identified while 43 biopsied had single organism infection recognized. Individual specimens were classified as showing evidence of superficial colonization, deep colonization and invasion for gram positive cocci, gram negative bacilli and fungi. Electron microscopy was utilized for the clinical diagnosis of infection or the identification of diseased tissue in autopsy material in 21 cases.

Table 1 identifies the source of admission of patients during calendar year 1979 and again the majority of patients were from the Continental United States. Table 2 summarizes the burn etiology and Table 3 summarizes the effect of age and total body surface injury on mortality. Table 4 lists mortality rate associated with increments of 10% total body surface burn involvement for the years 1976 through 1979. Table 5 through Table 7 summarize the mortality experience at the Institute of Surgical Research.

RECOMMENDATIONS

The major recommendation for the Clinical Division is in the area of improvement of the physical facility to bring this facility in line with the standards of the 20th century that provides for both safe and acceptable patient care. The lack of bathing and toilet facilities on the two burn center wards, making it necessary for men, women adults and children, all, to use the same bathroom and toilet facilities is unacceptable. In addition to the lack of bathing and toilet facilities the general lack of privacy for the severely injured patient, the lack of suitable family waiting and counseling areas, and the lack of professional office space all combine to present an unacceptable appearance of a world famous patient care facility. Immediate consideration need be given to either remodeling the existing facilities or building an entirely new facility to correct these serious shortcomings.

SUMMARY

A total of 273 patients were admitted to the U.S. Army Institute of Surgical Research and 267 dispositions were made during calendar year 1979. Infection continued to be the most common cause of mortality. As in the preceeding year no episode of upper gastrointestinal hemorrhage required operation in 1979. The highly successful management of a large number of patients burned at one time demonstrates that such success is made possible by the experience gained in the daily management and aeromedical transfer of acutely injured patients.

Table 1. Source of Admission, 1979

Area	A	AD	AF	AFD	N	ND	VAB	Other	TOTAL
1st Army	3	1	1	0	0	0	2	0	7
3rd Army	13	3	4	2	2	2	8	13	47
5th Army	13	16	8	9	2	2	19	85	154
6th Army	4	7	1	1	1	0	0	2	16
Germany	5	0	0	1	0	0	0	0	6
Brazil	0	0	0	0	0	0	0	1	1
Alaska	1	0	0	0	0	0	0	0	1
Honduras	0	0	0	0	0	0	0	1	1
Guam	0	0	0	0	0	0	0	1	1
Hawaii	0	0	0	0	0	0	1	0	1
Japan	0	0	0	0	23	0	0	0	23
Panama	0	0	1	0	0	0	0	0	1
Mexico	0	0	0	0	0	0	0	3	3
Spain	0	0	2	0	1	0	0	0	3
Dominican Republic	0	0	0	0	0	0	0	2	2
	39	27	17	13	29	4	30	108	267

A - Army
AF - Air Force
D - Dependent
N - Navy, Marine Corps & US Coast Guard
VAB - Veterans Administration Beneficiary
Other: Civilian Emergency
US Public Health Service Beneficiary
Bureau of Employees Compensation Beneficiary

Table 2. Burn Etiology, 1979 - 267 Dispositions

Causes	Number of Patients	% Disposition	Deaths	% Mortality
Gasoline, Diesel & Kerosene	82	30.7%	24	29.3%
Structural Fires	19	7.1%	10	52.6%
Motor Vehicle Accidents	22	8.2%	6	27.3%
Aircraft Accidents	4	1.5%	3	75.0%
Open Flames	13	4.9%	3	23.0%
Electrical	17	6.4%	0	0.0%
Hot Liquids	29	10.9%	5	17.2%
Chemical	6	2.2%	0	0.0%
Butane, Propane or Natural, Sewer Gas Exp.	28	10.5%	9	32.1%
Welding	8	3.0%	3	37.5%
Smoking Clothes Ignited	8	3.0%	5	62.5%
Bomb, Shell, Simulator Grenade, Gunpowder Exp.	14	5.2%	3	21.4%
Others	15	5.6%	3	20.0%
Contact	2	0.7%	0	0.0%
TOTAL	267		74	

Table 3. Age, Body Surface Involvement & Mortality, 1979

Age (Yrs)	Per Cent Burn										Total Cases	Total Deaths	% Mortality
	0-10	10-20	20-30	30-40	40-50	50-60	60-70	70-80	80-90	90-100			
0-1	0	1	0	1(1)	1	0	0	0	0	0	3	1	33.3
1-2	3	3	1	0	0	1(1)	1(1)	0	1(1)	0	10	3	30.0
2-3	1	1	1	0	0	0	0	0	0	0	3	0	0.0
3-4	0	0	0	1	0	0	0	0	0	0	1	0	0.0
4-5	0	1	0	0	1(1)	0	0	0	0	0	2	1	50.0
5-10	1	3	3	2	1(1)	0	1	0	0	0	11	1	9.1
10-15	3	3	1	0	1	0	0	0	0	0	8	0	0.0
15-20	12	4	5	0	3	6	2(2)	0	2(2)	1(1)	35	5	14.3
20-30	13	13	16	6	12(1)	10(3)	5(3)	1(1)	6(6)	1(1)	83	15	18.0
30-40	2	8	2	3	6(1)	4(1)	4(3)	3(3)	4(4)	2(2)	38	14	36.8
40-50	7	3	4	4(3)	2	2(2)	0	2(2)	0	1(1)	25	8	32.0
50-60	6	6	0	3(1)	1	1(1)	2(2)	3(3)	1(1)	3(3)	26	11	42.3
60-70	1	3(2)	2	2(2)	2(2)	4(2)	1(1)	1(1)	0	0	16	10	62.5
70-80	0	0	0	0	2(2)	1(1)	0	0	0	0	3	3	100.0
80-90	1	0	0	1(1)	0	0	0	0	1(1)	0	3	2	66.7
Total	50	49	35	23	32	29	16	10	15	8	267		
Deaths	0	2	0	8	8	11	12	10	15	8		74	
% Mortality	0	4	0	34.8	25	37.9	75	100	100	100			27.7

Note: Deaths shown in parentheses.

Table 4. Per Cent Body Surface Involvement and Mortality, 1976 - 1979

% Burn	0-10	10-20	20-30	30-40	40-50	50-60	60-70	70-80	80-90	90-100	Total
					(1976)						
No. Burned	28	49	39	30	31	27	19	15	13	9	260
Deaths	0	2	3	4	10	13	12	13	13	9	79
% Mortality	0	4.1	7.7	13.3	32.3	48.1	63.2	86.7	100	100	30.4
					(1977)						
No. Burned	37	35	32	46	24	20	18	12	6	4	234
Deaths	0	1	5	10	9	11	14	11	5	4	70
% Mortality	0	2.9	15.6	21.7	37.5	55	77.8	91.7	83.3	100	29.9
					(1978)						
No. Burned	48	49	46	37	27	17	20	12	6	6	268
Deaths	0	4	6	10	9	8	12	12	2	6	69
% Mortality	0	8.2	13.0	27.0	33.3	47.0	60.0	100	33.3	100	25.8
					(1979)						
No. Burned	50	49	35	23	32	29	16	10	15	8	267
Deaths	0	2	0	8	8	11	12	10	15	8	74
% Mortality	0	4.0	0	34.8	25.9	37.9	75.0	100	100	100	27.7

Table 5. Survival and Death by Year for Patients With Extensive Burns, 1957-1979

Year	Survivors (burns over 30%)			Deaths		
	No. Cases	Average % Burn		No. Cases	Average % Burn	
		Total	3°		Total	3°
1957	19	38.4	24.1	17	57.1	38.8
1958	15	42.3	21.6	23	56.5	35.3
1959	29	43.1	20.6	24	63.1	38.1
1960	17	44.2	20.1	30	57.8	37.3
1961	18	44.2	25.0	31	58.0	39.7
1962	18	42.7	21.4	54	59.1	46.2
1963	28	45.8	19.6	57	69.0	41.0
1964	40	41.8	14.8	37	65.0	42.4
1965	47	43.8	21.0	33	66.0	33.4
1966	68	41.5	14.9	59	59.9	31.3
1967	103	42.7	13.3	51	59.9	32.3
1968	143	44.2	12.6	38	54.6	24.6
1969	113	43.2	11.1	70	58.7	26.4
1970	92	39.4	10.7	70	51.9	32.6
1971	63	41.9	14.0	68	60.8	38.0
1972	62	42.0	17.2	103	56.7	35.9
1973	47	43.7	19.6	113	60.3	36.2
1974	55	43.9	12.2	97	60.8	35.9
1975	80	46.1	14.7	94	61.3	32.8
1976	69	45.5	15.0	79	64.2	31.1
1977	66	42.2	14.4	70	56.9	29.0
1978	67	45.7	14.8	69	55.2	33.0
1979	61	45.4	13.4	74	65	37

Table 6. Comparison of Burn Mortality Rates, 1962-1963 and 1964-1979

Years	Per Cent Burn														
	0-30			30-40			40-50			50-60			60-100		
	No. Pts.	No. Deaths	% Mortality	No. Pts.	No. Deaths	% Mortality	No. Pts.	No. Deaths	% Mortality	No. Pts.	No. Deaths	% Mortality	No. Pts.	No. Deaths	% Mortality
1962-63	140	6	4.3	36	16	44.4	36	22	61.1	23	18	78.3	55	49	89.1
1964-79	2185	73	3.3	641	123	19.2	537	170	31.7	368	180	48.9	683	579	84.8

Table 7. Cause of Death, 1979

Patient	Age	Sex	% Burn Total	% Burn 3°	PBD Death	Cause of Death
1	51	M	98	97	1	*98% total body surface burn and inhalation injury
2	36	M	96	92	1	*96% total body surface burn and inhalation injury
3	35	M	96	90	2	*96% total body surface burn and inhalation injury
4	53	M	94	62.5	2	94% total body surface burn, inhalation injury and fat embolus
5	49	M	93	84	7	93% total body surface burn, severe inhalation injury and Staphylococcal pneumonitis with septic embolization
6	22	M	93	45.5	14	*93% total body surface burn, severe inhalation injury and Pseudomonas pneumonitis bilaterally
7	54	M	92	79	4	*92% total body surface burn, severe inhalation injury
8	18	M	91	46.5	9	91% total body surface burn and bilateral bronchopneumonia
9	35	F	89	83	10	89% total body surface burn, Staphylococcal pneumonia, Staphylococcal septicemia, acute Staphylococcal endocarditis
10	24	F	89	33	11	Bilateral bronchopneumonia, severe fungal invasion of stomach with fungemia and Pseudomonas septicemia
11	22	M	88	65	2	Inhalation injury with subsequent pulmonary edema
12	31	F	88	38	54	Severe inhalation injury with subsequent acute Aspergillus pneumonitis and Aspergillus septicemia
13	26	M	88	8	24	Inhalation injury, invasive burn wound sepsis with Staphylococcus aureus and Pseudomonas aeruginosa, myocardial necrosis and bilateral interstitial pneumonia
14	29	M	87	34	37	*Inhalation injury, bilateral pneumonia with Staphylococcal septicemia
15	1 10/12	M	86	68	½	*86% burns and inhalation injury

* Autopsy not performed

Table 7. Cause of Death, 1979

Patient	Age	Sex	% Burn Total	% Burn 3°	PBD Death	Cause of Death
16	19	M	85.5	51	22	Bilateral pneumonia and Proteus septicemia with septic shock
17	51	M	84.5	77.5	2	84.5% total body surface burn and acute congestive heart failure
18	20	M	82	65	1	Inhalation injury with Klebsiella pneumonia
19	32	F	81	40.5	119	Acute bacterial endocarditis organism Pseudomonas aeruginosa
20	30	M	80.5	50.5	19	Burn wound sepsis mixed Staphylococcus aureus and Pseudomonas aeruginosa with septicemia
21	80	F	80.5	41	0	*80.5% total body surface burn, underlying cardiovascular disease pre-existent
22	17	M	80.5	30.5	12	Inhalation injury with subsequent bilateral bronchopneumonia and septicemia
23	22	M	80	54	9	*Inhalation injury, suppurative thrombophlebitis, Staphylococcal septicemia
24	31	M	77	56	8	Bilateral pneumonia and Staphylococcal septicemia
25	51	M	77	41	2	*Hypoxemia and bradyarrhythmia
26	22	M	76.5	56.5	12	Severe inhalation injury with bilateral Pseudomonas pneumonia and Pseudomonas septicemia
27	58	M	75.5	1	25	*Bilateral pneumonia, septicemia, acute congestive heart failure
28	60	M	75	23	10	Severe inhalation injury with subsequent pneumonia
29	56	M	75	16	15	Severe inhalation injury and pneumonia with Staphylococcal septicemia
30	32	M	74	56	45	Severe bilateral pneumonia with Pseudomonas aeruginosa and Staphylococcus aureus

* Autopsy not performed

Table 7. Cause of Death, 1979

Patient	Age	Sex	% Burn Total	% Burn 3°	PBD Death	Cause of Death
31	48	M	72.5	19	13	Severe inhalation injury with subsequent bronchopneumonia with Staphylococcus aureus and Staphylococcal septicemia and septic emboli
32	35	M	70	43	31	Inhalation injury with subsequent bilateral pneumonia and Pseudomonas burn wound invasion
33	40	M	70	31	68	*Staphylococcal burn wound invasion and Staphylococcal septicemia
34	21	M	69.5	44	6	Severe inhalation injury with bilateral bronchopneumonia and pulmonary edema
35	17	M	68	54	110	Severe bilateral Pseudomonas pneumonia and calcific cardiomyopathy
36	30	M	68	57	14	Severe inhalation injury, bronchopneumonia and pulmonary edema
37	33	M	68	35.5	15	Respiratory insufficiency from aspiration pneumonia and severe pulmonary edema
38	33	M	67.5	35.5	20	Inhalation injury, pulmonary embolism with subsequent infarction of the lungs
39	20	M	64	43	16	*Severe inhalation injury with pneumonia and cardiac arrhythmia
40	64	M	63	63	11	Severe arteriosclerotic heart disease, acute renal failure and cerebral hematoma
41	18	F	62	37	8	Severe bilateral pneumonia, suppurative thrombophlebitis superior vena cava and superior caval obstruction with mural thrombi of the right heart
42	55	M	62	25	21	Inhalation injury severe pneumonia with lung abscesses
43	20	M	62	5.5	18	Bilateral bronchopneumonia, Pseudomonas aeruginosa, Pseudomonas septicemia and myocardial necrosis with septic mural thrombus with Pseudomonas
44	55	M	61.5	7	12	Klebsiella pneumonia and septicemia, septic shock

* Autopsy not performed

Table 7. Cause of Death, 1979

Patient	Age	Sex	% Burn Total	% Burn 3°	PBD Death	Cause of Death
45	1 5/12	M	60	10	2	Severe inhalation injury with severe tracheobronchitis and pulmonary edema
46	46	M	58.5	12.5	58	*Pseudomonas burn wound sepsis with septicemia, inhalation injury with bilateral pneumonia
47	77	M	57	53	3	*Severe inhalation injury and acute congestive heart failure with cardiogenic shock
48	42	M	57	11	100	Probable meningitis and septicemia
49	21	M	56	46	54	*Severe inhalation injury with bilateral pneumonitis with Pseudomonas aeruginosa
50	25	M	56	0	49	Severe Pseudomonas pneumonitis and Pseudomonas burn wound sepsis with Pseudomonas septicemia
51	51	M	55.5	35	9	Severe inhalation injury, bilateral pneumonitis and septic shock
52	39	M	54.5	46.5	18	Invasive burn wound sepsis and septic shock
53	1 5/12	F	54.5	0	29	*Pseudomonas burn wound sepsis and septicemia
54	22	M	54	50	29	*Gram negative septicemia, disseminated intravascular coagulation and invasive burn wound sepsis
55	61	M	53	45	57	Severe inhalation injury, mediastinal empyema and gram negative septicemia
56	64	M	52	0	5	Severe inhalation injury with bilateral pneumonitis and septicemia with Pseudomonas and Klebsiella
57	74	M	49.5	46.5	8	*Acute myocardial infarction
58	20	M	49.5	46.5	34	Gram negative septicemia, bilateral pneumonia and acute pulmonary edema
59	73	M	49.5	27	16	Septicemia with marked necrotizing enterocolitis and acute renal failure

* Autopsy not performed

Table 7. Cause of Death, 1979

Patient	Age	Sex	% Burn Total	% Burn 3°	PBD Death	Cause of Death
60	5	M	49.5	2	17	Bilateral hemorrhagic pneumonia and acute peritonitis
61	4	F	48.5	0	3	Sudden infant death syndrome with pulmonary edema
62	31	M	47	25	70	Bilateral Pseudomonas pneumonitis with mixed septicemia
63	69	M	44.5	10	68	Pseudomonas pneumonitis bilateral and mid-brain infarction from arteriosclerotic peripheral vascular disease
64	60	M	44	36	25	Suppurative thrombophlebitis innominate and subclavian veins, organism Staphylococcus aureus, Staphylococcal septicemia
65	69	M	39.5	37.5	1	*Hyperkalemia secondary to subfascial and extensive thermal injury of extremity, acute renal failure with acute myoglobinuria and cardiac arrhythmia
66	68	F	39.5	31.5	2	*Severe inhalation injury and pulmonary edema
67	48	M	38	18	23	*Bilateral Pseudomonas pneumonitis with Pseudomonas and Staphylococcal septicemia and Pseudomonas and Staphylococcal burn wound invasion
68	10/12	F	36	0	33	Bilateral bronchopneumonia and pulmonary emboli secondary to Klebsiella pneumonia with Klebsiella pneumonia septicemia and burn wound infection
69	80	F	33	27.5	28	*Acute congestive heart failure secondary to underlying cardiovascular disease
70	57	F	32	30	72	Pulmonary edema and cardiac arrhythmia
71	49	M	31	0	17	*Pseudomonas burn wound sepsis
72	48	M	31	0	4	Acute pulmonary embolus
73	63	M	17.5	11	65	Gram negative septicemia from invasive burn wound sepsis
74	64	M	12.5	5	71	Severe inhalation injury with bilateral severe pulmonary edema and cardiac arrhythmia

*Autopsy not performed

PRESENTATIONS

McManus WF: Prehospital Advanced Life Support: Value and Limitations. Univ of TX Health Science Center San Antonio, San Antonio, TX 5 Jan 79.

Pruitt BA Jr: Burns. Uniformed Services University of the Health Sciences Bethesda, MD 5 Jan 79.

Hunter E: Burn Care. Paramedical personnel, Houston Fire Dept, Houston, TX 5 and 9 Jan 79.

The following presentations were made at the Academy of Health Sciences, Physical Therapy students, Fort Sam Houston, TX 9 Jan 79:

Spebar MJ: Treatment of Burns
Dunn MA: Nursing Care of the Burn Patient
Henderson NE: Physical Therapy for Burn Patients
Diaz HM: Splinting Devices Utilized in the Treatment of Burn Patients

Treat RC: Treatment of Burns. Officers Basic Course, Academy of Health Sciences, Fort Sam Houston, TX 9 Jan 79.

Treat RC: Modern Burn Therapy. Residents USAF Sch of Aerospace Med, Brooks AFB, TX 10 Jan 79.

Wilmore DW: Nutrition for the Hospitalized Patient. Seminar on Nutrition. Shawnee Medical Society, Topeka, KS 12 Jan 79.

Treat RC: Treatment of Burns. Officers Basic Course, Academy of Health Sciences, Fort Sam Houston, TX 17 Jan 79.

Pruitt BA Jr: General Care of Burns. International Symposium on Burns. Goiania, Brazil 17 Jan 79.

Pruitt BA Jr: 1) Electrolyte and Acid Base Alterations in Burn Patients and 2) Pulmonary Function Following Burn Injury. International Symposium on Burns, Goiania, Brazil 18 Jan 79.

Treat RC: Inhalation Injuries. Respiratory Therapy students, Brooke Army Medical Center, Fort Sam Houston, TX 18 Jan 79.

Wilmore DW: Parenteral Nutrition. Staff Peter Bent Brigham Hospital, Boston, MA 18 Jan 79.

Pruitt BA Jr: 1) Systemic Complications of Burn Injury; 2) Prevention and Correction of Sequelae; and 3) The University, The Burn Team, and The Burn Unit. International Meeting on Burns, Goiania, Brazil 19 Jan 79.

Wilmore DW: Use of Hyperalimentation in Injured Patients. Staff Department of Surgery, Univ of W. VA., Morgantown, WV 19-20 Jan 79.

Treat RC: Modern Burn Therapy. USAF Sch of Aerospace Med, Brooks AFB, TX 23 Jan 79.

Goldfarb IW: Nutritional Assessment. Seminar on Total Parenteral Nutrtion sponsored by Stanford Univ, San Francisco, CA 25 Jan 79.

Wilmore DW: The Metabolic Alterations of Critical Illness. Royal College of Surgeons of Canada, Montreal, Canada 7 Feb 79.

Spebar MJ: The Burn Patient. In Service for Social Service Brooke Army Medical Center, Fort Sam Houston, TX 7 Feb 79.

Terry J: The Mission of the Burn Unit. Red Cross Volunteers, Brooke Army Medical Center, Fort Sam Houston, TX 8 Feb 79.

Goodwin CW: Increased Renal Blood Flow in Acute and Convalescing Burn Patients. S. Texas Chapter American College of Surgeons Mtg, San Antonio, TX 9 Feb 79.

McManus WF: Classification of Burns. Intensive Care Nurse Clinician Course students, BAMC, Fort Sam Houston, TX 12 Feb 79.

Pruitt BA Jr: Treatment of Burns with Special Attention to Mustard Burns. Biomedical Laboratory, Edgewood Arsenal, Edgewood, MD 12 Feb 79.

Dunn MA and Hunter E: Burn Care. Intensive Care Nurse Clinician Course students, BAMC, Fort Sam Houston, TX 12 Feb 79.

Pruitt BA Jr: 1) Escharotomy-Fasciotomy in Electrical Injury and 2) Fluid Therapy and Respiratory Complications. ABA Burn Symposium, Portland, OR 14 Feb 79.

Pruitt BA Jr: 1) The Burned Hand - Disaster? and 2) Nutrition for the Burn Patient. ABA Burn Symposium, Portland, OR 15 Feb 79.

Dunn MA: Pathophysiology of Burns. Nursing students. Univ of TX Health Science Center, San Antonio, TX 19 Feb 79.

Dunn MA and Hunter E: Burn Care. Intensive Care Nurse Clinician Course students, BAMC, Fort Sam Houston, TX 20 Feb 79.

Hunter E: Burn Care. Nursing students. Univ of TX Health Science Center, San Antonio, TX 20 Feb 79.

McManus WF: Complications of Burns. Intensive Care Nurse Clinician Course students, BAMC, Fort Sam Houston, TX 23 Feb 79.

Dunn MA: Emergency Burn Care. Nursing students. Univ of TX Health Science Center, San Antonio, TX 26 Feb 79.

Pruitt BA Jr: A New Day in Burns. The University of Mississippi Postgraduate Surgical Forum VI, Jackson, MS 1 Mar 79.

Sirinek KR: Cimetidine Affords Protection Equal to Antacids in Prevention of Stress Ulceration Following Thermal Injury. Central Surgical Assn, Omaha, NE 2 Mar 79.

Hunter E: Burn Care. Nursing students. Univ of TX Health Science Center, San Antonio, TX 5 Mar 79.

The following presentations were made at the seminar "Thermal Update--Nursing Approaches to Burn Care" sponsored by the Department of Nursing, Brooke Army Medical Center, Fort Sam Houston, TX 7 Mar 79:

Treat RC: Treatment of Burns
Hunter EC: Nursing Care in the Acute Phase
Dunn MA: Nursing Care of the Burn Patient
McCandless SA: Physical Therapy Programs for Burn Victims

Pruitt BA Jr: Resuscitation with Crystalloid or Colloid Solutions. Annual Mtg, California Medical Association, Los Angeles, CA 10 Mar 79.

Hunter E: Burn Care. Nursing students. Univ of TX Health Science Center, San Antonio, TX 12 Mar 79.

The following presentations were made at the American Burn Assn Anl mtg in New Orleans, LA 15-17 Mar 1979:

Pruitt BA Jr: Summary of NIH Consensus Conference on Burn Patient Resuscitation, Plenary Session
Spebar MJ: Noncandida-Fungal Invasion of the Burn Wound
Goodwin CW: Cardiac Injury Following Electrical Burns
Goodwin CW: Thermal Necrosis of the Skull
Lam V: Body Temperature Correction of Arterial Blood Gas Studies Necessary in the Burned Patient
Sirinek KR: Cimetidine Controls Postburn Gastric Edema

Hunter E: Burn Care. Nursing students. Univ of TX Health Science Center, San Antonio, TX 19 Mar 79.

McManus WF: Emergency Care of Burns. 507th Air Ambulance Co (MAST) Emergency Medical Technicians, Fort Sam Houston, TX 21 Mar 79.

Hunter E: Burn Care. Nursing Students. Univ of TX Health Science Center, San Antonio, TX 29 Mar 79.

McManus WF: Treatment of Burns. Officers Basic Course, Academy of Health Sciences, Fort Sam Houston, TX 2 Apr 79.

Goldfarb IW: Total Parenteral Nutrition. Southwest Physicians Conference Dallas, TX 2 Apr 79.

Dunn MA: Emergency Burn Care. Nursing Service San Marcos Hospital, San Marcos TX 6 Apr 79.

McManus WF: Current Burn Therapy. Trauma Course of the American College of Surgeons, Las Vegas, NV 11 Apr 79.

Dunn MA: Pediatric Burn Patients. Staff Pediatric Unit, BAMC, Fort Sam Houston, TX 12 Apr 79.

Dunn MA: Nursing Care of Thermally Injured Patients. Nursing students Valparaiso Univ, Valparaiso, IN 16 Apr 79.
Marion College, Marion, IN 17 Apr 79
Capital University, Columbus, OH 18 Apr 79.
Indiana State University, Terre Haute, IN 19 Apr 79
Ball State University, Muncie, IN 19 Apr 79
Purdue University, West Lafayette, IN 20 Apr 79.

Goodwin CW: Early Care of the Burn Patient and 2) Visceral Metabolic Response to Large Burns. Med College of VA Richmond, VA 17 Apr 79.

Pruitt BA Jr: 1) Initial Treatment of Chemical Burns; 2) Care of Burn Victims in the Hospital. Symposium on Chemical Burns and Associated Injuries, 123rd US Army Reserve Command, Grand Rapids, MI 19 Apr 79.

Spebar MJ: Fungal Invasion of the Burn Wound. Southwest Surgical Congress, Las Vegas, NV 23 Apr 79.

Pruitt BA Jr: Burn Management, The Combat Environment. 27th Annual Symposium, Society of Air Force Clinical Surgeons, San Antonio, TX 23 Apr 79.

Treat RC: Burn Assessment and Management. BAMC Interns AMIC-ER, Fort Sam Houston, TX 23 Apr 79.

Goldfarb IW, McCandless SA, Dunn MA: Overview of Burn Care. Rehabilitation Nurses of the Chull Insurance Company of Texas. BAMC, 24 Apr 79.

Pruitt BA Jr: Presentation of American Trauma Society, Distinguished Service Award to Dr. Truman Blocker, Annual Mtg of the American Trauma Society., Chicago, IL 28 Apr 79.

Dunn MA: Overview of Burn Care. Nursing students, Ranger Junior College Ranger, Texas, BAMC, 26 Apr 79.

McManus WF: Current Burn Therapy. American Association of Occupational Physicians, 64th Annual Meeting, Anaheim, CA 1 May 79.

Treat RC: Inhalation Injuries. Univ of KY, Lexington, KY 4 May 79.

Pruitt BA Jr: Care of the Extensively Burned Patient. Professional Staff Conference, MEDDAC, Fort Knox, KY 8 May 79.

Lam V: Does Pulmonary Extravascular Water Vary with Colloid Oncotic Pressure After Burn Injury. American Thoracic Society mtg, Las Vegas, NV 13 May 79.

Treat RC: Inhalation Injuries. Medical College of Georgia, Augusta, GA 14 May 79.

Goldfarb IW: Total Parenteral Nutrition. Physicians Seminar Portland, OR 15 May 79.

Goldfarb IW: Total Parenteral Nutrition. Seattle, WA 16 May 79.

Goldfarb IW: Total Parenteral Nutrition. Department of Surgery, Baylor Hospital, Dallas, TX 24 May 79.

Dunn MA: Burn Care. Nursing students. Univ of Tex Health Science Center, San Antonio, TX 4 Jun 79.

McManus WF: Inhalation and Respiratory Problems in Fire Fighters. Redmond Foundation, San Diego, CA 11 Jun 79.

Dunn MA and Hunter E: Burn Care. Nursing students. Univ of Tex Health Science Center, San Antonio, TX 11 Jun 79.

Dunn MA and Hunter E: Burn Care. Nursing students. Univ of Tex Health Science Center, San Antonio, TX 18 Jun 79.

Treat RC: Burn Assessment and Management. BAMC Interns AMIC-ER, Fort Sam Houston, TX 20 Jun 79.

Dunn MA and Terry J: Brackenridge Hospital School of Nursing, Austin, TX 25 Jun 79.

Treat RC: Treatment of Burns. Officers Basic Course, Academy of Health Sciences, Fort Sam Houston, TX 27 Jun 79.

Treat RC: The Burn Patient. Physician's Assistant students. Academy of Health Sciences, Fort Sam Houston, 19 Jul 79.

Pruitt BA Jr: Surgical Infections and Antibiotics. Surgical Literature Conference, University of Texas Health Science Center at San Antonio, San Antonio, TX 25 Jul 79.

McManus WF: Burn Assessment and Management. BAMC Residents AMIC-ER, Fort Sam Houston, TX 30 Jul 79.

Pruitt BA Jr: Resuscitation of Burn/Trauma Patients. Current Concepts of Combat Casualty Resuscitation Symposium, Naval Medical Research Institute, Bethesda, MD 1 Aug 79.

Terry J: Wound Care. Nursing students. St. Phillips College, San Antonio, TX 1 Aug 79.

Pruitt BA Jr: Frontiers of Research in the Treatment of Traumatic Injuries. Annual Mtg of the American Trial Lawyers Association, Houston, TX 4 Aug 79.

McManus WF: Classification of Burns. Intensive Care Nurse Clinician Course students, BAMC, Fort Sam Houston, TX 6 Aug 79.

McManus WF: Treatment of Burns. Officers Basic Course, Academy of Health Sciences, Fort Sam Houston, TX 9 Aug 79.

Terry J: Burn Nursing. Intensive Care Nurse Clinician Course students, BAMC, Fort Sam Houston, TX 9 Aug 79.

McManus WF: Complications of Burns. Intensive Care Nurse Clinician Course students, BAMC, Fort Sam Houston, TX 10 Aug 79.

Terry J: Burn Nursing. Intensive Care Nurse Clinician Course students, BAMC, Fort Sam Houston, TX 13 Aug 79.

Goldfarb IW: Burn Assessment and Early Management. BAMC Interns AMIC-ER, Fort Sam Houston, TX 13 Aug 79.

Pruitt BA Jr: Mission/overview of USAISR. HPSP/USU Student Orientation. Fort Sam Houston, TX 5 Aug 79.

Goldfarb IW: Care of IV Catheters. Nursing Service ISR In Service Ft Sam Houston, TX 15 Aug 79.

Pruitt BA Jr: Fluid Resuscitation of Injured Man. Surgical Grand Rounds, University of Texas Medical School at Houston, Houston, TX 24 Aug 79.

McManus WF: Prehospital Advanced Life Support. EMT Paramedic students. San Antonio, TX 31 Aug 79.

Pruitt BA Jr: Different Local Treatments in the Acutely Burned. World Congress of Surgery, San Francisco, CA 4 Sep 79.

McManus WF: Principles of Wound Care. Nursing Service ISR In Service Fort Sam Houston, TX 5 Sep 79.

Pruitt BA Jr: Pre-hospital Care: A Military Perspective Josiah Macy Foundation Conference on Emergency Medical Services, Williamsburg, VA 11 Sep 79.

Becker RA: Are Critically Ill Trauma Patients Hypothyroid? American Association for the Surgery of Trauma, Chicago, IL 14 Sep 79.

McManus WF: Treatment of Burns. Officers Basic Course, Academy of Health Sciences, Fort Sam Houston, TX 20 Sep 79.

Pruitt BA Jr: 1) Nutrition and Metabolism in Pediatric Burn Patients; 2) High Voltage Electrical Injury. Fifth Annual Pediatric Burn Symposium, Keystone, CO 20-21 Sep 79.

Goodwin CW: Metabolic Assessment of Burn Patients. Ross Conference on Nutritional Assessment. Sante Fe, NM 24 Sep 79.

Pruitt BA Jr: 1) Pathophysiology and Emergency Treatment of Burns; 2) Prevention and Education in Burn Injury. Vanderbilt University Symposium on Management of the Severely Burned Patient. Nashville, TN 28 Sep 79.

Pruitt BA Jr: Fluid Resuscitation of Injured Man. Surgical Grand Rounds. Vanderbilt University, Nashville, TN 29 Sep 79.

Pruitt BA Jr: Pseudomonas Aeruginosa in Burn Wounds and Burn Infections. International Symposium on Pseudomonas Aeruginosa. Boston, MA 1 Oct 79.

Terry J: Nursing Care of the Burn Patient. Nursing faculty and students DePaul University School of Nursing, Chicago, IL 1 Oct 79.

Terry J: Nursing Care of the Burn Patient. Alverno College School of Nursing, Milwaukee, WI 2 Oct 79.

Terry J: Nursing Care of the Burn Patient. Nurses from Illinois Nurses Association. Highland Park, IL 3 Oct 79.

Terry J: Nursing Care of the Burn Patient. Nursing faculty and students Marian College of Fon du lac, Fon du lac, WI 4 Oct 79.

Terry J: Nursing Care of the Burn Patient. Nursing faculty and students Elmhurst College, Elmhurst, IL 5 Oct 79.

Seaman T and Bedard D: Emergency Care and Evacuation. Civil Air Disaster Rescue Team, Harlingen, TX 10-12 Oct 79.

McManus WF: History and Mission of the Institute of Surgical Research. Nursing Service ISR In Service. Fort Sam Houston, TX 17 Oct 79.

Pruitt BA Jr: Burn Management: Fluids and Laboratory Tests in the Burn Patient. Clinical Congress of the American College of Surgeons. Chicago, IL 23 Oct 79.

Terry J: Overview of Burn Care. Physical Therapists, Beach Pav PT Clinic, Fort Sam Houston, TX 28 Oct 79.

Benitez H: Burn Assessment and Early Management. BAMC Interns AMIC-ER, Fort Sam Houston, TX 29 Oct 79.

Goldfarb IW: Complete Care of the Injured Man. Clinical Pastoral Chaplain's Course, BAMC, Fort Sam Houston, TX 30 Oct 79.

Pruitt BA Jr: The Future of Burn Care. Baptist Medical Center, Oklahoma City, OK 5 Nov 79.

Pruitt BA Jr: 1) Early Excision and Grafting of the Burn Wound; 2) Metabolic Aspects of Burn Care. Annual Seminar of The Pine Tree Foundation for Burn Treatment. Bangor, ME 7 Nov 79.

Pruitt BA Jr: Care of the Burn Wound. Surgical Grand Rounds, Maine Medical Center, Portland, ME 8 Nov 79.

Pruitt BA Jr: Complications of Burn Injury. Department of Surgery Seminar Uniformed Services University of the Health Sciences, Bethesda, MD 16 Nov 79.

Goldfarb IW: Workshop in Hemodynamic Monitoring in Critical Care, Pittsburgh, PA 16 and 17 Nov 79.

Pruitt BA Jr: 1) Transport of Burn Victims; 2) Respiratory Distress Syndrome; 3) Stress Ulcer. Postgraduate Trauma Symposium, Department of Surgery, University of New Mexico School of Medicine. Albuquerque, NM 30 Nov - 1 Dec 79.

Pruitt BA Jr: 1) Diagnosis and Treatment of Cannula Related Intravenous Sepsis in Burn Patients. Annual Meeting of the Southern Surgical Association. Homestead, VA 3 Dec 79.

Pruitt BA Jr: Current Approach to Prevention and Treatment of Pseudomonas Aeruginosa Infection in Burned, Traumatized, and Surgical Patients. Walter Reed Army Institute of Research Symposium on Pseudomonas Aeruginosa. Washington, DC 7 Dec 79.

Pruitt BA Jr: 1) Monitoring the Burn Patient; 2) Gastrointestinal Complications of Burn Injury; 3) Renal Complications of Burn Injury; 4) Pulmonary Thermal Injuries; 5) Systemic Infection in the Burn Patient; 6) Unsolved Problems and Needs in Burn Care. International Society for Burn Injuries Postgraduate Course in Burn Care. Denver, CO 14-15 Dec 79.

PUBLICATIONS

1. Jacobson HR: Altered permeability in the proximal tubule response to cyclic AMP. Amer J of Physiology 236:F71-F79, Jan 79.

2. Sasaki TM, Welch GW, Herndon DN, et al: Burn Wound Manipulation-induced bacteremia. J Trauma 19:46-48, Jan 79.

3. Jacobson HR: Characteristics of Volume Reabsorption in rabbit superficial and juxtamedullary proximal convoluted tubules. J Clin Invest 63:410-418 Mar 79.

4. Levine BA, Sirinek KR, McLeod CG, et al: The role of cimetidine in the prevention of stress induced gastric mucosal injury. SG&O 148:399-402, Mar 79.

5. Aulick LH, Wilmore DW: Increased peripheral amino acid release following burn injury. Surg 85:560-656, May 79.

6. Price GH: Sulfonamide inhibition of human alkaline phosphatase. Clin Chim Acta 94:211-217, 1979.

7. Lescher TJ, Sirinek KR and Pruitt BA Jr: Superior mesenteric artery syndrome in thermally injured patients. J Trauma 19:567-571, Aug 79.

8. Langlinais, PC and Panke TW: Intrasinusoidal bodies in the livers of thermally injured patients. Arch of Path and Lab Med 103:499-504, Sep 79.

9. Merrill RH, McLeod CG, Jarstfer BS: The use of lyophilized vein grafts in vascular access for chronic hemodialysis. Artificial Organs 3: Aug 79.

10. Levine BA, Sirinek KR, Peterson HD and Pruitt BA Jr: Efficacy of tangential excision and immediate autografting of deep second degree burns of the hand. J Trauma 19:670-673, Sep 79.

11. McElwee HP, Sirinek KR, and Levine BA: Cimetidine affords protection equal to antacids in prevention of stress ulceration following thermal injury. Surg 86:620-626, Oct 79.

12. Mason AD Jr: Weight loss in burned patients. J Trauma 903-904, Nov 79.

13. Spebar MJ, Lindberg RB: Fungal infection of the burn wound. Amer J Surg 138:879-882, Dec 79.

14. Levine,BA, Schweisinger WH, Jones D and Sirinek KR: Histamine receptor control of gastric microvasculature in shock. J Surg Res 26:532-539, 1979.

15. McManus WF: Immediate emergency department care. In CP Artz, JA Moncrief and BA Pruitt, Jr (eds) Burns: A team approach, 1st ed WB Saunders Co., Philadelphia, pp 159-164, 1979.

EXHIBITS

The following exhibit was shown during the year 1979:

"Inhalation Injury: Diagnosis and Treatment" at the American College of Surgeons meeting, Chicago, IL 22-25 Oct 1979.

ANNUAL PROGRESS REPORT

PROJECT NO. 3S162772A814-00, APPLIED RESEARCH

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

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

1 January 1979 - 31 December 1979

Investigator:

Anton J. Jirka, MD, MPH, Colonel, MC

Reports Control Symbol MEDDH-288(R1)

Unclassified

ABSTRACT

PROJECT NO. 3S162772A814-00, APPLIED RESEARCH

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

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

Period covered in this report: 1 January 1979 - 31 December 1979

Investigator: Anton J. Jirka, MD, MPH, Colonel, MC

Reports Control Symbol MEDDH-288(R1)

In the period covered in this report, 554 anesthetics were administered to 161 patients, an average of 3.44 anesthetics per patient. This is the largest number of anesthetics administered at ISR since 1972. The most commonly used anesthetic agent was Ethrane^{R} (58.59%), followed by ketamine (25.86%), and nitrous oxide (6.87%). Due to the nature and combinations of procedures now performed, regional anesthesia is seldom used. An automatic oscillometric blood pressure monitor is presently used on all patients.

Anesthesia.

ANESTHESIOLOGY

PREOPERATIVE EVALUATION

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

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

PREOPERATIVE PREPARATION

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

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

PREMEDICATION

Most burn patients require some pain relief during the trip to the operating room, and most receive a narcotic such as morphine sulfate, 0.1 mg/kg, to a maximal dose of 10 mg, one hour prior to surgery. Glycopyrrolate (Robinul^{R}), 0.005 mg/kg to a maximal dose of 0.4 mg, is used to dry secretions. Both of these medications are delivered intramuscularly.

Glycopyrrolate (Robinul^{R}) in the above dosage, is used as premedication 30 minutes prior to ketamine anesthesia.

FLUIDS

All fluids except hyperalimentation solutions are changed to D_5RL or RL on arrival in the operating room. Hyperalimentation solutions are continued throughout operative procedures.

TYPES OF ANESTHESIA

The pattern of anesthetic administration has changed from previous years and involves a greater use of enflurane and ketamine and a lesser use of halothane and regional anesthesia. (The reasons for this change will be discussed under individual agent headings.)

TABLE 1. PRIMARY AGENTS

AGENT	1978		1979	
	NUMBER	%	NUMBER	%
ENFLURANE	211	48.5	324	58.59
KETAMINE	95	21.8	143	25.86
HALOTHANE	36	8.3	18	3.25
N_2O	58	13.3	38	6.87
LOCAL	28	6.4	29	5.24
OTHER	7	1.6	1	0.18

1. Enflurane (Ethrane^R)

Enflurane is a halogenated ether which has been commercially available for approximately the past six years. It has a rapid induction with good muscle relaxation. Biotransformation amounts to less than 2% of an inhaled dose, a fact which perhaps accounts for a few clinical toxic effects observed in spite of the fact that increased plasma fluoride ion concentrations have been observed after administration to patients taking hepatic enzyme inducing drugs. Plasma fluoride levels in hypermetabolic burn patients during and after Ethrane administration have been measured and found not to be in the toxic range. Enflurane is presently the most commonly used anesthetic agent at the USAISR.

2. Halothane^R (Fluothane)

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

3. Nitrous oxide

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

4. Ketamine

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

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

5. Subanesthetic Ketamine

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

6. Regional Anesthesia

Regional anesthesia is generally considered one of the safest methods available, but its use in the thermally injured patient is limited for several reasons: sepsis and infection of the skin over the site of injection are contraindications for use, and multiple-site operations also limit the practicality of this method. Axillary block is the most common regional technique used at USAISR. However the tendency toward multiple procedures has decreased the usefulness of this technique.

MONITORING TECHNIQUES

A. CIRCULATION

1. Precordial and/or esophageal stethoscope

2. Peripheral pulse

3. Blood pressure. Direct arterial lines have been used when necessary. The Dinamap^{R} blood pressure instrument is routinely used for intraoperative blood pressure monitoring with ability to be used over dressings and its non-invasive method of operation, it is a most practical method of monitoring blood pressure in our patient population.

4. CVP

5. Swan Ganz catheter

6. ECG

7. Sponge weight - rarely used

8. Urine output

B. RESPIRATION

1. Rate

2. Auscultation

3. Arterial blood gases

C. TEMPERATURE

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

1. Ambient temperature is maintained at 80-85oF. This is probably the most important method to reduce heat loss.

2. The anesthetic gases may be heated and humidified.

3. A circle system which allows partial rebreathing of warm expired gases may be used to minimize heat loss.

4. Radiant heat lamps.

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

COMPLICATIONS

A 45 year old caucasian male was admitted to the USAISR with a 45% TBS burn of which 10% was third degree. Past medical history was negative except for the recent onset of gout, diabetes and hypertension. The only medication taken by the patient was Diabinese 250 mg q.d. All admission laboratory, radiographic and EKG data were normal.

With the exception of some difficulty in controlling his blood glucose, the patient had an unremarkable preoperative course. Fifty two days after his admission the patient was brought to the operating room where under enflurane anesthesia a Blair knife debridement of both lower extremities and left upper arm was performed. The patient was neither hypo or hypertensive before, after or during the anesthetic. His fluid requirements during the procedure were not excessive. About twenty minutes post operatively the

patient developed clinical signs of pulmonary edema. EKG changes were consistent with an acute myocardial infarction as were serial serum enzymes. After a benign and uneventful course of appropriate treatment, it was decided that the patient would require autografting. Seventy three days after admission and twenty one days post operative the patient underwent grafting under enflurane anesthesia. The perioperative and post operative course were uneventful and the patient was discharged seventeen days later with his burn wounds healed.

TABLE 2. OVERALL PATIENT DATA, USAISR (1970-1979)

Year	No. of Patients	No. Patients Anesthetized (ISR Only)	No. Patients Anesthetized / No. Patients (x100)	Total Anesthetics (ISR Only)	Anesthetics No. Patients Anesthetized (x100)
1970	321	198	61.7	497	2.51
1971	301	179	59.5	475	2.65
1972	301	183	60.8	575	3.14
1973	273	141	51.6	377	2.67
1974	226	123	54.4	380	3.09
1975	254	142	55.9	490	3.45
1976	277	139	50.2	476	3.43
1977	242	129	53.3	344	2.67
1978	268	151	56.3	435	2.88
1979	267	161	60.3	554	3.44

TABLE 3. NATURE OF SURGERY, USAISR

PROCEDURE	1978		1979	
	NUMBER OF PROCEDURES	%	NUMBER OF PROCEDURES	%
EXCISION	90	19.3	212	30.15
AUTOGRAFT	269	59.9	372	52.91
ORTHOPEDIC	33	7.1	34	4.84
CHONDRECTOMY	4	0.9	1	0.14
EYE AND LID	6	1.3	21	2.99
INTRA-ABDOMINAL	6	1.3	8	1.13
PLASTIC	6	1.3	3	0.43
OTHER	50	10.8	52	7.39
TOTAL	464	100%	703	100%

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OG 6975	01 Oct 80	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
01 Oct 79	D. CHANGE	U	U	NA	NL	[X] YES [ ] NO	A. WORK UNIT

10. NO. CODES.	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62772A	3S162772A874	AF	161
b. [redacted]	62772A	3S161102B814	00	120
c. [redacted]	STOG 80 -	7 2-5		

11. TITLE (Precede with Security Classification Code)

(U) The Hemodynamic Response to Thermal Injury in Burned Soldiers (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
Oct 76	Cont	DA	C. In-House

17. CONTRACT/GRANT: Not Applicable

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 1980	.2	$ 45
	CURRENT 1981	1.0	$ 50

19. RESPONSIBLE DOD ORGANIZATION

NAME: US Army Institute of Surgical Research

ADDRESS: Ft Sam Houston, Texas 78234

RESPONSIBLE INDIVIDUAL

NAME: Basil A. Pruitt, Jr., MD, COL, MC

TELEPHONE: 512-221-2720

20. PERFORMING ORGANIZATION

NAME: US Army Institute of Surgical Research
Surgical Study Branch

ADDRESS: Ft Sam Houston, Texas 78234

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)

NAME: Cleon W. Goodwin, Jr., MD

TELEPHONE: 512-221-2968

SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS

NAME:

NAME:

21. GENERAL USE

FOREIGN INTELLIGENCE NOT CONSIDERED

DA

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Humans; (U) Dogs; (U) Resuscitation Fluids; (U) Echocardiography; (U) Burn Injury; (U) Cardiac Output; (U) Septic Shock; (U) Cardiovascular Hemodynamics

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) To evaluate systemic and cardiopulmonary changes in burned soldiers and the influence of fluid resuscitation. To study noninvasively myocardial function in burned and burned-infected patients. To assess use of vasoactive agents in burned soldiers.

24. (U) Hemodynamic flow and pressure changes and ventilation sensitivity are studied in burn patients during and after resuscitation. Cardiac output is studied by a standardized rebreathing indicator-dilution technique. Alterations in ventilatory sensitivity are measured by the CO_2 rebreathing technique.

25. (U) 7910 - 8009. Air sensitivity was determined serially on postburn days 1, 3, 5, 7, and 10 (expressed as $\Delta V_E/\Delta Pa\ CO_2$, L/min. torr). Ventilatory sensitivity increased linearly over this period of time (0.834, 1.413, 1.682, 2.275, and 2.800, respectively). Moderate progressive hypocapnia (PCO_2 down to 31 torr) and respiratory alkalosis (pH up to 7.48) accompanied the alterations in respiratory drive. Since these patients chronically maintained a state of moderate hypocarbia, ventilation appeared to be in excess of that required to eliminate the augmented quantities of CO_2 produced during postburn catabolism. This response is in part explained by increased central sensitivity to CO_2, possibly arising from elevated levels of circulating catecholamines.

*Available to contractors upon addressee's approval

DD FORM 1498 1 MAR 68 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 68 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

ANNUAL PROGRESS REPORT

PROJECT NO. 3S162772A814-00, APPLIED RESEARCH

REPORT TITLE: THE HEMODYNAMIC RESPONSE TO THERMAL INJURY IN BURNED SOLDIERS - INCREASING RESPIRATORY DRIVE ACCOMPANYING THE ONSET OF POSTBURN HYPERMETABOLISM

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

1 October 1979 - 30 September 1980

Investigators:

Cleon W. Goodwin, Jr., MD
Victor Lam, MD
Diane Martin, SP5
Basil A. Pruitt, Jr., MD, Colonel, MC

Reports Control Symbol MEDDH-288(RI)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3S162772A814-00, APPLIED RESEARCH

REPORT TITLE: THE HEMODYNAMIC RESPONSE TO THERMAL INJURY IN BURNED SOLDIERS - INCREASING RESPIRATORY DRIVE ACCOMPANYING THE ONSET OF POSTBURN HYPERMETABOLISM

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

Period covered in this report: 1 October 1979 - 30 September 1980

Investigators: Cleon W. Goodwin, Jr., MD
Victor Lam, MD
Diane Martin, SP5
Basil A. Pruitt, Jr., MD, Colonel, MC

Reports Control Symbol MEDDH-288(R1)

Following successful resuscitation of thermally injured patients, metabolic rate progressively increases, and this postburn hypermetabolism is accompanied by increased CO_2 production and hyperventilation (1). These alterations reach a plateau after several weeks and then slowly decline as the burn wound is closed by grafting and spontaneous healing. To those caring for these patients, it is a common clinical observation that respiratory rate and total ventilation increased during the initial weeks following burn injury and that these patients develop a sustained respiratory alkalosis. Earlier studies from this Institute have demonstrated that ventilation in burn patients is related to metabolic rate, as would be expected to allow adequate oxygen uptake and carbon dioxide elimination (2). Burn patients exhibit a higher level of ventilation for the given degree of carbon dioxide production than do uninjured exercising subjects and the sensitivity of ventilation to carbon dioxide in these patients may be altered. This suggestion is further supported by the frequent occurrence of hyperventilation and respiratory alkalosis.

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1. Wilmore, DW, Long JA, Mason, AD Jr, et al: Catecholamines: Mediator of the Hypermetabolic Response to Thermal Injury. Ann Surg 180: 653-668, 1974.

2. Petroff, PA, Hander, EW, and Mason, AD Jr: Ventilatory Patterns Following Burn Injury and Effect of Sulfamylon, J Trauma 15: 650-656, 1975.

A complex metabolic servomechanism involved in the control of respiration has been proposed by Grodins (3). A large number of factors affect this control mechanism, which encompasses not only brainstem reflexes but also peripheral chemoreceptors. In this schema, changes in carbon dioxide production would be expected to elicit alterations in ventilation. This feedback control has been proposed to act directly on the brainstem, which senses changes in local hydrogen ion content. In this preliminary study, we elected to assay the sensitivity of this servocontroller to increasing levels of carbon dioxide by varying CO_2 levels in the inspired gas and measuring the ventilatory response.

METHODS

We serially studied eight hemodynamically stable patients over the first ten days following injury. Their average age was 29 years, with a range of 17 to 52 years, and their burns covered an average of 37% of the body surface, with a range of 12 to 52%. None of these patients was bacteremic at the time of study, and no incidence of burn wound invasion occurred.

All patients were fasted over night. Intravenous fluids were changed to normal saline and were regulated at rates to maintain adequate hydration. In the early morning hours, arterial blood was obtained aseptically for blood gas and pH determinations and for culture. The patients were placed in an environmental chamber warmed to 31° C and allowed to rest for at least one hour. Wound manipulation was avoided before the study, and the administration of analgesics, which aalters the ventilatory response to inhaled-carbon dioxide, was scheduled so as to precede the studies by several hours.

The rebreathing apparatusconsists of a rapidly responding spirometer for ventilatory measurements and a mass spectrometer for gas analysis of the inspired and expired air (Figure 1). The spirometer is filled with a calibrated gas containing carbon dioxide at a concentration approximating the patient's resting arterial PCO_2 and oxygen to make up the balance. As such, any influence by hypoxia during the study is avoided. The subject quietly breaths through the mouth piece, and expired carbon dioxide accumulates in the spirometer. CO_2 concentration is measured breath by breath by a rapidly responding mass spectrometer and tidal volume by the spirometer. The analogue signals are recorded on photographic paper and are processed offline upon completion of the study.

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3. Grodins FS and Yamashiro, SM: In Respiratory Function of the Lung and Its Control. MacMillian Publishing Co.,Inc, New York 1978, Pg 6.

As the subject breathes into the spirometer, the inspired CO_2 concentration rises and induces a corresponding rise in ventilation, which is usually manifest in these patients as an increase in respiratory frequency rather than as an increase in tidal volume. Since the patients were fasted, RQ is usually around 0.7, and less carbon dioxide by volume is added to the spirometer than is lost by oxygen consumption. Thus, the volume trace slowly decreases with time (Figure 2). The mass spectrometer consumes only 50 ml. of gas per minute, which causes a negligible loss of volume from the 20 liter spirometer. The response to ventilation, $\dot{V}_e$, to inhaled carbon dioxide is described by this linear equation: $\dot{V} = S(P_ACO_2 - B)$. This slope, S, reflects the increment in ventilation to the increment in CO_2 concentration and reflects the physiologic sensitivity of certain aspects of the mechanisms which control respiration: $S = \Delta\dot{V}_e / \Delta P_ACO_2$. End tidal CO_2 is assumed to closely reflect arterial CO_2, a concept well documented in the literature. B is the calculated CO_2 concentration at zero ventilation.

The analogue signals were digitized and plotted, and the slope was calculated by a linear squares equation fitted to the data. In normal subjects and in our stable, non-bacteremic burn patients, the relationship of end expired CO_2 to ventilation was linear (Figure 3). The slope of the line, which reflects CO_2 sensitivity, was reproducible between measurements and overtime.

RESULTS

Figure 4 illustrates the serial changes in carbon dioxide sensitivity which occurred following the burn injury to these patients (Figure 4). Each point represents the group mean values, plus or minus the standard errors of the mean, of the eight patients on each day of study. Over the ten day interval, the respiratory drive, as reflected here by CO_2 sensitivity, increased more than threefold. When examined by one-way analysis of variance techniques, this increase is highly significant at the $p < .001$ level.

Table 1 correlates the serial changes in CO_2 sensitivity with the patient's arterial PCO_2 and pH. As other investigators have shown, we found a progressive fall in arterial PCO_2 and a corresponding rise in arterial pH as CO_2 sensitivity increased. Using analysis of covariance techniques, we could not relate any of these change to burn size in this initial group of patients.

DISCUSSION

Since these patients chronically maintain a state of moderate hypocarbia, ventilation appeared to be in excess of that required to eliminate the augmented quantitites of carbon dioxide produced during

the period of postburn catabolism. This response is in part explained by increased sensitivity to CO_2. A number of factors may contribute to this increased respiratory drive. We think we have eliminated the influence of a hypoxic stimulus by selecting patients who were not clinically hypoxemic and by using high concentrations of oxygen in the rebreathing mixture. We use Sulfamylon, a potent carbonic anhydrase inhibitor, as a topical antimicrobial agent. This agent may alter not only blood buffer systems but also may may alter similar enzyme systems in various chemoreceptors. As postburn hypermetabolism develops, cardiac output increases. A number of investigators have shown that increased blood flow; such as occurs in our patients, stimulates ventilation by an as yet undefined mechanism (4, 5). Finally, an attractive cause for the progressive rise in respiratory sensitivity is an increase in circulatory levels of the catecholamines. Numerous investigators have found that beta adrenergic agents stimulate ventilation and respiratory sensitivity and that beta receptor blockade blunts this response (6, 7, 8). Harrison, and later Wilmore, have convincingly demonstrated that the rise in catecholamine turnover in burn patients parallels the rise in metabolic rate, blood flow, and ventilation and that these alterations can be moderated by beta receptor blockade (9). Our current studies are directed at defining the relationship of catecholamine flux, acid base balance, and level of nutritional support to gas exchange in severely injured patients.

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4. Stremel, RW, Whipp, BJ, Casaburi, R et all: Hypopnea Consequent to Reduced Pulmonary Blood Flow in the Dog. J. Applied Physiology, 46: 1171-1177, 1979.

5. Wasserman, K, Whipp, BJ, and Castagna, J: Cardiodynamic Hyperpnea: Hyperpnea Secondary to Cardiac Output Increase. J. of Applied Physiology, 36: 457-464, 1974.

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8. Winn, R, Hildebrandt, JR, and Hildebrandt, J: Cardio-respiratory Responses Following Isoproterenol Injection in Rabbits, J. Applied Physiology, 47: 352-359, 1979.

9. Harrison, TS, Seaton JF, and Feller, I: Relationship of Increased Oxygen Consumption to Catecholamine Excretion in Thermal Burns. Ann Surg 165: 169, 1967.

Table I. Serial Changes in CO_2 Sensitivity, Arterial pH, and Arterial PCO_2 during The First Ten Days Postburn.

Postburn Day	1	3	5	7	10
$\dot{V}_e$/ $PaCO_2$	0.834±0.129	1.413±.295	1.682±.285	2.275±.271	2.800±.272
pCO_2	37±3	35±2	33±2	33±1	31±2
pH	7.40±.02	7.44±.01	7.46±.02	7.47±.02	7.48±.02

*p < .001 for Increasing Sensitivity

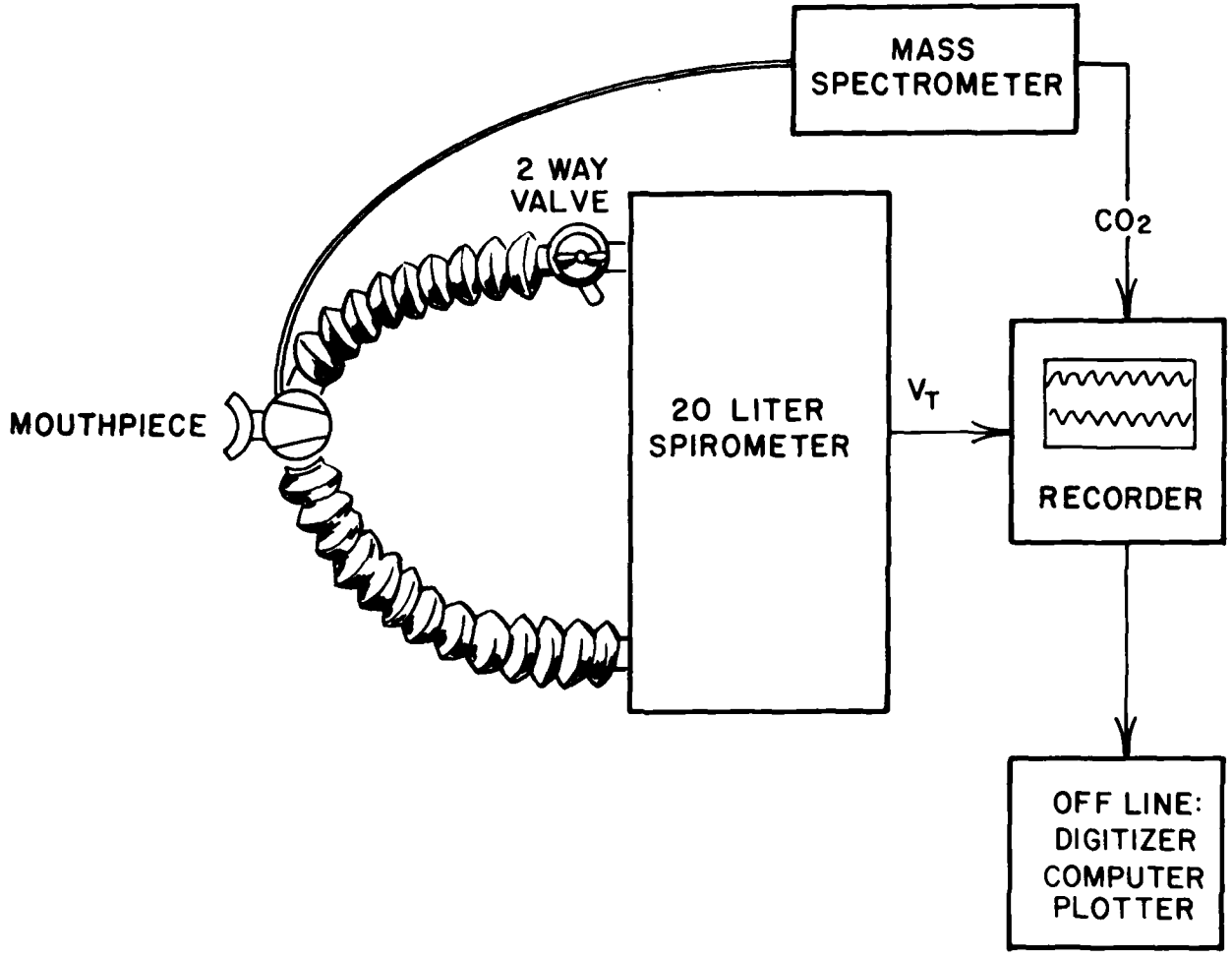

FIGURE 1. SCHEMATIC DIAGRAM OF APPARATUS TO MEASURE CO_2 SENSITIVITY

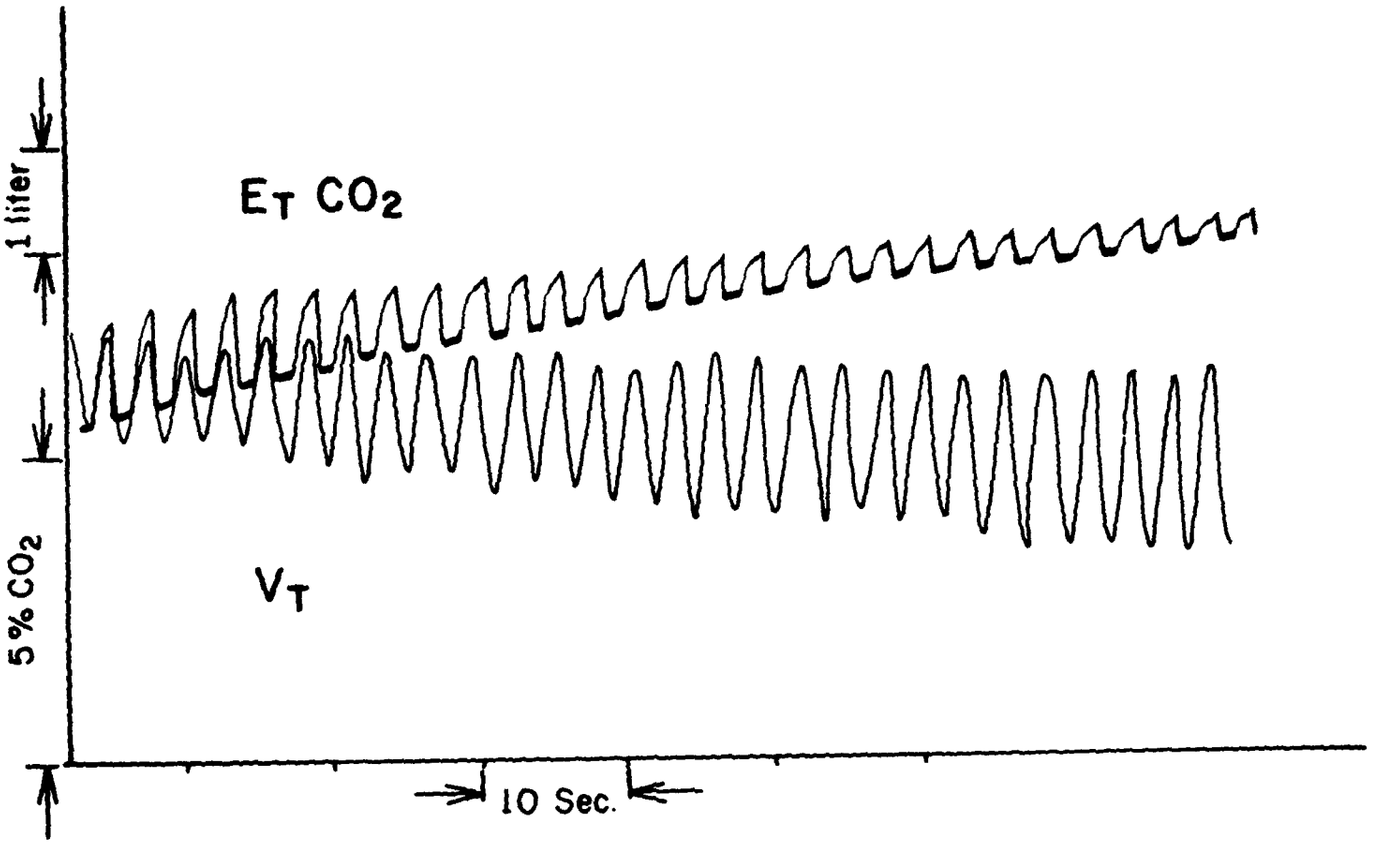

FIGURE 2. TYPICAL REAL TIME TRACE OF CHANGES IN VENTILATION AND END EXPIRED CARBON DIOXIDE AS PATIENT REBREATHS INTO A CLOSED SPIROMETER

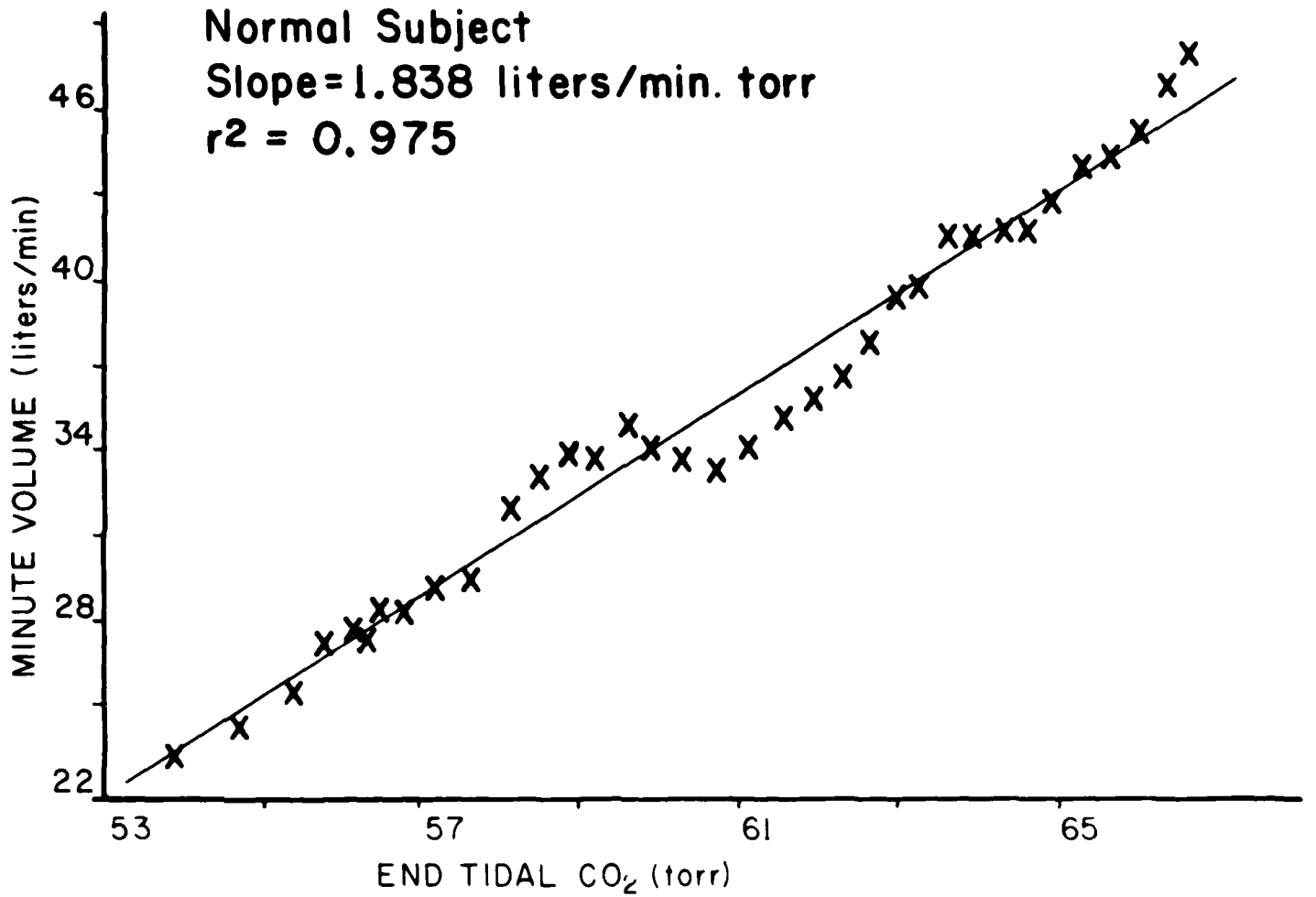

FIGURE 3. DIGITIZED OUTPUT RELATING CHANGE IN VENTILATION TO CHANGE IN TIDAL CO_2 AND A NORMAL SUBJECT.

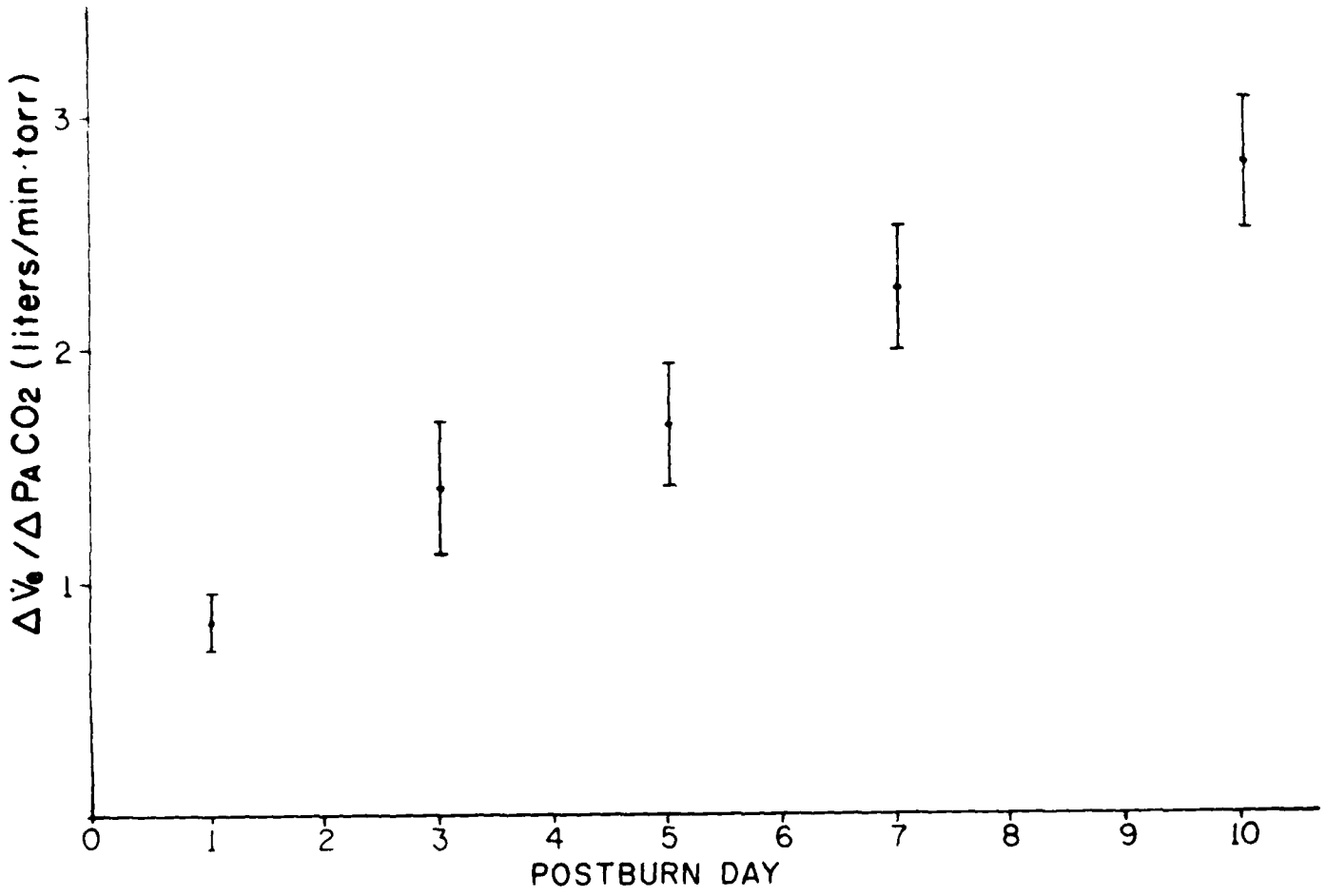

FIGURE 4. SERIAL CHANGES IN RESPIRATORY DRIVE IN 8 THERMALLY INJURED PATIENTS OVER THE FIRST 10 DAYS POSTBURN.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OG 6971	01 Oct 80	DD DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISS'N INSTR'N	8b. SPECIFIC DATA: CONTRACTOR ACCESS	9. LEVEL OF SUM
01 Oct 79	D. CHANGE	U	U	NA	NL	[X] YES [ ] NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62772A	3S162772A874	AF	162
b. [illegible]	62772A	3S162772A814	00	119
c. [illegible]	STOG 80 - 7.2:5			

11. TITLE (Precede with Security Classification Code)

(U) Evaluation of Burn Wound Care in Troops With Burn Injury (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
Oct 76	Cont	DA	C. In-House

17. CONTRACT/GRANT: Not Applicable

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
FISCAL YEAR	PRECEDING 1980	2.0	$ 69
	CURRENT 1981	2.0	$ 74

19. RESPONSIBLE DOD ORGANIZATION

NAME: US Army Institute of Surgical Research

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20. PERFORMING ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME:

NAME: DA

21. GENERAL USE

FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Burn Injury; (U) Topical Therapy; (U) Sulfamylon; (U) Wound Excision; (U) 5% Sulfamylon Acetate Solution; (U) Humans; (U) Autografts

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) The military relevance of improving burn wound care will be realized in increased troop survival following thermal injury. Newer methods under current investigation include the use of 5% aqueous Sulfamylon soaks, excision of eschar from burned soldiers, planned evaluation of cerium silver sulfadiazine, and the use of frozen homograft in burn wound care. Our objective is to further define the use of these methods.

24. (U) Patients admitted to the Institute of Surgical Research for care of thermal injuries receive burn wound care based on the specific injury. The 5% aqueous Sulfamylon soaks, excision of the eschar, and other modalities of wound care may be used.

25. (U) 7910 - 8009. Treatment with 5% aqueous Sulfamylon was utilized in 157 patients. Thirteen patients (8.3%) exhibited some form of allergic reaction. These 13 patients required no treatment of mild atopy. The low incidence of reactions and the clinical effectiveness of 5% aqueous Sulfamylon speaks for its continued use. Standard topical antimicrobial therapy of the burn wound is now sequential application of mafenide acetate and silver sulfadiazine every 12 hours to maximize the spectrum of antibacterial effectiveness and minimize the side effects of the respective agents.

*Available to contractors upon originator's approval

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 68 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

ANNUAL PROGRESS REPORT

PROJECT NO. 2S162774A814-00, APPLIED RESEARCH

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

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

1 October 1979 - 30 September 1980

Investigators:

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

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 2S162774A814-00, APPLIED RESEARCH

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

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

Period covered in this report: 1 October 1979 - 30 September 1980

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

Reports Control Symbol MEDDH-288(R1)

The improvement of wound care has been and continues to be a major goal of the Institute of Surgical Research. Methods under current investigation include the use of 5% aqueous Sulfamylon dressings. During this reporting period, 157 patients required 5% Sulfamylon soaked dressings for the care of their burn wounds. These dressings were employed either in final debridement of the wound or following a meshed cutaneous autograft procedure to prevent desiccation of the fresh skin. A 8.3% incidence of significant skin rash (atopy) was noted as the only adverse reaction. These results support the continued use of 5% Sulfamylon solution.

Burn injury
Topical therapy
5% Sulfamylon acetate solution
Humans

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

The evaluation of 5% Sulfamylon acetate solution for topical treatment of the burn wound has continued at this Institute. During the reporting period of 1 October 1979 through 30 September 1980 273 patients were admitted to the U.S. Army Institute of Surgical Research. Of these 273 patients, 157 had 5% aqueous Sulfamylon dressings employed for burn wound care. During this period, 426 split thickness skin autograft procedures were performed in 146 patients; 5% aqueous Sulfamylon soaked dressings were used in conjunction with the skin autografting procedures in 176 patients. The 5% Sulfamylon acetate soaked dressings are used either as continuous wet dressings or as wet to dry dressings to debride burn wounds. When mesh cutaneous autografts are applied dressings soaked with 5% Sulfamylon acetate solution are utilized to decrease the rate of bacterial growth and to keep the mesh cutaneous autograft moist until vascular ingrowth occurs.

Allergic reactions to the 5% aqueous Sulfamylon solution were noted in 13 of the 157 patients. This represents an incidence of 8.3% allergic reactions (atopy). In the 13 patients who developed allergic reactions rapid resolution of the reaction followed the administration of an antihistamine and discontinuing the 5% Sulfamylon soaked dressings. If 5% Sulfamylon soaked dressings were discontinued, saline or other aqueous topical antimicrobial agents were employed. No other adverse reactions were noted in this group of patients.

The use of 5% Sulfamylon acetate dressings has continued to be an important agent for the treatment of patients both in the preparation of the burn wound for cutaneous autografting or in the prevention of desiccation of freshly placed meshed cutaneous autografts. Its efficacy and low incidence of adverse side effects speak for its continued use.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OG 6970	01 Oct 80	DD-DR&E/AR1636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISS'N INSTR'N	8b. SPECIFIC DATA CONTRACTOR ACCESS	9. LEVEL OF SUM
01 Oct 79	D. CHANGE	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO. CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62772A	3S162772A874	AE	163
b. [redacted]	62772A	3S162772A814	00	114
c. [redacted]	STOG 80 - 7.2.5			

11. TITLE (Precede with Security Classification Code)

(U) Studies of The Neuroendocrine Abnormalities in Burn Injury (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
Oct 79	Cont	DA	C. In-House

17. CONTRACT GRANT — Not Applicable

a. DATES/EFFECTIVE — EXPIRATION

b. NUMBER

c. TYPE — d. AMOUNT

e. KIND OF AWARD — f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 1980	2.0	$ 100
	CURRENT 1981	2.0	$ 109

19. RESPONSIBLE DOD ORGANIZATION

NAME: US Army Institute of Surgical Research

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20. PERFORMING ORGANIZATION

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TELEPHONE: 512-221-5416

SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS

NAME: Richard A. Becker, M.D.

NAME: DA

21. GENERAL USE

FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Pineal; (U) Hypothalamus; (U) Thyroid; (U) Indoles; (U) Catecholamines

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) To determine the hormonal abnormalities resulting from burn injury, particularly in the context of the interaction between nervous structures and thyroid function.

24. (U) Nyctohemeral and chronic longitudinal profiles of pineal- and pituitary-related hormones after accidental burn injury in soldiers and other groups of thermally injured patients are being observed. We will use serially independent and serially dependent sampling in rats to assess pituitary and pineal-related abnormalities following burn injury. The pineal gland is being developed as an in vitro model of sympathetic nerve endings combined with an end-organ tissue in which to study the interaction of catecholamines, indolamines, and thyroid hormone.

25. (U) 7910 - 8009. Plasma cortisol rises dramatically as a result of burn injury in soldiers and other groups of thermally injured patients. This effect depends on the size of the burn and, in addition, is magnified if the soldier or other thermally injured is going to die from the injury. Although thyroid hormone levels fall prior to death, cortisol remains high and, in one case, we found it unsuppressed by exogenous steroid. Lack of correlation of cortisol with ACTH levels suggests some other injury-related mediator for cortisol. Studies of injecting radio-actively tagged thyroid hormones in rats have indicated strong uptake of T_3 in the pineal, pituitary (both greater than in muscle) and liver. At 4 h., pineal uptake is 75% that of liver. After injection of tagged catecholamines, strong in vivo uptake of both norepinephrine and epinephrine occurs in the pineal. Studies of rat pineals incubated with thyroid hormones are underway. Other in vitro studies indicate that pineals take up tagged norepinephrine and epinephrine and, in addition, convert tagged norepinephrine which is released into the medium.

(Available to contractors upon originator's approval)

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ANNUAL PROGRESS REPORT

PROJECT NO. 3S162772A814, APPLIED RESEARCH

PROJECT TITLE: STUDIES OF NEUROENDOCRINE ABNORMALITIES IN BURN INJURY - CORTISOL AND CORTICOTROPHIN AFTER BURN INJURY IN HUMANS

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

1 October 1979 - 30 September 1980

Investigators:

George M. Vaughan, M.D., Major, MC
Richard A. Becker, M.D.
John P. Allen, M.D.
Jennifer M. Tucker
Arthur D. Mason, Jr., M.D.
Basil A. Pruitt, Jr., M.D.

Reports Control Symbol MEDDH-288(R1)

Unclassified

ABSTRACT

PROJECT NO. 3S162772A814, APPLIED RESEARCH

REPORT TITLE: STUDIES OF NEUROENDOCRINE ABNORMALITIES IN BURN INJURY - CORTISOL AND CORTICOTROPHIN AFTER BURN INJURY IN HUMANS

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

Period covered in this report: 1 October 1979 - 30 September 1980

Investigators: George M. Vaughan, M.D., Major, MC
Richard A. Becker, M.D.
John P. Allen, M.D.
Jennifer M. Tucker
Arthur D. Mason, Jr., M.D.
Basil A. Pruitt, Jr., M.D.

Reports Control Symbol MEDDH-288(R1)

Although plasma cortisol (F) rises after burn injury, detailed examination of the time course and relationship to corticotrophin (ACTH) has not been reported. Therefore, we examined the pattern of plasma F and ACTH after burn injury in 22 male patients matched for age (18 to 20 years). Three groups were studied: controls (CONT, 8 patients) with minimal injury, total burn size (TBS) 2 to 7.5% of body surface; survivors (SURV, 10 patients) with larger burns (TBS 18 to 82%); and non-survivors (NSURV, 4 patients) with TBS 55 to 93%, expiring on postburn day (PBD) 6 to 54. Plasma F and ACTH were sampled between 0600 and 0800 h. on alternate days from PBD 3 to discharge or death. Mean (range) for individual values during the first month, when CONT samples were taken, are shown:

	CONT	SURV	NSURV
F (μg/dl)	8.4 (1-21)	22.5 (3-70)	39.0 (21-79)
ACTH (pg/ml)	98.4 (19-196)	118.0 (19-241)	86.1 (19-243)

Multiple regression analysis showed F to be a function of TBS ($r = 0.60$, $p < 0.001$) and patient group. For any given TBS, plasma F varied: NSURV > SURV > CONT ($p < 0.05$). Plasma F did not correlate with ACTH. Plasma ACTH was lower in NSURV than in CONT and SURV ($p < 0.05$). In SURV, F remained normal (≤ 21) or elevated after

one month, and in the six patients discharged between PBD 52 and 88, F was usually elevated until discharge. In NSURV, F remained elevated until the day of death. Conclusion: elevated postburn plasma F is related to burn size but not to simultaneously observed ACTH, suggesting some undetermined additional injury-related mediator for F production or for its sensitivity to ACTH.

Cortisol
Corticotrophin

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1 AGENCY ACCESSION	2 DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OG 6968	01 Oct 80	DD-DR&E-(AR)636

3 DATE PREV SUM'RY	4 KIND OF SUMMARY	5 SUMMARY SCTY	6 WORK SECURITY	7 REGRADING	8A DISP'N INSTR'N	8b SPECIFIC DATA CONTRACTOR ACCESS	9 LEVEL OF SUM
01 Oct 79	D. CHANGE	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10 NO CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	BB	303
b. ~~XXXXXXXX~~	61102A	3S161102BS05	00	090
c. ~~XXXXXXXX~~	STOC 80 - 7.2.5			

11 TITLE (Precede with Security Classification Code)

(U) Alteration of Host Resistance in Burned Soldiers (44)

12 SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 Clinical Medicine

13 START DATE	14 ESTIMATED COMPLETION DATE	15 FUNDING AGENCY	16 PERFORMANCE METHOD
Oct 76	Cont	DA	C. In-House

17 CONTRACT GRANT: Not Applicable

a. DATES/EFFECTIVE — EXPIRATION

b. NUMBER

c. TYPE — d. AMOUNT

e. KIND OF AWARD — f. CUM. AMT.

18 RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 1980	4.0	$ 125
	CURRENT 1981	4.0	$ 130

19 RESPONSIBLE DOD ORGANIZATION

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20 PERFORMING ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS

NAME:

NAME: DA

21 GENERAL USE

FOREIGN INTELLIGENCE NOT CONSIDERED

22 KEYWORDS (Precede EACH with Security Classification Code)

(U) Tissue Spreading Factors; (U) Rat Model; (U) Infection; (U) Immunostimulants; (U) Virulence Factors; (U) Plasmids; (U) Antibiotic Effects

23 TECHNICAL OBJECTIVE 24 APPROACH 25 PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) To define the microbial basis of opportunistic infection in susceptible burned soldiers. Identify specific mechanisms of decreased host resistance that are targeted by opportunistic pathogens. 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 immunostimulator therapies will be examined.

25. (U) 7910-8009. The in vitro transfer of a Pseudomonas antibiotic resistance plasmid (RP1) into Pseudomonas strain 59-1244 resulted in loss of rat burn wound virulence. The attenuation of the plasmid-containing strain was reversed by loss of the plasmid. Cefsulodin, an investigational cephalosporin antibiotic, was found to solidly protect rats against lethal infection with Pseudomonas aeruginosa using a 10-day treatment at 50 mg/kg/day i.p. This effective dose was tenfold less than the previously reported most effective antibiotic carbenicillin. An investigational trial in burned patients is scheduled during FY 1981.

*Available to contractors upon originator's approval

DD FORM 1498 1 MAR 68 — PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 68 AND 1498-1 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

ANNUAL PROGRESS REPORT

PROJECT NO. 3S161102BS05-00, BASIC RESEARCH

REPORT TITLE: ALTERATION OF HOST RESISTANCE IN BURNED SOLDIERS

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

1 October 1979 - 30 September 1980

Investigators:

Albert T. McManus, Ph.D., Major, MSC
Arthur D. Mason, Jr., M.D.
William J. Northam, SP5
Camille L. Filip, M.A.

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3S161102BS05-00, BASIC RESEARCH

REPORT TITLE: ALTERATION OF HOST RESISTANCE IN BURNED SOLDIERS

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

Period covered in this report: 1 October 1979 - 30 September 1980

Investigators: Albert T. McManus, Ph.D., Major, MSC
Arthur D. Mason, Jr., M.D.
William J. Northam, SP5
Camille L. Filip, M.A.

Reports Control Symbol MEDDH-288(R1)

The in vitro transfer of a Pseudomonas antibiotic resistance plasmid RP1 into Pseudomonas strain 59-1244 resulted in loss of rat burn wound virulence. The attenuation of the plasmid-containing strain was reversed by loss of the plasmid. Cefsulodin, an investigational cephalosporin antibiotic, was found to solidly protect rats against lethal infection with Pseudomonas aeruginosa using a 10-day treatment at 50 mg/kg/day i.p. This effective dose was tenfold less than the previously reported most effective antibiotic carbenicillin. An investigational trial in burned patients is scheduled for FY 1981.

Tissue spreading factors
Rat model
Infection
Immunostimulants
Virulence factors
Plasmids
Antibiotic effects

ALTERATIONS OF HOST RESISTANCE IN BURNED SOLDIERS

EFFECT OF PLASMID RP1 ON PSEUDOMONAS VIRULENCE

Experimental Pseudomonas aeruginosa surface infection of the rat remains the principal laboratory tool for in vivo evaluation of anti-Pseudomonas therapies. This model demonstrates both burn injury induced increased susceptibility to infection and the conditions necessary for pathogenicity of normally saphrophytic Pseudomonas aeruginosa. The animal component of this host-parasite interaction has been held uniform by breeding management. The bacterial component has been, for the most part, maintained by ultra-low temperature storage at the clinical time of isolation. Thus the prototype strain 1959-1244, although a burn patient blood isolate, is representative of strains infecting burn patients more than 20 years ago, which was prior to the clinical use of most topical chemotherapies and current antibiotics. Strain 1244 is sensitive in vitro to current drugs, such as Sulfamylon, silver-sulfadiazine, carbenicillin, colistin, amikacin, gentamicin, sulfadiazine and tetracycline. This fact does not negate the experimental value of 1244 in that an effective drug must first overcome the physical and physiological alterations of the host prior to controlling the infection. It would seem, however, that a more contemporary challenge strain would add to the clinical relevance of an effective drug.

Efforts have been initiated to modify strain 1244's antibiotic resistance pattern to include resistances to current clinically used drugs. Possession of rat virulent substrains of 1244 would allow more meaningful investigation of new drugs and also continue the accumulation of knowledge gathered with this prototype strain.

Experiments were conducted to infect strain 1244 with the antibiotic resistance plasmid RP1. This plasmid was initially isolated in the Birmingham Burns Unit and confers resistance to carbenicillin, neomycin/kanamycin and tetracycline (1). RP1 is a well characterized R factor and has broad host range which includes both Pseudomonas species and the Enterobacteriaceae. A donor strain was provided by Dr. R. H. Olsen, University of Michigan Medical School. The donor strain PAO-2 Ser^- (RP1) is a Pseudomonas aeruginosa strain which is auxotrophic for serine and contains the plasmid RP1. Prior to attempts to transfer RP1 into 1244, an identifiable genetic marker was required in 1244 so that it could be identified and selected after mating. A streptomycin resistant mutant 1244S was isolated by direct plating of concentrated 1244 onto plates containing 1000 µg/ml streptomycin. The original minimal inhibitory concentration (MIC) of streptomycin for 1244 was 50 µg/ml. The direct selection of streptomycin resistance in Pseudomonas aeruginosa has previously been

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1. Lowbury EJ, Lilly JA, Kidson A, et al: Sensitivity of Pseudomonas aeruginosa to antibiotics: emergence of strains highly resistant to carbenicillin. Lancet 2:448-452, 1969.

shown to be the result of a mutation of a gene located on the bacterial chromosome (2). The streptomycin MIC for PAO-2 (RP1) was found to be 50 µg/ml.

For transfer, strains 1244S and PAO-2 Ser$^-$ (RP1) were mixed and incubated in broth (TSB) overnight at 37^o C. Following incubation, the mixture was diluted and plated onto media containing 1000 µg/ml streptomycin and 500 µg/ml carbenicillin. This combination was intended to kill all bacteria that were not resistant to both drugs. Strain 1244S MIC for carbenicillin was 31.25 µg/ml and PAO-2 Ser$^-$ (RP1) was > 1250 µg/ml. The resulting strain (pool of five colonies) was designated 1244S (RP1). To confirm that this strain was truly a 1244 derivative and not a streptomycin-resistant mutant of PAO-2 Ser$^-$ (RP1), 1244S (RP1) was examined for its serotype and the requirement of serine for growth. 1244S (RP1) was found to have identical O-serotype with the parent 1244, which is distinct from PAO-2 and did not require serine. As an additional control, a strain of 1244S (RP1) which had lost RP1 resistance markers was selected by replica plating isolated colonies from nonselective agar onto plates containing 250 µg/ml carbenicillin. Testing of 400 clones resulted in four isolates sensitive to 250 µg carbenicillin. These clones also lost the other plasmid markers. The clones were pooled to form strain 1244S (RP1)$^-$.

The effect of plasmid RP1 on *in vitro* antibiotic sensitivity is presented in Table 1. As can be seen, strains containing RP1 demonstrated high resistance to the three presented drugs. Examination of other antibiotic markers using the Kirby-Bauer disc technique showed no other antibiotic resistances associated with RP1. Strain 1244S (RP1) remained sensitive to gentamicin, amikacin and sulfonamides.

Table 1. Effect of plasmid RP1 on *in vitro* sensitivity (MIC)

	MIC (µg/ml)				
	1244	1244S	PAO-2 (RP1)	1244S (RP1)	1244S (RP1)$^-$
Carbenicillin	125.0	31.2	> 1250	> 1250	62.5
Kanamycin	62.5	62.5	> 250	> 250	62.5
Tetracycline	15.5	15.5	> 250	> 250	7.78

2. Holloway BW, Krishnapillai V, Morgan AF: Chromosomal genetics of Pseudomonas. Microbiol Rev 43:73-102, 1979.

The rat virulence of 1244 (RP1) was examined in 30% scalded 350 gram rats. Control strains included 1244, 1244S, PAO-2 (RP1), 1244S (RP1) and 1244S $(RP1)^-$. All strains were inoculated at 10^8 CFU/rat. Mortality was recorded for 28 days postinoculation. Results are presented in Table 2. RP1 reversibly suppressed virulence of strain 1244 ($P < 0.01$).

Table 2. Relative burn rat virulence of strain 1244S (RP1)

	Experiment 1				Experiment 2		
	1244	1244S	1244S (RP1)	PAO-2 (RP1)	1244	1244S	1244S $(RP1)^-$
Lived	0	2	12	10	0	0	1
Died	15	13	3	5	10	10	9

H_o 1244 = 1244S N.S.
H_o 1244S = 1244S (RP1) $P < 0.001$
H_o 1244S = 1244S $(RP1)^-$ N.S.

Postmortem cultures of spleens taken from the three animals that died following 1244S (RP1) yielded Pseudomonas aeruginosa. Antibiotic sensitivity testing of these isolates showed two of the three isolates were sensitive to carbenicillin, neomycin and tetracycline and resistant to streptomycin. It is interesting to speculate that these two isolates are the result of in vivo loss of RP1. The third isolate had identical antibiotic resistances to the inoculated 1244S (RP1). Attempts will be made to passage this invasive drug resistant isolate in the hope of establishing a stable drug resistant but virulent substrain of 1244.

EXAMINATION OF ANTI-PSEUDOMONAS AERUGINOSA ACTIVITY OF CEFSULODIN

Most cephalosporin antibiotics, despite broad antibacterial spectra, are not active against many strains of Pseudomonas aeruginosa. Recently, a semisynthetic cephalosporin (cefsulodin) has been developed that demonstrates high activity against clinical isolates of Pseudomonas aeruginosa. The drug also shows significant activity against staphylococci, Group A beta hemolytic streptococci, pneumococci, and Neisseria. With the increasing incidence of resistance with the clinical use of the anti-Pseudomonas drugs carbenicillin and aminoglycosides, cefsulodin may offer the next step in the evolution

of effective agents. We have investigated the activity of cefsulodin *in vitro* against current burn ward Pseudomonas strains and also tested its activity *in vivo* in the burn rat model.

For *in vitro* assay, 81 strains of Pseudomonas from the host resistance culture collection were examined. The strains represent samples taken over the past 2 years. *In vitro* assay was by standard agar overlay Kirby-Bauer disc technique or by broth dilution tube assay. Cefsulodin was provided by CIBA-Geigy Corporation and Abbott Laboratories. The *in vitro* disc sensitivity pattern for cefsulodin and other selected antibiotics is presented in Table 3. A 30 µg disc was used for cefsulodin, and sensitivity interpretation was set at a zone of 15 mm or greater. Other antibiotic sensitivities were measured to manufacturer's criteria. Cefsulodin was the most active drug, with 93% of strains sensitive. Broth dilution data are presented in Figure 1. MIC data confirmed the disc sensitivity data, with 94% of strains sensitive to 16 µg/ml or less. This level is the sensitivity cut-off established by the manufacturer.

Table 3. Antibiotic sensitivity patterns* in 81 *Pseudomonas aeruginosa* burn ward isolates prior to the clinical use of cefsulodin

	% Sensitive	% Resistant
Gentamicin	28	72
Tobramycin	27	73
Amikacin	52	48
Kanamycin	1	99
Chloramphenicol	1	99
Carbenicillin	33	67
Cefsulodin	93	7

* Sensitivity was determined by the Kirby-Bauer agar overlay disc technique.

The *in vivo* effectiveness of cefsulodin was investigated in 200-gram rats with 20% scalds infected with 1244. Drug treatment was initiated 24 hours after burning and infecting. Treatments of 10, 20, or 50 mg/kg/day were investigated using 10 rats at each dose. Animals were inoculated i.p. once per day. Daily treatment with Silvadene^{R} was done in a group of 10 burned-infected rats as a positive treatment control, and 10 burned-infected rats were not treated for a negative treatment control. Results are presented in Table 4. As can be seen, cefsulodin showed a dose-dependent increase

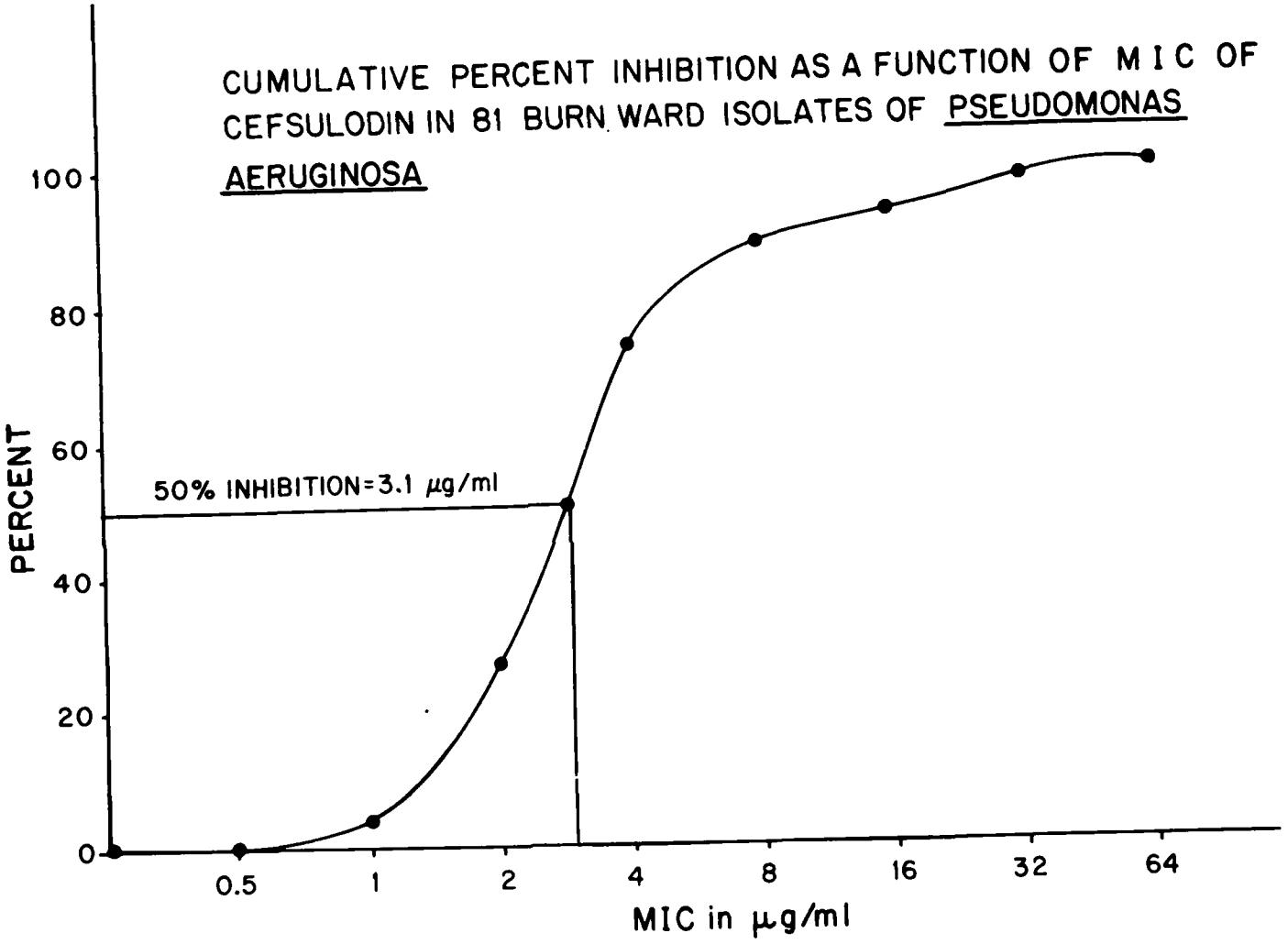
CUMULATIVE PERCENT INHIBITION AS A FUNCTION OF MIC OF CEFSULODIN IN 81 BURN WARD ISOLATES OF PSEUDOMONAS AERUGINOSA
PERCENT
100
80
60
40
20
0
50% INHIBITION=3.1 µg/ml
0.5
1
2
4
8
16
32
64
MIC in µg/ml

Figure 1

in protection. The dose of 50 mg/kg/day which gave solid protection is approximately equal to the human recommended dose: 4 g/70 kg/day = 57 mg/kg/day. This dose is 1/10 the recommended human dose of carbenicillin: 500 mg/kg/day. Also, 50 mg/kg/day is 1/10 the effective dose reported for 1244 infected rats (3).

Table 4. Effect of cefsulodin treatment* in strain 1244 burned-infected rats

Treatment	Survival rate
Cefsulodin, 10 mg/kg	4/10
" 20 mg/kg	6/10
" 50 mg/kg	10/10
Silvadene	10/10
No drug	0/10

* Cefsulodin was injected once per day i.p.
Silvadene was applied once per day.

A human trial is scheduled during the next reporting period.

3. McManus WF, Mason AD Jr, Pruitt BA Jr: Subeschar antibiotic infusion in the treatment of burn wound infection. J Trauma 20:1021-1023, 1981.

PRESENTATIONS

McManus AT: Studies on the mechanisms of in vitro resistance to silver sulfadiazine. Annual Meeting, American Burn Association, San Antonio, Texas, 28 March 1980.

McManus AT: Decreased virulence in experimental Proteus mirabilis burn wound sepsis associated with motility deficient mutants. Annual Meeting, American Society for Microbiology, Miami, Florida, 11-16 May 1980.

PUBLICATIONS

McManus AT, Moody EE, Mason AD: Bacterial motility: a component in experimental Pseudomonas aeruginosa burn wound sepsis. Burns 6: 235-239, 1980.

None.

ANNUAL PROGRESS REPORT

PROJECT NO. 3S161102BS05-00, BASIC RESEARCH

REPORT TITLE: *ALTERATION OF HOST RESISTANCE IN BURNED SOLDIERS -- EXPERIMENTAL FUNGAL SURFACE INFECTION*

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

1 October 1979 - 30 September 1980

Investigators:

Charles G. McLeod, Jr., Lieutenant Colonel, VC
Albert T. McManus, Major, MSC
Harrel L. Walker, M.S.
Arthur D. Mason, Jr., SES

Reports Control Symbol MEDDH-288 (RI)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3S161102BS05 00, BASIC RESEARCH

REPORT TITLE: ALTERATION OF HOST RESISTANCE IN BURNED SOLDIERS -- EXPERIMENTAL FUNGAL SURFACE INFECTION

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

Period covered in this report: 1 October 1979 - 30 September 1980

Investigators: Charles G. McLeod, Jr., Lieutenant Colonel, VC
Albert T. McManus, Major, MSC
Harrel L. Walker, M.S.
Arthur D. Mason, Jr., SES

Reports Control Symbol MEDDH-288 (R1)

A burned rat model of cutaneous fungal colonization and infection has been developed. Animals were burned on the tail by scalding the terminal 6 cm tail segment with boiling water for three seconds. The resulting injury was histopathologically a deep partial thickness injury. Scalded tails were inoculated with phycomycete (Mucor or Rhizopus) spores suspended in tryptic soy broth containing 10%$^{W}/_{V}$ Ficoll (400 K (M.W.)). The tails were then occlusively enclosed in plastic tubes. Animals were returned to their cages and resulting infection was examined sequentially.

Fungal infection
Burned rat
Phycomycetes
Mucor species
Rhizopus species

EXPERIMENTAL FUNGAL SURFACE INFECTION

With the development of topical antibacterial therapy, the incidence of opportunistic fungal infection in severely burned patients has increased Adequate topical chemoprophylactic and chemotherapeutic agents for fungal infections have not been developed. These infections, particularly those caused by the Phycomycetes group and Aspergillus species are managed at present by excision of the infected wound, amputation of limbs, or by treatment with systemic antifungal agents. This protocol was designed to develop an acceptable animal model of fungal infection. Such a model would be useful in studying the pathophysiology of fungal infections as well as for testing antifungal agents.

METHODS

Anesthetized rats (200 g) were subjected to a deep 2nd degree scald burn of a segment of their tails. A spore suspension of either Mucor or Rhizopus species was applied topically to the burned tails which were then enclosed in an occlusive plastic tube. The inoculum contained approximately 10^7 fungal spores in 0.5 cc of TSB and Ficoll. The incidence of burn wound colonization and/or invasive infection was studied histologically.

RESULTS

Experiments were conducted utilizing clinical isolates of Mucor and Rhizopus species. As expected, the incidences of infection and colonization varied with the different strains. Several experiments yielded disappointingly low incidences (20 to 30% of animals examined), however modification of the topical inoculum with Ficoll which made it more adherent to the burn skin gave better results (colonization incidence 87% and invasive infection 39%) in a group of 23 rats.

The sequential development of colonization and focal fungal invasion of viable tissue was studied microscopically. Intraeschar colonization was followed by a suppurative response at the eschar base and at sites of hyphal invasion. Vascular invasion and thrombosis were observed in some rats. Thrombi also developed in arteries which had no microscopic evidence of fungal invasion. Fungal infections were always self-limiting, apparently because of a successful histiocytic and giant cell response to the infection. The reproducible and characteristic lesions of this infection model may be useful in future development and testing of topical antifungal agents.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OD 6978	01 Oct 80	[illegible]

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8A. DISP'N INSTR'N	8B. SPECIFIC DATA CONTRACTOR ACCESS	9. LEVEL OF SUM
01 Oct 79	B. CHANGE	U	U	NA	NL	X YES ___ NO	A. WORK UNIT

10. NO. CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	BB	505
b. [illegible]	61102A	3S161102BS05	00	081
[illegible]	STOG 80-T-253			

11. TITLE (Precede with Security Classification Code): (U) Evaluation of Synthetic Sheeting as Operating Room Drape Material For Use in a Military Burn Unit (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 005500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
Jul 70	Cont	DA	C. In-House

17. CONTRACT/GRANT: Not Applicable
a. DATES/EFFECTIVE: EXPIRATION:
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c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT:

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR — PRECEDING 1980	0.2	$ 10
FISCAL YEAR — CURRENT 1981	0.2	$ 12

19. RESPONSIBLE DOD ORGANIZATION
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NAME: Robert E. Lindberg, Ph.D.
NAME:

21. GENERAL USE
DISCLOSURE INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Military Burn Unit; (U) Operating Room Based Infections; (U) Surgical Drapes; (U) Surgical Gowns.

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) Evaluation in terms of draping characteristics, absorbency, physician acceptance, and bacterial barrier qualities of a Spunbonded Olefin-cellulosic laminated sheeting as surgical drapes and gowns. A decrease in bacterial seeding of operative wounds via drapes will minimize postoperative wound infections decreasing subsequent morbidity and mortality in injured troops.

24. (U) Laboratory assessment of bacterial barrier properties of synthetic sheeting. Clinical use of drapes on burn patients to determine surgeon acceptability. Photographic documentation of draping characteristics, absorbency, and "run-off". Pre-and post-operative cultures at margin of operative field. Temperature monitoring to determine heat transmission characteristics.

25. (U) 7910 - 8009. The bacterial barrier property of eleven synthetic drape materials has been assessed using the testing method developed in this laboratory. Significant differences between both drape penetration and organism penetrating ability were identified. The Pseudomonas test strain penetrated more consistently than the other four test organisms. The least resistant drape material permitted penetration of bacteria at 96.2% of test sites while bacterial penetration occurred at only 17.6% of test sites in each of the two best drape materials. Statistical analysis identified six materials showing "least penetration" and five materials showing "most penetration" with the mean occurrence rate of penetration being 31.5% in the former group and 89.1% in the latter group. Drape characteristics were the predominant determinant of bacterial penetration. All of the synthetic sheeting tested at this time demonstrated inadequate bacterial barrier function either as a result of the sheeting material itself or the currently employed fabrication process.

*Available to contractors upon originator's approval

DD FORM 1 MAR 66 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 68 AND 1498-1, 1 MAR 68, FOR ARMY USE ARE OBSOLETE.

ANNUAL PROGRESS REPORT

PROJECT NO. 3S161102BS05, MILITARY BURN RESEARCH

REPORT TITLE: EVALUATION OF SYNTHETIC SHEETING AS OPERATING ROOM DRAPE MATERIAL FOR USE IN A MILITARY BURN UNIT

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

1 October 1979 - 30 September 1980

Investigators:

Basil A. Pruitt, Jr., MD, FACS, Colonel, MC
Robert B. Lindberg, PhD
Arthur D. Mason, Jr., MD

Reports Control Symbol MEDDH-288(RI)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3S161102BS05, MILITARY BURN RESEARCH

REPORT TITLE: EVALUATION OF SYNTHETIC SHEETING AS OPERATING ROOM DRAPE MATERIAL FOR USE IN A MILITARY BURN UNIT

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

Period covered in this report: 1 October 1979 - 30 September 1980

Investigators Basil A. Pruitt, Jr., MD, FACS, Colonel, MC
Robert B. Lindberg, PH.D.
Arthur D. Mason, Jr., M.D.

Reports Control Symbol MEDDH-288(RI)

The bacterial barrier properties of 11 types of non-woven synthetic surgical drape material were assessed using test methods developed at this Institute. The transmission of each of five different bacteria (Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Klebsiella pneumonia and Serratia marcescens) suspended in liquid culture media and inoculated on samples of each of the 11 drape materials was determined in seven replicate trials of each material.

Penetration rates differed significantly between drape materials. Penetration of individual drape materials also varied according to the test organism. The penetration rate of Pseudomonas accounted for the significance of the difference between organisms. The materials could, on the basis of bacterial penetration, be divided into two groups, i.e., those six materials showing least penetration and those five materials showing the most penetration. Within the group of materials showing the least penetration, there were two materials which allowed bacterial transmission at less than 20 percent of test sites, while penetration of the other four materials ranged from 20.5 percent to 64.3 percent. In those materials showing the highest rate of penetration, one sample permitted transmission of bacteria at 96.2 percent of all inoculation sites. Mean penetration in the group of materials showing the least penetration was 31.3 percent and the mean penetration in those materials having the least effective bacterial barrier function was 89.4 percent. The composition and the processing of the materials thus appeared to be critical factors determining the rate of microbial penetration through these materials as assessed by this testing procedure.

EVALUATION OF SYNTHETIC SHEETING AS OPERATING ROOM DRAPE MATERIAL FOR USE IN A MILITARY BURN UNIT

Bacteria and other microorganisms readily penetrate standard surgical drapes made of muslin or cotton, once the drapes become moistened in the course of an operation. Such drape material serves as an inadequate barrier to microbial migration into the surgical field and the operative wound. Synthetic drape materials are now used in more than 50 percent of all operations, largely on the basis of decreased linting, easy disposability, and some earlier studies showing improved bacterial barrier properties. The poor draping characteristics of the synthetic drape material and the fact that such material permitted quantitative runoff of liquids from the operative field onto the surgeon limited the acceptance of such materials. Consequently, the manufacturing processes used in the production of these materials has been modified to improve their draping characteristics and "soften" the material. Alteration in the density of the non-woven materials may also improve their draping characteristics but adversely affect their bacterial barrier function. Testing of newer forms of "softened" non-woven spunbonded drape material, both treated and untreated with water repellency compounds, has been carried out to evaluate their adequacy as microbial barriers.

Methods

Discs of the synthetic drape materials, 90 mm in diameter, were cut, gas-sterilized and placed on the surface of blood agar culture plates. The top-bottom orientation designated for each sample of drape material was observed with the upper surface of the disc corresponding to the side of the drape which would not be in contact with the surface of the patient when used. Six drops of an overnight TSB broth culture of each bacterial strain used for testing were placed at equidistant intervals on each disc. The spacing permitted differentiation of individual areas of growth of the test organism, if it penetrated the disc material. The drops of bacterial-containing broth were left in position on the drape material for four hours at room temperature, following which any remaining culture liquid was removed using a micro pippette. The disc of draping material was then removed and the plate incubated overnight at $37^{o}C$. Penetration of the drape material was considered to have occurred if growth of the bacterial test strain was apparent at any site where it had rested on the material. If confluent growth occurred which encompassed two inoculation sites, each of the sites involved was considered as a site of penetration although it would be theoretically possible for organisms from a single site of penetration to spread over adjacent sites under the disc of drape material. Each of 11 drape material samples was tested for penetration by Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, and Serratia marcescens. Seven replicate trials of each drape material for each organism were carried out - a total of 2,310 inoculation sites.

Results

The percentage of penetration of each drape material by all test strains is shown in the Table. There is identifiable microbial genus-specific variation in penetrability within drape materials, but a greater difference is observed between materials in terms of overall bacterial penetration. The percentage of

inoculation sites at which microbial penetration occurred was least in two materials, No. 77-104-1 and No. 77-104-3, with bacterial transmission at 17.6 percent of inoculation sites on both materials. The percent of inoculation site penetration in the other materials ranged from 20.5 percent to 96.2 percent, with the latter highest rate of penetration characteristic of material 77-104-9.

TABLE I

PENETRATION OF NON-WOVEN DRAPE MATERIAL
INOCULATION SITES BY TEST BACTERIA

Test Organism and Fraction of Test Sites Penetrated

Drape Materials Sample Number	Pseudo. aerug	Staph. aureus	E. coli	Klebsiella Pneumoniae	Serratia Marces.	Totals	Percent Penetration
77-104-1	4/42	5/42	5/42	16/42	7/42	37/210	17.6
77-104-2	9/42	10/42	4/42	17/42	3/42	43/210	20.5
77-104-3	6/42	3/42	7/42	11/42	10/42	37/210	17.6
77-104-4	42/42	39/42	39/42	42/42	29/42	191/210	91.0
77-104-5	20/42	3/42	5/42	19/42	8/42	55/210	26.2
77-104-6	39/42	35/42	31/42	38/42	33/42	176/210	83.8
77-104-7	30/42	42/42	29/42	6/42	28/42	135/210	64.3
77-104-8	29/42	13/42	21/42	11/42	14/42	88/210	41.9
77-104-9	42/42	42/42	38/42	38/42	42/42	202/210	96.2
77-104-10	42/42	39/42	40/42	37/42	39/42	197/210	93.8
77-104-11	39/42	25/42	36/42	41/42	32/42	173/210	82.4
TOTAL	302/462	256/462	255/462	276/462	245/462	1334/2310	
Total Percentage of Sites Penetrated	65.4	55.4	55.2	59.7	53.0	57.75	

Discussion

The 11 samples of drape material which were tested showed considerable variation in microbial penetration, which ranged from 17.6 percent for materials

77-104-1 and 77-104-3, to 96.2 percent for material 77-104-9. Statistical analysis indicated that there were significant differences both between drape material samples and between organism penetrability. The increased capacity for drape material penetration of Pseudomonas aeruginosa accounted for the significance of the difference between the penetration of the test organisms. A Friedman non-parametric two-way analysis of variance identified two groups of drape materials, i.e., six of the 11 which permitted "least penetration" and five of the 11 which permitted "most penetration." The rate of penetration within the materials showing "least penetration" ranged from 17.6 percent to 64.3 percent. In addition to the two samples allowing penetration at only 17.6 percent of inoculation sites, there were two other drape materials which allowed penetration of less than 30 percent of test sites, i.e., material No. 77-104-2 showing 20.5 percent penetration, and material No. 77-104-5 showing 26.2 percent penetration. The rate of penetration in the samples showing "most penetration" ranged from 82.4 percent to 96.2 percent. The mean incidence of penetration in the materials showing "least penetration" was 31.3 percent while the mean penetration in the samples showing "most penetration" was 89.4 percent.

In summary, the results of these studies confirm earlier tests indicating that while drape composition is of importance in terms of bacterial barrier properties, processing to alter the draping and softness characteristics of the material can adversely influence the ability of the material to resist bacterial penetration. On the whole, none of the materials tested in this group performed as well as certain previously tested samples and little progress in producing a drape possessing satisfactory bacterial barrier properties has been made. In fact, it appears as if all of the non-woven drape material samples tested at this time have inadequate bacterial barrier function either as a result of the sheeting material itself, or the processing of the material to improve its draping characteristics and "softness." Further testing is planned to determine whether the processing causes microscopic disruption in the sheeting or whether the non-woven material used for the softened sheeting has been altered to increase the size of the material's pores to a point where bacteria are freely transmissible.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OG 6972	01 Oct 80	DD DR&E/AR(6)6

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8A. DISB'N INSTR'N	8B. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
01 Oct 79	D. CHANGE	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO. CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	BB	301
b. [illegible]	61102A	3S161102BS05	00	088
c. [illegible]	STOG 80-7	2:5		

11. TITLE (Precede with Security Classification Code)
(U) Studies of Infection and Microbiologic Surveillance of Troops With Thermal Injury(44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS
003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
Oct 76	Cont	DA	C. In-House

17. CONTRACT/GRANT: Not Applicable
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER:
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 1980	4.0	$ 2[illegible]
	CURRENT 1981	3.0	$ 178

19. RESPONSIBLE DOD ORGANIZATION
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SOCIAL SECURITY ACCOUNT NUMBER
ASSOCIATE INVESTIGATORS
NAME:
NAME: DA

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Pseudomonas (U) Klebsiella, (U) Staphylococci, (U) Wound Infection, (U) Antibiotic Resistance, (U) Sepsis, (U) Topical Chemotherapy, (U) Humans

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) Burns constitute a large component of military injuries sustained in combat. Military relevance of this research lies in the fact that infection and ensuing sepsis are major problems among burned soldiers. Control of surface infection is a major objective, and species of organisms causing sepsis, epidemiology, response of significant species to topical chemotherapy modalities, and relation of antibiotics to sepsis control are major study areas.

24. (U) Culture 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 used to assess experimental drugs, both topical and systemic.

25. (U) 7910 - 8009. Epidemic Ps. aeruginosa was demonstrated. Its incidence was decreased in a reflection. Epidemic patterns altered, when a 37 patient increment was admitted following a major catastrophe. Acinetobacter spp. was recovered from wounds to an unprecedented extent, although it did not incite sepsis, while Pseudomonas sepsis decreased markedly. Fluctuations in methicillin sensitivity changed, with Staphylococci mainly resistant to axacillin but sensitive to methicillin and Nafcillin. Experimental chemotherapy with new Mn, Co, Cd, and Ce sulfonamides showed promise for wound infection prophylaxis, and reduction of sub-eschar bacterial counts was marked. This index of relative efficacy serves to point out most promising experimental agents. Serotyping of Ps. aeruginosa has been refined to a level where epidemic patterns were delineated accurately so that nosocomial epidemics could be more accurately traced and evaluated. Sensitivity of epidemic strains to Sulfamylon showed a return to significant sensitivity after a previous year of resistant Pseudomonas. Enzyme production by Ps. aeruginosa and its relation to virulence was studied further, and the metabolism was related to virulence. Counter immune electrophoresis as a means of detecting type specific antigen in tissue and body fluids was initiated and refinement of technic is being carried forward.

*Available to contractors upon originator's approval

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 68 AND 1498-1 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

ANNUAL PROGRESS REPORT

PROJECT NO. 3S161102BS05-00, BASIC RESEARCH

REPORT TITLE: STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE OF TROOPS WITH THERMAL INJURY -- ANTIBIOTIC SENSITIVITY OF CURRENT MILITARY BURN PATIENT FLORA

US ARMY INSTITUTE OF SURGICAL RESEARCH
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1 October 1979 - 30 September 1980

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Susan J. Constable, SSG
Gloria Bailey, SP5

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3S161102BS05-00, BASIC RESEARCH

REPORT TITLE: STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE OF TROOPS WITH THERMAL INJURY -- ANTIBIOTIC SENSITIVITY OF CURRENT MILITARY BURN PATIENT FLORA

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

Period covered in this report: 1 October 1979 - 30 September 1980

Investigators: Robert B. Lindberg, Ph.D.
Jack R. Henderson, Ph.D.
Susan J. Constable, SSG
Gloria Bailey, SP5

Reports Control Symbol MEDDH-288(R1)

Fourteen species of bacteria, and a total of 250 strains, were tested for antibiotic sensitivity by MIC technic in 1979-1980. Sources included primarily blood culture and wound biopsy, but other clinical sources also contributed to this number. Predominant in blood stream invasion were Staphylococcus aureus and Pseudomonas aeruginosa. Significant trends and changes in susceptibility of burn wound pathogens to antibiotics were observed. Staphylococcus aureus strains once more became gentamicin and tobramycin sensitive. Methicillin sensitivity became almost complete, and nafcillin-sensitive strains also predominated. Tetracyclines and cephalothin continued active against staphylococci. With P. aeruginosa, gentamicin sensitivity reappeared after a 6-year period of aminoglycoside resistance. Tetracycline-sensitive strains predominated, and carbenicillin and ticarcillin sensitivity was the rule. Klebsiella, Escherichia and Enterobacter spp. were susceptible to gentamicin and tetracyclines. Providencia stuartii reappeared in the burn ward population and was totally antibiotic resistant.

Burns
Antibiotic sensitivity
Pseudomonas
Staphylococci

STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE OF TROOPS WITH THERMAL INJURY -- ANTIBIOTIC SENSITIVITY OF CURRENT MILITARY BURN PATIENT FLORA

Antibiotics used in the treatment of bacterial infection are rightly viewed as having constituted a revolution in the relationship between man and infectious disease. Specific bacterial diseases, such as streptococcal infections or infections due to Neisseria spp., have yielded to appropriate antibiotics; but the opportunistic pathogens, which are the major problem in infection in burn patients, have assumed a far larger role in the totality of bacterial infection. With these organisms, antibiotic treatment has been less than completely successful, and nosocomial infections are now a major challenge in burn patient care. An ongoing scrutiny of bacterial isolates associated with sepsis in burn patients is of basic importance in understanding the dynamics of wound infection and offers an immediate aid in selection of antibiotics prior to individual minimum inhibitory concentration (MIC) determinations.

The incidence of bacteremia, and the number of isolates from biopsies, was considerably reduced in 1979-80 in comparison with preceding years. There is no obvious explanation for this gratifying change. It connotes a lessened extent of sepsis in the population of burn patients.

STRAINS TESTED AND ANTIBIOTICS EMPLOYED

There were 14 species of bacteria and 250 strains tested by macro tube dilution for MIC. The number of isolates tested was much smaller than had been the case in preceding years. One reason was that fewer patients were admitted this year than was the case in previous years. In addition, a large group of patients admitted as a single group after being injured in Japan showed fewer systemic infections than would have been anticipated with comparable numbers of patients admitted in the usual course of events. Since the number of strains tested is directly related to the severity of infection in a given patient, a reduced incidence of sepsis meant fewer strains being tested.

The incidence of most frequently tested species is shown in Table 1. Totals for the past 4 years are shown. Staphylococcus aureus and P. aeruginosa were the species most frequently recovered in blood and biopsy of wounds, and hence were the species most frequently tested. Enterobacter cloacae, which occurred in epidemic scale in 1977, had disappeared as an invasive species in 1978-79, but once again appeared in septicemia in 1979-80. Its occurrence was only briefly epidemic, but its potential for severe systemic infection calls for prompt and thorough study of this species whenever it is recovered from the blood stream. Klebsiella pneumoniae had previously caused epidemic sepsis in the burn ward, but its incidence was also reduced from that seen 3 years earlier. Acinetobacter spp. and Serratia marcescens were not

Table 1. Species of Bacteria Tested for Antibiotic MIC: 1976-1980

Species	Year and No. of Strains Tested			
	1976-77	1977-78	1978-79	1979-80
Staphylococcus aureus	75	345	245	78
Staphylococcus epidermidis	66	35	25	20
Streptococcus spp.	31	31	13	10
Pseudomonas aeruginosa	90	71	166	84
Acinetobacter spp.	0	0	4	0
Klebsiella pneumoniae	32	25	15	13
Enterobacter cloacae	41	19	0	12
Escherichia coli	23	45	16	11
Serratia marcescens	10	4	5	0
Proteus spp.	19	20	8	6
Providencia stuartii	1	0	0	6

recovered in sepsis in 1979-80 but are listed because, though rare, they have shown the capacity for generating epidemic sepsis in burn populations.

TESTING SYSTEM FOR ANTIBIOTICS

The test battery of antibiotics in this burn research institute is shown in Table 2. The antibiotics selected cover the current available spectrum of categories, including penicillins, aminoglycosides, cephalothins, polypeptides, and macrolides. The battery has not been changed in the past 2 years, but is subject to review and augmentation as new developments in drugs or attributes of infecting organisms indicate.

Dilutions for all antibiotics but carbenicillin and ticarcillin were set from 25 mcg/ml to 0.78 mcg/ml. Carbenicillin and ticarcillin were tested at concentrations from 1250 mcg/ml to 4.5 mcg/ml. The upper limit of concentration designating sensitivity is 6.2 mcg/ml for gram-positive bacteria and 12.5 mcg/ml for gram-negative forms. The sensitivity upper limit for carbenicillin and ticarcillin is 312 mcg/ml.

SENSITIVITY OF BURN PATIENT FLORA TO ANTIBIOTICS

As has been the case for the past 3 years, S. aureus was numerically one of the two most important species causing septicemia in burn patients. The number of isolates tested, however, was strikingly fewer than had been the case in recent years. The sensitivity of strains of S. aureus to antibiotics of the current test series is summarized in Table 3. The two aminoglycosides were each inhibitory for about half

Table 2. Antibiotics Used in MIC Assessment of Sensitivity
1 October 1979 - 30 September 1980

GRAM-POSITIVE ORGANISMS		GRAM-NEGATIVE ORGANISMS	
Antibiotic	Symbol	Antibiotic	Symbol
Gentamicin	G	Gentamicin	G
Tobramycin	To	Tobramycin	To
Oxacillin	Ps	Kanamycin	K
Methicillin	Sc	Amikacin	Ak
Nafcillin	U	Minocin	M
Minocin	M	Vibramycin	Vb
Vibramycin	Vb	Keflin	Kf
Keflin	Kf	Colistin	Co
Vancomycin	Va		
Clindamycin	Cl		
For *Pseudomonas aeruginosa*:		Carbenicillin	Cb
		Ticarcillin	Ti

of the strains tested, at the upper limit of potential dosage. The semi-synthetic penicillins showed striking differences in activity against this staphylococcal population. Oxacillin inhibited two-thirds of the strains at 6.2 mcg/ml, but with further dilution, the proportion of strains inhibited fell quickly. To a lesser degree, the same pattern appeared with nafcillin. However, with methicillin, concentrations as low as 1.5 mcg/ml were still inhibitory for 87% of strains tested. The staphylococcal population had clearly changed markedly in the past year; it was, overall, markedly more methicillin sensitive.

The tetracyclines, Minocin and Vibramycin, were consistently effective against staphylococci. This pattern has persisted since these drugs were first tested on the burn wound flora. Keflin has been consistently active against most of the strains of staphylococci for many years, and in this period continued that pattern. Vancomycin, which has been a mainstay among anti-staphylococcal antibiotics, continued to be highly active against the strains tested. It should be noted, however, that a small but growing number of resistant strains have appeared with this antibiotic. Clindamycin, an antibiotic with which staphylococci have fluctuated markedly during successive years, was extremely active in its anti-staphylococcal properties. Even in low concentrations, most strains were inhibited.

A comparison of the anti-staphylococcal activity of the antibiotic battery now in use, over the past 8 years, is shown in Table 4. Conspicuous was the reappearance of activity on the part of aminoglycosides,

Table 3. Staphylococcus aureus: Cumulative Inhibitory Levels for 78 Strains
1 October 1979 - 30 September 1980

Antibiotic Level mcg/ml	Antibiotic and % Inhibited									
	G	To	Ps	Sc	U	M	Vb	Kf	Va	Cl
> 25.0	100	100	100	100	100	100	100	100	100	100
25.0	71.4	69.3	92.2	96.1	82.0	100	97.4	97.4	100	98.7
12.5	71.4	66.6	89.6	94.8	78.2	98.7	96.1	91.0	98.7	98.7
6.2	61.0	53.3	66.2	94.8	75.6	92.3	92.3	79.4	96.1	96.1
3.1	36.3	36.0	54.5	93.5	53.8	74.3	67.9	71.7	90.9	90.9
1.5	16.8	14.6	41.5	87.1	42.3	47.4	41.0	61.5	63.6	79.2
< .78	15.5	10.6	11.6	75.6	28.2	38.4	15.3	53.8	22.0	71.4
Total tested	77	75	77	78	78	78	78	78	77	77

G: Gentamicin; To: Tobramycin; Ps: Oxacillin; Sc: Methicillin; U: Nafcillin; M: Minocin; Vb: Vibramycin; Kf: Keflin; Va: Vancomycin; Cl: Clindamycin

Table 4. Comparison of Sensitivity of Staphylococcus aureus to Antibiotics, 1973 - 1980

Antibiotic	Year and % of Strains Inhibited by 6.2 mcg/ml							
	1973	1974	1975	1976	1977	1978	1979	1980
Gentamicin	67.9	92.2	38.3	50.0	30.6	7.4	17.2	61.0
Tobramycin	....	....	88.0	100.0	65.4	16.7	6.2	53.3
Oxacillin	69.7	82.6	73.6	70.5	65.1	31.0	75.9	66.2
Methicillin	50.0	65.2	21.8	23.5	35.7	77.9	34.6	94.8
Nafcillin	62.3	83.3	85.6	49.5	1.8	0.5	0.4	75.6
Minocin	84.1	96.0	46.5	92.8	93.9	95.3	96.3	92.3
Vibramycin	....	....	78.3	94.2	96.9	42.2	98.0	92.3
Keflin	72.1	90.4	97.2	94.0	97.1	96.9	78.4	79.4
Vancomycin	....	....	100.0	100.0	100.0	99.6	98.8	94.8
Clindamycin	40.7	95.8	98.0	95.6	97.1	14.4	73.1	94.8

after 2 to 3 years of predominance of resistant forms. Among the three semisynthetic penicillins, oxacillin was over the years the most consistent in its inhibitory capacity. Only in 1978 was there a marked drop in efficacy. Methicillin has twice become relatively ineffective against staphylococci -- in 1975-77 and again in 1979. The percentage of strains inhibited in 1979-80 was the highest that has been seen for this antibiotic. Nafcillin, which for 4 years was an effective anti-staphylococcal agent, became virtually inactive against staphylococci from 1977 through 1978-79. It was active at 6.2 mcg/ml against 75% of strains tested in 1979-80, which level made it roughly equivalent to oxacillin in activity. The tetracyclines are the one category of antibiotics against which staphylococcal resistance has appeared least often. There was one year, 1975, in which Minocin was less active, and in 1978, Vibramycin similarly was less active than in the other years. Keflin has been consistently high in anti-staphylococcal activity, and vancomycin has remained the most consistent anti-staphylococcal antibiotic in the armamentarium. Clindamycin was for several years consistent in a high level of inhibitory activity, but in 1978 it was virtually ineffective. Since 1979, sensitivity to clindamycin has again increased.

Staphylococcus epidermidis. Early postburn bacteremia due to S. epidermidis has occurred with increasing frequency in recent years. The number of patients with positive blood cultures suggests this trend:

Year and number of patients with blood culture positive for S. epidermidis

	1973	1974	1975	1976	1977	1978	1979	1980
No. patients positive	5	17	16	6	28	39	28	20

There appears to be no obvious reason for this increasing incidence, but the number of blood stream isolates prompts continued evaluation of the antibiotic sensitivity of this ubiquitous species. Table 5 presents the sensitivities of this organism. The suggestion is frequently made that these strains are simply contaminants. However, the fact that they so often are found within the first 72 hours after injury and are rare later renders this suggestion less plausible. Contaminants would appear in a more random fashion.

Streptococci. Streptococcal bacteremia was relatively infrequent. Nine patients yielded a total of 10 strains from blood cultures. The streptococci were heterogeneous, and no Group A streptococci were recovered. Four were Strep. viridans, two Strep. fecalis, two Strep. durans, and two strains could not be speciated precisely. They were designated as "alpha hemolytic streptococci, not Group A, B, or D."

The sensitivities of these strains are summarized in Table 6. The numbers recovered were too small to make a cumulative tabulation meaningful. The aminoglycosides were relatively low in effectiveness against these streptococci. The semisynthetic penicillins varied widely in effect; methicillin was highly active, and oxacillin and nafcillin less effective, in that order. Strains varied widely in their response to tetracyclines. Four out of 10 strains were Keflin resistant, and two vancomycin-resistant strains were found. Clindamycin varied widely in its effect on this heterogeneous group of organisms.

Pseudomonas aeruginosa. The major gram-negative bacterium associated with focal and systemic infection was P. aeruginosa. This has been the case for each year since 1970, when the last major epidemic of enteric forms was encountered. Thirty-six patients yielded 85 strains of P. aeruginosa which were tested for antibiotic sensitivity. Table 7 shows the inhibitory activity against these strains. The aminoglycosides varied in extent of effectiveness against Pseudomonas. Gentamicin and amikacin were inhibitory for the major portion of the isolates. Tobramycin, however, only inhibited 29% of the strains at 12.5 mcg/ml, and kanamycin was virtually ineffective. These wide discrepancies appeared with different aminoglycosides, but a significant proportion of strains were sensitive to at least two aminoglycosides. The two tetracyclines, Minocin and Vibramycin, both inhibited at least 70% of the strains. Keflin was inactive, as it has been in the past. Colistin was the most active anti-pseudomonal agent, as it had been in previous years. The in vitro effectiveness of this antibiotic did not, unfortunately, connote its effectiveness in therapy.

Carbenicillin and ticarcillin were inhibitory in the same degree, with a slight increase in activity of ticarcillin in the higher dilution range of potential clinical effectiveness.

A review of the proportion of P. aeruginosa inhibited over the past several years offers a useful perspective. There is a tendency

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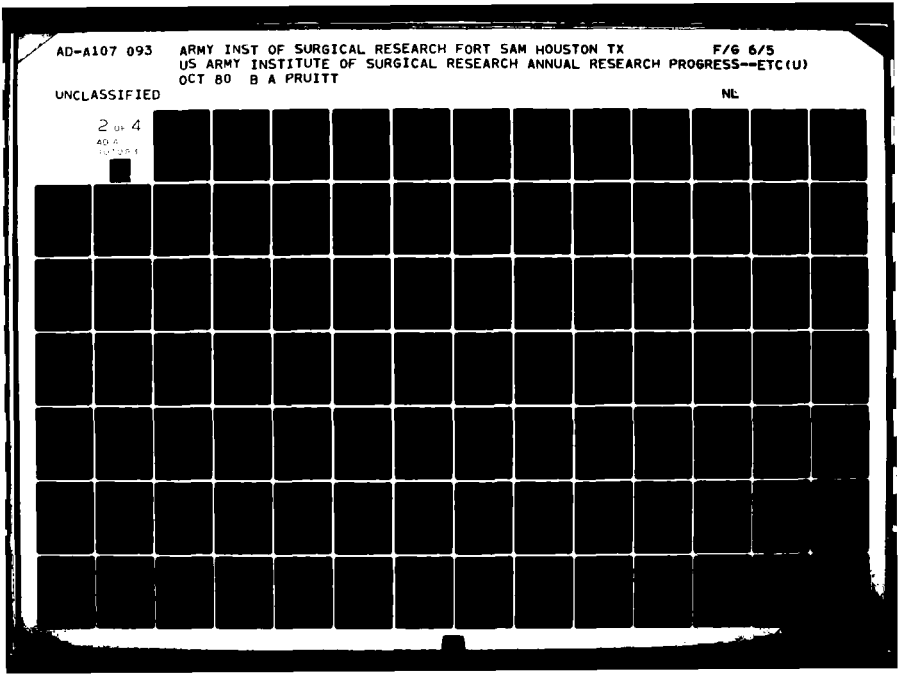

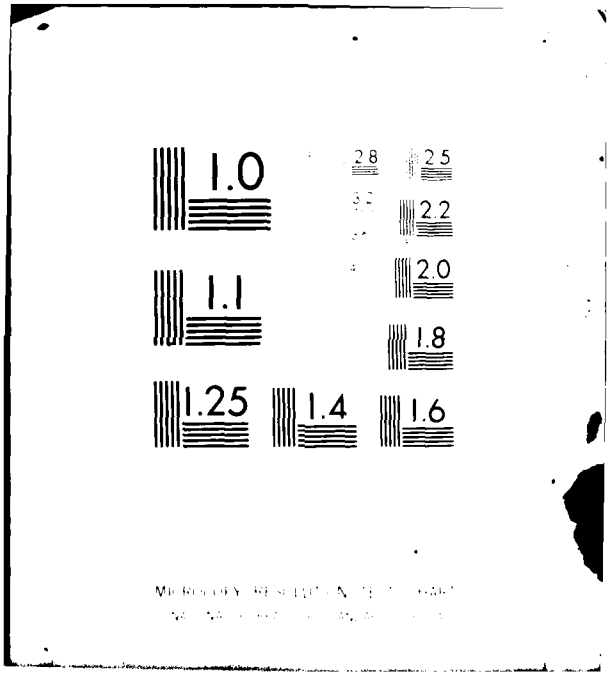
1.0
2.8
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1.1
1.8
1.25
1.4
1.6

Table 5. Staphylococcus epidermidis: Cumulative Inhibitory Levels for 20 Strains
1 October 1979 - 30 September 1980

Antibiotic Level mcg/ml	Antibiotic and % Inhibited									
	G	To	Ps	Sc	U	M	Vb	Kf	Va	Cl
> 25.0	100				100				100	
25.0	95				94.7				95	
12.5	95	100		100	47.3		100	100	95	100
6.2	95	94.4	100	94.7	42.1		90	90	95	94.7
3.1	95	94.4	95	88.4	36.8	100	85	90	80	84.2
1.5	90	83.3	85	94.2	36.8	95	70	85	60	47.3
< .78	80	77.7	76	68.4	26.3	90	55	75	25	31.5
Total strains	20	18	20	19	19	20	20	20	20	19

G: Gentamicin; To: Tobramycin; Ps: Oxacillin; Sc: Methicillin; U: Nafcillin; M: Minocin; Vb: Vibramycin; Kf: Keflin; Va: Vancomycin; Cl: Clindamycin

Table 6. Streptococcus Species: Sensitivity of 10 Strains from Blood Culture 1 October 1979 - 30 September 1980

Antibiotic Level mcg/ml	Antibiotic and Number Inhibited									
	G	To	Ps	Sc	U	M	Vb	Kf	Va	Cl
> 25.0	1	2	0	0	0	0	0	1	0	0
25.0	2	3	1	0	2	0	1	0	0	1
12.5	2	2	2	0	1	1	1	4	2	2
6.2	1	0	2	1	2	2	1	1	0	0
3.1	1	0	0	1	2	2	1	1	0	2
1.5	0	2	0	1	3	0	2	0	2	0
< .78	3	1	4	6	0	4	3	3	6	5
Total	10	10	9	9	10	9	9	10	10	10

G: Gentamicin; To: Tobramycin; Ps: Oxacillin; Sc: Methicillin; U: Nafcillin; M: Minocin; Vb: Vibramycin; Kf: Keflin; Va: Vancomycin; Cl: Clindamycin

Table 7. Pseudomonas aeruginosa: Cumulative Inhibitory Levels for 85 Strains
1 October 1979 - 30 September 1980

Antibiotic Level mcg/ml	Antibiotic and % Inhibited of Strains								Conc. mcg/ml	Cb	Ti
	G	To	K	Ak	M	Vb	Kf	Co			
> 25.0	100	100	100	100	100	100	100	100	>1250	100	100
25.0	85.7	56.4	11.7	91.0	93.8	96.8	2.9	96.9	1250	90.1	100
									625	84.3	100
12.5	70.2	29.4	4.8	74.6	76.9	69.8	0.0	96.9			
6.2	57.1	14.0	0.0	62.6	64.6	28.5	0.0	95.4	312	78.4	93.6
3.1	39.2	10.5	0.0	40.2	24.6	6.3	0.0	93.9	156	76.4	82.9
1.5	21.5	3.5	0.0	19.4	4.6	3.1	0.0	93.9	78	68.6	68.0
< .78	4.7	1.1	0.0	11.9	3.0	1.5	0.0	80.3	39	60.7	48.9
									19	35.2	44.6
									9	9.8	21.2
									< 4.5	5.8	10.6
Total strains tested	84	85	82	67	65	63	67	66		51	47

G: Gentamicin; To: Tobramycin; K: Kanamycin; Ak: Amikacin; M: Minocin; Vb: Vibramycin; Kf: Keflin; Co: Colistin; Cb: Carbenicillin; Ti: Ticarcillin

to assume that Pseudomonas is uniquely capable of developing antibiotic resistance, and that once established it is fixed and unchanging. The observations recorded here do not support this pessimistic view. Table 8 presents a comparison of levels over an 8-year period. Gentamicin, which on its first appearance was extremely active against Pseudomonas, fell steadily in proportion of strains inhibited from 1973 through 1978. Then an increase in sensitivity appeared and, in 1979-1980, 70% of isolates were sensitive at the levels stated. Tobramycin, which when first tested was far less active than gentamicin, had one year in which it was inhibitory for 61% of strains, but it subsequently fell to a very low level and has not been highly active against P. aeruginosa since that time. Amikacin, which was almost completely active when first introduced, showed an initial drop in proportion of sensitive strains, but in the past year it remained inhibitory for three-fourths of the strains tested. The tetracycline Minocin was for 3 years, from 1973 through 1975, minimally active against Pseudomonas. Subsequent isolates have been relatively susceptible, and three-fourths of the strains tested in 1980 were inhibited by 12.5 mcg/ml or less. Vibramycin has been slightly less active in the 6 years it has been included in the test battery. Colistin has been consistently highly active against P. aeruginosa during the entire period being recorded. Similarly, carbenicillin and, subsequently, ticarcillin have remained relatively effective anti-pseudomonal agents. Only in two years, 1976 and 1977, were less than 60% of the strains tested sensitive to carbenicillin.

Table 8. Sensitivity of Pseudomonas aeruginosa to 3 Aminoglycosides, 2 Tetracyclines, Colistin and 2 Semisynthetic Penicillins Over an 8-Year Period

Antibiotic	Year and % of Strains Inhibited by 12.5 mcg/ml						
	1973	1974	1975	1976-77	1978	1979	1980
Gentamicin	84.3	61.8	40.0	19.1	19.7	25.9	70.2
Tobramycin	....	....	18.5	61.6	17.0	4.0	29.4
Amikacin	....	....	....	98.3	60.0	73.5	74.6
Minocin	31.3	15.7	16.8	58.9	72.2	86.7	76.9
Vibramycin	....	....	20.0	43.6	63.6	60.8	69.8
Colistin	86.2	93.3	86.3	89.3	91.3	94.6	96.9
Carbenicillin*	80.4	70.8	68.8	58.6	62.0	86.0	78.4
Ticarcillin*	....	....	....	....	....	89.4	93.6

* For carbenicillin and ticarcillin, the upper limit of sensitivity is set at 312 mcg/ml, rather than 12.5 mcg/ml used for the other antibiotics in this set.

Klebsiella pneumoniae. The incidence of sepsis due to this opportunistic pathogen was very low in 1979-80. Only two strains were recovered from two patients in blood culture. The 13 strains tested included four from biopsies and six from sputum. The sensitivities are summarized in Table 9. The strains were essentially sensitive to the whole spectrum of test antibiotics. The principal change from the previous year, when 15 isolates were tested, was an increase in the number of strains recorded as sensitive to the aminoglycosides. Keflin also was more active against these isolates than it had been in 1978-1979.

Escherichia coli. With E. coli, as was the case with Klebsiella, there was minimal involvement with sepsis in burn patients. There were 11 strains tested, but only three of these were recovered in blood. Eight strains were recovered from biopsies. The sensitivity pattern is summarized in Table 10. The strains were most sensitive to the tetracyclines and to colistin. Among the aminoglycosides, gentamicin was the most active. Tobramycin, amikacin and kanamycin were decreasingly active, in that order. Most of the strains were inhibited by 12.5 mcg/ml or less of the aminoglycoside. Keflin was highly active against these E. coli isolates. The most potent antibiotic against E. coli was colistin.

Enterobacter cloacae. As one of the enteric species which has in the past caused epidemic sepsis in burn patients, Entero. cloacae was of special interest during this monitoring study. Only 12 strains were tested, but seven of these were recovered from blood cultures of five patients. Thus Entero. cloacae exhibited, even in this small sample, an invasive potential not shared by the two preceding enteric species. Three additional strains were recovered from biopsies, and two from sputum. One strain of Entero. aerogenes and one of Entero. agglomerans were also recovered, the former from blood and the latter from sputum. The sensitivity of Entero. cloacae strains is summarized in Table 11. With this group of strains, all aminoglycosides were highly inhibitory. The tetracyclines Minocin and Vibramycin were almost identical in inhibitory action. Keflin was almost inert, and colistin was highly active against most strains. With this sample of strains, resistance for Entero. cloacae was not a matter of concern, in that some antibiotics remained active against this species.

Providencia stuartii. Providencia stuartii has been given attention that would not be merited by its incidence during this 12-month period but because of its history. The last year in which Prov. stuartii was a major cause of sepsis in burn patients was 1974. During 1974, there were 29 patients with Providencia bacteremia, and 75 strains from blood cultures were tested for MIC of antibiotics. Not one strain was sensitive to any antibiotic tested. At that time, gentamicin, Kantrex, Minocin, ampicillin, Keflin and colistin were available. Not only were all strains resistant, but the organism was recognized as a major cause of death on the basis of postmortem tissue bacteriology. This was

Table 9. Klebsiella pneumoniae: Cumulative Inhibitory Levels for 13 Strains from Blood, Sputum and Biopsy, 1 October 1979 - 30 September 1980

Antibiotic Level mcg/ml	Antibiotic and % Inhibited							
	G	To	K	Ak	M	Vb	Kf	Co
> 25.0	100	100	100	100	100	100	100	100
25.0	92.3	92.3	76.9	84.6	92.3	92.3	76.9	92.3
12.5	92.3	84.6	61.5	84.6	92.3	69.2	76.9	92.3
6.2	76.9	69.2	61.5	61.5	69.2	69.2	61.5	92.3
3.1	69.2	69.2	53.8	46.1	53.8	53.8	53.8	84.6
1.5	61.5	30.7	38.4	7.6	30.7	23.0	30.7	69.2
< .78	15.3	7.6	23.0	7.6	0.0	0.0	15.3	53.8

G: Gentamicin; To: Tobramycin; K: Kanamycin; Ak: Amikacin; M: Minocin; Vb: Vibramycin; Kf: Keflin; Co: Colistin

Table 10. Escherichia coli: Cumulative Inhibitory Levels for 11 Strains from Blood and Biopsy, 1 October 1979 - 30 September 1980

Antibiotic Level mcg/ml	Antibiotic and % Inhibited							
	G	To	K	Ak	M	Vb	Kf	Co
> 25.0	100	100	100	100	100	100	100	100
25.0	100	90.9	54.5	81.8	100	71.7	90.9	100
12.5	100	72.7	54.5	72.7	81.8	45.4	90.9	100
6.2	90.9	54.5	45.4	36.3	45.4	45.4	36.3	100
3.1	72.7	27.2	45.4	18.1	45.4	45.4	9.0	100
1.5	9.0	0.0	9.0	0.0	45.4	45.4	9.0	100
< .78	0.0	0.0	9.0	0.0	45.4	45.4	0.0	72.7

G: Gentamicin; To: Tobramycin; K: Kanamycin; Ak: Amikacin; M: Minocin; Vb: Vibramycin; Kf: Keflin; Co: Colistin

Table 11. Enterobacter cloacae: Cumulative Inhibitory Levels for 12 Strains 1 October 1979 - 30 September 1980

Antibiotic Level mcg/ml	Antibiotic and % Inhibited							
	G	To	K	Ak	M	Vb	Kf	Co
> 25.0	100	100	100	100	100	100	100	100
25.0	100	100	91.8	100	96.6	96.6	16.6	91.6
12.5	91.6	91.6	72.7	100	83.3	83.3	8.3	91.6
6.2	83.3	83.3	72.7	100	83.3	75.0	0.0	83.3
3.1	83.3	75.0	72.7	91.6	72.7	41.6	0.0	75.0
1.5	75.0	58.3	54.5	41.6	0.0	8.3	0.0	75.0
< .78	37.3	25.0	18.1	16.6	0.0	0.0	0.0	41.6
No. of strains	12	12	11	12	12	12	12	12

G: Gentamicin; To: Tobramycin; K: Kanamycin; Ak: Amikacin; M: Minocin; Vb: Vibramycin; Kf: Keflin; Co: Colistin

the last year of a prolonged epidemic problem; in 1975, only one strain was recovered in blood. From 1975 to 1979, the organism disappeared completely from ISR burn wards. In 1979, this species reappeared, on 17 August. It persisted fortunately in a small number of patients to whom it was in all probability transferred by patient-attendant-patient sequence, until 26 October 1979. Since that time, no strains have been recovered. The behavior of these strains toward antibiotics was identical with that last seen in 1974. At this time the antibiotic battery included tobramycin, amikacin and Vibramycin. Ampicillin had been dropped. However, the results were equally disturbing because of the degree of resistance seen in the current population of Providencia. Table 12 summarizes these results. In contrast to other enteric species. these strains were minimally sensitive. One was inhibited at 6.2 mcg/ml of amikacin. In view of the previous performance of Prov. stuartii, when it was initially but briefly sensitive to some degree to several antibiotics, this result with amikacin can hardly be described as reassuring. The disappearance of Prov. stuartii from the burn ward was fortunate, but it did not result from any specific eradication effort, and its future potential remains a matter of concern.

Minor Gram-negative Species. There were three genera that appeared in patients in the burn ward in small numbers, as far as blood culture or tissue invasion were concerned. Acinetobacter anitratus (two strains) and Acineto. lwoffii (two strains) were tested. One strain of Acineto. anitratus was from blood culture. The strains showed scattered resistance and sensitivity patterns; resistance to Keflin was complete, and some were sensitive to the remaining antibiotics. Proteus mirabilis was tested with five strains, two from blood culture and three from biopsy. All were resistant to Vibramycin and colistin, and three-fifths were resistant to tobramycin, vancomycin, and amikacin. All were Keflin sensitive. Three strains of Aeromonas hydrophila and one of Achromobacter xylosoxidans were recovered, from blood culture. The Aeromonas strains were sensitive to the four aminoglycosides, highly sensitive to tetracyclines and resistant to Keflin and colistin. The Achromobacter species was aminoglycoside resistant, tetracycline sensitive, Keflin sensitive and colistin resistant. It was sensitive to carbenicillin and ticarcillin.

DISCUSSION

The 1979-1980 12-month period showed a continuation of the situation presented in the previous reporting period: sepsis, as reflected in blood stream invasion, was primarily due to S. aureus and to P. aeruginosa. Sepsis due to Klebsiella, Enterobacter and Escherichia, each of which genera has been the cause of epidemic sepsis in the past, did not reach significant levels, and these episodes were actually less extensive than they had been in the previous year. Staphylococci were sensitive to a significant extent to aminoglycosides, were highly sensitive to tetracyclines, to cephalothin and to vancomycin, and were

Table 12. Providencia stuartii: Antibiotic Sensitivity Results on 7 Strains 1979

Concentration mcg/ml	Antibiotic and No. Strains Inhibited							
	G	To	K	Ak	M	Vb	Kf	Co
> 25.0	7	7	7	1	7	7	7	7
25.0	0	0	0	2	0	0	0	0
12.5	0	0	0	3	0	0	0	0
6.2	0	0	0	1	0	0	0	0
3.1	0	0	0	0	0	0	0	0
1.5	0	0	0	0	0	0	0	0
< .78	0	0	0	0	0	0	0	0

G: Gentamicin; To: Tobramycin; K: Kanamycin; Ak: Amikacin; M: Minocin; Vb: Vibramycin; Kf: Keflin; Co: Colistin

far more sensitive to methicillin than had been the case in the previous year. Pseudomonas strains shifted to a renewed sensitivity to gentamicin, remained sensitive to a significant degree to amikacin, and were also still sensitive to tetracyclines. Carbenicillin and ticarcillin remained relatively effective in 1979-1980. There were no minor epidemic outbreaks in which resistant forms were a problem. Thus, for the present, the status of patients in the burn ward is one in which potential effective control of systemic infection by available antibiotics may be expected. There was no evidence by in vitro testing of uncontrollable strains or species playing a dominant role. However, continued scrutiny of the potentially invasive bacterial population is emphatically indicated. Without continued systematic monitoring, there would be no warning of the emergence of resistant forms. The reappearance of a small epidemic outbreak of Prov. stuartii was an example of the type of potential emergence of an epidemic resistant form that historically has great potential for causing a major problem of sepsis in burn patients.

PRESENTATIONS

Lindberg RB: Antibiotic sensitivity monitoring in burn patients. Presented at Symposium on Current Status of Antibiotic Sensitivity Monitoring, American Society of Microbiology Annual Meeting, Miami, Florida, 11-16 May 1980.

PUBLICATIONS

None

ANNUAL PROGRESS REPORT

PROJECT NO. 3S161102BS05-0C, BASIC RESEARCH

REPORT TITLE: STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE OF TROOPS WITH THERMAL INJURY -- SEROLOGIC TYPES OF PSEUDOMONAS AERUGINOSA FOUND IN BURNED SOLDIERS

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1 October 1979 - 30 September 1980

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ABSTRACT

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Period covered in this report: 1 October 1979 - 30 September 1980

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Serotyping of Pseudomonas aeruginosa was carried out on 507 isolates from 97 patients. Principal sources were wounds and sputum. Blood cultures were less numerous than had been the case during the past 3 years. There were 10 different types, both with single factor and with multiple factor antigenic structure, which appeared in some degree as epidemic strains. Types 4,11 and 15 were, as had been observed in earlier years, the major epidemic types. Strain-specific epidemics characterize P. aeruginosa infection in burn wards.

Pseudomonas
Serotype
Burns
Infection
Epidemic
Humans

STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE OF TROOPS WITH THERMAL INJURY -- SEROLOGIC TYPES OF PSEUDOMONAS AERUGINOSA FOUND IN BURNED SOLDIERS

Pseudomonas aeruginosa has over at least the last 20 years been a prominent feature of the bacteriologic flora of burn wounds. Pseudomonas sepsis resulting from invasion of unburned tissue has been largely controlled by topical medication, and the spectrum of antibiotics effective in some degree against Pseudomonas systemic infection has been enlarged. Nevertheless, P. aeruginosa continues to be the most frequently encountered gram-negative species of organism in severely burned patients, and the presence of microepidemics due to this organism in burn wards is a frequent observation. Outbreaks of cross-infection imply the presence of individual predominant strains as causative organisms, and the differentiation of such strains is essential if the epidemiology of Pseudomonas infection in a burn ward is to be elucidated. Type differentiation of individual strains was initially done with a bacteriophage typing set, developed in this laboratory and refined to the point where it became an internationally accepted typing set. However, phage typing demands virtually full-time effort of a highly trained technician, and the difficulty of maintaining such expertise prompted an evaluation of the international serotyping set, which has been used since 1976.

Serotyping is a less precise technic for differentiating strains of P. aeruginosa than is phage typing, but its accuracy is of an order that permits recognition of epidemic transmission of strains within a burn ward. There is a distinct time advantage in serotyping: colonies can be typed even from the primary culture plate of a specimen. Such a procedure can reveal an epidemic outbreak relatively early in its course; although no unequivocal successes in rapid extinction of such episodes have been reported, it remains a valid objective, which can only be pursued if precise identity of the offending strains is known. Further, strain differentiation has revealed the phenomenon of separate types being recovered at the same time from wound, from sputum, and from blood. Even more frequent is the demonstration of separate types from the urinary tract and other sites on the burned patient.

The differentiation into major and minor groups of P. aeruginosa, as described in the Institute of Surgical Research Annual Research Progress Report, 1978-79, p 124, was further refined in view of accrued data. The major set comprised types 3, 4, 6, 8, 9, 10, 11, 15, and 16. The less frequently encountered factors were 1, 2, 5, 7, 12, 13, 14, and 17. Typing was carried out with major factors first; negative reactors were typed with the minor set of factors. Typing in this year was carried out on live-cell suspensions from blood agar plates. Autoclaved suspensions, tested in random comparisons, confirmed the adequacy of the live-cell testing technic.

TYPES OF PSEUDOMONAS AERUGINOSA OBSERVED

Five hundred and seven strains of P. aeruginosa were typed during the 12-month observation period. The strains were collected from 97 patients. Type distribution has been summarized for each year since 1976; the results are shown in Table 1. In 1976-77, 10 types were found. In 1977-78, there were 22; in 1978-79, 25 types; and in 1979-80, the total of types recognized was 33. The increase in number of types seen was initially related to increasing technical skill in typing this species, but for the past 2 years no changes in technic have been introduced.

An effective serotyping technic for Pseudomonas would be expected to differentiate types which appear only for short periods of time, as well as some which occupy long periods of predominance. There were two types that appeared only in 1976-77. Seven others were found only in 1977-78, and six in 1978-79. In 1979-80, the unique category was much extended. Fifteen types which had not earlier appeared were found. Table 2 presents these type patterns. Patterns of association of major antigenic factors were apparent. Combinations including factors 4 and 10 appeared in seven different patterns over a 3-year period. Factors 11 and 15 appeared in three different patterns in the same period. Factor 16, although it was not very common as a single factor, was present in nine types in 1979-80. Combinations of factors are apparently quite labile, and it is probable that new combinations will continue to appear. There was no unequivocal evidence, on the basis of chronologic sequence, for the assumption that these multiple factor types were derived from antecedent related types.

Over the 4-year period covered here, there have been 11 different types that have occurred often enough to be regarded as epidemic in incidence. These were types 3; 4; 4,9,10; 4,9,10,11; 4,10; 8; 9,10; 10; 11 and 15. Of these types, only types 4 and 15 have been observed in each year, as causing nosocomial outbreaks. Types 3; 4,9,10,11; 8; and 9,10 were each seen in epidemic intensity in one of the past 4 years.

In assessing the behavior of P. aeruginosa as an infecting agent in burns, there is implicit in type differences the suggestion that virulence may be type related. Thus the types recovered from blood have in previous years been more homogeneous than the types recovered from sputum. The types recovered from blood, wound, and sputum are summarized in Table 3. There were only two types, 11 and 15, that appeared in blood of more than one patient, in contrast to the preceding year, when five types were recovered in more than one blood culture. In this sense, the divergence between blood stream isolates and the flora of the wound and respiratory tract was marked in 1979-80. There were fewer septicemias due to P. aeruginosa than there were in previous years. Wound and sputum type distributions were parallel and more resembled the patterns observed in previous years.

Table 1. Serotypes of Pseudomonas aeruginosa from Burn Patients 1976-1980

Type	No. of Isolates 1976-77	1977-78	1978-79	1979-80
1				1
1,9	2			1
1,2,3,4,9,10		7		
2,3,6,15	3			
3		9	2	
3,4,9,10		1		
3,8,9,14		2		
3,10			2	2
3,15			2	
4	110	183	42	102
4,6,9,10,16				2
4,8,9,11,12,14		2		
4,9				1
4,9,10	20	12		2
4,9,10,11				11
4,9,11				1
4,10			21	20
4,10,11				1
4,10,13		2		
4,10,15			1	
4,10,16				1
4,11		1	1	3
4,11,15,16			1	
4,15		2	2	
4,16				2
5		1	1	
5,8,16				1
6	38		5	11
6,16				3
7		3		
8		17	1	3
8,9		2		
8,11			1	
8,11,15,16				2
8,12		1	1	
8,15,16				1
9	2	2		1
9,10	2	10	2	3
9,16				1
10	3	16	6	31
10,11,15				1
10,15			1	
10,15,16			1	1
10,16				1
11		35	89	69
11,15			3	1
11,15,16			1	
11,16				4
12		1	2	
14	7			
15	239	119	158	171
15,16			1	9
16		4	11	6
NT*	3	21	43	37
TOTAL	429	453	401	507

* NT: Non-typable; non-reactive with each of 17 different type sera.

Table 2. *Pseudomonas aeruginosa* Types Which Have Appeared in Only a Single Year Since 1976

1976-77	1977-78	1978-79	1979-80
2,3,6,15	1,2,3,4,9,10	3,15	1
14	3,4,9,10	4,10,15	4,6,9,10,16
	3,8,9,14	8,11	4,9
	4,8,9,11,12,14	8,12	4,9,10,11
	4,10,13	10,15	4,9,11
	7	11,15,16	4,10,11
	8,9		4,10,16
			4,16
			5,8,16
			6,16
			8,11,15,16
			8,15,16
			10,11,15
			10,16
			11,16

Table 3. Serotypes of *Pseudomonas aeruginosa* from Blood, Wound, and Sputum of Burn Patients, 1979-1980

Type	Source and No. of Isolates Recovered		
	Blood	Wound	Sputum
4		49	43
6		1	8
8		2	1
10		6	15
11	4	31	14
15	10	64	91
4,10	1	13	7
8,11,15,16	1	3	
10,15,16		3	3
15,16		6	2

Epidemic patterns in P. aeruginosa burn patients as reflected in types recovered in at least two patients in a single month are summarized in Table 4. The continuity of transmission of a strain from patient to patient is suggested by the peaks of incidence, which occur in a relatively circumscribed period, often with no subsequent high incidence. Thus type 10 was really epidemic only in the first of the 12 months covered. Type 15,16 only appeared during one month, when it caused a significant number of infections. Type 4 exhibited a peak incidence in November and December 1979, and again in June and July 1980. Type 11 achieved a peak during the September-December period, with a tapering off from January to March. Type 15 was present but not a major feature of the 1979 epidemic period. However, it rose to a striking peak in 1980, from May through September. There were 10 types which, under the criteria set down, occasioned epidemic outbreaks in some degree during this 12-month period. The major epidemic-producing types have varied over the past 3 years as follows:

Year	Major epidemic-producing types
1977-78	4; 6; 8; 11; 15
1978-79	4; 11; 15
1979-80	4; 10; 11; 15; 15,16

Thus far, types 4, 11, and 15 have been the major causative forms for micro-epidemic outbreaks on the burn wards. The proportion of epidemic-producing types to the total of strains collected during a succession of 12-month intervals gives a further insight into the proportion of P. aeruginosa strains which can be involved in individual monotype outbreaks of nosocomial infections. The eight types that have, over the past 4 years, caused epidemic episodes were recovered as shown in Table 5. Types 4 and 15 have been recovered in each of the 4 years. In all but one year, these two types were numerically predominant. Type 11, in 1978-79, exceeded the incidence of type 4. The eight types which had shown the capability to set up epidemic patterns of transmission were all observed between 1976 and 1979. No additional epidemic types appeared in 1979-80; each type appearing in that year had been observed earlier.

DISCUSSION

The feasibility of differentiating strains of P. aeruginosa with the commercially available international typing set of sera has been demonstrated. Although the major epidemic types (4, 10, 11, and 15) make up the numerical preponderance of strains, there have been several other types, monovalent and polyvalent, which have behaved in epidemic manner for brief periods of time. If a serious attempt is to be made to minimize cross-infection with P. aeruginosa by eliminating offending strains, the recognition and tracing of such strains would be valuable. Whether such control can be achieved will depend upon attempts at effective reverse isolation and detailed attention to the minutiae of

Table 4. Pseudomonas aeruginosa Strains Involving Two or More Patients in at Least One Month 1979-1980

Type	No. of Patients - No. of Isolates by Month											
	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep
4		7-15	6-27	3-4	3-8	1-3	1-2	3-16	4-15	4-12		
4,10	1-2	5-16				1-1					1-1	
6		1-1		2-6	1-4							
10	7-15	1-2		2-4	1-8			1-1			1-1	
10,16	1-2	2-2										
11	6-9	8-11	6-16	3-4	3-12	2-9		1-2		1-1	2-5	
11,16	1-1	2-3										
15	2-6	4-8	2-3		4-6	3-8		6-32	11-49	6-9	9-15	11-35
15,16		5-9										
16	1-2	2-2										
NT*	3-4	1-1	1-1		1-1	1-1		4-5	7-12	4-10	1-1	1-1

* NT: Non-typable.

Table 5. Epidemic Outbreaks of Monotype Infection with Pseudomonas aeruginosa in Burn Patients, 1976-1980

Type	Incidence and % of Annual Total Isolates			
	1976-77	1977-78	1978-79	1979-80
4	110 (27.9%)	183 (38.3%)	42 (10.3%)	102 (20.1%)
4,9,10	20 (5.0%)	12 (2.5%)		
4,10			21 (5.2%)	20 (3.9%)
6	38 (7.9%)			
8		17 (4.2%)		
10		16 (3.3%)		31 (5.1%)
11		35 (7.7%)	89 (21.9%)	69 (13.6%)
15	239 (60.6%)	119 (24.9%)	158 (39.0%)	171 (34.0%)

patient handling practices. This experiment has not yet been attempted. The value of serotyping to date, as with bacteriophage typing in the past, has been to delineate chronologically the anatomy of epidemic spread of P. aeruginosa in burn patients.

PRESENTATIONS/PUBLICATIONS - None

ANNUAL PROGRESS REPORT

PROJECT NO. 3S161102BS05-00, BASIC RESEARCH

REPORT TITLE: STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE OF TROOPS WITH THERMAL INJURY -- PATHOGENESIS OF BURN WOUND INFECTION: BACTERIAL FLORA OF BURN WOUNDS OF MILITARY PERSONNEL RECEIVING SULFAMYLON OR SILVER SULFADIAZINE TREATMENT

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1 October 1979 - 30 September 1980

Investigators:

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Bacterial infection has been the principal cause of morbidity and death in severely burned patients for the past several decades, but the causative organisms have shifted in identity with the passage of time and the influence of antimicrobial therapy. Continued monitoring of causative species in burn patients has included assessment of the bacterial flora of the burn wound, the respiratory and urinary tracts, and especially the flora involved in septicemia. During 1979-1980, the principal species causing sepsis were, as they had been for the preceding 2 years, Staphylococcus aureus and Pseudomonas aeruginosa. Enterobacteriaceae species were recovered in significant numbers, but failed to establish epidemic patterns as had been observed between 1969 and 1977. Providencia stuartii, which had been for several years a major lethal species, had been entirely absent in the past 2 years. During the past year, this species has reappeared as part of the burn ward flora, although it has not reestablished lethal sepsis on an epidemic scale. Forty-four species of bacteria and eight species of yeasts were recovered during the 1979-1980 observation period.

Burns
Staphylococci
Pseudomonas
Sepsis
Candida
Humans

STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE OF TROOPS WITH THERMAL INJURY -- PATHOGENESIS OF BURN WOUND INFECTION: BACTERIAL FLORA OF BURN WOUNDS OF MILITARY PERSONNEL RECEIVING SULFAMYLON OR SILVER SULFADIAZINE TREATMENT

Wound and systemic infection in burn patients is the major cause of morbidity and death. This circumstance prevails even with maximum use of antibiotics and with the whole spectrum of available topical chemotherapeutic agents. The problem of such hospital-acquired or nosocomial infections is focused on opportunistic bacteria, rather than on specific human pathogens. Only one offending species, Pseudomonas aeruginosa, causes a morphologically distinctive burn wound invasion, but enteric species typical of the normal gut flora, the genitourinary tract, the perineal area and the oropharyngeal and nasal flora are frequently encountered. Among these, individual species may serve as the cause of epidemic outbreaks which can persist for months or even years in the burn ward. These outbreaks are opportunistic in nature and have in many instances disappeared abruptly after persisting for months or even years. During the years since 1978, epidemics of sepsis have most often been due to Staphylococcus aureus and P. aeruginosa, with enteric species less frequent as the causative agents. The incidence of sepsis appeared to decline during the period of this report, since the proportion of patients from whom blood cultures were collected and who exhibited bacteremia was markedly reduced from that seen in the preceding 4 years. An important factor in the patient load for this year, and a change from preceding years, was the admission of a large increment of 37 patients injured at one time in an explosion of gasoline vapor in the Marine barracks in Japan. Such an input of recently injured individuals required emergency rearrangement of treatment facilities for these patients. It is a distinct possibility that this alteration of management caused an alteration in the rate of bacterial colonization of these patients. There appeared to be an extended period during which the rate of colonization of patients admitted during the following several months was slowed, in contrast to rates observed over the preceding 4 years.

Detailed precise identification of bacterial isolates has been the subject of continuing study, and the precision of final identification has been improved.

ANTEMORTEM BACTERIOLOGY OF BURN PATIENTS, 1979-1980

Bacterial and yeast species recovered during this 12-month period are listed in Table 1. The source and number of isolates are presented.

There were 296 patients admitted during the reporting period. Of these, 286 or 96% had at least one culture taken. Wound cultures were taken from 54.8% of all patients cultured, contact plates from 58.3%, blood cultures from 75.8%, throat cultures from 26.2%, sputums from

Table 1. Antemortem Bacteriology of Burn Patients, 1 October 1979 - 30 September 1980

Organism	Source and Number of Isolates												
	Wound Surface			Respiratory Tract			Catheter Tips						
	Swab	CP	Blood	Throat	Sputum	Urine	IV	Foley	Biopsy	Grafts	Stool	CSF	Total
S. aureus	147	179	36	65	314	47	7	4	38	10	8	0	855
S. epidermidis	31	141	15	43	57	29	5	0	4	4	19	0	348
S. saprophyticus	0	0	1	0	0	0	0	0	0	0	0	0	1
Micrococcus sp.	0	1	0	0	0	0	0	0	0	0	0	0	1
Strep. viridans	16	21	5	240	283	5	1	1	2	0	17	0	591
Non-hemol. Strep. not Gp D	10	4	2	79	91	4	1	2	0	1	11	0	205
Beta Strep. not Gp A, B or D	0	0	1	28	17	1	0	0	0	0	1	0	48
Gp D Strep. not Enterococcus	3	4	6	21	27	24	1	5	0	0	17	0	108
Strep. pneumoniae	1	0	1	15	13	0	0	0	0	0	2	0	32
Gp B Strep.	0	0	0	3	1	0	0	0	0	0	1	0	5
Bacillus sp.	5	208	1	4	31	3	0	0	3	0	3	0	258
Corynebacterium sp.	0	20	0	0	0	0	0	0	0	1	21	0	42
Neisseria sp.	2	0	0	8	18	0	1	0	0	0	0	0	29
P. aeruginosa	97	147	22	16	354	66	10	5	50	9	6	0	782
P. fluorescens	0	0	0	0	1	0	0	0	0	2	0	0	3
P. putida	1	0	0	0	0	0	0	0	0	3	0	0	4
P. maltophilia	0	0	0	0	2	0	0	0	0	1	0	0	3
P. cepacia	0	0	1	0	3	0	0	0	0	1	0	0	5
Group 2K-1	0	1	0	0	0	0	0	0	0	0	0	0	1
Alcaligenes sp.	0	0	1	0	3	0	0	0	0	1	0	0	5
Group 5E-1	0	1	0	0	0	0	0	0	0	0	0	0	1
Flavobacterium sp.	0	1	0	0	0	0	0	0	0	0	0	0	1
Group M-3	0	0	0	0	1	0	0	0	0	0	0	0	1
Achromo. xylosoxidans	0	0	1	0	0	0	0	0	0	0	0	0	1
Acineto. anitratus	5	23	1	19	88	4	1	0	4	1	8	0	154
Acineto. lwoffii	0	8	0	0	6	0	0	0	0	0	0	0	14
E. coli	19	9	4	33	99	45	1	1	12	6	99	0	328
E. coli (A-D)	0	0	0	0	0	3	0	0	0	0	0	0	3
Citro. freundii	2	1	0	1	3	1	0	0	0	1	0	0	9
Citro. diversus	1	2	0	0	10	2	0	0	0	1	0	0	16
Kleb. pneumoniae	16	26	1	24	169	39	2	5	3	1	60	0	346
Kleb. oxytoca	3	0	2	2	28	0	0	0	2	0	2	0	39
Kleb. ozaenae	1	3	0	1	7	2	0	0	0	0	2	0	16

Table 1. Antemortem Bacteriology of Burn Patients, 1 October 1979 - 30 September 1980 (cont.)

Organism	Source and Number of Isolates												
	Wound Surface			Respiratory Tract			Catheter Tips						
	Swab	CP	Blood	Throat	Sputum	Urine	IV	Foley	Biopsy	Grafts	Stool	CSF	Total
Entero. cloacae	9	24	8	11	30	2	0	0	4	3	13	0	104
Entero. aerogenes	5	14	1	3	39	3	1	0	1	0	12	1	80
Entero. agglomerans	0	12	0	1	18	0	0	0	0	2	4	0	37
Entero. gergoviae	0	0	0	0	1	0	0	0	0	0	0	0	1
Serratia marcescens	2	3	0	4	5	0	0	0	0	0	0	0	14
Proteus vulgaris	0	2	0	1	0	0	0	0	0	0	3	0	6
Proteus mirabilis	5	18	3	1	44	22	1	1	5	0	14	0	114
Proteus rettgeri	0	0	0	0	0	0	0	0	0	0	4	0	4
Morganella morganii	3	0	1	0	4	2	1	0	1	0	5	0	17
Providencia stuartii	0	5	1	1	31	2	1	1	1	0	0	0	43
Aeromonas hydrophila	2	0	1	2	0	0	0	0	0	0	1	0	6
Yeast-like organism	0	2	0	1	5	6	0	0	1	0	0	0	15
Candida albicans	6	19	17	8	105	120	2	5	5	0	0	0	287
Candida rugosa	6	43	10	1	2	9	3	0	12	1	0	0	87
Candida tropicalis	4	0	3	0	26	38	6	0	2	0	0	0	79
Candida krusei	0	0	0	0	1	0	0	0	0	0	0	0	1
Candida parapsilosis	0	0	0	0	1	0	0	0	0	0	0	0	1
Torulopsis glabrata	0	0	1	0	0	0	1	0	0	0	0	0	2
Trichosporon beigelii	0	0	0	0	4	0	0	0	1	0	0	0	5
Curvularium sp.	0	0	0	0	0	0	0	0	3	0	0	0	3
Diplosporium sp.	0	0	0	0	0	0	0	0	1	0	0	0	1
Fusarium sp.	0	0	0	0	0	0	0	0	4	0	0	0	4
Mucor sp.	0	0	0	0	0	0	0	0	3	0	0	0	3
Aspergillus sp.	0	0	0	0	0	0	0	0	21	0	0	0	21
Alternaria sp.	0	1	0	0	0	0	0	0	7	0	0	0	8
Penicillium sp.	0	0	0	0	1	0	0	0	2	0	0	0	3
Mycelia sterilia	0	0	0	0	0	0	0	0	2	0	0	0	2
Number isolates:	402	943	147	636	1943	479	46	30	194	49	333	1	
Number of specimens:	582	831	1228	551	902	900	112	29	287	92	147	6	
Number of patients:	157	167	217	75	125	174	51	24	75	45	63	4	

Total isolates: 5203; total specimens: 5667

Total patients on whom one or more cultures were done: 286

43.7%, urine cultures from 60.8%, and biopsies from 26.2% of all patients from whom cultures were collected. From wounds, 402 isolates were recovered, while contact plates yielded 943 isolates. There were some qualitative differences in recovery with these two methods. Staphylococcus epidermidis was far more often recovered with contact plates than with swabs, as was also the case with Acinetobacter anitratus. For most other species, the recovery rates reflected the number of specimens collected, rather than the technic of collection. From blood cultures, 147 strains were recovered. Staphylococcus aureus and P. aeruginosa were the most common species recovered, and S. epidermidis and Candida albicans were recovered in significant numbers. From all sources, the most frequently encountered species were S. aureus (855) strains, P. aeruginosa (782), Streptococcus viridans (591), S. epidermidis (348), Klebsiella pneumoniae (346), and Escherichia coli (328). Candida albicans was the most commonly isolated species of yeast. Other numerically important species were C. rugosa and C. tropicalis. Acinetobacter anitratus isolates totaled 154, which was a sharp rise from the previous year's total. In contrast, Serratia marcescens, an opportunist of intermittent importance, was recovered only 14 times. Providencia stuartii, a species of great importance in epidemic burn sepsis for several years, was entirely absent for the previous 2 years but reappeared in burn patients in 1980. Forty-three strains had been recovered by 1 October. The organism spread readily to newly admitted patients, but had not appeared in blood cultures.

There were 44 species of bacteria and eight of yeasts recognized during this year. This number corresponds to that reported in 1978-79.

The total of strains collected does not show their relative importance in potential epidemic situations in the burn patient population, and hence a resumé of the number of patients colonized or invaded is summarized in Table 2. Only the most important species numerically are shown. Numerically the important species included S. aureus, S. epidermidis, P. aeruginosa, Acineto. anitratus, E. coli, and K. pneumoniae. Enterobacter cloacae, at times an important epidemic invasive species, was recovered from blood in six patients, which made it the most important enteric species involved in sepsis. Five patients harbored C. tropicalis in blood; the significance of such candidemia was not apparent in terms of specific sepsis or outcome of injury.

The percentage of patients cultured for each site and positive for each major species is shown in Table 3. The numerically important species in blood culture were S. aureus, S. epidermidis and P. aeruginosa. Staphylococcus aureus and P. aeruginosa were the most significant species in biopsies; the S. epidermidis incidence in these samples was low.

BURN WOUND BACTERIOLOGY

The infected burn wound is an obvious basis for sepsis in the severely burned patient. Monitoring burn flora is an important aspect

Table 2. Antemortem Bacteriology of Burn Patients: Principal Species
1 October 1979 - 30 September 1980

Organism	Source and Number of Patients Positive in Culture										
	Wound Surface		Respiratory Tract				Catheter Tips				
	Swab	CP*	Blood	Throat	Sputum	Urine	IV	Foley	Biopsy	Stool	CSF
S. aureus	68	82	24	35	85	22	7	4	22	7	0
S. epidermidis	26	78	15	24	31	19	5	0	4	15	0
Strep. viridans	13	14	5	60	91	5	1	1	2	11	0
Non-hemol. Strep. not Gp D	10	3	2	34	52	4	1	2	0	8	0
Gp D Strep. not Enterococcus	0	5	1	27	23	5	0	0	0	2	0
Gp D Enterococcus	3	3	5	16	19	15	1	5	0	14	0
P. aeruginosa	48	57	16	12	53	33	8	5	22	6	0
Acineto. anitratus	5	12	1	11	36	4	1	0	2	6	0
Escherichia coli	12	7	4	15	37	30	1	1	5	46	0
Kleb. pneumoniae	15	20	1	17	42	20	2	4	2	41	0
Entero. cloacae	7	15	6	7	17	2	0	0	3	11	0
Entero. aerogenes	4	10	1	3	17	3	1	0	1	49	1
Proteus mirabilis	5	15	3	1	13	12	1	1	3	12	0
Candida albicans	4	9	9	7	27	22	2	2	5	0	0
Candida tropicalis	4	0	2	0	11	10	4	0	1	0	0
Candida rugosa	6	14	4	1	2	5	3	0	7	0	0
Total No. Patients Sampled	157	167	217	75	125	174	51	24	75	63	4

* CP: Contact plate.

Table 3. Percentage of Patients Cultured Positive for Major Species at Sites of Major Significance 1 October 1979 - 30 September 1980

Organism	Source and % of Cultured Patients Positive			
	Wound Swab	Biopsy	Blood	Sputum
S. aureus	43	29	11	68
S. epidermidis	17	5	7	25
P. aeruginosa	31	29	7	42
Escherichia coli	12	7	2	30
Kleb. pneumoniae	10	3	0.5	34
Entero. cloacae	4	4	3	14
Acineto. anitratus	3	3	0.5	29

of care of such patients. The colonizing flora is undoubtedly affected by the topical chemotherapeutic regimen employed. A frequent procedure employed alternate treatments with Sulfamylon burn cream and silver sulfadiazine at 12-hour intervals. Extensive use of 5% Sulfamylon soaks in treatment of healing burns has extended the exposure of burn wound flora to this agent.

The microbial flora of the burn wound surface is shown in Table 4. The principal species in terms of isolates and of patients positive were S. aureus, S. epidermidis, and P. aeruginosa. Enteric species in significant numbers were K. pneumoniae, Entero. cloacae, and Proteus mirabilis, but none of these species occurred in large numbers. There were 34 species, including groups of streptococci and three species of yeasts.

A long-standing surveillance for Group A streptococci has been conducted in this laboratory. This organism, although readily controlled by appropriate antibiotic, has frequently caused dangerous and rapidly advancing wound infections in burns. This species did not appear in any burn patient during 1979-80. It has been 2 years since Group A streptococci were recovered from patients in the Institute of Surgical Research burn wards.

RESPIRATORY TRACT FLORA IN BURN PATIENTS

Pneumonia is a frequent problem in the severely burned patient, and the respiratory tract flora is of major importance in the pathogenesis of burn injury. Table 5 presents the principal species recovered in sputum and Luken's tube cultures. As with wound flora, staphylococci and P. aeruginosa were a major part of the sputum flora. Streptococcus viridans and other streptococci were conspicuous, but probably not of

Table 4. Burn Wound Surface Flora in 226 Patients
1 October 1979 - 30 September 1980

Organism	No. of Strains	No. of Patients Positive	% of Cultured Patients Positive
S. aureus	326	150	66
S. epidermidis	172	104	46
Micrococcus sp.	1	1	0.4
Strep. viridans	37	27	12
Non-hemol. Strep. not Gp D	14	13	6
Gp D Strep. not Enterococcus	5	5	2
Gp D Enterococcus	7	6	3
Strep. pneumoniae	1	1	0.4
Corynebacterium sp.	20	16	7
Neisseria sp.	2	2	0.8
P. aeruginosa	244	105	46
P. putida	1	1	0.4
Gp 2K-1	1	1	0.4
Gp 5E-1	1	1	0.4
Acineto. anitratus	28	17	8
Acineto. lwoffii	8	7	3
Flavobacterium sp.	1	1	0.4
Escherichia coli	28	19	8
Citro. freundii	3	3	1
Citro. diversus	3	3	1
Klebsiella pneumoniae	42	35	15
Klebsiella oxytoca	3	2	0.8
Klebsiella ozaenae	4	4	2
Enterobacter cloacae	33	22	10
Enterobacter aerogenes	19	14	7
Enterobacter agglomerans	12	10	4
Serratia marcescens	5	3	1
Proteus mirabilis	23	20	9
Providencia stuartii	5	4	2
Morganella morganii	3	3	1
Aeromonas hydrophila	2	2	0.8
Candida albicans	25	13	6
Candida rugosa	49	20	9
Candida tropicalis	4	4	2

Table 5. Principal Species of Bacteria Recovered from Respiratory Tract of 158 Patients, 1 October 1979 - 30 September 1980

Organism	No. of Isolates	No. of Patients Positive	% of Cultured Patients Positive
S. aureus	379	120	78
S. epidermidis	100	55	35
Strep. viridans	523	151	96
Non-hemol. Strep. not Gp D	170	86	54
Beta-hemol. Strep. not A, B, or D	45	23	15
Gp D Strep. not Enterococcus	89	50	32
Gp D Enterococcus	48	35	22
Strep. pneumoniae	28	22	14
Bacillus sp.	35	14	9
Neisseria sp.	26	18	11
P. aeruginosa	370	65	41
Acineto. anitratus	107	47	30
Escherichia coli	132	52	33
Klebsiella pneumoniae	193	59	37
Klebsiella oxytoca	30	13	8
Enterobacter cloacae	41	24	15
Enterobacter aerogenes	42	20	13
Enterobacter agglomerans	19	8	5
Proteus mirabilis	45	14	9
Providencia stuartii	34	5	3
Candida albicans	113	34	22
Candida tropicalis	26	11	7

significance in pulmonary infection. Acinetobacter anitratus was far more frequent in occurrence; 47 out of 158 patients were positive for this usually infrequent species. Klebsiella pneumoniae and E. coli were other species recovered in significant numbers of patients.

SEPTICEMIA IN BURN PATIENTS

The most significant aspect of bacterial infection in burns is blood stream invasion. In 1979-80, 217 burn patients had blood cultures drawn. Of these, 108 had at least one culture positive. All positive blood cultures were not necessarily recovered from seriously ill patients, but multiple positive cultures were inevitably associated with sepsis. Table 6 shows the total of strains of each of 29 species recovered, and the number and percent of patients who were cultured and

Table 6. Blood Culture Isolates from 217 Burned Patients
1 October 1979 - 30 September 1980

Organism	Total No. Isolates	No. Patients Positive	% of Cultured Patients Positive
S. aureus	36	24	11
S. epidermidis	15	15	7
S. saprophyticus	1	1	0.5
Strep. viridans	5	5	2
Non-hemol. Strep. not Gp D	2	2	0.9
Beta-hemol. Strep. not Gp A, B, or D	1	1	0.5
Gp D Strep. not Enterococcus	1	1	0.5
Gp D Enterococcus	6	5	2
Strep. pneumoniae	1	1	0.5
Bacillus sp.	1	1	0.5
P. aeruginosa	22	16	7
P. cepacia	1	1	0.5
Alcaligenes sp.	1	1	0.5
Achromo. xylosoxidans	1	1	0.5
Acinetobacter anitratus	1	1	0.5
Escherichia coli	4	4	2
Klebsiella pneumoniae	1	1	0.5
Klebsiella oxytoca	2	1	0.5
Enterobacter cloacae	8	6	3
Enterobacter aerogenes	1	1	0.5
Proteus mirabilis	3	3	1
Proteus morganii	1	1	0.5
Providencia stuartii	1	1	0.5
Aeromonas hydrophila	1	1	0.5
Candida albicans	17	9	4
Candida rugosa	10	4	2
Candida tropicalis	3	2	0.9
Torulopsis glabrata	1	1	0.5

Underlined species represent numerically important organisms

were positive for each species. The most important species, numerically, were S. aureus, S. epidermidis, P. aeruginosa, and C. albicans. Of the enteric species, E. coli and Entero. cloacae were the most significant numerically. The S. epidermidis strains were each recovered once from a patient, but in no instance was there indication of sepsis associated with this species. The Candida species were similarly not specifically associated with symptoms of sepsis. The remaining species were not recovered in numbers large enough to suggest

any epidemic presence of the organism.

The typical course of events for septic burned patients has in previous years been one of multiple blood stream invasions with more than one species being recovered. During 1979-80, this circumstance changed to a marked degree. One hundred patients each yielded only one species from blood (Table 7). In contrast (Table 8), only eight patients had more than one species recovered from single or successive cultures during their illness. The decrease in mixed blood stream infections in contrast to that seen 3 years or more earlier was striking, but no recognizable factors to explain such a change were detected.

Table 7. Bacteremia with Only One Species of Bacteria Recovered
1 October 1979 - 30 September 1980

Organism	No. Patients with One Species Recovered	Average No. of Positive Blood Cultures Per Patient	No. Deaths	% Mortality for One Species Bacteremia
S. aureus	23	1.5	11	48
S. epidermidis	17	1.0	0	0
S. saprophyticus	1	1.0	0	0
Strep. viridans	3	1.0	0	0
Strep. pneumoniae	1	1.0	1	100
Beta-hemol. Strep. not A, B, or D	1	1.0	1	100
Gp D Enterococcus	4	1.0	3	75
Escherichia coli	3	1.0	2	66
Entero. cloacae	5	1.7	3	60
Entero. aerogenes	1	1.0	1	100
Klebsiella oxytoca	1	1.0	1	100
Proteus mirabilis	1	1.0	1	100
Morganella morganii	1	1.0	1	100
Providencia stuartii	1	1.0	1	100
Aeromonas hydrophila	1	1.0	0	0
Acineto. anitratus	1	1.0	0	0
P. aeruginosa	15	1.4	12	80
P. cepacia	1	1.0	0	0
Alcaligenes faecalis	1	1.0	1	100
Achromo. xylosoxidans	1	1.0	1	100
Bacillus sp.	1	1.0	1	100
Candida albicans	8	1.5	5	63
Candida rugosa	4	2.3	4	100
Candida tropicalis	3	1.0	0	0
Torulopsis glabrata	1	1.0	0	0

Underlined species represent numerically important organisms

Table 8. Blood Culture Isolates in Patients with Mixed Infections
1 October 1979 - 30 September 1980

Organisms	No. of Patients
S. aureus, Gp D Enterococcus	2
S. aureus, Strep. viridans	1
Strep. viridans, non-hemol. Strep. not Gp D	1
Klebsiella pneumoniae, P. aeruginosa	1
Klebsiella oxytoca, Proteus mirabilis	1
Proteus mirabilis, Escherichia coli, Gp D Enterococcus	1
Candida rugosa, Candida albicans	1
Number of patients with:	
S. aureus	3
Strep. viridans	2
Non-hemol. Strep. not Gp D	1
Gp D Enterococcus	2
Klebsiella pneumoniae	1
Klebsiella oxytoca	1
Proteus mirabilis	2
Escherichia coli	1
Candida rugosa	1
Candida albicans	1

With staphylococci, Pseudomonas, E. coli and Entero. cloacae, a relationship between species and mortality was suggested. Staphylococcal sepsis was associated with a fatal outcome in one-half of the cases. With the enteric species, two-thirds of the patients expired, and Pseudomonas sepsis was associated with an 80% mortality.

BIOPSIES OF BURN WOUNDS

The importance of wound biopsies in diagnosis and prognosis has become well established. The flora of such samples, collected from 75 patients, is shown in Table 9. The summation includes fungi as well as bacteria and yeasts. There has been a gradual increase in the number of biopsy specimens in which both bacteria and fungi were recovered, and the characterization of sepsis has shown a parallel increase in observation of both groups of organisms in microscopy of the biopsied tissue. Numerically, S. aureus and P. aeruginosa were by far the most commonly occurring species. Aspergillus sp. and two species of Candida were recovered in significant numbers of patients. No species of Enterobacteriaceae was recovered in significant numbers of patients.

Table 9. Bacterial Flora of Biopsies of Burn Wounds of 75 Patients
1 October 1979 - 30 September 1980

Organism	No. of Patients Positive	% of Patients Positive	No. of Patients with Positive Cultures Who Expired	% of Patients Who Expired
S. aureus	22	29	15	68
S. epidermidis	4	5	2	50
Strep. viridans	2	3	1	50
Bacillus sp.	2	3	2	100
P. aeruginosa	22	29	16	73
Acineto. anitratus	2	3	1	50
Escherichia coli	5	7	4	80
Klebsiella pneumoniae	2	3	2	100
Klebsiella oxytoca	1	1	1	100
Entero. cloacae	3	4	3	100
Entero. aerogenes	1	1	1	100
Proteus mirabilis	3	4	2	67
Morganella morganii	1	1	0	0
Providencia stuartii	1	1	1	100
Candida albicans	5	7	5	100
Candida rugosa	7	9	6	86
Candida tropicalis	1	1	1	100
Trichosporon beigelii	1	1	0	0
Curvularium sp.	1	1	0	0
Diplosporium sp.	1	1	1	100
Fusarium sp.	4	5	1	25
Mucor sp.	2	3	1	50
Aspergillus sp.	10	13	8	80
Alternaria sp.	5	7	4	80
Penicillium sp.	2	3	1	50
Mycelia sterilia	2	3	2	100

Number of specimens: 287 Number of specimens per patient: 3.8

This result contrasts with the situation prevailing over several years ending in 1977; during that time, literal epidemics of burn wound sepsis were associated with strains of commonly encountered Enterobacteriaceae.

CATHETER TIPS AND BACTERIAL CONTAMINATION

Intravenous catheters are an essential facet of the treatment of severely burned patients, but they also constitute a potential avenue for bacterial invasion of the vascular tissues and a consequent source of infection and thrombophlebitis. There were 51 patients from whom catheter tips were cultured at the time of removal from the patient (Table 10). Only P. aeruginosa and S. aureus were recovered from significant numbers of patients. There were 14 species of bacteria and four of yeasts recovered. Of historical importance is the recovery of one strain of Providencia stuartii. This species had not been recovered in the past 2 years.

Table 10. Bacterial Flora of IV Catheter Tips
1 October 1979 - 30 September 1980

Organism	No. of Isolates	No. of Patients Positive	% Total Patients Positive
S. aureus	7	7	14
S. epidermidis	5	5	10
Strep. viridans	1	1	2
Non-hemol. Strep. not Gp D	1	1	2
Gp D Enterococcus	1	1	2
Neisseria sp.	1	1	2
P. aeruginosa	10	8	16
Acineto. anitratus	1	1	2
Escherichia coli	1	1	2
Klebsiella pneumoniae	2	2	4
Entero. aerogenes	1	1	2
Proteus mirabilis	1	1	2
Morganella morganii	1	1	2
Providencia stuartii	1	1	2
Candida albicans	2	2	4
Candida rugosa	3	3	6
Candida tropicalis	6	4	8
Torulopsis glabrata	1	1	2

Number of patients cultured: 51 Number of cultures: 112

URINARY TRACT BACTERIOLOGY

Urinary tract infection is a common development among severely burned patients in whom indwelling urinary catheters are extremely common. The results of urine cultures on 174 patients are summarized in Table 11. As would be expected, enteric species were seen more frequently than they were in cultures from wound or sputum. The most commonly encountered species was P. aeruginosa, with E. coli and K. pneumoniae present less frequently in 30 and 20 patients respectively. Staphylococcus aureus was recovered with the same frequency as were the enteric species. Staphylococcus epidermidis was almost as frequent in occurrence, being found in 19 patients. The overall incidence of bacteria and yeasts resembled that recovered from other sites, with the difference being a higher incidence of enteric species. Urine cultures represented the only marked divergence from the overall pattern of distribution of the burn wound flora.

XENOGRAFT (PORCINE SKIN) CULTURES

Xenograft, in the form of sheets of pig skin which have been collected by dermatome from freshly killed, thoroughly cleaned hogs, has become a widely used biologic dressing in the treatment of burns. The skin is cleaned but not sterilized, and was originally shipped and stored in antibiotic solution. Although there is no detailed information accompanying the pig skin, the possibility has arisen that in recent months the antibiotic (usually a tetracycline) may have been reduced in concentration. In any event, cultures of small portions of pig skin and of its transport fluid began, in 1979-80, to show increased incidence of bacterial contamination. Samples from lots of xenograft assigned to 45 patients were cultured. As shown in Table 12, 18 species were recovered, with 49 strains recovered. Thus in most instances only one species was recovered. Staphylococcus aureus, P. aeruginosa and E. coli were the most commonly encountered species. It cannot be assumed that these organisms were all present in the xenograft samples when they reached the hospital; the sampling technics present the possibility that some were introduced during the sampling process.

The recovery of uncommon species of Pseudomonas, even in small numbers, suggests that these organisms at least were present on the pig skin. Pseudomonas fluorescens, putida, maltophilia and cepacia are not unknown in the flora of burn patients in this Institute, but they were relatively rare during this year. Subsequent cultures, not included in Table 11, enlarged the role of Pseudomonas species other than P. aeruginosa as contaminants of pig skin.

POSTMORTEM MICROBIAL FLORA

Quantitative and qualitative cultures have been carried out from autopsy tissues since 1961. In previous years, over 20 species

Table 11. Urine Cultures on 174 Patients
1 October 1979 - 30 September 1980

Organism	No. of Isolates	No. of Patients Positive	% Total Patients Positive
S. aureus	47	22	13
S. epidermidis	29	19	11
Strep. viridans	5	5	3
Non-hemol. Strep. not Gp D	4	4	2
Beta-hemol. Strep. not Gp A, B, or D	1	1	0.6
Gp D Strep. not Enterococcus	5	5	3
Gp D Enterococcus	24	15	9
Bacillus sp.	3	3	2
P. aeruginosa	66	33	19
Acineto. anitratus	4	4	2
Escherichia coli	45	30	17
Escherichia coli (A-D)	3	1	0.6
Citro. freundii	1	1	0.6
Citro. diversus	2	1	0.6
Klebsiella pneumoniae	39	20	11
Klebsiella ozaenae	2	1	0.6
Entero. cloacae	2	2	1
Entero. aerogenes	3	3	2
Proteus mirabilis	22	12	7
Morganella morganii	2	2	1
Providencia stuartii	2	2	1
Candida albicans	120	22	13
Candida rugosa	9	5	3
Candida tropicalis	38	10	6

were recorded in an annual collection. Table 13 summarizes the species and strains recovered from tissues of 47 autopsies during 1979-1980. Staphylococcus aureus, P. aeruginosa, E. coli, and K. pneumoniae were recovered in significant numbers. There were 34 bacterial species and 12 of yeasts and fungi retrieved in cultures of the spectrum of tissues sampled. Six hundred ninety-two isolates were retrieved and characterized. The spectrum of bacterial species was more diverse than in the two previous years. One significant finding was the retrieval of 13 isolates of Providencia stuartii, an opportunist species that had been totally absent for at least 2 years. Future spread of this once lethal opportunist will merit close scrutiny.

Table 12. Xenograft (Porcine) Cultures
1 October 1979 - 30 September 1980

Organism	No. of Isolates	No. of Samples Positive	% Total Samples Positive
S. aureus	10	8	18
S. epidermidis	4	2	4
Non-hemol. Strep. not Gp D	1	1	2
Corynebacterium sp.	1	1	2
P. aeruginosa	9	7	16
P. fluorescens	2	1	2
P. putida	3	2	4
P. maltophilia	1	1	2
P. cepacia	1	1	2
Alcaligenes sp.	1	1	2
Acineto. anitratus	1	1	2
Escherichia coli	6	6	13
Citro. fruendii	1	1	2
Citro. diversus	1	1	2
Klebsiella pneumoniae	1	1	2
Entero. cloacae	3	3	7
Entero. agglomerans	2	2	4
Candida rugosa	1	1	2

Patients cultured: 45

Lung and wound were the principal sources of fungi recovered in autopsy. Aspergillus sp. was the most frequently encountered. Fusarium sp., once the most common genus, was only recovered nine times. Mucor sp., representing the most dangerous genus for burn wounds, was recovered eight times, from lung and burn wound. Typical invasive phycomycosis, the most inexorable of tissue-destroying fungal infections, was not observed during this year.

The reappearance of Providencia stuartii in burn patients during this year occasioned a prompt scrutiny of isolates. Since the species had shown a potential for establishing a serious epidemic pattern, differentiation of strains was investigated. Thus if a pervasive strain emerged, means for recognizing it would be available. As an initial approach, the biochemical utilization and dissimilation patterns were assessed for 95 isolates of Providencia stuartii. Distinctive biotypes were recognizable in the second week of the study, and a total of four biotype patterns have been delineated. Patterns are shown in Table 14. The classical differentiating reactions for this species include oxidase (negative), no lactose fermentation, lysine

Table 13. Postmortem Bacteriology of 47 Burn Patients, 1 October 1979 - 30 September 1980

Organism	Total Isolates Recovered at Autopsy	Source and Number of Isolates							
		Liver	Spleen	Lung	Blood	Blood Thrombus	IV Tip	Burn Wound	Heart
S. aureus	110	5	10	61	4	2	5	22	1
S. epidermidis	16	2	1	3	1	1	2	4	2
S. saprophyticus	2	0	0	2	0	0	0	0	0
Strep. viridans	23	1	1	11	2	0	3	3	2
Non-hemol. Strep. not Gp D	12	1	1	6	1	0	1	2	0
Beta-hemol. Strep. not A, B, or D	2	0	0	2	0	0	0	0	0
Strep. pneumoniae	2	0	0	1	1	0	0	0	0
Gp D Strep. not Enterococcus	10	1	2	4	1	0	1	1	0
Gp D Enterococcus	16	0	2	3	4	0	2	5	0
Gp B streptococcus	3	0	0	1	1	0	0	1	0
Bacillus sp.	2	0	0	0	1	0	0	1	0
Corynebacterium sp.	2	0	0	1	0	0	0	1	0
P. aeruginosa	117	6	2	45	4	1	7	50	2
P. fluorescens	3	0	1	1	0	0	0	1	0
P. putida	2	0	0	1	0	0	0	1	0
P. maltophilia	2	0	0	1	0	0	0	1	0
P. stutzeri	1	0	1	0	0	0	0	0	0
Achromobacter Biotype 2	1	0	0	0	0	0	0	1	0
Acinetobacter anitratus	9	0	0	3	1	0	2	2	1
Acinetobacter lwoffii	2	0	0	0	0	0	1	1	0
Escherichia coli	57	6	8	16	5	3	3	11	5
Citrobacter diversus	1	0	0	0	0	0	1	0	0
Citrobacter freundii	1	0	0	0	0	0	1	0	0
Klebsiella pneumoniae	80	7	8	39	8	1	4	12	1
Klebsiella oxytoca	3	0	0	0	2	0	0	1	0
Klebsiella ozaenae	2	0	0	1	1	0	0	0	0

Table 13. Postmortem Bacteriology of 47 Burn Patients, 1 October 1979 - 30 September 1980 (cont.)

Organism	Total Isolates Recovered at Autopsy	Source and Number of Isolates							
		Liver	Spleen	Lung	Blood	Blood Thrombus	IV Tip	Burn Wound	Heart
Enterobacter cloacae	16	0	1	7	2	0	3	2	1
Enterobacter aerogenes	8	0	0	5	2	0	0	1	0
Enterobacter agglomerans	1	0	0	0	0	0	0	1	0
Group 5A-2	1	0	0	0	0	0	0	1	0
Proteus vulgaris	7	1	0	5	0	0	0	0	1
Proteus mirabilis	13	1	0	5	1	1	3	0	2
Morganella morganii	8	0	1	4	2	0	0	0	1
Providencia stuartii	13	0	1	7	1	0	2	1	1
Unidentified yeast-like	3	1	0	0	0	0	1	1	0
Candida albicans	40	0	4	17	1	1	1	15	1
Candida rugosa	22	1	1	2	0	1	0	17	0
Candida tropicalis	22	3	2	12	0	0	0	4	1
Alternaria sp.	8	1	0	3	0	0	0	4	0
Mucor sp.	8	1	0	3	0	0	0	4	0
Aspergillus sp.	18	2	0	1	0	0	0	15	0
Fusarium sp.	9	1	1	3	0	1	0	3	0
Geotrichum sp.	1	0	0	1	0	0	0	0	0
Trichosporon beigelii	1	0	0	0	0	0	0	1	0
Penicillium sp.	1	0	0	1	0	0	0	0	0
Nigrosporum sp.	1	0	0	0	0	0	0	1	0
Rhizopus sp.	1	0	0	0	0	0	0	1	0
Mycelia sterilia	9	1	0	4	0	0	0	4	0
TOTAL	692	42	48	282	46	12	43	197	22

Table 14. Biochemical Reactions Differentiating Providencia stuartii Biotypes

Reaction	Biotype A	B	C	D
ONPG	-	-	-	+
Ornithine	-	-	-	+
Citrate	+	+	+	+
Tryptophane deaminase	+	+	+	+
Indole	+	+	+	+
Glucose	+	+	+	+
Amygdalin	-	-	-	+
Inositol	+	+	+	+
Rhamnose	-	-	-	+
Mannitol	-	-	+	+
Sorbitol	-	-	+	+
Sucrose	-	+	-	-
Arabinose	-	-	+	-

deaminase positive, urease negative, indole positive, ornithine negative, glucose and inositol fermented, citrate utilized, and tryptophane deaminase positive. The differentiating reactions used to distinguish biotypes include, in addition to ONPG, ornithine, citrate, urea and glucose, the sugars amygdalin, inositol, mannitol, sorbitol and arabinose. The patterns designated B through D represent an ascending level of activity from A.

Table 15 shows the distribution of biotypes observed over an 11-week period. The most consistent pattern was Biotype A, but pattern B was recovered in 5 separate weeks and D during 3 weeks. As yet the Providencia stuartii has not assumed a clinically significant role, but strain differentiation remains possible.

Sources of the biotypes are shown in Table 16. The respiratory tract flora remained the principal site of involvement with Providencia. Biotypes C and D were rare but real. The potential of these strains for invasive infection will be evaluated as they continue to appear.

The introduction of 37 casualties from a major thermal accident in Japan offered an opportunity to study acquisition of infection in such a population in this physical environment. The patients were transported to the Institute of Surgical Research within 60 hours after the accident, but had been hospitalized in several Japanese medical facilities immediately after the accident. Wounds were cultured sequentially using both swabs and contact plates (CP). The flora recovered in the entire period of hospitalization in the ISR is summarized in Table 17. It is assumed that this cohort of patients

Table 15. Distribution of Biotype by Week of Isolation

Week (1980)	No. Patients Prov. stuartii Isolated	No. Strains Isolated	Biotypes Isolated
17 Aug	2	6	A
24 Aug	1	1	B
31 Aug	1	10	A
7 Sep	1	13	A,D
14 Sep	2	6	A,D
21 Sep	1	2	A,B
28 Sep	2	12	A,B,D
5 Oct	5	9	A,B,C
12 Oct	4	8	A,C
19 Oct	3	27	A,B
26 Oct	4	11	A

Table 16. No. Isolates by Specimen Source & Biotype

Specimen	Biotype A	B	C	D
Blood	5	0	0	0
Sputum	62	6	2	3
Urine	3	0	0	0
Skin	13	1	0	0
IV catheter	4	0	0	0
Foley catheter	0	1	0	0

Table 17. Antemortem Bacteriology on 37 Marine Mass Casualty Patients

Organism	No. Isolates/No. Patients Positive										
	Wound Surface			Respiratory Tract			Catheter Tips				Total Strains
	Swab	CP	Blood	Throat	Sputum	Urine	IV	Foley	Biopsy	Xeno-grafts	Recovered
S. aureus	35/16	29/17	3/2	51/20	21/13	2/1	0	1/1	7/4	2/2	151
S. epidermidis	19/14	46/22	7/7	30/17	8/4	6/3	2/2	0	0	0	118
S. saprophyticus	0	1/1	1/1	0	0	0	0	0	0	0	2
Strep. viridans	10/7	9/6	2/2	207/37	34/14	1/1	1/1	0	1/1	0	265
Non-hemol. Strep. not Gp D	6/6	3/2	0	68/30	11/5	1/1	1/1	0	0	1/1	91
Beta-hemol. Strep. not A, B, or D	0	0	0	23/9	2/1	0	0	0	0	0	25
Gp D Strep. not Enterococcus	0	2/2	0	44/18	2/2	0	0	0	0	0	48
Gp D Enterococcus	1/1	3/2	0	17/12	8/8	0	0	2/2	0	0	31
Strep. pneumoniae	1/1	0	0	12/10	1/1	0	0	0	0	0	14
Gp B Strep.	0	0	0	3/3	1/1	0	0	0	0	0	4
Corynebacterium sp.	0	16/13	0	0	0	0	0	0	0	0	16
Neisseria sp.	2/2	0	0	8/8	5/3	0	1/1	0	0	0	16
P. aeruginosa	29/13	46/17	4/3	12/7	28/8	4/2	3/2	0	8/5	2/1	136
P. fluorescens	0	0	0	0	1/1	0	0	0	0	0	1
P. putida	1/1	0	0	0	0	0	0	0	0	0	1
Gp 2K-1	0	1/1	0	0	0	0	0	0	0	0	1
Alcaligenes sp.	0	0	0	0	2/2	0	0	0	0	0	2
Acineto. anitratus	4/4	20/9	0	16/8	17/8	3/3	1/1	0	3/1	0	64
Acineto. lwoffii	7/6	0	0	0	0	0	0	0	0	0	7
Gp 5E-1	0	1/1	0	0	0	0	0	0	0	0	1
Escherichia coli	2/2	5/3	1/1	28/11	13/8	1/1	0	0	0	4/4	54
Citro. freundii	0	1/1	0	1/1	3/3	0	0	0	0	0	5
Citro. diversus	0	1/1	0	0	0	0	0	0	0	0	1

Table 17. Antemortem Bacteriology on 37 Marine Mass Casualty Patients (cont.)

	No. Isolates/No. Patients Positive										
	Wound Surface			Respiratory Tract			Catheter Tips				Total Strains
Organism	Swab	CP	Blood	Throat	Sputum	Urine	IV	Foley	Biopsy	Xeno-grafts	Recovered
Klebsiella pneumoniae	4/4	16/12	0	15/8	10/5	5/2	1/1	0	0	0	51
Klebsiella oxytoca	0	0	0	1/1	2/1	0	0	0	0	0	3
Klebsiella ozaenae	0	3/3	0	1/1	1/1	0	0	0	0	0	5
Entero. cloacae	5/5	17/12	2/2	4/3	2/2	0	0	0	0	1/1	31
Entero. aerogenes	3/2	13/9	1/1	3/3	6/3	2/2	0	0	1/1	0	29
Entero. agglomerans	0	10/8	0	0	0	0	0	0	0	0	10
Serratia marcescens	1/1	3/1	0	4/4	1/1	0	0	0	0	0	9
Proteus vulgaris	0	2/1	0	1/1	0	0	0	0	0	0	3
Proteus mirabilis	0	2/2	0	0	2/2	0	0	0	0	0	4
Aeromonas hydrophila	2/2	0	1/1	2/2	0	0	0	0	0	0	5
Yeast-like organism	0	0	0	0	0	3/2	0	0	1/1	0	4
Candida albicans	1/1	1/1	6/3	4/3	4/4	6/2	0	0	2/2	0	24
Candida rugosa	0	0	0	0	0	0	0	0	4/1	0	4
Candida tropicalis	2/2	0	3/2	0	1/1	8/1	1/1	0	2/1	0	17
Diplosporium sp.	0	0	0	0	0	0	0	0	1/1	0	1
Fusarium sp.	0	0	0	0	0	0	0	0	2/2	0	2
Mucor sp.	0	0	0	0	0	0	0	0	1/1	0	1
Aspergillus sp.	0	0	0	0	0	0	0	0	3/1	0	3
Alternaria sp.	0	0	0	0	0	0	0	0	2/2	0	2
Penicillium sp.	0	0	0	0	1/1	0	0	0	0	0	1

Total isolates: 1263

with a unique experience prior to admission and which was segregated in a new treatment area for the first 4 weeks of treatment, might exhibit a distinctive pattern of bacterial seeding and colonization. If there were differences between this group of patients and the remainder of the burn patient population, they were minor. An increase in Acinetobacter anitratus over that seen in the remainder of the year was noted. This strain may have been brought back with the patients from Japan. Staphylococcus aureus and P. aeruginosa were the principal species encountered. Escherichia coli and K. pneumoniae were the most common species. The rate of colonization in this population was delayed beyond that typically seen in the burn ward, but the ultimate bacterial pattern was not significantly altered.

PUBLICATIONS

Lindberg RB, Mason AD, Jr, Pruitt BA, Jr: Epidemiologic and cultural evidence for existence of epidemic strains of Enterobacteriaceae in burn patients. Fed Proc 39:778, 1980.

PRESENTATIONS

Lindberg RB: Epidemiologic and cultural evidence for existence of epidemic strains of Enterobacteriaceae in burn patients. Presented at American Association of Immunologists Annual Meeting, Anaheim, California, 15 April 1980.

ANNUAL PROGRESS REPORT

PROJECT NO. 3S161102BS05-00, BASIC RESEARCH

REPORT TITLE: STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE OF TROOPS WITH THERMAL INJURY -- THE ROLE OF FUNGI IN BURN WOUND INFECTION: OBSERVATIONS ON BIOPSY AND AUTOPSY TISSUES FROM SERIOUSLY BURNED SOLDIERS

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

1 October 1979 - 30 September 1980

Investigators:

Robert B. Lindberg, Ph.D.
Jack R. Henderson, Ph.D.
Susan J. Constable, SSG
Gloria Bailey, SP5

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ABSTRACT

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Biopsy and autopsy tissue samples from burn patients were cultured on Sabouraud's agar, and the fungi recovered were classified to the level of genus. Fungal isolates were scattered in time; there were no peaks of incidence to suggest that an epidemic incidence had occurred. Ten genera of fungi were recognized, seven from biopsy and eight from autopsy samples. Five genera -- Alternaria, Mucor, Aspergillus, Fusarium, and Penicillium -- were recovered from both sources. Pathogenic genera included two phycomycetes -- Mucor and Rhizopus.

Fungi
Phycomycosis
Burns
Humans

STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE OF TROOPS WITH THERMAL INJURY -- THE ROLE OF FUNGI IN BURN WOUND INFECTION: OBSERVATIONS ON BIOPSY AND AUTOPSY TISSUES FROM SERIOUSLY BURNED SOLDIERS

Fungal infections in burn patients have presented a recurrent problem. Fungi are frequently recovered from burn wounds, both in surface and in biopsy samples, and also from burn wound tissues at autopsy. Whether these strains are of clinical significance in burn infection pathogenesis is not always clear. Their identities parallel those found in the burn patients' environment, and in many instances they could plausibly represent nonpathogenic colonization of the burn.

FUNGI IN BIOPSY SPECIMENS

During this observation period, biopsy specimens from 75 patients were cultured for presence of fungi. Following the technic developed in this laboratory, a small portion of biopsy sample was plated on the surface of a screw-capped tissue culture bottle containing a layer of Sabouraud's agar. Results of this series are summarized in Table 1. Seven genera were differentiated, and two strains could not be identified due to absence of fruiting bodies in culture. The predominant genus was Aspergillus, of which 21 strains were recovered. Alternaria and Fusarium were also present in significant numbers. The number of genera recovered has varied in recent years from four to ten. A comparison of recoveries from successive years is shown in Table 2. This series gives a better insight into the long-term incidence of fungi. Three genera -- Aspergillus, Fusarium, and Alternaria -- were recovered in every year. Cephalosporium was almost as consistent in occurrence; it was recovered in every year but one of the comparison period. Trichophyton was found in one year only. It is an interesting fact that dermatophytes have been extremely rare among the genera of fungi recovered from burn wounds. One would expect the ubiquitous dermatophytes to grow on burn wounds, but evidently they do not. The Phycomycetes were represented by two genera: Mucor was recovered in four of the eight years, while Rhizopus, the other genus recovered from cases of phycomycosis, was only recovered in one year.

FUNGI RECOVERED AT AUTOPSY

Autopsy specimens cultivated included burned tissue, liver, spleen, and lung. The genera recovered were set down in two categories: wounds, which refers to tissue blocks selected in the burn wound, and viscera, to include liver, spleen, and lung tissue. Table 3 summarizes the results obtained in 1979-1980. There were eight genera of fungi recovered, six from each of the two categories. Geotrichum and Penicillium were recovered only from burn wounds, while Rhizopus and Nigrospora were found only in lung tissue. Liver and spleen yielded no fungi. When compared to biopsy fungi, there were two genera -- Curvularia and Diplosporium -- which were found only in biopsies. Three genera -- Rhizopus,

Table 1. Fungi Recovered from Biopsy Samples - 1979-1980

Organism (Genus)	No. Patients Positive	No. of Strains Recovered
Curvularia	1	3
Diplosporium	1	1
Fusarium	4	4
Mucor	2	3
Aspergillus	10	21
Alternaria	5	7
Penicillium	2	2
Mycelia sterilia	2	2

No. of patients cultured 75
No. of genera recovered 8
No. of strains of fungi isolated 43

Table 2. Fungi Recovered from Burn Wound Biopsies - 1973-1980

Genus	Year and No. of Strains Recovered						
	1973	1974	1975	1976-7	1977-8	1978-9	1979-80
Aspergillus	17	5	2	5	23	28	21
Cephalosporium	5	5	1	4	5	5	0
Fusarium	23	17	2	4	4	1	4
Sepodonium	1	0	0	3	0	0	0
Penicillium	1	3	0	1	0	1	2
Alternaria	2	3	1	3	6	1	7
Trichophyton	0	0	0	1	0	0	0
Mucor	2	0	0	1	4	0	3
Rhizopus	2	0	0	0	0	0	0
Curvularium	2	3	0	0	0	2	3
Helminthosporium	9	2	0	0	0	1	0
Geotrichum	0	4	0	0	10	0	0
Coccidioides	0	0	0	0	0	1	0
Diplosporium	0	0	0	0	0	0	1
Mycelia sterilia	0	0	0	0	0	2	2
No. patients cultured	106	135	63	113	61	78	75
No. genera	10	8	4	8	6	9	8
No. strains recovered	64	42	5	22	52	42	43

Table 3. Fungi Recovered from Burn Wounds and Viscera at Autopsy, 1979-1980

Genus	Wounds		Viscera	
	Patients Positive	No. of Strains	Patients Positive	No. of Strains
Alternaria	4	4	4	4
Mucor	2	4	2	4
Rhizopus	0	0	1	1
Aspergillus	3	3	10	15
Fusarium	5	5	3	3
Geotrichum	1	1	0	0
Penicillium	1	1	0	0
Nigrosporium	0	0	1	1
Mycelia sterilia	4	5	3	4

Geotrichum and Nigrosporium were found only in autopsy tissues. Five genera were found in both categories. As has been the case for the past 3 years, Aspergillus was the predominant genus for the observation period. The distribution of genera recovered reinforces the concept that burn wound fungi are indeed true opportunists. Only the Phycomycetes, including Mucor, Rhizopus and Absidia, have been recovered from invasive burn wound mycosis.

PUBLICATIONS AND/OR PRESENTATIONS - None.

FINAL REPORT

PROJECT NO. 3S161102BS05-00, BASIC RESEARCH

REPORT TITLE: STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE OF TROOPS WITH THERMAL INJURY -- DETECTION OF ENDOTOXIN IN BURNED SOLDIERS WITH SEPSIS

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

1 October 1979 - 30 September 1980

Investigators:

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ABSTRACT

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A variety of technics have been applied to the problem of detection of endotoxin in blood for the rapid diagnosis of endotoxemia. It was shown that no significant correlation between positive Limulus amoebocyte lysate (LAL) reaction and endotoxemia can be made. The LAL reaction remains a useful tool for other laboratory applications, but the diagnosis of endotoxemia by this procedure is not valid.

Pseudomonas
Endotoxin
Burns
Humans

STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE OF TROOPS WITH THERMAL INJURY -- DETECTION OF ENDOTOXIN IN BURNED SOLDIERS WITH SEPSIS

The detection of endotoxin in picogram concentrations has been accomplished by use of the Limulus amoebocyte gelation reaction. This procedure has had widespread, effective use. It was proposed at this Institute that search be made for endotoxin in the blood of patients severely ill with sepsis as a consequence of severe thermal injury. Many individuals in this category exhibit a syndrome virtually identical to the classic entity designated "endotoxic shock."

Beginning in 1971, a large number of patients was examined for presence of endotoxin, or at least of amoebocyte-reactive material, in the peripheral blood. Examinations were conducted on plasma, serum, whole blood, and even triturated whole blood clot. In a separate study, endotoxin was sought in liver and spleen samples of tissue collected at autopsy from patients dying with extensive burns. Technics for extracting endotoxin included trichloracetic acid extraction, heat extraction, direct examination of whole serum and plasma, and assay by extraction of endotoxin on resin beads. With each of these methods, endotoxin was demonstrable in nanogram and even picogram concentrations, both in clinical samples and in experimental samples prepared by dissolving endotoxin in serum or plasma. However, demonstrating a clinically useful reaction, to be used as a diagnostic or prognostic test, was not possible. The correlation examined was that between blood cultures positive for gram-negative aerobic bacilli and a positive Limulus amoebocyte lysate (LAL) reaction. Positive correlation of bacteremia with the LAL reaction would make this reaction valuable as a diagnostic and prognostic procedure. However, when a series including both patients with gram-negative bacteremia and patients without positive blood cultures was tested, no correlation between bacteremia and positive LAL reaction could be demonstrated. Endotoxin and bacteremia were associated in a random fashion. A small number of patients had demonstrable endotoxemia and positive blood cultures. Two larger groups were distinguished. In one, the LAL reaction was negative and bacteremia was present. In the other, the LAL reaction was positive but blood cultures were negative. The fourth and largest group had both LAL and blood cultures negative. Thus, it was evident that, although endotoxin can be demonstrated in the blood of some patients with septicemia, its presence is not consistent or correlated with positive blood culture. The hypothesis on which this protocol to demonstrate endotoxin in burned soldiers was based was not provable. The LAL test does not merit further pursuit as a diagnostic test for or prediction of septicemia in burned patients. This protocol is hereby terminated.

PUBLICATIONS/PRESENTATIONS - None.

ANNUAL PROGRESS REPORT

PROJECT NO. 3S161102BS05-00, BASIC RESEARCH

REPORT TITLE: STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE OF TROOPS WITH THERMAL INJURY -- EMERGENCE AND DISAPPEARANCE OF METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS IN BURNED MILITARY PERSONNEL

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1 October 1979 - 30 September 1980

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ABSTRACT

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The behavior of Staphylococcus aureus in a burn ward population was assessed by comparing sensitivity and resistance to methicillin, oxacillin and nafcillin over a succession of monthly intervals. Reaction to each of these antibiotics revealed a pattern of emergence of resistant forms followed, often with an abrupt change, by a sensitive population. Strain identities remained constant, and the changes occurred without the intrusion of a significant degree of use of these antibiotics in the host population. The extreme fluctuations in sensitivity possible in an epidemic population of bacteria imply an intrinsic factor or factors and make the problem of antibiotic resistance a function of endogenous variation, rather than exogenously imposed selection.

Staphylococcus
Burns
Septicemia
Infections
Antibiotics

STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE OF TROOPS WITH THERMAL INJURY: EMERGENCE AND DISAPPEARANCE OF METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS IN BURNED MILITARY PERSONNEL

Staphylococcus aureus has for at least the past 20 years constituted a major part of the bacterial flora causing infection, morbidity and death in severely burned soldiers. Although antibiotics which are active in vitro against this ubiquitous pathogen have not been lacking, it has remained at all times one of the two most important species involved in burn sepsis. Its elimination from burn patients by even the most stringent reverse isolation technic is probably impossible, since it can be transferred from patient to patient not only by intermediate carriers but also by air-borne passage.

Previous studies have shown that monotype epidemics of S. aureus are typical of wards housing burn patients. Phage typing, serotyping and biotyping, together and separately, have confirmed this tendency, but type identification of strains alone does not clarify the dynamics of antibiotic resistance in this bacterial species. It has been virtually axiomatic that the bacterial population of burn patients tends to become more and more resistant as selection occurs in response to antibiotic use. When the annual totals of staphylococci were sorted on the basis of proportion of isolates inhibited by 6.2 mcg/ml of antibiotic, it was observed that extreme fluctuations between sensitive and resistant populations occurred. Table 1 summarizes the annual totals for sensitivity to methicillin, oxacillin and nafcillin over an 8-year period. During some periods, methicillin was highly active against staphylococci, while in other years the proportion of strains inhibited fell below 25% of the total. Oxacillin fluctuations were less frequent but did occur. Nafcillin was the antibiotic to which staphylococci responded over the broadest range of variation. Years in which 85% of strains were sensitive were followed by years in which less than 1% of strains were sensitive.

Table 1. Sensitivity of Staphylococcus aureus to Three Semisynthetic Penicillins, 1972 - 1980

	Year and % of Isolates Inhibited at 6.2 mcg/ml								
Antibiotic	1972	1973	1974	1975	1976	1977	1978	1979	1980
Methicillin	13.1	50.0	65.2	21.8	23.5	35.7	77.9	34.6	92.3
Oxacillin	18.8	69.7	82.6	73.6	70.5	65.1	31.0	75.9	66.2
Nafcillin	26.0	62.3	83.3	85.6	49.5	1.8	0.5	0.5	75.6

Since the methicillin group of antibiotics is of major importance in the armamentarium of anti-staphylococcal drugs, detailed analysis of the fluctuations which occurred was made. Grouping strains by the month in which they were isolated proved to be an effective system for uncovering the nature of the appearance and disappearance of antibiotic sensitivity in a population of staphylococci recovered from burn patients.

The nature of the current variations in sensitivity of staphylococci to antibiotics of the methicillin group is shown in the following three charts. Chart 1 summarizes the behavior of staphylococci to methicillin between January 1979 and September 1980. The organisms recovered in January 1979 were entirely methicillin resistant. From February through April 1979, up to half of the isolates were resistant. The strains listed as sensitive were at the upper range of sensitivity, inhibited by 6.2 mcg/ml. In September 1979, almost all isolates were methicillin resistant. Then in November and December 1979, an abrupt shift to highly sensitive strains appeared. Strains were inhibited by less than 0.78 mcg/ml. In 1980, resistant strains once more predominated, from February to April. After that time, the strains were almost entirely highly sensitive.

Oxacillin differed markedly from methicillin, as seen in Chart 2. From January to May 1979, staphylococci were roughly divided between sensitive and resistant. From June to December, the isolates were essentially sensitive to oxacillin. This situation prevailed from January to July 1980. In August, a shift to resistance began.

Nafcillin displayed a striking variation of activity with the passage of time (Chart 3). In January 1979, strains were all resistant to nafcillin. This situation persisted for 11 months. Only in December did sensitive strains become predominant. This sensitive state persisted from February through August 1980. The degree of sensitivity increased during the latter part of this interval. In July and August 1980, no strains required more than 3.1 mcg/ml for inhibition. In previous observations, such sharp shifts in sensitivity occurred in 1977 and 1978.

The observations on methicillin-resistant staphylococci in 1979-1980 extend the point made earlier that, starting in 1977, there was a rather abrupt swing from sensitive to resistant with these three variants of penicillin. Methicillin itself was seldom used during this period, and the phemonenon of appearance, disappearance and reappearance of resistance appeared to occur as a variable essentially independent of an external stimulus. The implications of this phenomenon are far-reaching. The anthropocentric view that resistance is induced by use of an antibiotic in a given population, and that a resistant population may be removed by the expedient of suspending use of that antibiotic for a period, may not always apply. The incursion of resistant populations and their subsequent replacement by antibiotic sensitive strains

Chart 1. Number of Staphylococcus aureus Strains Inhibited at Test Levels of Methicillin
January 1979 - September 1980

Inhibited by mcg/ml	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
1979												
> 25.0	0	3	0	0	2	0	1	1	0	1	0	0
25.0	8	2	4	5	3	3	0	0	2	0	0	0
12.5	8	14	8	8	3	12	1	9	4	1	0	0
6.2	0	8	12	8	2	5	1	15	1	0	0	0
3.1	0	0	0	0	1	8	1	0	0	0	0	0
1.5	0	0	0	0	0	3	0	0	0	2	2	11
< .78	0	0	0	0	0	0	0	0	0	0	0	0
1980												
> 25.0	0	1	1	2	0	0	0	0	0			
25.0	0	0	1	0	0	0	0	0	0			
12.5	0	6	5	1	1	1	0	0	0			
6.2	0	2	1	0	1	0	1	0	0			
3.1	0	0	0	1	0	0	1	0	0			
1.5	0	0	0	2	3	3	1	3	0			
< .78	0	0	0	0	1	1	7	16	0			

Chart 2. Number of Staphylococcus aureus Strains Inhibited at Test Levels of Oxacillin
January 1979 - September 1980

Inhibited by mcg/ml	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
1979												
> 25.0	0	3	7	2	1	0	0	0	0	1	1	1
25.0	2	2	2	2	2	1	1	0	1	1	0	1
12.5	2	4	9	2	3	0	0	0	0	0	0	2
6.2	2	13	5	1	1	0	1	0	0	0	0	0
3.1	5	2	2	3	1	9	1	3	3	0	0	1
1.5	5	5	5	10	3	9	0	10	2	0	0	5
< .78	0	1	0	0	0	7	0	12	1	2	1	1
1980												
> 25.0	0	0	0	1	0	0	0	0	0			
25.0	0	0	1	0	0	0	0	1	0			
12.5	0	0	0	0	0	0	0	1	0			
6.2	0	0	0	0	1	0	1	3	0			
3.1	0	1	2	2	0	1	2	4	0			
1.5	0	0	2	1	2	1	4	7	0			
< .78	0	8	3	2	3	1	3	4	0			

Chart 3. Number of Staphylococcus aureus Strains Inhibited at Test Levels of Nafcillin
January 1979 - September 1980

Inhibited by mcg/ml	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
1979												
> 25.0	17	29	18	15	11	21	3	20	6	0	2	1
25.0	0	1	7	4	0	2	0	4	0	1	0	1
12.5	0	0	6	1	0	3	1	0	1	0	0	1
6.2	0	0	0	0	0	0	0	0	0	0	0	4
3.1	0	0	0	0	0	0	0	0	0	0	0	4
1.5	0	0	0	0	0	0	0	0	0	0	0	0
< .78	0	0	0	0	0	0	0	0	0	0	0	0
1980												
> 25.0	0	0	0	0	0	0	0	0	0			
25.0	0	0	0	0	0	0	0	0	0			
12.5	0	0	0	0	0	0	0	0	0			
6.2	0	4	3	1	2	1	0	0	0			
3.1	0	3	3	0	1	1	2	5	0			
1.5	0	2	0	1	0	0	1	7	0			
< .78	0	0	1	2	3	1	6	7	0			

suggests that these fluctuations may be, and in the instances here shown are, fortuitous. They were not driven by the consistent use of methicillin, nor was the disappearance of resistant forms associated with suspension of use of methicillin. Instead, the species acted as though the organism, in a collective sense, was unaware of or oblivious to our presence.

From the viewpoint of management of burn patients, the continued scrutiny of staphylococcal populations is more than ever a basic requirement. Aside from individual strain sensitivity, there is a need to know the pattern and trend of resistant-sensitive reactions, to offer optimal guidance for therapy.

PRESENTATIONS

Lindberg RB: Naturally occurring reversals of methicillin resistance of Staphylococcus aureus in burn patients. Presented at American Society of Microbiology Annual Meeting, Miami, Florida, 14 May 1980.

Lindberg RB: Mechanism of emergence and disappearance of methicillin resistance in Staphylococcus aureus in burn ward patients. Presented at American Burn Association Annual Meeting, San Antonio, Texas, 28 March 1980.

PUBLICATIONS

None.

ANNUAL PROGRESS REPORT

PROJECT NO. 3S161102BS05-00, BASIC RESEARCH

REPORT TITLE: STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE OF TROOPS WITH THERMAL INJURY -- SENSITIVITY TO SULFAMYLON OF PSEUDOMONAS AERUGINOSA RECOVERED FROM BURNED SOLDIERS

US ARMY INSTITUTE OF SURGICAL RESEARCH
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1 October 1979 - 30 September 1980

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

Period covered in this report: 1 October 1979 - 30 September 1980

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In 1979-1980 the sensitivity of Pseudomonas aeruginosa to Sulfamylon^{R} was assessed for 461 strains. The strains were more sensitive than a group of 715 tested in 1978-1979. Most strains of the 1979 group had emanated from two epidemic episodes of resistant strains. The greater percentages of current strains of P. aeruginosa were inhibited in a range of from 0.625 to 0.156 gm/dl of Sulfamylon, peaking at 0.312 gm/dl of the drug. With the exception of the increases of resistant strains seen in 1972 and 1979, current sensitivity levels of P. aeruginosa are consistent with those for the previous nine years.

Pseudomonas
Sulfamylon
Burns
Topical therapy
Humans

STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE OF TROOPS WITH THERMAL INJURY -- SENSITIVITY TO SULFAMYLON OF PSEUDOMONAS AERUGINOSA RECOVERED FROM BURNED SOLDIERS

Pseudomonas aeruginosa has continued to be the major organism which colonizes and infects burns. The incidence of unequivocal primary burn wound sepsis was grea reduced by the use of Sulfamylon burn cream, and this and other established topical agents are an essential part of management of severe burns. However, systemic infection with P. aeruginosa still occurs in severely burned patients, and its persistence is such that continued assessment of this species is essential. The sensitivity of P. aeruginosa strains to Sulfamylon is of fundamental importance, since development of resistance to any significant degree by P. aeruginosa would remove a significant part of the anti-pseudomonal armamentarium. Sulfamylon is not merely a sulfonamide but a methylated sulfonamide. However, since sulfonamide resistance is readily acquired, it is of continued interest that Sulfamylon susceptibility of P. aeruginosa be monitored. Virtually all strains of this organism which appear on burn patients are exposed extensively to Sulfamylon on the Institute of Surgical Research burn wards. The fact that during prolonged periods of observation Sulfamylon resistance has not appeared in P. aeruginosa is itself an unusual phenomenon. Continued exposure of any species would be expected to give rise to resistant strains, and the fact that this has not happened is an unusual circumstance which merits documentation.

SENSITIVITY OF PSEUDOMONAS AERUGINOSA TO SULFAMYLON

A total of 461 strains of P. aeruginosa from burn patients were tested for Sulfamylon sensitivity from 1 October 1979 to 30 September 1980. The testing technic, devised in this laboratory, has been described earlier in detail. It is based on incorporating Sulfamylon in trypticase soy agar plates in concentrations from 5% to 0.019%. The agar plates are seeded with 1000-cell inocula of a 20-hour broth culture of strains being tested. Growth is read at 24 hours, with sensitivity expressed as the concentration which inhibits growth.

The total sensitivity of strains to individual levels is shown in Table 1. The progression of sensitivity with increasing concentrations is apparent, as is the fact that three-fourths of the strains were inhibited by 0.312% Sulfamylon or less. The level of inhibition is within the range that is considered to be sensitive; the concentration of Sulfamylon acetate is not less than 11% in the burn cream and 5% in Sulfamylon soak solution. Thus the 0.312% level is 1/32 of the concentration in Sulfamylon burn cream.

Sensitivity of P. aeruginosa to Sulfamylon over the past nine years is summarized in Table 2. The 1979-1980 increment was unique

Table 1. Sensitivity to Sulfamylon of Pseudomonas aeruginosa
1 October 1979 - 30 September 1980

No. of Strains	Concentration Required for Inhibition (gm/dl)	% of Total Tested
30	1.250	6.5
98	0.625	21.3
178	0.312	38.6
68	0.156	14.8
45	0.078	9.8
25	0.039	5.4
13	0.019	2.8
4	< 0.019	0.8
Total 461		

in this period, in that the largest group of strains was that inhibited by 0.312%. The largest groups of strains which clustered even higher, at 0.625%, were those of 1972 and 1978-1979.

The variations in sensitivity of P. aeruginosa to Sulfamylon are most strikingly exhibited when sensitivity levels are arranged on a cumulative basis, annually. This information is shown in Table 3. The comparative data cover the years since 1968. There are fluctuations from year to year, but there are some long-term trends. Through 1971, the number of strains requiring more than 0.312% for inhibition was very small. Indeed, in 1970, no strains required more than 0.312% for inhibition. Since 1972, a few strains required more than 0.625% for inhibition, although this number was not large. In fact, it was larger in 1972 than it was in any subsequent year until 1979-1980, when the percentage tolerating 0.625% was 6.5%. The pattern of upper limit resistance was similar in 1979-1980 to that seen in the previous year. However, there was a significant difference in the lower dilutions. Half of the strains were inhibited in 1978-1979 by 0.156%; in 1979-1980, this proportion was 33%. There has thus been a slow, irregular upward creep in sensitivity, but it is not near a level that would preclude anticipated value of Sulfamylon as a therapeutic agent.

A final basis for comparison of the sensitivity of strains from successive annual collections of P. aeruginosa is the median level of inhibitory activity: the concentration at which 50% of all strains are inhibited (see Table 4). This value for 1979-1980 is compared with results from each year since 1968. The two extremely high values occurred in 1972 and 1978-1979. There were no obvious or even plausible

Table 2. Inhibiting Concentrations of Sulfamylon for Pseudomonas aeruginosa, 1971-1980

Year	No. of Strains	Concentration of Sulfamylon in gm/dl; No. & % of Strains Inhibited								
		2.5	1.25	0.625	0.312	0.156	0.078	0.039	0.019	< 0.019
1971	280	0	0	48 17.1	41 14.6	56 20.0	57 20.4	65 22.2	13 4.6	0
1972	463	0	29 6.3	212 45.8	46 9.9	88 19.0	31 6.7	37 8.0	15 3.2	5 1.1
1973	285	0	4 1.4	14 4.9	85 29.8	85 29.8	52 18.3	32 11.2	12 4.2	1 0.4
1974	437	0	5 1.1	59 13.5	78 17.9	97 22.2	97 22.2	86 19.7	11 2.5	4 0.9
1975	656	0	13 2.0	133 20.3	108 16.4	155 23.6	68 10.4	147 22.4	28 4.3	4 0.6
1976-77	698	0	4 0.6	118 16.9	135 19.3	295 42.3	95 13.6	18 2.6	23 3.3	10 1.4
1977-78	141	0	16 11.4	17 12.1	16 11.4	26 18.4	48 34.0	12 8.5	5 3.5	1 0.7
1978-79	715	0	78 11.0	307 43.0	193 27.0	59 8.2	47 6.6	16 2.2	1 0.1	14 2.0
1979-80	461	0	30 6.5	98 21.3	178 38.6	68 14.8	45 9.8	25 5.4	13 2.8	4 0.8
TOTAL	4136	0	179 4.3	1006 24.3	880 21.3	929 22.5	540 13.1	438 10.6	121 2.9	43 1.0

Table 3. Cumulative Sensitivity to Sulfamylon of Pseudomonas aeruginosa, 1968-1980

Year	No. of Strains	Concentration of Sulfamylon in gm/dl; % of Strains Inhibited							
		1.25	0.625	0.312	0.156	0.078	0.039	0.019	< 0.019
1968	294	100	100	95.1	60.4	45.8	14.1	1.7	0
1969	385	100	100	96.5	50.0	26.9	7.7	0.5	0
1970	296	100	100	100	78.0	49.9	21.9	2.0	0
1971	280	100	100	82.9	68.3	48.3	27.9	4.7	0
1972	463	100	93.7	48.0	38.0	19.0	12.3	4.3	1.1
1973	285	100	98.1	81.3	57.0	33.5	16.1	3.2	0.4
1974	437	100	99.0	85.5	67.5	45.3	23.1	2.4	0.9
1975	656	99.8	97.8	80.1	63.2	38.9	24.2	5.0	0.6
1976-77	698	100	99.4	82.5	63.2	21.0	7.3	4.7	1.4
1977-78	141	100	98.1	83.5	64.3	34.3	17.9	4.5	0.9
1978-79	715	100	95.8	71.5	52.2	28.7	15.2	3.8	1.1
1979-80	461	100	93.5	72.2	33.6	18.8	9.0	4.4	0.8

Table 4. Median Value of Pseudomonas aeruginosa Sensitivity to Sulfamylon, 1968-1980

Year	No. of Strains Tested	Median Inhibitory Level (gm/dl)
1968	294	0.136
1969	385	0.176
1970	296	0.068
1971	280	0.125
1972	463	0.316
1973	285	0.111
1974	437	0.086
1975	656	0.125
1976-77	698	0.117
1977-78	141	0.089
1978-79	715	0.324
1979-80	461	0.198

circumstances discernible that would account for such a shift -- apparently it was a normal fluctuation in the population of Pseudomonas that can occur at irregular intervals. The years with highest and lowest median levels of sensitivity did not correspond to changes in the incidence of clinical sepsis. Episodic decreases in sensitivity could be the forerunner of emerging resistance, but judging by past experience, this untoward event appears to be unlikely.

PRESENTATIONS AND/OR PUBLICATIONS - None.

ANNUAL PROGRESS REPORT

PROJECT NO. 3S161102BS05-00, BASIC RESEARCH

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

1 October 1979 - 30 September 1980

Investigators:

Robert B. Lindberg, Ph.D.
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Patients are studied with specific attention to unusual oxidative and fermentative gram-negative bacilli, since several of such species have shown the capacity to cause institutional epidemic outbreaks. The appearance of new species and their invasive capability are phenomena to be continuously observed. There were 23 species recovered that fitted the epithet "unusual." This is a marked increase in comparison with the previous totals observed.

Burns
Oxidative microorganisms
Acinetobacter
Pseudomonas

STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE OF TROOPS WITH THERMAL INJURY: NON-FERMENTATIVE AND OTHER GRAM-NEGATIVE BACILLI IN BURNED SOLDIERS: NEW POTENTIAL OPPORTUNISTIC PATHOGENS

The long-term experience with burn wound infections has made it apparent that, with substantial control of Pseudomonas aeruginosa by appropriate topical therapy, there is no individual species of gram-negative bacteria that can behave consistently as a burn wound pathogen. Instead, there is increasing evidence that the burn patient is primarily at risk from opportunistic infecting species, which include both fermentative gram-negative fecal bacteria and oxidative species which in many instances have their basic habitat in soil and surface water. A continued scrutiny of burn wound, respiratory tract, autopsy material and other sources of cultures from burned patients has been carried out. The species discussed below represent the return from this search.

UNUSUAL GRAM-NEGATIVE BACILLI ON BURNED PATIENTS

The unusual gram-negative species recovered in 1979-1980 are summarized in Table 1. There were five species of the genus Pseudomonas recovered, none of which was present in large numbers. Pseudomonas fluorescens was far less common than had been the case in some preceding years. One strain of P. cepacia was recovered from blood culture, but none of those strains established an infection with sepsis such as has often been seen with P. aeruginosa. Acinetobacter anitratus (formerly designated as Mima vaginicola) was far more common than it had ever been in the past 19 years. Strains were numerous on 37 burn patients, United States Marines, injured in a gasoline accident in Japan. Acinetobacter anitratus strains were found on their burns at the time of admission, 48 hours post-injury, and it has been conjectured that this high incidence stemmed from the initial seeding in Japan. Recovery of strains of Acinetobacter was more common in Japan, during the Korean War, than from patients in the United States during the 1960s (personal experience, RBL). Most of the Acinetobacter strains came from this group of patients, but other patients in the burn wards also became seeded. There were 14 strains of the other species, A. lwoffii, recovered. This incidence was consistent with recoveries of this organism made in recent years. It is probably essentially an endogenous species.

Two relatively unusual species of Klebsiella, K. oxytoca and K. ozaenae, were recovered in relatively large numbers in sputum. Klebsiella oxytoca was twice recovered from blood. This species was, until the last 5 years, extremely rare in the experience of this Institute. It has become relatively more common in incidence and occasionally is recovered in septicemia.

A species of Enterobacter, E. agglomerans, which was extremely rare in burn patients prior to 1978, was recovered in the highest incidence yet seen in this Institute. Enterobacter agglomerans is a part

Table 1. Unusual Gram-Negative Species Recovered from Clinical Bacteriology Specimens
1 October 1979 - 30 September 1980

	Source and Number of Isolates											
	Wound			Respiratory Tract			Catheter			Xeno-		
	Swab	CP*	Blood	Throat	Sputum	Urine	IV	Foley	Biopsy	grafts	Stool	Total
Pseudomonas fluorescens	0	0	0	0	1	0	0	0	0	2	0	3
Pseudomonas putida	1	0	0	0	0	0	0	0	0	3	0	4
Pseudomonas maltophilia	0	0	0	0	2	0	0	0	0	1	0	3
Pseudomonas cepacia	0	0	1	0	3	0	0	0	0	1	0	5
Pseudomonas paucimobilis (2K-1)	0	1	0	0	0	0	0	0	0	0	0	1
Alcaligenes sp.	0	0	1	0	3	0	0	0	0	1	0	5
Flavobacterium sp.	0	1	0	0	0	0	0	0	0	0	0	1
Gp M-3 (Moraxella-like)	0	0	0	0	1	0	0	0	0	0	0	1
Achromobacter xylosoxidans	0	0	1	0	0	0	0	0	0	0	0	1
Acinetobacter anitratus	5	23	1	19	88	4	1	0	4	1	8	154
Acinetobacter lwoffii	0	8	0	0	6	0	0	0	0	0	0	14
Gp 5E-1 (Pseudomonas-like)	0	1	0	0	0	0	0	0	0	0	0	1
Escherichia coli (A-D)	0	0	0	0	0	3	0	0	0	0	0	3
Citrobacter freundii	2	1	0	1	3	1	0	0	0	1	0	9
Citrobacter diversus	1	2	0	0	10	2	0	0	0	1	0	16
Klebsiella oxytoca	3	0	2	2	28	0	0	0	2	0	2	39
Klebsiella ozaenae	1	3	0	1	7	2	0	0	0	0	2	16
Enterobacter agglomerans	0	12	0	1	18	0	0	0	0	2	4	37
Enterobacter gergoviae	0	0	0	0	1	0	0	0	0	0	0	1
Proteus rettgeri	0	0	0	0	0	0	0	0	0	0	4	4
Morganella morganii	3	0	1	0	4	2	1	0	1	0	5	17
Providencia stuartii	0	5	1	1	31	2	1	1	1	0	0	43
Aeromonas hydrophila	2	0	1	2	0	0	0	0	0	0	1	6
Total	18	57	9	27	206	16	3	1	8	13	26	384

* Contact plate

of the normal enteric flora, but it also occurs as a water contaminant. Its relative persistence in the burn patients in this observation period had no obvious explanation, but it was evident that it was transmitted from patient to patient in the burn wards.

In observations made prior to 1970, unusual oxidative gram-negative bacteria were very frequently recovered in burn tissues at autopsy. The autopsy isolates are summarized in Table 2. The species range in 1979-1980 was parallel to the overall collection for this period. There was one major exception: no strains of Acinetobacter anitratus were recovered in autopsy tissues. This species was unusually frequent in antemortem samples, in comparison to previous years, but it failed to survive to the point where autopsy sampling confirmed its presence.

The recovery of Providencia stuartii was very low when compared to the epidemic occurrence of this species. It was, however, significant in contrast to the past 4 years. This species will be observed with especial care, since it has shown exceptional capacity for causing serious epidemic outbreaks.

PRESENTATIONS/PUBLICATIONS - None

Table 2. Unusual Gram-Negative Species Recovered from Autopsy Specimens
1 October 1979 - 30 September 1980

Organism	No. of Patients	Source	No. of Isolates
Pseudomonas fluorescens	2	Spleen, lung, wound	3
Pseudomonas putida	1	Lung, wound	2
Pseudomonas maltophilia	2	Lung, wound	2
Pseudomonas stutzeri	1	Spleen	1
Achromobacter Bio-2	1	Wound	1
Citrobacter diversus	1	IV tip	1
Citrobacter freundii	1	IV tip	1
Klebsiella oxytoca	2	Blood, wound	3
Klebsiella ozaenae	1	Blood, wound	2
Enterobacter agglomerans	1	Wound	1
Gp 5A-2	1	Wound	1
Morganella morganii	2	Blood, spleen, lung, heart	8
Providencia stuartii	3	Blood, spleen, lung, wound, IV tip, heart	13

ANNUAL PROGRESS REPORT

PROJECT NO. 3S161102BS05-00, BASIC RESEARCH

REPORT TITLE: STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE OF TROOPS WITH THERMAL INJURY -- ENZYME PRODUCTION AND VIRULENCE OF PSEUDOMONAS AERUGINOSA RECOVERED FROM SOLDIERS WITH THERMAL INJURY

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

1 October 1979 - 30 September 1980

Investigators:

Virginia C. English, M.A.
Robert B. Lindberg, Ph.D.

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ABSTRACT

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A study of enzyme production by Pseudomonas aeruginosa was instituted in 1978-1979. The study was continued in 1979-1980 with the addition of a technic to detect lecithinase production by P. aeruginosa. Tests were carried out on 461 strains of P. aeruginosa to detect their production of caseinase, lipase, amylase and elastase. Tests to detect lecithinase production were carried out on 353 strains of P. aeruginosa. Production of enzyme and toxin production by P. aeruginosa have been described as associated with virulence. The current study has been designed to determine if differences of enzyme production by P. aeruginosa can be related to the pathogenesis of Pseudomonas infection in the burn patient.

Several attempts to demonstrate collagenase and hyaluronidase by P. aeruginosa have proved unsuccessful. Efforts to develop reliable and reproducible technics to demonstrate the production of these enzymes by P. aeruginosa will be continued.

Burns
Pseudomonas
Virulence
Enzymes
Humans

STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE OF TROOPS W TH THERMAL INJURY -- ENZYME PRODUCTION AND VIRULENCE OF PSEUDOMONAS AERUGINOSA RECOVERED FROM SOLDIERS WITH THERMAL INJURY

The mechanism by which bacterial invasion of burn patients is effected has not been elucidated. Infection due to Pseudomonas aeruginosa is a major cause of morbidity and death in severe burns, and study of possible mechanisms of invasive infection by this organism has been under way.

Bacterial enzymes, and particularly proteases, have been thought to play a role in the pathogenesis of P. aeruginosa infections. In a previous report (1), some of the enzymes of P. aeruginosa with pathologic implications have been summarized.

During 1979-1980, 461 isolates of P. aeruginosa from burn patients were examined for their ability to produce caseinase, amylase, elastase and lipase. Lecithinase production was assessed on 353 isolates of P. aeruginosa. Plate assay technics were used in detection of enzyme production. This procedure is relatively simple, and also reliably duplicable. The technic involves a base medium which will support optimal growth of the species being tested. A suitable substrate for detection of the enzyme being sought is incorporated in the base medium. The presence of enzyme is demonstrated by detecting degraded substrate. During this study period, lecithinase was added to the list of enzymes sought. Substrate of Tryptic Soy Agar (Difco) containing enriched egg yolk (Difco) 10% v/v showed an almost chalky zone of opacity around a point inoculum of lecithinase-producing organism. The media and substrates used in plate assays of caseinase, lipase, elastase, and amylase have been detailed previously (1).

The results of tests performed on 461 P. aeruginosa organisms are shown in Table 1. Caseinase, elastase and lipase were produced by 98%, 86% and 96%, respectively, of the isolates tested. Lecithinase was produced by 93% of 353 organisms tested. Amylase was not produced by any of the organisms tested. Comparison of individual isolates failed to show a predominant pattern of enzyme production. Those isolates which failed to produce one or more enzymes (excluding amylase) were distributed at random among the 461 isolates tested. Isolates from an individual patient showed a tendency toward like patterns of enzyme production, but dissimilar patterns were found also.

Bacto-Pseudomonas Aeruginosa Antigen, types 1-17 (Difco), were included in this study. It is noted, from Table 2, that only host strain number 14 failed to show caseinase production. Host strain

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1. English VC, Lindberg RB: Enzyme production and virulence of Pseudomonas aeruginosa recovered from soldiers with thermal injury. USAISR Annual Report FY 1979, BAMC, Ft Sam Houston, Texas, pp 179-183.

Table 1. Summary of Enzyme Production by 461 Pseudomonas Isolates 1979-1980

	Enzyme				
	Caseinase	Amylase	Elastase	Lipase	Lecithinase
No. of isolates tested	461	461	461	461	353
No. of tests positive	454	None	400	445	330
% of isolates positive	98	0	86	96	93

number 17 was the only non-lecithinase producer. Twelve of the 17 host strains failed to show elastase production. None of the host strains produced amylase or lipase.

Attempts to establish a plate assay method for hyaluronidase production have not been successful. Attempts to utilize the basic concept of a colorimetric technic in a simpler plate assay are now under investigation.

Insoluble collagen (Sigma), suspended in warm Tryptic Soy Agar (Difco) prior to solidification, was used as a medium for the detection of collagenase production. The collagen particles were not degraded by the Pseudomonas tested or a control organism. Tryptic Soy Broth (Difco) containing semi-soluble collagen (Millipore Corporation) was also used as a substrate in an attempt to detect collagenase production. None of the Pseudomonas or control organisms degraded the collagen.

The agar plate assays used to detect amylase, lipase, caseinase, elastase and lecithinase are simple, yet reliable and reproducible. A technic for demonstration of hyaluronidase production will be soon available. Thus far, production of collagenase has not been demonstrated by plate methods devised in this laboratory. Methods other than agar plate technic for collagen production are under investigation.

Virulence studies are planned for selected isolates from the collection of Pseudomonas aeruginosa assayed for enzyme production.

PRESENTATIONS/PUBLICATIONS - None.

Table 2. Pattern of Enzyme Production by Pseudomonas aeruginosa Antigens - Serotypes 1-17

	Enzyme				
Serotype	Caseinase	Lipase	Amylase	Elastase	Lecithinase
1	+	-	-	+	+
2	+	-	-	+	+
3	+	-	-	-	+
4	+	-	-	-	+
5	+	-	-	-	+
6	+	-	-	-	+
7	+	-	-	-	+
8	+	-	-	-	+
9	+	-	-	-	+
10	+	-	-	-	+
11	+	-	-	+	+
12	+	-	-	-	+
13	+	-	-	-	+
14	-	-	-	-	+
15	+	-	-	-	+
16	+	-	-	+	+
17	+	-	-	+	-

ANNUAL PROGRESS REPORT

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Counterimmunoelectrophoresis (CIE) has been found to be a valuable diagnostic tool in several infectious diseases. Presence of bacteria has been demonstrated by this method in body fluids and in some instances in broth culture supernates examined far sooner than the time at which bacteria could be demonstrated. Bacterial antigen determination by CIE has been successful with body fluids including urine, saliva, and cerebrospinal, pleural, joint, peritoneal and pericardial fluids. Among bacterial antigens, Pseudomonas aeruginosa has been thus demonstrated. The CIE technic has been applied to detection of Pseudomonas antigen in experimental burn wound sepsis. The objective is the development of an effective, rapid, sensitive and specific diagnostic technic for clinical illness. Pseudomonas aeruginosa was detected using the 17 antisera used in the international typing system. Proposed directions of investigation include diagnostic detection of antigen in body fluids, determination of early antibody appearance and, in appropriate circumstances, identification of clinical isolates.

Burns
Pseudomonas
Gel precipitations
Serology
Counterimmunoelectrophoresis

STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE OF TROOPS WITH THERMAL INJURY -- APPLICATION OF COUNTERIMMUNOELECTROPHORESIS FOR DETECTION OF PSEUDOMONAS AERUGINOSA IN BURNED SOLDIERS

Counterimmunoelectrophoresis (CIE) has been described as a promising diagnostic tool for rapid diagnosis of infectious diseases (1). The method is rapid, simple, economical and accurate. Identification of bacteria is based on the detection of bacterial antigen present in both culture supernatants and in body fluids. Detection of microbial antigens by CIE in body fluids such as serum, tears, saliva, urine, cerebrospinal fluid and pleural, joint, peritoneal and pericardial fluids has been successful (1-3). Identification of microbial antigens has included those associated with Streptococcus pneumoniae, several groups of Neisseria meningitidis. Haemophilus influenzae, type B, Klebsiella pneumoniae, Escherichia coli, Staphylococcus aureus, Streptococcus, groups B and D, and Pseudomonas aeruginosa (1-4). The usefulness of the technic is not, however, limited to the detection of bacterial antigen. A variety of microbiological applications are possible by simple modification of the procedure (5). The basic methodology of CIE described by Hill et al (6) was employed in this laboratory in a preliminary study for detection of P. aeruginosa antigen. If the method can be verified in Pseudomonas sepsis, it could be of great value in rapid diagnosis of sepsis in burned patients.

MATERIALS AND METHODS

Bacto-Pseudomonas Aeruginosa Antisera for types 1-17 (Difco) were used with appropriate Pseudomonas Aeruginosa Antigens (Difco) to

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1. Anhalt JP, Kenny GE, Rytel MW: Detection of microbial antigens by counterimmunoelectrophoresis. Cumulative Techniques & Procedures in Clinical Microbiology (CUMITECH) No. 8, TL Gavan, coordinating ed. American Society for Microbiology, Washington, DC, 1978, pp 1-11.
2. Ogunbi O, Odugbemi TO: Counterimmunoelectrophoresis technique in laboratory diagnosis of bacterial meningitis. Trop Geogr Med 28: 141-144, 1976.
3. Bartram CE, Crowder JG, Beeler B, White A: Diagnosis of bacterial diseases by detection of serum antigens by counterimmunoelectrophoresis, sensitivity, and specificity of detecting Pseudomonas and pneumococcal antigens. J Lab Clin Med 83:591-598, 1974.
4. Durfee KK, Marymont JH, Sarachek A, Smith JP: Detection of soluble group A streptococcal antigen in broth culture. Am J Clin Pathol 72:836-840, 1979.
5. Moody GJ: Methodology and applications of counterimmunoelectrophoresis in microbiology. Laboratory Practice 25:575-580, 1976.
6. Hill HR, Riter ME, Men[illegible] SK, Johnson DR, Matsen JM: Rapid identification of group B streptococci by counterimmunoelectrophoresis. J Clin Microbiol 1:188-191, 1975.

demonstrate antigen-antibody reaction. CIE was carried out on glass microscope slides (1" x 3") which had previously been covered with 3 ml of 1% agarose (Fisher Scientific Company) in barbital buffer (pH 8.8; Gelman). Wells of 2 mm diameter were cut in parallel rows to contain antigen and antibody. The wells were separated by 2 mm of agar. Antisera were diluted 1:10, 1:20 and 1:40. Aliquots of 10 µl of each dilution were added to the wells nearest the cathode. Figure 1 depicts a slide prepared for CIE. The electrophoresis chamber was filled to a depth of approximately 1/2" with barbital buffer. Strips of Whatman No. 1 filter paper were used for wicks. Electrophoresis was carried out in a Gelman electrophoresis apparatus at room temperature for 30 minutes using 5 to 7 mA per slide. After electrophoresis was completed, the agarose was flooded with saline and cooled to 4° C.

RESULTS

Oblique and dark-field illuminations were used for detection of the precipitin lines indicating antibody-antigen reactions. The precipitin lines were detected more easily with a magnifying lens. Located between the wells of antigen and corresponding antibody, the line of precipitin was either straight or slightly arced. As Table 1 indicates, an antibody dilution of 1:10 was capable of detecting the corresponding antigen in each of the 17 serotypes used. The precipitin lines were heavy, and no difficulty was encountered in detecting them. Antisera dilutions of 1:20 were capable of detecting antigen of serotypes 1, 11, and 14. There was no visible precipitate at dilutions greater than 1:20

Table 1. Precipitin Reactions of Pseudomonas Antigen-homologous Antisera (Types 1-17) as Determined by Counterimmunoelectrophoresis

	Antisera Dilution		
	1:10	1:20	1:40
Serotypes which gave precipitin reaction with antigen	1-17	1,11,14	None

DISCUSSION

Application of CIE to the microbiology of the burn patient requires certain modifications of the basic technic. Generally, any fluid may be tested directly for antigen. If antigen is demonstrable

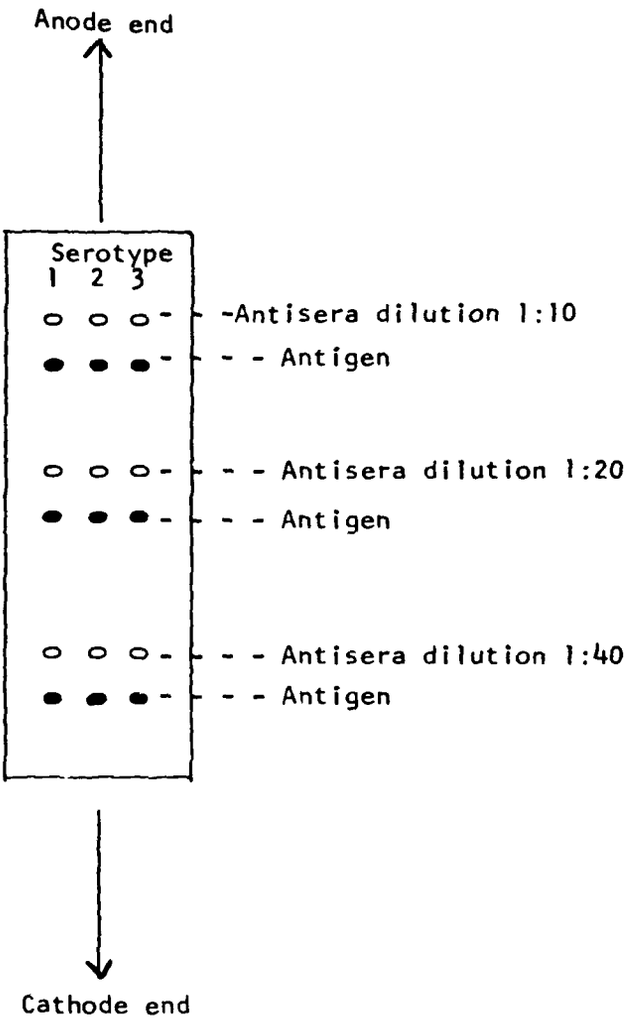

Figure 1. Diagram of an agarose covered slide prepared for CIE.

under confirmed conditions of clinical illness, rapid diagnosis is possible. Antigens present in body fluids below the sensitive level may be detected, when the causative organism is present, in the supernatant of a 3/4-hr broth culture of the specimen. Utilizing a known antigen, quantitative antibody titer determination of bacterial antigen in patient sera should be possible using CIE. The role of P. aeruginosa antigen or specific antibody in sera of burn patients has not been clearly established. The correlation of recovery rates with the presence and amount of antibody in patient sera may prove a valuable diagnostic tool. Finally, morbidity and mortality may be predictable through correlation with the presence and quantity of serum antigen as measured by CIE.

PRESENTATIONS/PUBLICATONS - None.

ANNUAL PROGRESS REPORT

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Metal-sulfonamide compounds were shown to rank in order of therapeutic effectiveness of metallic ion: chromium, manganese, zinc, copper, and, in last place, cerium. Silver sulfadiazine as a topical agent was less effective than the other metals. Quantitative tissue assays showed suppression of bacterial proliferation in subeschar tistue to occur with all five metal-sulfadiazine compounds. Comparison of virulence tests showed a marked drop in killing ability of strains collected in 1978-1979, when compared to lethality of Pseudomonas aeruginosa isolated in 1961-1965. This drop in virulence was paralleled with less invasive behavior. The 17-strain international typing set was tested for virulence; three highly virulent types and six types with moderate virulence were demonstrated.

Pseudomonas
Topical therapy
Burns
Burn wound sepsis

STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE OF TROOPS WITH THERMAL INJURY -- DEVELOPMENT OF PROPHYLACTIC TOPICAL THERAPY FOR USE ON BURN WOUNDS OF MILITARY PATIENTS: SEARCH FOR IMPROVED FORMULATIONS

Study of metal-sulfonamide complexes as potential agents for topical therapy in control of invasive burn wound infection has been continued. Five compounds -- zinc sulfadiazine, chromium sulfadiazine, copper sulfadiazine, chromium sulfadiazine and manganese sulfadiazine -- have been prepared and made up in 1% concentration in a standard water dispersible cream formulation to be tested as topical agents on burned rats. The animals were seeded with appropriate challenge strains of Pseudomonas aeruginosa. In terms of relative effectiveness, the metal ions ranked as follows: chromium first, then manganese, zinc, copper and cerium. Strain differences associated with specific challenge strains were found and were most extensive with the highly virulent challenge strains. At this Institute, strain 12-4-4 has been extensively used as a challenge in a wide variety of chemotherapy studies. With strain 12-4-4, all metal-sulfadiazine compounds tested were effective, in a range from 96.7% survival for manganese to 88% survival for cerium. However, when more virulent challenge strains were used, differences were much wider. With strain 8-28-3, survival ranged from 93% for chromium to 33% for cerium. When the highly virulent VA-134 was used, survival ranged from 64% for chromium to zero for cerium.

Silver-sulfadiazine, used as a therapeutic control for these compounds, achieved survival rates of 86.3% for strain 12-4-4, 84.7% for 8-28-3, and 38% for strain VA-134. Thus it was less effective than the optimal experimental formulations which were prepared for testing in this series.

This work was extended to assessment of therapeutic activity by determining bacterial content of rat wound tissues and viscera at intervals post-seeding and post-treatment. The tissues selected were subeschar muscle frum the scapular region, and a sample of liver tissue. Tissues were collected from 3 to 12 days post-seeding. Treatment was started at 24 hours post-burn and post-seeding.

Results of these quantitative studies are thus far consistent with the data on survival rates of metal-sulfadiazine treated rats. However, not enough samples have yet been collected for analysis, due to a prolonged interruption in animal studies while renovation of experimental animal quarters was carried out. These studies are to be completed during the next year, and at the same time, blood-metal ion levels are to be obtained. This will answer some of the questions regarding safety that will arise before a valid clinical trial of new compounds can be proposed.

CHANGES IN VIRULENCE OF PSEUDOMONAS AERUGINOSA FOR THE BURNED RAT

The only animal model that has permitted study of Pseudomonas burn wound sepsis and made possible methods for its control has been the

burned, seeded rat. This model made possible the development of Sulfamylon burn cream, evaluation of other modalities for control of burn wound sepsis, and evaluation of chemotherapeutic agents and immunologic approaches to the control of Pseudomonas infection. One critical feature of this procedure is the availability of stable, virulent challenge strains of P. aeruginosa which would kill predictable numbers of seeded, unprotected animals at a consistent rate. As a corollary of recovering such challenge strains, an intermittent sampling of P. aeruginosa strains recovered from burn patients has been carried on. Not only the incidence of invasive infection but qualitative differences in pathogenesis of the infectious lesion in the burned rat have been elucidated by this procedure. In the past 5 years, major, long-term changes in the animal virulence of populations of P. aeruginosa strains from patients on the burn wards have been demonstrated. Results of virulence tests for the years 1977-1979 are shown in this report, and a comparison with the behavior of strains tested from 1959 to 1965 is shown.

The assessment of virulence was made by seeding the surface of a 20% body surface scald burn on the clipped dorsum of 200 gram rats. The strains to be tested were fresh isolates from clinical specimens, with emphasis on blood stream, sputum and tissue biopsy recoveries. The strains were frozen at -70° C and stored in sterile milk at -70° C in multiple aliquots. A thawed strain was inoculated into trypticase soy broth and grown 20 to 22 hours. The inoculum was in the amount of 10^8 cells of the culture, spread over the burned area with a cotton swab. The animals were observed for survival for 21 days post-burn.

The sources of the strains tested are shown in Table 1. Of the 182 strains tested, 77 came from blood cultures, 28 from sputum or post-mortem lung tissue, and 37 from biopsy or post-mortem subeschar burn wound tissue.

In Table 2, the lethality of 189 test strains, over the 1977-1979 period, is summarized. The largest percentage in each year was entirely avirulent; no seeded rats died. In 1977, the next largest increment was the zone in which the strains were lethal for 11% to 20% of the rats. This comprised 26.1% of the strains in 1977, 18.1% in 1978; and in 1979, only 4.9% of the strains tested were in this group. Smaller numbers of strains fell into groups of increasing virulence, up to 61%-70% lethality. There were no strains that killed 71% to 90% of the animals. Seven strains in the 3-year period would qualify as completely lethal; the zone heading was 91%-100%, but these strains were almost completely lethal.

The drop in proportion of strains which would be rated as highly lethal was in some degree associated with a variability in test results which played an increasing role with the passage of time. Initially, strains which killed no animals were rechecked once. If no deaths occurred on the second test, they were classified as nonvirulent. Strains which killed one or more rats on the initial five- or six-rat test group were retested. The percentage of lethality was assessed on

Table 1. Sources of Isolates of Pseudomonas aeruginosa Assessed for Virulence, 1977-1979

Source	Year 1977	1978	1979	Totals
Blood	26	25	26	77
Sputum	9	2	7	18
Biopsy	3	3	11	17
Wound surface	6	6	11	23
Urine	2	1	3	6
Post-mortem:				
Blood	4	1	1	6
Liver & spleen	5	0	0	5
Lung	8	2	0	10
Wound	18	1	1	20
Totals	81	41	60	182*

* A total of seven strains above this number were tested, but information regarding source was not available.

the basis of two or more tests; hence an initial virulent result could be diluted down if in subsequent tests killing did not occur. This process occurred with some strains. With others, initial virulence was demonstrated readily on repeat tests. Table 3 presents patterns of the two modes of behavior.

It may be seen from Table 3 that the "consistent" cultures gave essentially identical lethality on successive tests. The "inconsistent" ranged in successive tests from complete virulence to complete avirulence. The strain was not altered in storage, and the reconstituted challenge did not vary. Further study of this phenomenon is planned. Such fluctuations in virulence would diminish the validity of many of the therapeutic applications of the virulence test as it has been applied here.

It was pointed out earlier that recent tests for virulence of P. aeruginosa have not shown any resemblance to earlier experiences with this procedure. Table 4 summarizes part of the experience with this procedure. Results of virulence tests on 72 strains examined between 1961 and 1965 are compared with the findings on examining 189 strains between 1977 and 1979. The marked difference in incidence of virulent and nonvirulent strains is at once obvious. Only 11 out of 72 strains in the earlier group were entirely nonvirulent. In 1977-1979,

Table 2. Lethality of Pseudomonas aeruginosa Isolates from Burned Rats, 1977-1979

Year	Percent died in test group: No. of strains and % of total										
	0	1-10	11-20	21-30	31-40	41-50	51-60	61-70	--	91-100	Total
1977											
No. of strains	40	2	22	2	3	6	5	2	--	2	84
% of total	47.6	2.3	26.1	2.3	3.5	7.1	5.9	2.3		2.3	
1978											
No. of strains	26	2	8	0	3	2	2	1	--	0	44
% of total	59.0	4.5	18.1		6.8	4.5	4.5	2.2			
1979											
No. of strains	44	5	3	1	2	0	1	0	--	5	61
% of total	72.1	8.1	4.9	1.6	3.2		1.6			8.1	
3-year total	110	9	33	3	8	8	8	3	--	7	189
% of total	58.2	4.7	17.4	1.5	4.2	4.2	4.2	1.5		3.7	

Table 3. Examples of Consistent and Inconsistent Behavior of Virulence Tests of *Pseudomonas aeruginosa* on Burned Rats

Behavior pattern	Year	Strain No.	Deaths/Total per test	Total	% Lethality
Consistent	1977	1-17-27:	3/7; 3/6; 3/7	9/20	45.0
		4-1-36:	3/7; 4/7; 3/5	10/19	52.6
	1979	10-12-10:	5/5; 8/9; 4/4	17/18	94.4
Inconsistent	1977	5-14-1:	4/5; 3/5; 0/6	7/16	43.7
		6-14-2:	5/6; 1/5; 2/6; 3/7; 1/5; 1/5	13/34	38.2
	1978	12-15-1:	3/6; 5/5; 5/6; 1/5; 0/5; 0/5	14/32	43.7
	1979	2-17-5:	4/4; 0/6; 0/5	4/15	26.6
		9-17-29:	4/5; 0/9; 0/5	4/19	21.0

Test conditions: Subcultures from blood agar quick-frozen and stored in glass at -70° C. Reconstituted in broth, cultured 20 hours to seeding.

Table 4. Virulence of Pseudomonas aeruginosa: 72 Strains from 1961-1965; 189 Strains from 1977-1979

Degree of virulence	Year								
	1961	1962	1963	1964	1965	--	1977	1978	1979
100% virulence	1	2	4	5	10	--	2	0	5
Highly virulent 50%-99%	2	2	3	4	7	--	7	3	1
Moderately virulent 1%-49%	2	0	3	5	11	--	35	15	11
Nonvirulent	3	0	0	6	2	--	40	26	44
Totals	8	4	10	20	30	--	84	44	61

110 out of 189 strains were completely nonvirulent. In the completely virulent category, 22 out of 72 strains were 100% virulent, while only seven out of 189 were in this category in 1979.

It was evident that a marked change in incidence of virulence had occurred between these two time intervals. An explanation for this difference is not presently available. Various attributes of the strains are being studied to assess the possibility that a demonstrable difference can be associated with the virulence-nonvirulence classification. Such a factor, or factors, has not been demonstrated up to the present time. It is, of course, presumed that the difference is based on strain differences. A change in basic elements of the test, including rat susceptibility, is another possibility.

A group of cultures which have been handled in a manner quite removed from the procedure described for burn ward isolates is the collection of International Pseudomonas aeruginosa Typing Cultures. The virulence of these strains was assessed by the technic used for other Pseudomonas strains. The variations in death rates with successive samplings are shown in detail in Table 5. The degree of virulence in these strains, which had been carried on routine culture media for years in some instances, was unexpected. Only five of the 17 strains, types 9, 12, 15, 16, and 17 were nonvirulent. A low level of virulence was apparent with types 1, 4, 5, 6, 8, and 10. With these strains, the highest kill level occurred with one experiment with type 1, in which three of six rats died. There were four experiments in which all rats survived. The other low virulence strains had not more than one

Table 5. Virulence of *Pseudomonas aeruginosa* International Serotype Strains for Burned Rats

Type	Experiment no. and deaths/total tested							Totals	
	1	2	3	4	5	6	7	No. died/total	%
1	3/6	1/10	0/6	0/6	1/9	0/4	0/5	5/46	10.8
2	4/6	0/5	0/5	-	-	-	-	4/16	25.0
3	6/6	6/7	6/6	9/9	5/5	5/5	-	37/38	97.3
4	0/5	1/5	0/6	-	-	-	-	1/16	6.25
5	0/5	1/5	0/6	-	-	-	-	1/16	6.25
6	0/5	1/6	-	-	-	-	-	1/11	9.0
7	1/3	1/6	5/5	2/9	0/5	0/5	-	9/33	27.2
8	0/5	1/5	0/5	-	-	-	-	1/15	6.6
9	0/4	0/6	-	-	-	-	-	0/10	0
10	0/5	1/5	0/5	0/7	-	-	-	1/22	4.5
11	4/5	3/5	3/6	-	-	-	-	10/16	62.5
12	0/4	0/4	-	-	-	-	-	0/8	0
13	3/4	1/5	4/6	2/4	-	-	-	10/19	52.6
14	1/5	0/4	5/6	0/6	0/5	0/5	-	6/31	19.3
15	0/5	0/5	-	-	-	-	-	0/10	0
16	0/4	0/5	-	-	-	-	-	0/9	0
17	0/6	0/6	-	-	-	-	-	0/12	0
Controls Strain 12-4-4-59	3/4	5/6	7/7	5/5	5/5	5/5	4/5	34/37	91.8

animal die in a single experiment. Types 2, 7, and 14 showed total kills in the 20% to 30% range. When the lethality of individual experiments was considered, a high degree of variation occurred. With type 2, four of six animals died in one trial. With type 7, all animals died in one trial, while in three other trials one or two animals died. Type 14 had one trial with five of six animals dead, but n four other trials, none died. A high level of virulence was seen with types 3, 11, and 13. Type 3 was completely lethal in five of six trials. Type 11 killed in the 50% to 80% range. Type 13 showed a fluctuation in kill rate, from 75% of one set of rats down to 20% in another. The fluctuations in survival in successive experiments resembled, with several strains, the pattern that was observed in clinical isolates from 1977 through 1979. The similarity in pattern with such diverse strains suggests that a factor is present which may represent variation in the test substrate, i.e., the burned rat itself.

The persistence of virulence in long-established strains, such as serotype 3 and 11, is significant. It suggests that virulence may be due to more than one factor: a labile factor which can be readily lost by minimal adverse environment, and a stable factor which tolerates extensive subculturing and, essentially, neglect. The fact of virulence loss in recent populations of P. aeruginosa is evident, and calls for an explanation. Monitoring will continue using this protocol; there is as yet no more reliable method for detecting virulent strains. Variations in handling of P. aeruginosa strains which have been held at -70° C are being set up, to determine if possible a manipulative basis for some of the changes observed.

PUBLICATIONS/PRESENTATIONS - None.

AD-A107 093 ARMY INST OF SURGICAL RESEARCH FORT SAM HOUSTON TX F/G 6/5
US ARMY INSTITUTE OF SURGICAL RESEARCH ANNUAL RESEARCH PROGRESS--ETC(U)
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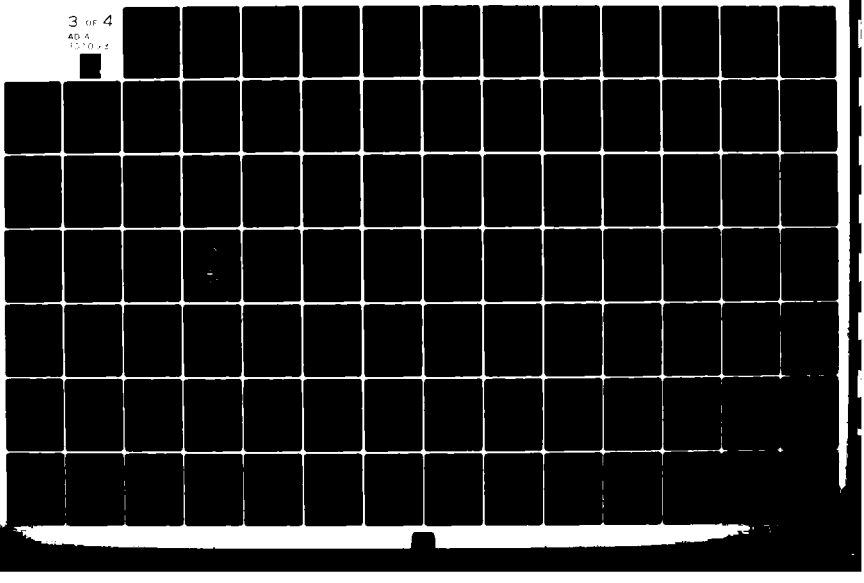

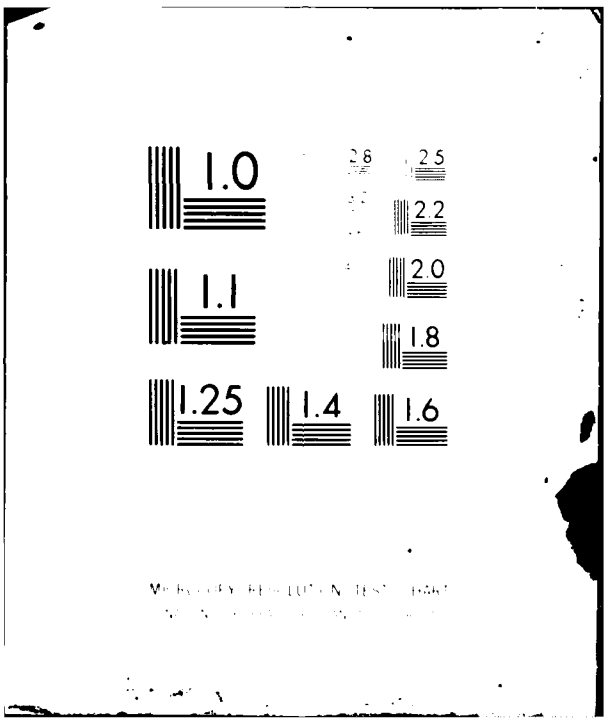
1.0
2.8
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2.2
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2.0
1.8
1.25
1.4
1.6

animal die in a single experiment. Types 2, 7, and 14 showed total kills in the 20% to 30% range. When the lethality of individual experiments was considered, a high degree of variation occurred. With type 2, four of six animals died in one trial. With type 7, all animals died in one trial, while in three other trials one or two animals died. Type 14 had one trial with five of six animals dead, but in four other trials, none died. A high level of virulence was seen with types 3, 11, and 13. Type 3 was completely lethal in five of six trials. Type 11 killed in the 50% to 80% range. Type 13 showed a fluctuation in kill rate, from 75% of one set of rats down to 20% in another. The fluctuations in survival in successive experiments resembled, with several strains, the pattern that was observed in clinical isolates from 1977 through 1979. The similarity in pattern with such diverse strains suggests that a factor is present which may represent variation in the test substrate, i.e., the burned rat itself.

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PUBLICATIONS/PRESENTATIONS - None.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OG 6969	01 Oct 80	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. RTG/DNG	8b. DISP'N INSTR'N	8c. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
01 Oct 79	D. CHANGE	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	BB	302
b. XXXXXXXXX	61102A	3S161102BS05	00	087
c. XXXXXXXXX	STOG - 80-7.2:5			

11. TITLE (Procede with Security Classification Code)
(U) The Study of Metabolism and Nutritional Effects of Burn Injury in Soldiers (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS
003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
Oct 76	Cont	DA	C. In-House

17. CONTRACT/GRANT: Not Applicable
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER:
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
PRECEDING FISCAL YEAR 1980	6.0	$ 264
CURRENT FISCAL YEAR 1981	6.0	$ 315

19. RESPONSIBLE DOD ORGANIZATION
NAME: US Army Institute of Surgical Research
ADDRESS: Ft Sam Houston, Texas 78234

RESPONSIBLE INDIVIDUAL
NAME: Basil A. Pruitt, Jr., MD, COL, MC
TELEPHONE: 512-221-2720

20. PERFORMING ORGANIZATION
NAME: US Army Institute of Surgical Research
Surgical Study Branch
ADDRESS: Ft Sam Houston, Texas 78234

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME: Cleon W. Goodwin, Jr., M.D.
TELEPHONE: 512-221-2968
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME:
NAME: DA

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Nitrogen Balance; (U) Burn Injury; (U) Temperature Regulation; (U) Metabolism; (U) Humans; (U) Animal Model

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) To identify afferent and efferent mediators of postinjury hypermetabolism and altered thermoregulation in burned soldiers. To define alterations and control of blood flow to the wound and various organs of the body. To describe the effects of thermal injury on endocrine function and metabolism of proteins, carbohydrates and fats.

24. (U) An environmental chamber serves as an experimental laboratory to monitor thermoregulatory and metabolic alterations o. burn patients. Limb plethysmography is employed to assess the control of wound circulation in patients. An injured animal model was also developed to characterize the control of peripheral circulation after limb trauma. Arterial and venous blood analysis are conducted in both patients and models to measure turnover rate of various substrates across different regional beds.

25. (U) 7910 - 8009. Studies of splanchnic blood flow in thermally injured patients reveals that hepatic glucose production rises with injury, increases even more when the patient becomes bacteremic and falls markedly with progressive clinical deterioration. An increase in hepatic glucogenic and gluconeogenic uptake presumably support the rise in liver glucose output. Splanchnic perfusion is elevated in the hypermetabolic burn patient and not significantly altered by the advent of systemic infection. These data suggest that alterations in hepatic glucose metabolism is not a function of variations in regional perfusion. Preliminary work has shown that a full thickness burn covering 25% of the total body surface causes an increase in metabolic rate and catecholamine excretion of 20-40 ng goats. As these responses are comparable to those of the burn patient, additional studies are planned to more completely describe the characteristics of this model. A large animal respiration chamber, currently under construction, will permit long term metabolic studies under controlled environmental conditions (temperature, humidity, light and noise).

(U) Unclassified when separated from classified portion.

ANNUAL PROGRESS REPORT

PROJECT NO. 3S161102BS05-00, BASIC RESEARCH

PROJECT TITLE: THE STUDY OF METABOLISM AND NUTRITIONAL EFFECTS OF BURN INJURY IN SOLDIERS - STUDIES OF DISTURBANCE OF PROTEIN TURNOVER IN BURNED TROOPS: USE OF AN ANIMAL MODEL

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

1 October 1979 - 30 September 1980

Investigators:

Wanda L. Brown, M.S.
Eleanor G. Bowler, Ph.M.
Arthur D. Mason, Jr., M.D.

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3S161102BS05-00, BASIC RESEARCH

PROJECT TITLE: THE STUDY OF METABOLISM AND NUTRITIONAL EFFECTS OF BURN INJURY IN SOLDIERS - STUDIES OF DISTURBANCE OF PROTEIN TURNOVER IN BURNED TROOPS: USE OF AN ANIMAL MODEL

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

Period covered in this report: 1 October 1979 - 30 September 1980

Investigators: Wanda L. Brown, M.S.
Eleanor G. Bowler, Ph.M.
Arthur D. Mason, Jr., M.D.

Reports Control Symbol MEDDH-288(R1)

The mean water content of whole untreated 20% wounds of burned rats (Group BU) increased rapidly after injury. The rate of increase slowed from 6 to 12 hours postburn, then rose to a maximum at 24 hours postburn. Afterwards the mean water content decreased slowly, but at 144 hours postburn was still 4.3 ml greater than that of whole untreated wounds of sham rats (Group SU).

The mean water content of wounds treated with hyaluronidase (Group BHY) increased very little after 6 hours postburn.

The mean water contents of untreated sham wounds (Group SU) and of hyaluronidase-treated sham wounds (Group SHY) were similar over the 144-hour period of study.

The mean water contents of wounds of burned rats (BU + BHY) were significantly higher than of those of unburned controls (SU + SHY) throughout the time of study ($p < 0.001$).

The mean dry weight of wounds of Group BU was significantly higher than that of Group BHY at 24 and 72 hours postburn but not different at other times.

The mean dry weight of the wounds of Group SHY was significantly lower than that of Group SU at 72 and 144 hours postburn but not different at the other times of measurement.

The mean dry weight of wounds of control animals (SU + SHY) was lower than that of burned animals (BU + BHY) throughout the time of study ($p < 0.001$).

The mean water content of the unburned skin of rats in Group BU showed a small, but statistically significant, increase over that of Group SU at 36 hours postburn. At other times the mean water contents of the unburned skin of the two groups were not significantly different.

The mean dry weight of the unburned skin of rats in Group BU was significantly lower than that of Group SU at 24 hours postburn but not different at other times.

We conclude that the speed and magnitude of the postburn increase in interstitial fluid volume is sufficiently great to increase the interstitial pressure to the point at which wound compliance increases and interstitial resistance to further volume increase is markedly diminished.

Dilution of the concentration of long chain hyaluronic acid molecules in the connective tissue and depolymerization of the molecules by hyaluronidase loosen the network of the mucopolysaccharide gel of the connective tissue with the result that movement of fluid through the interstitium is facilitated. Consequently, less fluid was retained in the wounds of the hyaluronidase-treated burned rats.

STUDIES OF DISTURBANCE OF PROTEIN TURNOVER IN BURNED TROOPS: USE OF AN ANIMAL MODEL

An earlier part of this study reported the results of measurements of water contents and dry weights of the wounds of burned and sham burned rats at 6, 24, and 48 hours postburn (1). In this report, we show results obtained from measurements over the interval from 1 hour to 144 hours postburn using the same experimental models.

Groups of 180-200 g Sprague-Dawley rats, anesthetized with sodium pentobarbital, were subjected to 20% body surface full-thickness scald burns or sham burns. At 1 hour postburn, some of the rats from each group were given subcutaneous injections of 0.2 ml hyaluronidase into each of five sites of the wound (total dose 150 N.F. units in 1 ml 0.15 M NaCl). The four treatment groups were:

Group SU: Sham untreated

Group SHY: Sham hyaluronidase-treated

Group BU: Burned untreated

Group BHY: Burned hyaluronidase-treated

The rats were housed in individual cages and permitted free access to food and water.

At the selected time postburn, each rat was anesthetized with methoxyflurane. The tissue within the margins of the burn wound, or within the inked outline of the sham wound, was excised through the paniculus carnosum to fascia. The entire sample, approximately 68 cm^2 surface area at the time of injury, was used for determination of total water and dry weight. This sample will be referred to below as wound tissue whether from burned or sham burned rats.

The tissues were weighed immediately after excision and dried to constant weight at 70° C. Total water was determined from the difference between the wet and dry weights.

CALCULATIONS

In studies of edema, it has been customary to report the water content of tissues as percentages or as the quantity of water per gram of fat-free dry weight in an attempt to compensate for the changes in

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1. Brown WL, Bowler EG, Mason AD Jr: Studies of disturbance of protein turnover in burned troops: Use of an animal model. USAISR Annual Research Progress Report, FY 1977, pp. 103-106.

wet weight which occur. When these values are used as a basis for comparison of burned with normal tissues, error is introduced because the dry weight and fat content as well as the wet weight of the burned tissue vary with time after injury. Because the burned tissue is swollen it is difficult to obtain comparable samples from burn and sham wound using biopsy technics. Because of this we have chosen to use the area of tissue delineated by the margins of the opening in the burning mold at the time of injury as the unit for comparison of the fluid volume changes in the tissue.

Significance of the differences between treatment groups was determined by Analysis of Variance using a computer program which permits comparison of groups of unequal size.

RESULTS

Total water in wound

The rate of increase in water content of the wounds of burned rats was greatest during the first half-hour postburn (Fig. 1). At that time, the wounds of rats of Group BU contained a mean of 3.2 ml more water than did the sham wound. This volume is equivalent to approximately one-third of the normal plasma volume of a 200 g rat.

The increase in water content of wounds of Group BU slowed from 6 to 12 hours postburn before it again began to rise. The maximum water content was attained at 24 hours postburn when the wound of Group BU contained a mean of 8.8 ml more water than the wound of Group SU. The plasma volumes (not shown) of both groups were normal at this time.

After 24 hours postburn the water content of the wounds of Group BU decreased slowly, but at 144 hours postburn the wounds still contained 4.3 ml more water than did the wounds of Group SU.

The mean water content of the wounds of Group BHY increased very little after 6 hours postburn. It was significantly lower than that of Group BU at 24, 48, and 72 hours postburn.

The mean water contents of wounds of sham burned rats (SU and SHY) were similar except at 144 hours postburn when the mean water content of wounds of Group SHY showed a small, but statistically significant decrease.

Mean water contents of the wounds of burned rats (BU + BHY) was significantly higher than that of unburned controls (SU + SHY) throughout the time of study ($p < 0.001$).

Dry weight of wound

The mean dry weight of the wounds of rats in Group BHY was significantly lower than that of Group BU at 24 and 72 hours postburn, but was similar at other times (Fig. 2).

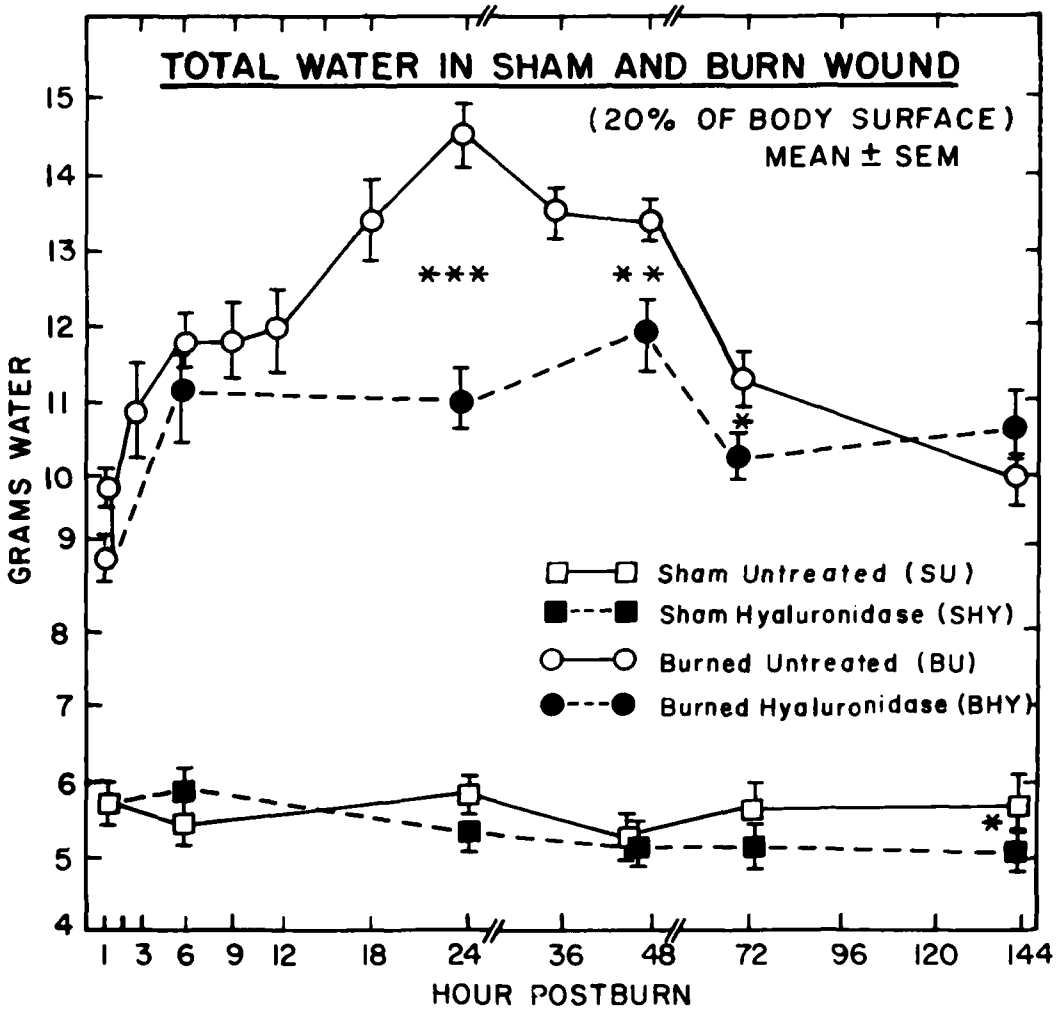
TOTAL WATER IN SHAM AND BURN WOUND
(20% OF BODY SURFACE)
MEAN ± SEM
GRAMS WATER
HOUR POSTBURN
Sham Untreated (SU)
Sham Hyaluronidase (SHY)
Burned Untreated (BU)
Burned Hyaluronidase (BHY)
***
**
*
*
15
14
13
12
11
10
9
8
7
6
5
4
1 3 6 9 12 18 24 36 48 72 96 120 144

Figure 1

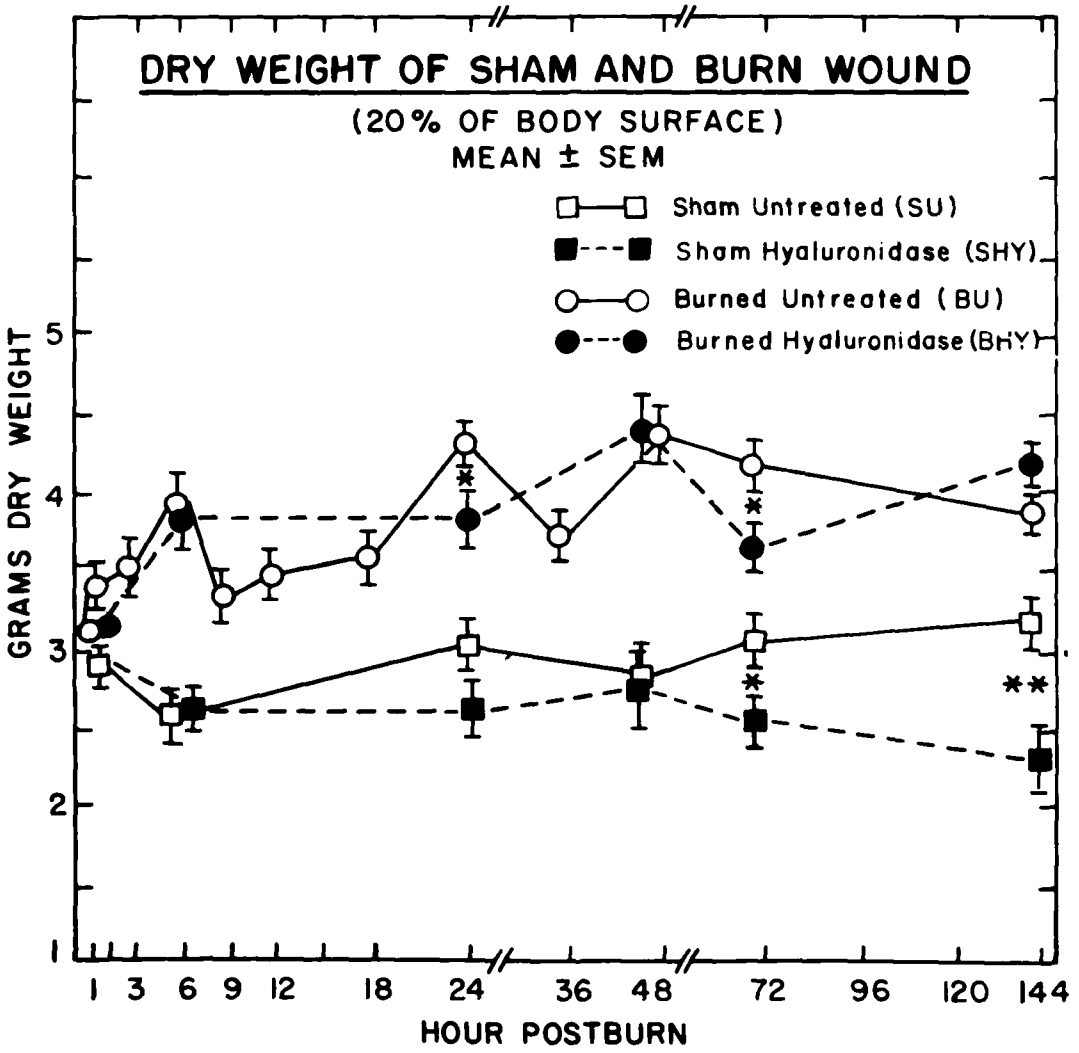
DRY WEIGHT OF SHAM AND BURN WOUND
(20% OF BODY SURFACE)
MEAN ± SEM
Sham Untreated (SU)
Sham Hyaluronidase (SHY)
Burned Untreated (BU)
Burned Hyaluronidase (BHY)
GRAMS DRY WEIGHT
1
2
3
4
5
1 3 6 9 12 18 24 36 48 72 96 120 144
HOUR POSTBURN

Figure 2

The mean dry weight of the wounds of Group SHY was significantly lower than that of Group SU at 72 and 144 hours but not different at the other times of measurement.

The mean dry weight of wounds of unburned controls (SU + SHY) was lower than that of burned rats (BU + BHY) at each time measured ($p < 0.001$).

Unburned skin

The water content of the unburned skin of rats in Group BU was similar to that of rats in Group SU except at 36 hours postburn when the unburned skin of Group BU showed a small, but statistically significant increase in water content (Fig. 3).

The dry weight of the unburned skin of rats in Group BU was significantly lower than that of Group SU at 24 hours postburn but not different at other times.

DISCUSSION

Although "increased capillary permeability" is generally considered to be the underlying cause of edema following burn injury, recent studies have demonstrated that the connective tissue of the interstitium also plays an important role in controlling fluid volume shifts.

This connective tissue has been characterized as a two-phase system in which the mucopolysaccharides form a tight gel which water, but not protein, can penetrate (2). Plasma proteins in the interstitium are contained in free fluid vesicles between the mucopolysaccharide complexes. The two phases are in osmotic equilibrium.

Guyton et al. have shown that interstitial pressure increases in proportion to the increase in fluid volume until the pressure increases 8 to 10 mm Hg. Further increase in volume elicits very little increase in pressure, with the result that fluid moves into the interstitium freely by gravity (3). This point is reached rapidly when fluid volume increases at a rate exceeding that at which it can be removed as lymph. This appears to happen almost instantaneously following burn injury.

The sieving effect of the gel phase is highly dependent upon the

---

2. Gersh I, Catchpole HR: The nature of ground substance of the connective tissue. Perspect Biol Med 3:282, 1960.

3. Guyton AC, Taylor AE, Granger HJ: Pressure volume curves of the interstitial fluid spaces. In Guyton AC, et al. (eds.), Circulatory Physiology II. Dynamics and Control of the Body Fluids, pp 71-86, Philadelphia, W.B. Saunders, 1975.

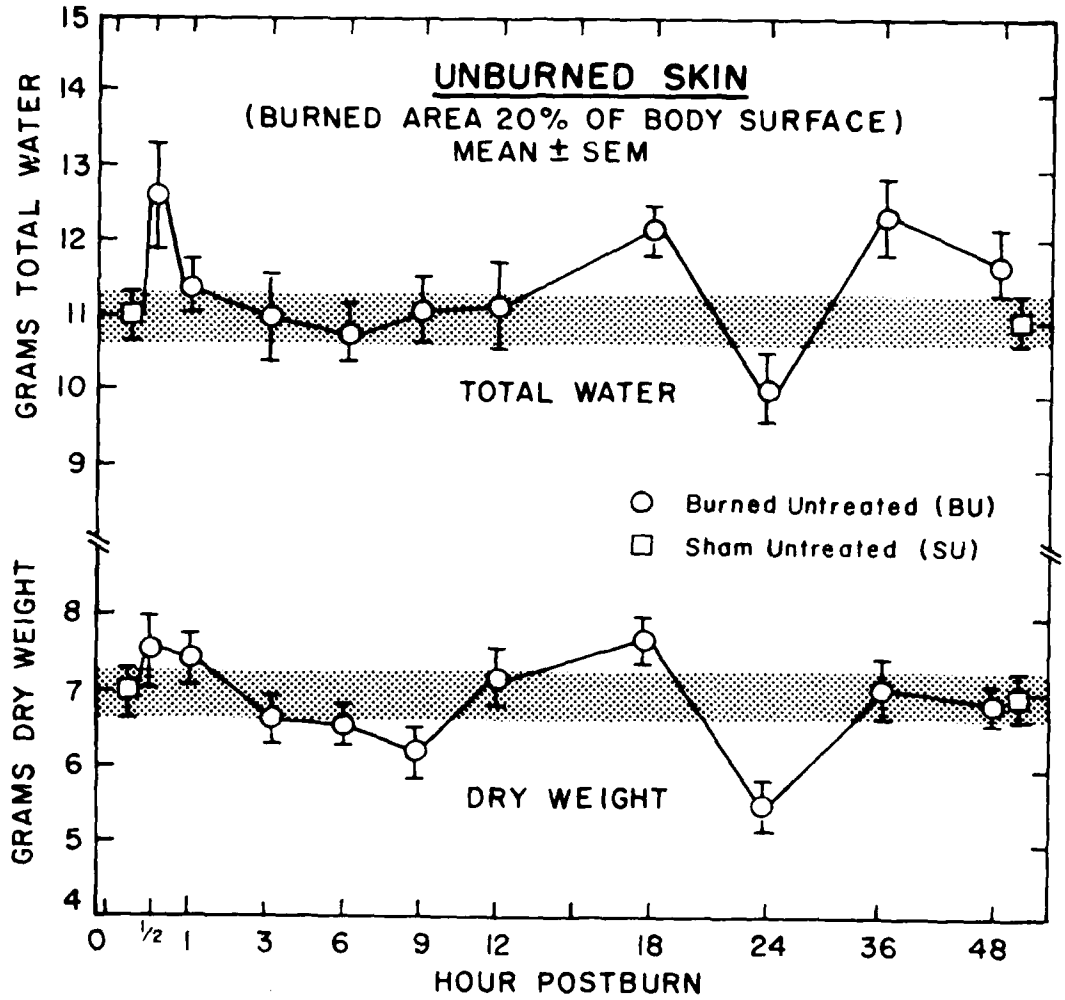
UNBURNED SKIN
(BURNED AREA 20% OF BODY SURFACE)
MEAN ± SEM
GRAMS TOTAL WATER
GRAMS DRY WEIGHT
TOTAL WATER
DRY WEIGHT
Burned Untreated (BU)
Sham Untreated (SU)
HOUR POSTBURN
0
½
1
3
6
9
12
18
24
36
48

Figure 3

concentration of mucopolysaccharide in the tissue. When the concentration of long chain hyaluronic acid molecules is decreased either by dilution (edema) or by depolymerization (by action of hyaluronidase), movement of water through the interstitium is facilitated (4). The free fluid vesicles coalesce to form pools of fluid through which molecules may freely diffuse. Until the concentration of hyaluronic acid is restored to normal, either by removal of fluid or replacement of the hyaluronic acid by synthesis, there is little or no resistance to fluid flow through the interstitium. The prolonged elevation of the water content of the tissue of the burn wound is probably a result of such changes in the physical characteristics of the interstitial tissue. The lower water content of the hyaluronidase-treated burn wound probably reflects accelerated transport of water through the interstitium because there is less restriction to diffusion through the gel in which the concentration of long chain hyaluronic acid molecules has been reduced.

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4. Wiederhielm CA, Fox JR, Lee DR: Ground substance mucopolysaccharides and plasma proteins: Their role in capillary water balance. Am J Physiol 230:1121-1125, 1976.

PRESENTATIONS/PUBLICATIONS - None.

ANNUAL PROGRESS REPORT

PROJECT NO. 3S161102BS05, BASIC RESEARCH

PROJECT TITLE: THE STUDY OF METABOLISM AND NUTRITIONAL EFFECTS ON BURN INJURY IN SOLDIERS - CONTROL OF BLOOD FLOW IN A LARGE SURFACE WOUND

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

1 October 1979 - 30 September 1980

Investigators:

L. Howard Aulick, Ph.D., LTC, MSC
Wallace B. Baze, D.V.M., CPT, VC
Charles G. McLeod, Jr., D.V.M., Ph.D., LTC, VC
Douglas W. Wilmore, M.D.

Reports Control Symbol MEDDH-288(R1)

Unclassified

ABSTRACT

PROJECT NO. 3S161102BS05-00, BASIC RESEARCH

REPORT TITLE: THE STUDY OF METABOLISM AND NUTRITIONAL EFFECTS ON BURN INJURY IN SOLDIERS - CONTROL OF BLOOD FLOW IN A LARGE SURFACE WOUND

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

Period covered in this report: 1 October 1979 - 30 September 1980

Investigators: L. Howard Aulick, Ph.D., LTC, MSC
Wallace B. Baze, D.V.M., CPT, VC
Charles G. McLeod, Jr., D.V.M., Ph.D., LTC, VC
Douglas W. Wilmore, M.D.

Reports Control Symbol MEDDH-288(R1)

To study the factors which control the increased blood flow to a large granulating wound, Doppler flow probes were implanted around the external iliac arteries bilaterally in 20 to 40 kg goats. Following operative recovery and basal measurements, skin was excised from one hind limb. Blood flow in the injured leg of five awake, resting goats rose above that of the uninjured leg by the fourth postoperative day and plateaued at 70 to 90% above uninjured leg flows for the next two weeks. The increase in injured leg blood flow was associated in time with the formation of a highly vascularized wound. This increased blood flow to the injured leg persisted in 11 anesthetized goats studied 9 to 12 days postinjury (186 ± 27 ml/minute versus 107 ± 19, $p < 0.01$, mean $\pm$ SEM). Substrate turnover revealed that elevated blood flow to the injured leg was not the result of increased oxygen consumption, but was associated with increased glucose uptake (7.8 ± 1.1 mg/minute versus 2.7 ± 0.6, $p < 0.001$) and lactate release (3.6 ± 1.3 mg/minute versus 1.1 ± 0.7, $p < 0.05$). Limitations in oxygen delivery failed to explain the increased blood flow to the injured leg, since raising arterial PO_2 or exposing the leg to a high oxygen environment had no effect on limb perfusion. Although lactate and potassium, both potential vasodilators, were elevated in the femoral vein blood from the injured leg, a series of cross perfusion studies failed to reduce vascular resistance in another leg on the same or a second uninjured animal. Additional studies revealed that changes in leg vascular resistance were markedly diminished in the injured leg following hemorrhage, spinal anesthesia, or intravenous infusion of

epinephrine or norepinephrine. These studies of large granulating wounds reveal: (1) elevated injured leg flow is not the result of local hypoxia, (2) any wound vasodilators have no impact on systemic circulation, (3) the wound vasculature appears relatively insensitive to circulating and neurogenic vasomotor drives.

This project has been completed, and a paper under the same title has been published in Ann Surg 191:249-253, 1980. No further work in this area is anticipated.

FINAL REPORT

PROJECT NO. 3S161102BS05-00, BASIC RESEARCH

REPORT TITLE: THE STUDY OF METABOLISM AND NUTRITIONAL EFFECTS ON BURN INJURY IN SOLDIERS--STUDIES OF HEPATIC BLOOD FLOW AND SUBSTRATE TURNOVER FOLLOWING THERMAL INJURY

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

1 October 1979 - 30 September 1980

Investigators:

Douglas W. Wilmore, M.D.
Cleon W. Goodwin, Jr., M.D.
L. Howard Aulick, Ph.D., LTC, MSC
Michael C. Powanda, Ph.D., MAJ, MSC
Arthur D. Mason, Jr., M.D.
Basil A. Pruitt, Jr., M.D., COL, MC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3S161102BS05-00, BASIC RESEARCH

REPORT TITLE: THE STUDY OF METABOLISM AND NUTRITIONAL EFFECTS OF BURN INJURY IN SOLDIERS--STUDIES OF HEPATIC BLOOD FLOW AND SUBSTRATE TURNOVER FOLLOWING THERMAL INJURY

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

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Reports Control Symbol MEDDH-288(R1)

To characterize the role of the liver and kidney in the metabolic response to injury and infection, selective chatetherization of the hepatic (42 veins) and renal veins (21 veins) was performed in 31 burn patients (mean burn size: 51% TBS), studied 4 to 129 days postinjury. Blood flow was determiend by standard clearance techniques (ICG and PAH), and simultaneous arterial and hepatic and/or renal vein blood was obtained for oxygen, glucose, lactate, pyruvate, and amino acids. Patients studied in the first to third weeks postinjury were classified as "noninfected" (8 patients), "bacteremic" (8 patients), or "bacteremic with complications" (5 patients). There was no difference in age, weight, mean burn size, pulse rate, blood pressure, rectal temperature, total body oxygen consumption, or cardiac index among these groups. Estimated hepatic blood flow (EHBF) and hepatic substrate balance of these patients were compared with postabsorptive normal subjects as reported in the literature (mean $\pm$ SEM or range).

	Normal	Noninfected	Bacteremic	Complicated Bacteremic
Blood Flow ($l/min \cdot m^2$)	0.63 - 0.85	1.54 ± 0.12	1.74 ± 0.17	1.19 ± 0.18
Oxygen Uptake ($ml/min \cdot m^2$)	34 - 40	68 ± 4	66 ± 5	73 ± 3
Glucose Output ($\mu M/min \cdot m^2$)	350 - 450	635 ± 35	835 ± 54	362 ± 60
Lactate Uptake ($\mu M/min \cdot m^2$)	130 - 160	377 ± 77	431 ± 107	268 ± 108
Alanine Uptake ($\mu M/min \cdot m^2$)	30 - 45	124 ± 31	213 ± 40	42 ± 11

Thermal injury alone resulted in marked increases in EHBF, hepatic oxygen uptake, and glucogenesis. The added insult of bacteremia significantly increased hepatic glucose output; as clinical sepsis progressed, glucose output decreased sharply. The kidney consistently demonstrated a net uptake of glucose in all studies. The changes in hepatic glucose output in bacteremic patients occurred without significant differences in EHBF, oxygen utilization or lactate uptake, but were associated with marked alterations in amino acid uptake.

THE STUDY OF METABOLISM AND NUTRITIONAL EFFECTS OF BURN INJURY IN SOLDIERS-- STUDIES OF HEPATIC BLOOD FLOW AND SUBSTRATE TURNOVER FOLLOWING THERMAL INJURY

The metabolic response to major injury is characterized by hypermetabolism (1,2), increased hepatic glucose production (3,4,5), accelerated ureagenesis, and increased urinary nitrogen excretion (1,6). Multiple trauma or severe injury is frequently complicated by infection. As the septic process progresses, organ dysfunction occurs resulting in increased morbidity and mortality. To gain further understanding of the metabolic alterations which occur following trauma and trauma complicated by infection, we studied splanchnic and renal blood flow, regional oxygen consumption, and substrate exchange in patients with extensive thermal injury who were free of infection, in burned patients with bacteremia, and in burned patients with sepsis associated with severe organ dysfunction.

MATERIALS AND METHODS

Subjects

Twenty-nine male and two female burn patients were studied (mean burn size: 51% total body surface, range: 41 to 83.5%). Patients had no known pre-existing disease prior to injury. While most were studied between the first and third weeks postinjury, some patients were studied as early as the fourth postburn day or as late as 127 days postinjury. Serial measurements were performed on 6 patients to evaluate the effect of time and septic complications on posttraumatic

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circulation and metabolism. Patients studied between the fourth and twenty-ninth postburn days were matched for burn size and placed into one of three categories defined prior to study and based on clinical and laboratory criteria (Table 1).

TABLE 1. CHARACTERISTICS OF PATIENTS (MEAN ± SEM)

	Noninfected Burn Patients	Bacteremic Burn Patients	Bacteremic Burn Patients With Complications
Number of Patients	7	8	4
Number of Studies	8	8	5
Age (Years)‡	26 ± 2	26 ± 2	33 ± 3
Weight (kg)	74.5 ± 4.7	67.2 ± 5.5	83.9 ± 4.0
Body Surface Area (m^2)	1.90 ± 0.07	1.81 ± 0.07	2.03 ± 0.06
Per Cent Total Body Surface Burn*	58.0 ± 5.0	62.0 ± 3.0	64.5 ± 4.0
Per Cent 3^o Burn*	32.0 ± 6.0	14.0 ± 6.0	22.5 ± 7.5
Post Burn Day Studied	10 ± 1	13 ± 2	15 ± 6
Positive Blood Cultures Before Day of Study	0	6/8"	5/5"
Positive Blood Cultures on Day of Study	0	8/8†	5/5§
Died	1/7	3/8	4/4

*As determined by the clinical assessment to the closest 0.5%.

† Staphylococcus aureus was recovered in four cultures, and gram negative organisms were identified in the remaining (three Pseudomonas aurogenosa and one Enterobacterclocca).

‡ Age and burn size were considered only once in the description of group characteristics.

§ Staphylococcus aureus was recovered in four studies and Pseudomonas aurogenosa was found on the other.

"These cultures represented similar findings to those observed on the day of study. Approximately half of the cultures grew Stephylococcus aureus and the remaining gram negative organisms.

Noninfected patients. These patients were: (1) normotensive and hemodynamically stable after an uneventful resuscitation; (2) in a normal state of hydration with hematocrits greater than 30 and without abnormalities in serum osmolality, pH, or concentrations of electrolyte, blood urea nitrogen, or creatinine; (3) free of systemic infection prior to and including the day of study, as determined by clinical symptoms and signs, chest x-rays, and urine and blood cultures; and (4) alert, cooperative and able to participate in the study.

Bacteremic patients. The subjects met the first two criteria of the noninfected patients but had signs of infection as characterized by changes in mental status (6/8 patients) ileus (5/8 patients), glucosuria (6/8 patients), and previous positive blood stream cultures (6/8 patients). All patients in this group had bacteria cultured from their bloodstream at the time of the investigation and were receiving systemic antibiotics. Since they were studied shortly after the onset of infection, however, no clinical or biochemical evidence of specific organ dysfunction or multiorgan failure was present in this group.

Bacteremic patients with complications. Although these patients had apparently been successfully resuscitated, they became septic (as documented by positive bloodstream cultures) early in their posttraumatic course and developed evidence of multiorgan failure. At the time of the study, all had undergone alterations in mentation as characterized by confusion (2/5 patients) or obtundation (3/5 patients), and three required mechanical ventilatory support. Renal impairment, as documented by serum creatinine greater than 1.5 mg/dl, was present in four subjects. All of these subjects maintained adequate circulation and cardiovascular stability for several days before and during the study.

Subject Preparations

All patients were treated in a similar manner. Patients studied within the first three weeks of injury had not undergone primary wound excision or other operative treatment requiring general anesthesia. Most wounds were treated by the exposure method, using either silver sulfadiazine cream (Silvadine®) or 11% mafenide acetate (Sulfamylon®). In a few individuals, small wound areas were covered with dressings soaked with 5% mafenide solution.

Patients received vigorous nutritional support during their hospitalization. Those who could not eat received tube feedings or parenteral nutrition. Nutrient intake for at least three days before each study satisfied at least 80% of the patients' metabolic requirements and at least half of the administered calories were carbohydrate. Body weight was generally stable during the week before the study, and no patient studied within three weeks of injury exhibited a body weight loss exceeding 5% of preinjury weight at the time of initial study.

Study Design

Patients were studied in the early morning after fasting since midnight. Those who required intravenous fluid to maintain a normal state of hydration received 0.04 molar nutrient free sodium chloride infusions for six hours before and throughout the study. While routine clinical care continued in the morning, patient manipulation was minimized for at least six hours before the study. Patients who were not able to rest during this period of time were not studied.

Subjects were taken to a nearby x-ray suite where a #7 J-catheter was advanced under fluoroscopic control through the femoral vein and inferior vena cava to deep within the right hepatic vein (3 to 4 cm from the wedge position). In selected patients, the catheter was first directed into the right renal vein and blood samples obtained before proceeding to the hepatic vein. Once proper position was established in the hepatic vein, the catheter was secured in the groin with a silk suture and adhesive tape and the subject moved to an environmental chamber.[7] Chamber temperature was maintained at 30C. and relative humidity between 40 and 50%. Under local anesthesia, an arterial catheter (a #21 polyvinyl tubing) was inserted into the left femoral artery and, if not present, a venous catheter (#18) was inserted into a large peripheral vein. Catheter patency was maintained by slow infusion of 0.04 molar sodium chloride solution (a syringe pump maintained this patency of the arterial catheter while gravity infusion was used for the intravenous lines). Total time required for catheter insertion and initial preparation was 1 to 1.5 hours. Following this period, the subjects were allowed to rest for at least one hour in the semi-dark, warm, quiet room.

After the equilibration period, blood samples were drawn simultaneously from arterial and hepatic venous catheters and subsequently analyzed for oxygen content, whole blood glucose and plasma lactate, pyruvate, and amino acid concentrations. A bolus injection of indocyanine green dye (ICG: 0.5 mg/kg) was then given

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via the peripheral venous catheter and simultaneous arterial and hepatic venous blood samples obtained at two, four, six, ten, and twelve minutes postinjectin. The rate of plasma ICG clearance over this time period provided a measure of splanchnic or estimated hepatic blood flow (8). This technique was selected over the more common constant infusion method because, in preliminary studies, steady state arterial ICG concentrations could not be achieved in four of six patients using the high-dose infusion rate suggested in the literature (9). When lower doses were used, including those recommended for patients with cirrhosis (10), completely unpredictable results were obtained. The bolus clearance technique also provided another advantage, since a marked reduction in the hepatic venous extraction with constant infusion signaled the development of back diffusion of the dye from the hepatocyte (11). In these patients, however, back diffusion did not occur in the first 12 to 15 minutes postinjection.

Cardiac output was then determined using the standard ICG dye dilution technique (12). Three to five determinations were performed and an average value obtained. A canopy hood was then placed over the subject's head and oxygen consumption determined by the open circuit technique over the next 15 to 20 minutes (13). Oxygen consumption of patients on ventilators was determined by Douglas bag techniques.

Patients usually slept throughout the 1.5- to 2-hour study period. At the end of each study, pulse rate, blood pressure, and rectal temperature were obtained. X-ray confirmation of hepatic vein catheter position was routinely performed initially and in selected individuals throughout the study.

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Study Methods

Heparinized blood samples were analyzed for oxygen content (Lex-O_2-Con, Lexington Instrument Corporation, Dallas, Texas). While blood glucose was measured by the glucose oxidase method (12) lactate by enzymatic technique, and plasma amino acids by standard chromatography (14). Hematocrits were determined on all samples and were within 5% for each matched sample set. All measurements were performed in triplicate, and an average value reported. Indocyanine green dye concentrations were determined using a spectrophotometer (Gilford Model 240, Gilford Instrument Laboratories, Inc., Oberlin, Ohio), and splanchnic blood flow calculated from the proportionality constant for plasma ICG disappearance, the hepatic ICG extraction ratio, and hematocrit (8). Extrapolation of the arterial ICG disappearance curve to time zero provided an estimate of plasma volume, which compared favorably with simultaneous ^{131}I-albumin plasma volume determinations performed in five individuals. Splanchnic substrate exchange and oxygen consumption were calculated by multiplying splanchinc blood flow by arterial-hepatic venous concentration differences.

Paired and unpaired t-tests were used when appropriate and significance was considered at the $p < 0.05$ level. When comparing the three groups of patients, the Scheffe technique for multiple group comparisons was used. Normal values were taken from the literature (12,15,16,17,18,19).

RESULTS

The three groups of patients had similar ages, weights, body surface areas, and burn sizes, and were studied at similar times following their injury (Table 1). The systemic responses to injury were comparable in all three groups, as reflected by similar rectal

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temperatures, pulse rates, blood pressure, cardiac indices, and total body oxygen consumption (Table 2). Because of the extensive injuries, cardiac output and oxygen consumption approached near maximal levels. Arterial concentrations of oxygen, glucose, lactate, and pyruvate were not significantly different among groups (Table 3). The mean arterial-hepatic vein oxygen content differences ($A\text{-}HV_{O_2}$) were 4.6 and 4.1 ml/dl in the uninfected burn patients and those with bacteremia, similar to the range of 4 to 5 ml/dl reported in normals (18). However, the bacteremic patients with complications had an expanded $A\text{-}HV_{O_2}$ difference of 6.7 ml/dl, significantly greater than the bacteremic patients. The arterial-hepatic vein gradient for glucose, in all three groups was similar to the 0.4 to 0.5 mM/L (8 to 10 mg/dl) reported in normal postabsorptive man (17), although the arterial-hepatic vein concentration difference for lactate and pyruvate appeared increased when compared with normals. The arterial-hepatic vein alanine difference in the critically ill burn patients with complications was sharply reduced when compared to the bacteremic group.

TABLE 2. SYSTEMIC RESPONSES (Mean $\pm$ SEM)

Systemic Responses	Noninfected Burn Patients	Bacteremic Burn Patients	Bacteremic Burn Patients With Complications
Rectal Temperature (^{o}C)	38.5 ± 0.3	38.6 ± 0.2	38.0 ± 0.5
Pulse (beats/min)	125.5 ± 5	115 ± 5	124 ± 7
Blood Pressure (mmHg)	$\frac{132 \pm 4}{70 \pm 4}$	$\frac{141 \pm 6}{77 \pm 3}$	$\frac{138 \pm 10}{68 \pm 4}$
Cardiac Index ($L/min \cdot m^2$)	8.17 ± 0.33	8.78 ± 0.41	7.67 ± 0.72
Oxygen Consumption ($ml/min \cdot m^2$)	228 ± 9	238 ± 8	244 ± 12

TABLE 3. BLOOD CONCENTRATION (MEAN ± SEM)

	Normal	Noninfected Burn Patients	Bacteremic Burn Patients	Bacteremic Burn Patients With Complications
Arterial Oxygen, ml/100 ml	15-18	14.1±0.7	13.8±0.7	14.0±0.8
A-HV_{O_2}, * ml/100 ml	4-5	4.6±0.5	4.1±0.5	6.7±1.0 †
Arterial Glucose Concentration, mM/L	4.0-5.0	5.56±0.22	7.11±1.28	6.28±0.56
A-HV Glucose, mM/L	-0.4- -0.5	-0.44±0.05	-0.50±0.05	-0.33±0.05
Arterial Lactate Concentration, mM/L	0.5-0.7	1.022±0.089	1.444±0.256	1.533±0.389
A-HV Lactate, mM/L	0.18-0.24	0.244±0.044	0.278±0.078	0.211±0.067
Arterial Pyruvate Concentration, mM/L	0.06-0.07	0.090±0.006	0.106±0.008	0.118±0.017
A-HV Pyruvate, mM/L	0.010-0.020	0.012±0.005	0.011±0.004	0.012±0.005
Arterial Alanine Concentration, mM/L	0.250-0.400	0.345±0.051	0.376±0.062	0.170±0.021
A-HV Alanine, mM/L	0.080-0.10	0.119±0.028	0.196±0.036	0.058±0.020 †

* A-HV: arterial-hepatic vein concentration

† Bacteremic burn patients versus bacteremic burn patients with complications, $p < 0.05$.

The proportionality constants for green dye disappearance in the noninfected patients and in the bacteremic group were in the high normal range. This value was significantly decreased in those individuals with complications (Table 4). No alterations in indocyanine green dye extraction were noted among groups. Estimated splanchnic blood flow ranged between 1 and 2 liters/min $\cdot m^2$. Splanchnic blood flow accounted for 15 to 20% of cardiac index, a finding similar to previous reports in burn patients (9). Splanchnic oxygen consumption was twice normal in all three patient groups, with the splanchnic bed accounting for approximately 25 to 30% of the total oxygen consumed. No differences in splanchnic oxygen consumption were observed among patient groups.

The basal rate of splanchnic glucose output was approximately 50% above normal in the noninfected burn patients and increased significantly above this level in the bacteremic burn patients (Table 4). However, glucose production was significantly less in the bacteremic patients with complications when compared with the other two patient groups; the rate of glucose output in the patients with complications was comparable to rates reported for normal postabsorptive subjects.

All burn patients demonstrated splanchnic uptake of lactate and pyruvate greater than rates reported for normals, but there were no differences between patient groups in arterial concentrations, percent extraction, or hepatic uptake of these three-carbon glucose precursors. Assuming complete hepatic conversion of lactate and pyruvate to glucose in the injured subjects, these two substrates accounted for 30 to 50% of the glucose produced by the liver.

Marked differences were noted between groups with respect to the splanchnic exchange of amino acids. Of the 17 amino acids studied, consistently positive arterial-hepatic venous concentration differences (A-HV), indicating net uptake, were demonstrated in both the non-infected and bacteremic burn patients but not those patients with complications (Table 5). Gluconeogenic precursors predominated as the amino acids taken up by the liver. In the noninfected and bacteremic burn patients, these included alanine, glycine and tyrosine. A significant uptake of threonine and methionine was also observed in the noninfected burn patients and serine, proline, isoleucine, phenyl-alanine and lysine were taken up by the bacteremic burn patients.

TABLE 4. SPLANCHNIC BLOOD FLOW AND RATES OF SUBSTRATE EXCHANGE (RANGE OR MEAN ± SEM)

	Normal	Noninfected Burn Patients	Bacteremic Burn Patients	Bacteremic Burn Patients With Complications
ICG and ESBF				
k/min	0.2-0.3	0.328±0.027	0.273±0.030	0.141±0.021*
Indocyanine green dye per cent extraction	75-90	67±4	50±5	46±8
Blood volume, ml/kg	70-80	82.4±4.8	81.9±6.3	104.8±14.1
Estimated splanchnic blood flow, μ L/min · m^2	0.63-0.85	1.54±0.12	1.74±0.17	1.19±0.18
Splanchnic blood flow as per cent of cardiac index	22-28	19.1±1.8	20.1± 2.1	16.1±2.5
Hematocrit per cent	39-46	34±1	33±1	33±1
Splanchnic Exchange				
Splanchnic VO_2, ml/min · m^2	34-40	68±4	66±5	73±3
Splanchnic VO_2 as a per cent of total VO_2	20-25	29.8±1.5	27.8±2.2	30.3±1.5
Glucose production, mM/min · m^2	0.35-0.45	0.635±0.035	0.835±0.054*	0.362± 0.060*†
Lactate uptake, mM/min · m^2	0.13-0.16	0.377±0.077	0.431±0.107	0.268±0.108
Per cent of glucose from lactate	20-24	30.5±6.7	28.6±7.4	45.5±21.9
Pyruvate uptake, mM/min · m^2	0.005-0.010	0.019±0.008	0.018±0.007	0.011±0.004
Per cent glucose from pyruvate	1-3	1.52±0.66	1.20±0.44	1.32±0.40
Alanine uptake, mM/min m^2	0.030-0.045	0.124±0.031	0.213±0.040	0.042±11†
Per cent of glucose from alanine	5-9	9.2±2.3	13.2±2.0	6.3± 1.5

*Noninfected burn patients versus bacteremic burn patients, $p < 0.05$.

†Bacteremic burn patients versus bacteremic burn patients with complications, $p < 0.05$.

TABLE 5. ARTERIAL CONCENTRATIONS (A), ARTERIAL-HEPATIC VENOUS DIFFERENCES (A-HV), PER CENT HEPATIC EXTRACTION, AND HEPATIC EXCHANGE OF AMINO ACIDS (MEAN ± SEM)

	Noninfected Burn Patients				Bacteremic Burn Patients				Bacteremic Burn Patients with Complications			
	A, μM/L	A-HV, μM/L	% Extr.	Hepatic Exchange, μM/min·m²	A μM/L	A-HV, μM/L	% Extr.	Hepatic Exchange μM/min·m²	A μM/L	A-HV μM/L	% Extr.	Hepatic Exchange, μM/min·m²
Taurine	45 ±15	-7 ± 6	-47 ± 35	-7.3 ± 5.3	63 ±21	1 ± 6	30 ±43	0.9 ± 6.0	44 ±30	10 ±12	57 ±41	5.6 ± 7.2
Threonine	126 ±17	50 ±12*	36 ± 7	48.8 ±10.6	115 ±23	54 ±23	37 ±12	57.5 ±28.1	95 ±16	35 ±11	37 ±10	27.6 ± 8.7
Serine	136 ±29	10 ±18	-53 ± 65	12.8 ±17.5	194 ±31	88 ±16*	47 ± 5	93.8 ±13.1 †	79 ±18 ‡	26 ±14	22 ±15 ‡	18.7 ±10.8 ‡
Proline	239 ±53	14 ±35	18 ± 21	9.5 ±37.5	175 ±36	69 ±17* †	45 ±11 †	68.9 ±12.3	29 ±29 ‡	-12 ± 9	- 3 ± 3	- 1.6 ± 1.6
Glycine	269 ±46	71 ±33*	25 ± 7	66.5 ±26.4	318 ±41	128 ±48*	38 ±12	138.0 ±61.0	158 ±47	35 ±35	-12 ±43	29.7 ±23.3
Alanine	345 ±51	119 ±28*	34 ± 5	124.0 ±31.0	376 ±63	196 ±36*	53 ± 4	213.0 ±40.0	170 ±21	58 ±20	33 ±10 ‡	42.0 ±11.0 ‡
Valine	143 ±40	- 12 ± 17	- 3 ± 11	-17.9 ±19.7	213 ±28	31 ±43	14 ±16	65.0 ±50.0	167 ±27	38 ± 52	5 ± 35	34.0 ±42.0
Cystine	23 ± 2	4 ± 3	27 ±18	5.4 ± 3.8	41 ±16	21 ±20	-18 ±43	18.0 ±18.6	48 ±14	27 ± 6	66 ±16	24.2 ± 8.3
Methionine	27 ± 3	10 ± 2*	38 ± 9	9.8 ± 2.2	37 ± 9	15 ± 8	22 ±15	15.6 ±10.0	99 ±61	77 ±64	43 ±16	85.2 ±78.0

Isoleucine	81 ± 7	3 ± 8	1 ±12	5.0 ± 6.4	85 ±16	34 ±12*	36 ±10	38.3 ±15.2	43 ±12	4 ± 6	4 ±21	5.2 ± 5.8
Leucine	136 ±16	2 ±13	0 ±11	2.6 ±10.8	102 ±20	41 ±17	30 ±10	50.0 ±24.1	96 ±13	5 ±25	-8 ±26	8.8 ±18.8
Tyrosine	70 ± 9	18 ± 6*	22 ±10	19.3 ± 6.4	66 ±15	32 ±10*	31 ±19	35.7 ±11.9	62 ±19	- 6 ±11	-20 ±38	4.8 ±11.8
Phenylalanine	138 ±19	26 ±20	12 ±16	32.2 ±22.1	142 ±21	63 ±17*	40 ± 9	67.9 ±21.1	94 ±35	27 ±11	26 ± 9	21.5 ± 9.8
Ornithine	112 ±34	21 ±25	10 ±13	28.9 ±32.6	77 ±34	- 4 ±22	26 ±13	12.4 ±10.3	77 ±33	18 ±51	- 73 ±88	35.2 ±53.4
Lysine	160 ±17	32 ±17	19 ±10	35.3 ±19.2	217 ±48	109 ±34*	47 ± 8	119.3 ±46.9	131 ±25	52 ±38	15 ±37	40.6 ±35.1
Histidine	68 ± 9	2 ±12	3 ±16	- 6.7 ±14.0	76 ±15	10 ±16	0 ±62	9.3 ±16.6	53 ± 9	9 ±11	13 ±24	11.8 ±10.7
Arginine	69 ±11	- 11 ±16	- 9 ±22	12.2 ±17.4	28 ±11	- 1 ± 4	- 35 ±33	- 0.3 ± 5.2	30 ±12	- 9 ±23	-149 ±93	1.0 ±23.0

* Arterial and hepatic venous concentrations significantly different by paired t-test, $p < 0.05$.

† Noninfected burn patients versus bacteremic burn patients, $p < 0.05$.

‡ Bacteremic burn patients versus bacteremic burn patients with complications, $p < 0.05$.

An increased splanchnic exchange of amino acids occurred in the noninfected burn patient when compared to hepatic amino acid uptake in postabsorptive normals. Alanine, which quantitatively is a major nitrogen transport compound from skeletal muscle to liver and provides a three-carbon skeleton as a glucose precursor, was taken up at an average of 124 $\mu M/min \cdot m^2$ in the noninfected burn patients, rates three to four times those reported for postabsorptive normals (Tables 4 and 5). Since arterial concentrations of alanine in this group were within the normal range and the per cent amino acid extracted was comparable to levels reported in normals (approximately 36% (19)), the mechanism for this augmented alanine uptake was dependent on the increased delivery of the amino acids to the liver via the elevated splanchnic blood flow.

In the bacteremic patients, splanchnic uptake of amino acids increased markedly when compared with the noninfected burn patients. The total amino acid nitrogen taken up by the liver in the bacteremic burn patients averaged 131 ± 24 μM nitrogen/$min \cdot m^2$, two to three times the uptake observed in the noninfected burn subjects (48.1 ± 10, $p < 0.01$). Since the arterial concentrations of the amino acids were similar in the two groups and blood flow was comparable, the augmented splanchnic amino acid uptake observed in the bacteremic patient was solely a consequence of increased fractional extraction by the splanchnic bed. The average per cent extraction for all the 17 amino acids studied was $26 \pm 6\%$ for the bacteremic burn patients, significantly greater than the $8 \pm 6\%$ ($p < 0.05$) in the noninfected burn group.

In the bacteremic burn patients with complications, the uptake of amino acids was reduced. Alanine exchange, for example, was significantly decreased in these individuals when compared to the bacteremic subjects. In most instances, the plasma amino acid arterial concentrations were less in the complicated bacteremic burn patients than in the other two groups, and the average extraction for all amino acids was only $3 \pm 3\%$.

Renal arterial-venous differences demonstrated a widened A-RV_{O_2} in the noninfected burn patients when compared to the bacteremic patients (Table 6). The kidney consistently consumed glucose in all patients studied. Renal vein catheterization was performed in only two individuals with bacteremia with complications. In these patients, the extraction of oxygen and glucose was similar to those values observed in the bacteremic group.

TABLE 6. THE ARTERIAL-RENAL VEIN DIFFERENCES* FOR OXYGEN, GLUCOSE AND LACTATE (MEAN ± SEM)

	Normal	Non-Infected† Burn Patients	Bacteremic‡ Burn Patients
Oxygen (ml/100ml)	1.6-1.8	2.41 ± 0.14	0.92 ± 0.18§
Glucose (mM/L)	0-0.056	0.222 ± 0.056	0.056 ± 0.056§
Lactate (mM/L)	0 - -0.001	- 0.044 ± 0.008	-0.066 ± 0.018

* Only two of the complicated bacteremic burn patients underwent renal vein catheterization. The A-RV difference results were similar to those reported for the bacteremic patients. Renal blood flow, however, was decreased and averaged 0.447 ± 0.048 L/min · m^2.

† Renal blood flow measured in six subjects averaged 0.693 ± 0.074 L/min · m^2. Normal = 0.552 ± 0.037.

‡ Renal blood flow measured in three subjects averaged 1.970 ± 0.380 L/min · m^2.

§ $p < 0.05$ when compared with noninfected burn patients.

DISCUSSION

This current investigation provides direct evidence of altered glucogenesis which occurs following major injury. In the noninfected burn patients, rates of glucose production were one and one-half times greater than values reported in normal postabsorptive subjects. While the normal individual produces approximately 200 g of glucose/day, the thermally injured, noninfected patient releases approximately 320 g of glucose/day. This measurement of increased net splanchnic glucose production is consistent with data derived from tracer studies which suggest increased glucogenesis following injury (4). This increased rate of glucose production is even more striking in face of the slightly negative calorie balance sustained by all patients during the time following injury and the fact that hepatic glycogen stores were probably partially depleated. Comparable studies in control individuals with some degree of caloric restriction are not available, but the study of Garber et al. demonstrated that with only three days of starvation there is a marked fall in hepatic glucose production, to approximately half the quantity of glucose produced in postabsorptive man (20). Finally, renal catheterization data demonstrated that the kidney does not participate in the increased glucose production following injury and that this function is solely the responsibility of the liver.

20. Garber AJ, Menzel PH, Boden G. Owen OE: Hepatic ketogenesis and gluconeogenesis in humans. J Clin Invest 54:981-989, 1974.

In addition to the increase in glucose produced, these data provide evidence of altered gluconeogenesis following injury. First, the net splanchnic uptake of lactate and pyruvate appear greater than observed in control subjects (19), suggesting increased Cori cycle activity following burn injury. This observation agrees well with the finding of increased glucose uptake and lactate release across injured, but not uninjured, extremities (12). Approximately 80% of the glucose consumed by the burn wound is converted to lactate, and previous estimates of peripheral lactate production are quite comparable to these measurements of splanchnic lactate uptake (12,21).

Secondly, the enhanced uptake of alanine and other glucogenic amino acids in the noninfected burn patients is further evidence of an accelerated rate of hepatic gluconeogenesis following injury. Because plasma and not whole blood amino acids were measured, the total splanchnic uptake of amino acids is probably under-estimated (22). However, Chiasson and associates demonstrated that 90-95% of the alanine exchanged across the hepatic bed was transported in serum, and thus alanine can be followed as an index of skeletal muscle-hepatic exchange of amino acids (23). Alanine exchange in the noninfected burn patients was three to four times the splanchnic uptake observed in normal man (24). Moreover, splanchnic alanine exchange rates of 200-220 μM/min in the noninfected burn patients compare favorably with the estimates of peripheral alanine release previously reported (14). Alanine generally accounts for 30-50% of the new glucose derived from amino acids (24), but in these injured patients 100% conversion of this gluconeogenic amino acid to new glucose may not occur. This is based on the observation of Long and associates, who administered C^{14} alanine to critically ill patients and found that as much as 32% of the tagged carbon rapidly appeared as expired CO_2 (25). However, assuming complete conversion

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21. Wilmore DW, Aulick LH: Metabolic changes in burn patients. Surg Clin North Am 58:1173-1187, 1978.

22. Aoki TT, Muller WA, Brennan FM, Cahill GF, Jr: Blood cell and plasma amino acid levels across forearm muscle during a protein meal. Diabetes 22:768-775, 1973.

23. Chaisson JL, Liljenquist JE, Sinclair-Smith BC, Lacy WW: Gluconeogenesis from alanine in normal postabsorptive man: Intrahepatic stimulatory effect of glucagon. Diabetes 24:574-584, 1975.

24. Felig P: Amino acid metabolism in man. Annual Rev Biochem 44:933-955, 1975.

25. Long CL, Kinney JM, Geiger JW: Nonsuppressability of gluconeogenesis by glucose in septic patients. Metabolism 25:193-201, 1976.

of glugogenic amino acids to new glucose, as much as 20 to 30% of the glucose produced in the noninfected burn patients could be derived from the carbon skeletons of these amino acids. This value compares favorably with theoretical calculations of glucose derived from amino acids based on the quantity of nitrogen excreted in the urine.* In contrast to postabsorptive normals in whom only 20 to 25% of hepatic glucose output can be accounted for by gluconeogenesis (19), noninfected burn patients could derive approximately half of their glucose from three carbon precursors.

Finally, it is important to note that the increased hepatic uptake of glucose precursors closely matched the peripheral release of these substances, and thus serum substrate concentrations were maintained at near normal levels. Because the per cent extractions of lactate, pyruvate and gluconeogenic amino acids from the blood in the noninfected burn patients were comparable to normal values, the increased splanchnic uptake of these substances following injury was the consequence of greater substrate delivery provided by the increased splanchnic blood flow.

With the onset of bacteremia, hepatic glucose production increased. While the exchange of lactate and pyruvate was not altered when compared to the noninfected burn patient, the uptake of amino acids was significantly increased in the bacteremic patients. The increased hepatic utilization of these glucose precursors with bacteremia could be a consequence of either greater substrate availability or augmented hepatic extraction of circulating substrate. Because blood flow and substrate concentrations did not change between these two groups, there is little evidence of increased amino acid availability in the bacteremic patient. However, there was increased extraction of amino acids in the bacteremic patients when compared with the noninfected burn subjects. These data suggest that the augmented hepatic uptake of gluconeogenic amino acids is the consequence of altered intrahepatic metabolism as a consequence of sepsis, as opposed to an increased availability of precursor substrate. The fact that serum concentrations were maintained at levels comparable to those observed in the noninfected subjects, support the thesis that this increased hepatic amino acid uptake was matched by augmented peripheral release. Finally, all

*It has been suggested that 4.66 grams of nitrogen from catabolized protein should yield approximately 16 grams of glucose (26). Since these patients excrete 20 to 30 grams N/day, approximately 80 grams of glucose are theoretically derived from nitrogen containing compounds each day. Thus approximately 25% of the total 320 grams glucose/day produced in the noninfected burn patient can be accounted for by amino acids.

26. Owen OE, Felig P, Morgan AP, et al: Liver and kidney metabolism during prolonged starvation. Clin Invest 48:574-583, 1969.

of these alterations occurred without changes in regional blood flow or oxygen utilization. The usual response to infection in previously healthy individuals is to increase oxygen consumption, cardiac output, and splanchnic blood flow (27, 28,29), but these alterations were not observed in the infected burn patients when compared with the noninfected burn subjects, presumably because of the near maximal total body metabolic and circulatory responses to burn injury attained before the onset of infection.

In contrast to the first two groups of patients, the septic patients with complications demonstrated diminished hepatic glucose production, reduced amino acid exchange, but comparable lactate uptake. It is well known that alterations in hepatic production and tissue uptake of glucose occur in association with severe infection (30,31). The most dramatic symptom complex observed is hypoglycemia in the newborn associated with gram negative sepsis (32). While animal studies suggest that severe infection impairs hepatic glucose production (33,34), increased clearance (tissue uptake) of glucose has also recently been implicated (35). It has been suggested that endotoxin blocks hepatic glucose production (34), although the precise role of this and other bacterial products in the metabolic response to infection is unknown. However, the commonly used

27. Albrecht M, Clowes GHA, Jr.: The increase of circulatory requirements in the presence of inflammation. Surgery 56: 158-171, 1964.

28. Bradley SE, Chasis H, Goldring W, Smith HW: Hemodynamic alterations in normotensive and hypertensive subjects during the pyrogenic reaction. J Clin Invest 24:749-758, 1945.

29. Gump FE, Price JB, Jr, Kinney JM: Whole body and splanchnic blood flow and oxygen consumption measurements in patients with intraperitoneal infection. Ann Surg 171:321-328, 1970.

30. Beisel WR: Metabolic response to infection. Ann Rev Med 26:9-20, 1975.

31. Wilmore, DW, Mason AD, Jr, Pruitt BA, Jr: Impaired glucose flow in burn patients with gram-negative sepsis. Surg Gynecol Obstet 143:720-724, 1976.

32. Young CY: Hypoglycemia in neonatal sepsis. J Pediatr 77:812-817, 1970.

33. LaNoue KF, Mason AD, Jr, Daniels JP: The impairment of gluconeogenesis by gram negative infection. Metabolism 17:606-611, 1968.

34. McCallum RE, Berry LJ: Effects of endotoxin on gluconeogenesis, glycogen synthesis and liver glycogen synthase in mice. Infect Immun 7:642-654, 1973.

35. Wolfe RR, Elahi D, Spitzer JJ: Glucose and lactate kinetics following endotoxin administration in dogs. Am J Physiol 232:E180-E185, 1977.

liver function tests did not reflect these severe functional abnormalities, characterized by a reduced glucose production and a diminished amino acid exchange, seen in the complicated bacteremic burn patients. In contrast, however, the clearance of indocyanine green dye was abnormal. This and previous work suggests that this test may be used as a measure of hepatic dystunction in critically ill patients (11,36). If hepatic amino acid uptake was impaired and skeletal muscle amino acid release continued at previous rates, the arterial serum amino acid concentrations would rise. Because low, not elevated, amino acid levels were observed in the septic patients with complications, the data suggests that mechanisms which regulate skeletal muscle amino acid release are also altered. Although hormone concentrations were not obtained in these patients, previous investigations in similar individuals have demonstrated an excess, not a lack, of counterregulatory hormones which stimulate gluconeogenesis in burn patients with sepsis and complications (2,37,38). Thus, the exact mechanisms for this altered glucose output in the severely ill patients are not precisely known, but the metabolic impact appears to affect both the liver and skeletal muscle.

Blood oxygen and substrate concentrations are measured with a high degree of precision and accuracy. Splanchnic blood flow, however, is not as reliable a measurement (8). The crux of the evidence demonstrating altered net splanchnic glucose production in infected, critically ill subjects rests on the calculation of hepatic glucose production rate which requires an estimate of splanchnic blood flow. This flow measurement is based on the hepatic uptake of indocyanine green dye and the kinetics of this inert dye are disturbed following infection and endotoxemia (11,36). Thus, as patients develop complications, indocyanine green dye uptake decreases and these alterations may add to variability of the splanchnic blood flow measurement. However, there are several lines of evidence that support the validity of these regional flow measurements. Although burn size, time of study, and other patient characteristics are not identical to the patients studied by Gump et al., the per cent of cardiac output directed to the splanchnic bed in this study is comparable to a previous measurement in burn patients (9). The splanchnic bed accounts for a large portion of the oxygen consumed by the body and in nonexercising patients this quantity is roughly proportional to the total body oxygen consumption. In this study, the total body oxygen consumption was similar in the patient groups, and splanchnic oxygen

36. McDougal WS, Wilmore DW, Pruitt BA, Jr: Glucose-dependent hepatic membrane transport in nonbacteremic and bacteremic thermally-injured patients. J Surg Res 22: 697-708, 1977.

37. Wilmore DW, Aulick LH, Pruitt BA, Jr: Metabolism during the hypermetabolic phase of thermal injury. Adv Surg 12: 193-225.

38. Wilmore DW, Lindsey CA, Moylan JA, et al: Hyperglucagonaemia after burns. Lancet 1: 73-75, 1974.

consumption (calculated using the blood flow measurement) was also comparable. Moreover, in the infected patients with complications, as the splanchnic blood flow fell, the A-HV_{O_2} correspondingly increased.

Studies in these and comparable patients have quantitated blood flow to the extremities (12) and skeletal muscle (39). These flow studies combined with the present measurements account for most, if not all, of the increased cardiac output following burn injury. Thus, the splanchnic blood flow and regional oxygen consumption measurements presented in this report together with similar studies across other regional beds adequately account for the cardiac output and the total body oxygen consumption which occurs in the severely burned patient.

In summary, these studies indicate that: (1) hepatic glucose production increases following major injury; (2) bacteremia in severely injured patients further augments gluconeogenesis by the increased hepatic uptake of amino acids; (3) with septic complications, hepatic glucose production and amino acid uptake decreases; and (4) these changes occur without alterations in splanchnic blood flow, oxygen utilization or lactate uptake.

39. Aulick LH, Wilmore DW, Mason AD, Jr, Pruitt BA, Jr: Muscle blood flow following thermal injury. Ann Surg 188:778-782, 1978.

PUBLICATIONS

Wilmore DW, Goodwin CW, Aulick LH, Powanda MC, Mason AD, Jr, Pruitt BA, Jr: Effect of injury and infection on visceral metabolism and circulation. Ann Surg 192:491-504, 1980.

ANNUAL PROGRESS REPORT

PROJECT NO. 3S161102BS05-00, BASIC RESEARCH

REPORT TITLE: THE STUDY OF METABOLISM AND NUTRITIONAL EFFECTS OF BURN INJURY IN SOLDIERS -- A NEW APPROACH TO THE STUDY OF THE HYPERMETABOLIC RESPONSE TO THERMAL INJURY

US ARMY INSTITUTE OF SURGICAL RESEARCH
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1 October 1979 - 30 September 1980

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An open and closed respiration chamber is described which, when constructed, will permit long-term monitoring of respiratory gas exchange in unrestrained large animals (sheep, goats, dogs, etc.). This system is designed to extend the limited period of observation available in the classical "confinement" method by permitting sequential confinement periods automatically separated by short periods of chamber ventilation. Additional features will include good control of ambient temperature, humidity, air velocity and noise. Once the chamber has been constructed and validated, experiments will begin which characterize the metabolic and thermoregulatory responses of goats to a full thickness, 25 percent total body surface burn. Based on the acceptability of this model, investigations are planned to determine the afferent mediators of these responses.

Metabolic response
Thermoregulatory response

A NEW APPROACH TO THE STUDY OF THE HYPERMETABOLIC RESPONSE TO THERMAL INJURY

The hypermetabolic response to thermal injury has been well characterized, but the afferent limb of this stress reflex is poorly understood. Afferent signals presumably originate in the burn wound itself, since the degree of hypermetabolism is primarily determined by the extent of injury and only disappears when the wound is covered and healed. The hypermetabolism of the burn patient is apparently not dependent on afferent nervous activity from the affected area. (1,2,3) Numerous potential circulating afferents are known to be present in the burn wound as well as in the lymphatic and venous drainage from the injured part (4,5,6), but the normal constraints of clinical research have precluded systematic appraisal of their impact on total body metabolism.

1. Wilmore DW: Hormonal responses and their effects on metabolism. In Surgical Clinics of North America, ed by GA Clowes, Jr. Philadelphia: W.B. Saunders, Vol 5, p 999, 1976.

2. Wilmore DW and Aulick LH: Metabolic changes in burned patients. In Surgical Clinics of North America, ed by JA Boswick, Jr. Philadelphia: W.B. Saunders, Vol 58, p 1173, 1978.

3. Taylor JW, Hander EW, Skreen R and Wilmore DW: The effect of central nervous system narcosis on the sympathetic response to stress. J Surg Res 20:313, 1976.

4. Wilmore DW: Studies of the effect of variations of temperature and humidity on energy demands of the burn soldier in the controlled metabolic room. U.S. Army Institute of Surgical Research Annual Research Progress Report, 1 July 1975 - 30 June 1976.

5. Arturson G: Prostaglandins in human burn wound secrection. Burns 3:112, 1978.

6. Anggard E, Johnson, DE: Efflux of prostaglandins in lymph from scalded tissue. Acta Physiol Scand 81:440, 1971.

Efforts to develop an appropriate small animal model have been only partially successful, since they were hampered both by the animal's size and the relatively limited metabolic response to injury (7,8,9). Preliminary work just completed has shown that a full-thickness, thermal burn covering 25 percent of the total body surface resulted in a 20 to 40 percent rise in the resting oxygen consumption of large goats (10). This was a more pronounced metabolic response than that of small animals with the same size burn wound (7,8,9). Associated with this rise in energy turnover was a three- to four-fold increase in urinary catecholamine excretion similar to the sympathoadrenal response of burn patients (11). Unlike thermally injured man, however, the goats did not become febrile. Therefore, while the thermoregulatory adjustment of this large animal model remains uncertain, the combined neuroendocrine and metabolic responses of the goat to thermal injury strongly suggest that it is a suitable model for further research. To extend these observations and seek a better understanding of the afferent drives for burn hypermetabolism, a new measurement system is currently under development at the Institute.

An Open and Closed Respiration Chamber. The new measurement system consists primarily of a large animal, open and closed respiration chamber which will permit long-term monitoring of the respiratory gas exchange in confined but unrestrained goats. The basic design was developed by Blaxter et al (12) for metabolic studies in sheep and cattle,

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7. Caldwell FT, Jr., Osterholm JL, Sower ND and Moyer CA: Metabolic responses to thermal trauma of normal and thyroprivic rats at three environmental temperatures. Ann Surg 150: 976, 1959.

8. Herndon DN, Wilmore DW, Mason AD Jr.: Development and analysis of a small animal model simulating the human postburn hypermetabolic response. J Surg Res 25: 394, 1978.

9. Moyer CA: The metabolism of burned mammals and its relationship to vaporization heat loss and other parameters. In Research in Burn, ed by CP Artz, Philadelphia and Washington: FA Davis Co. and American Institute of Biological Sciences, 1962.

10. Aulick LH, Baze WB, Johnson AA, Wilmore DW and Mason AD Jr.: A large animal model of postinjury hypermetabolism. U.S. Army Institute of Surgical Research Annual Research Progress Report, 1 October 1979 - 20 September 1980.

11. Goodall McC, Stone C, Haynes BW Jr.: Urinary output of adrenaline and noradrenaline in severe thermal burns. Ann Surg 145: 479, 1957.

12. Blaxter KL, Brockway JM and Boyne AW: A new method for estimating the heat production of animals. Quarterly J of Exper Physiol 57: 60, 1972.

but substantial modifications have been made to meet our specific research requirements. The operation of this new system is an extension of the classical "confinement" method where an animal was placed in an airtight compartment and its metabolism determined by measuring the change in respiratory gas volumes over brief periods of confinement. In this new open and closed system, the chamber can be automatically ventilated between consecutive periods of confinement thereby permitting a series of metabolic measurements over an extended period of time.

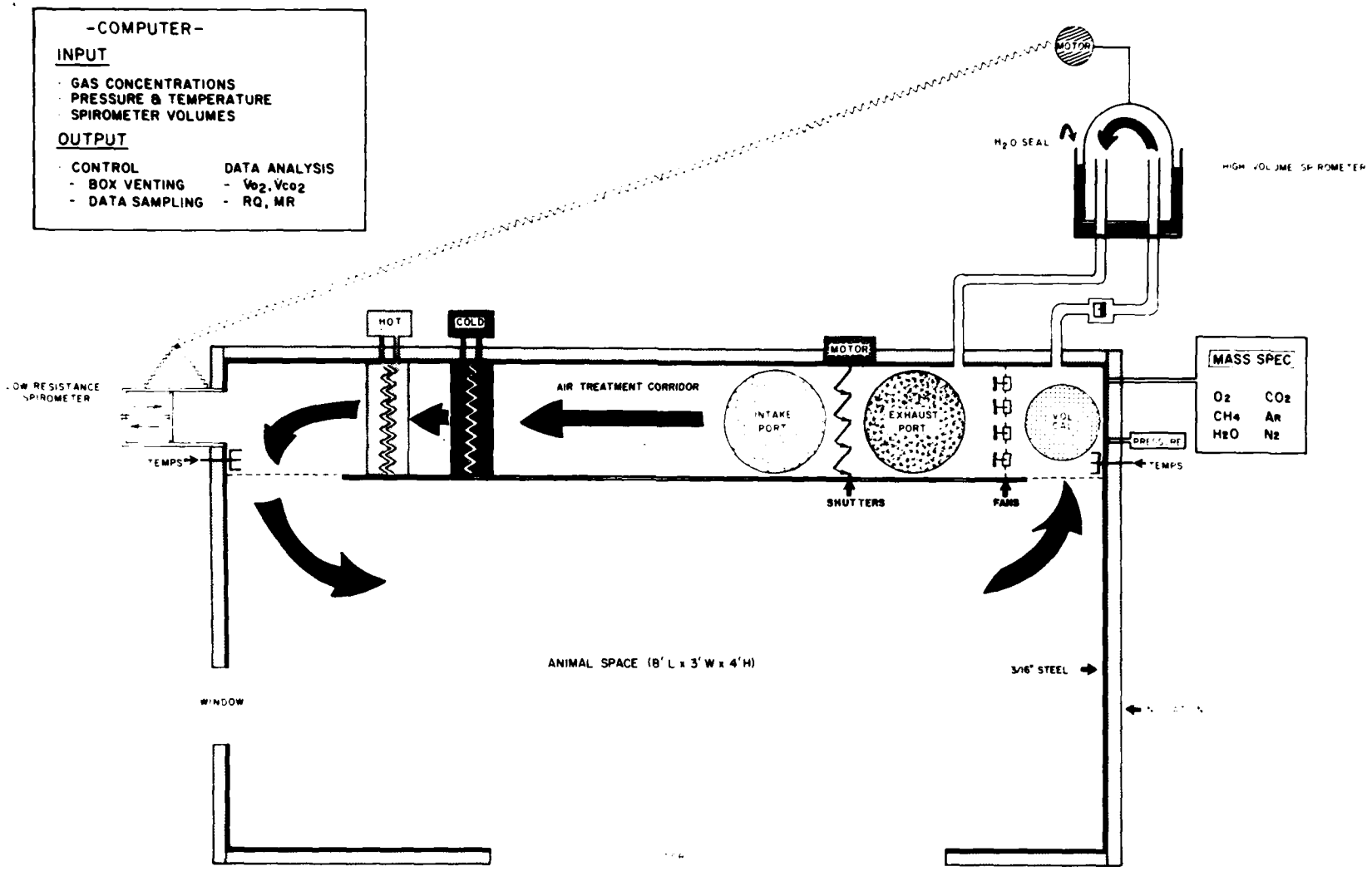
The major features of this new system are illustrated in Figure 1. The 8x4x4 foot chamber is constructed of 3/16 inch steel covered by a 1 inch layer of thermal insulation. The door and window are made of 1/2 inch plexiglas which has been treated with an opaque screen to permit undetected observation of the animal. The goat is free to move about in the animal space but does not have access to an air treatment corridor located at the back of the chamber. The chamber is airtight when large intake and exhaust ports located on the roof of the air treatment corridor are closed. Four fans continuously circulate the air as indicated by the large arrows. When the chamber is closed, air moves through opened shutters and past cold and hot coils, where it is first dehumidified and then brought back to the desired temperature. By opening the two ventilating ports and closing the shutters, air flow is redirected and chamber air is rapidly replaced with fresh outside air. A low resistance spirometer monitors changes in the volume of gas in the system while the chamber is closed. If these changes exceed the limits of this 9-liter spirometer, the excess or deficit volume will be corrected by transfering air to or from a motor-driven, high volume spirometer. Chamber temperature is maintained by a proportional controller which compares the air temperature with a predetermined set-point value and varies the output of the heating coils accordingly. Barometric pressure is monitored continuously by an electronic barometer.

Respiratory gas exchange of the animal is determined by measuring the changes in respiratory gas volumes over the period when the chamber is closed. As soon as the ventilating ports are closed, gas concentrations are measured by the mass spectrometer. The low resistance spirometer is calibrated (VOLCAL), and the volume of each gas is determined by multiplying its fraction times the total gas volume of the chamber corrected to standard conditions. The chamber remains closed until CO_2 concentration reaches 0.9%. (At anticipated rates of CO_2 production, the length of a typical run should be around two hours.) Gas analysis and volume calibrations are then repeated and the chamber ventilated. The rates of oxygen consumption ($\dot{V}_{O_2}$) and carbon dioxide production ($\dot{V}_{O_2}$),

respiratory quotient (RQ) and metabolic rate (MR) are then calculated. This process of repeated metabolic determinations can continue indefinitely, being interrupted only periodically for 3 to 5 minutes of chamber ventilation. Operation of the chamber and all data analysis are controlled by a computer. Current plans are to perform 24-hour studies.

The open and closed respiration chamber is unique and offers many advantages over other conventional approaches. First, it provides for long-term measurement of energy metabolism in conscious, unrestrained animals. This will eliminate much of the physiological and experimental variations associated with isolated, short-term studies. For example, similar work in sheep has shown that day-to-day variation in metabolism of these animals was only 3% (12). If this same degree of baseline stability is also present in the goat, the metabolic impact of injury can be very well defined. Prolonged studies also enable one to characterize the time course of metabolic adjustments to injury better and thereby identify the responses to superimposed experimental manipulations. Second, this system provides excellent control of environmental temperature, humidity, air movement, and noise, all factors which could have a major impact on the metabolism of thermally injured animals. Third, the relative simplicity of design and operation of this system eliminates most of the expense and technical difficulties associated with the more complicated flow-through chambers.

FIGURE 1.



OPEN AND CLOSED RESPIRATION CHAMBER

ANNUAL PROGRESS REPORT

PROJECT NO. 3S161102BS05-00, BASIC RESEARCH

PROJECT TITLE: THE STUDY OF METABOLISM AND NUTRITIONAL EFFECTS ON BURN INJURY IN SOLDIERS - A LARGE ANIMAL MODEL OF POSTINJURY HYPERMETABOLISM

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1 October 1979 - 30 September 1980

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Reports Control Symbol MEDDH-288(R1)

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Further research on postburn hypermetabolism is limited by the constraints of patient studies and the reduced responses of small animal models. To test the validity of a large animal model, oxygen uptake ($\dot{V}_{O_2}$), urinary excretion of epinpehrine (E), norepinephrine (NE) and dopamine (DA), and rectal temperature were monitored in conscious 20 to 40 kg goats for three weeks following a 25% total body surface burn. While $\dot{V}_{O_2}$ and catecholamine output remained at control levels in sham burned animals, $\dot{V}_{O_2}$ rose from 5.57 ± 0.23 to 6.66 ± 0.31 ml/min · Kg (mean $\pm$ SEM) in one group of 7 injured animals at 8 to 10 days postinjury and from 4.58 ± 0.17 to 6.30 ± 0.07 in another group ($n = 6$) studied at 19 to 21 days postinjury. Excretion of E and NE in four injured goats was three to four times that in four sham burned animals at 7, 14 and 21 days postburn. Dopamine output was comparable in the two groups. There was no measurable change in rectal temperature after injury. Injured animals maintained body weight and did not become bacteremic. The hypermetabolic and neuroendocrine responses of the injured goat make this large animal an appropriate model for further research.

Hypermetabolism
Animal model

A LARGE ANIMAL MODEL OF POSTINJURY HYPERMETABOLISM

The hypermetabolic response to injury was first described in patients with long bone fractures in 1930 (1). Since that time, numerous investigators have confirmed and extended these initial observations to demonstrate that the increase in metabolic rate is a) common in a wide variety of surgical patients, b) related to the extent of injury, and c) greatest following severe burns (2, 3).

Many basic components of postinjury hypermetabolism, however, continue to elude clinical investigators due to the constraints of human research. Attempts to develop small animal models have been hampered both by the animals' size and their relatively limited response to injury (4,5,6). Sheep (7) and dogs (8) have been utilized to study the acute cardiovascular adjustments to burn injury, but the associated metabolic responses of these animals have not been recorded. The purpose of this study is to determine whether thermal injury causes a reproducible and, therefore, predictable increase in resting oxygen consumption of the goat. Because hypermetabolic burn patients are febrile and excrete increased quantities of epinephrine and norepinephrine, additional efforts to validate the goat model include measurements of core temperature and urinary excretion of catecholamines.

1. Cuthbertson DP: The disturbance of metabolism produced by bony and non-bony injury, with notes of certain abnormal conditions of bone. Biochem J 24:1244,1930.

2. Moore FD: *Metabolic care of the surgical patient*. Philadelphia: WB Saunders Co, 1959, p 16.

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5. Farkas LG, McCain WG, Birch JR, James J.: The effects of four different chamber climates on oxygen consumption and healing of severely burned rats. J Trauma 13:911, 1973.

6. Herndon DN, Wilmore DW, Mason AD Jr.: Development and analysis of a small animal model simulating the human postburn hypermetabolic response. J Surg Res 25:394, 1978.

7. Traber DL, Bohs CT, Carvajal HF, Linares HA, Miller TH, Larson DL: Early cardiopulmonary and renal function in thermally injured sheep. Surg Gynecol Obstet 148:753, 1979.

8. Moncrief JA: Effect of various fluid regimens and pharmacologic agents on the circulatory hemodynamics of the immediate postburn period. Ann Surg 164:723, 1966.

MATERIALS AND METHODS

Young, healthy, castrated male and non-pregnant female goats of mixed breeds were utilized as experimental animals. Upon arrival, they were given anthelmintics and housed individually in outdoor runs for at least two weeks. Animals weighted between 20 and 40 kilograms, were fed Wayne® Ruff 'N Redi 12 Complete Horse Feed and alfalfa hay, and given water ad libitum.

After the initial period of adjustment, goats were moved into the laboratory where room temperature was maintained between 25 and 28° C. Over the next three weeks, the animals were conditioned to stand quietly for one hour in a small stand. The goat stood in a nylon mesh sling to prevent the animal from stepping off the stand, and the horns were tethered to an overhead bar to limit head movement. At the end of each training session, rectal temperature was taken with a standard glass thermometer, and the animal was weighed on a platform balance.

Once conditioned, each animal was placed in one of two basic protocols. In one, oxygen consumption was measured before and after receiving a 25% total body surface burn. In the other, urinary catecholamine excretion was determined before and after the same injury. Uninjured animals served as controls in both protocols.

Oxygen consumption was determined in tracheostomized goats by closed circuit spirometry (9). Measurements were performed once or twice daily for three days beginning the day after tracheostomy. On the day of study, a disposable cuffed, tracheostomy tube was inserted, the cuff inflated and the animal left undisturbed on the stand for 30 to 45 minutes prior to spirometry (Figure 1). The tracheostomy tube was then attached to a two-way, low-resistance Rudolph valve which was, in turn, connected to a calibrated, 9-liter, Collins spirometer* by large-bore respiratory tubing. The valve permitted unidirectional air flow between the goat and the spirometer so that the animal inhaled 100% oxygen from the spirometer and exhaled through a separate line into a carbon dioxide absorber and back into the spirometer. The rate of decrease in oxygen volume was recorded for 15 to 20 minutes and this slope used to calculate the animal's oxygen uptake. All gas volumes were corrected to standard conditions, and oxygen consumption was expressed in ml O_2 per minute per kilogram body weight.

The tracheostomy tube was removed immediately after each study, and the animal's rectal temperature and body weight were then recorded.

*W.E. Collins, Inc., Braintree, Massachussetts

9. Consolazio CF, Johnson RE, Pecora LJ. In: Physiological Measurements of Metabolic Function in Man. New York: McGraw-Hill Book Co., 1963.

The frequency of testing depended on the general activity level of the animal. Most stood quietly and were studied once daily. Two animals had to be dropped from the study due to their inability to accept the experimental setup and stand quietly.

Following control studies, anesthesia was induced in 13 animals by intravenous methohexital sodium (10 to 15 mg/kg) and maintained at a surgical plane with a mixture of methoxyfluorane and 100% oxygen. Hair was clipped from the back and both sides, and a third degree flame burn was created over 20 to 25% of the total body surface. Six goats were anesthetized and the hair clipped as described, but these goats were not injured. They were designated the "sham burned" animals and treated in exactly the same manner as the injured animals over the course of study.

The goats were allowed to recover spontaneously without fluid or electrolyte administration. For the remainder of the study, all animals received a daily supplement of 1000 calories (Ensure®, Ross Laboratories, Columbus, Ohio) by gavage in addition to the regular diet. The daily conditioning program continued as before. In one group of seven injured animals (Burn Group I) and six sham burned animals (Sham Burn Group I), a second tracheostomy was performed distal to the previous site on the seventh day postinjury. Oxygen consumption measurements were repeated in these two groups on the eighth, ninth, and tenth days postinjury. In another group of six injured goats (Burn Group II), the second tracheostomy was performed on the 18th day postburn and spirometry conducted over the next three days.

No systemic or topical antibiotic therapy was used. Venous blood samples were obtained the last two days of study, and bacteriological cultures were performed. Animals were euthanatized at the end of the study, necropsies performed and wound biopsies obtained for histological examination.

Urinary excretion of catecholamines was determined in eight goats following the same general protocol. Four injured animals made up Burn Group III and four sham burned animals were designated Sham Burn Group II. Four control studies were performed on each animal, and single studies were repeated at 7, 14 and 21 days postinjury or sham burn. To avoid the stress of urethral catheterization, a catch-pan was constructed for the goat stand, and urine was collected when the animal voided normally. Urine samples were acidified immediately upon collection, and epinephrine, norepinephrine and dopamine contents were determined by reverse-phase, high-pressure liquid chromatography with electrochemical detection (10).

10. Riggin RM, Kissinger PT: Determination of catecholamines in urine by reverse-phase liquid chromatography with electrochemical detection. Analyt Chem 49:2109, 1977.

Prior to the control runs, each goat was given a liter of water by gavage to promote urine production. On subsequent studies, one liter of the dietary supplement was used instead of water. Depending on the initial level of hydration and resultant urinary frequency, the collection periods ranged from 3 to 14 hours. While the animal may have voided more than once during this time, excretion rates were determined as the average for the entire period and expressed in nanograms per minute per kilogram of body weight.

All data are presented as group mean $\pm$ SEM. The student t-test for paired data was used to evaluate changes in oxygen consumption following injury or sham burn. An analysis of variance was performed on the catecholamine data.

RESULTS

The 25% total body surface burn was well-tolerated by all animals. During the first one or two days of recovery, injured goats drank liberal quantities of water but appetite was usually depressed. By the third or fourth day postinjury, they began eating normally and, in general, maintained body weight over the period of observation (Table 1). No animal became bacteremic or developed histological evidence of burn wound invasion. Three goats had to be excluded from study due to pulmonary complications* rather than any direct result of the burn itself.

Oxygen consumption ($\dot{V}_{O_2}$) remained at control levels in the sham burned animals but was increased in the two groups of injured goats (Table 2). The average increase in $\dot{V}_{O_2}$ was 19.6% above control levels in the group of seven animals studied 8 to 10 days postinjury (Burn Group I) and 37.6% in the six goats of Burn Group II retested three weeks postburn. This difference in the percent increase in $\dot{V}_{O_2}$ following injury developed as a result of lower control values in Burn Group II rather than any absolute difference in postburn $\dot{V}_{O_2}$ of the two groups.

Over the three-week period of observation, catecholamine excretion remained at control levels in the sham burned animals, but in the injured goats, epinephrine and norepinephrine outputs rose above preinjury values by the seventh day postinjury and remained elevated at 14 and 21 days postburn (Table 3). The elevated rates of

*Two uninjured goats developed excessive soft tissue swelling around the tracheostomy site; the third developed a lung abscess.

catecholamine excretion were reasonably stable over this period of time. While all injured animals maintained increased norepinephrine excretion rates, one of the four failed to elevate epinephrine output above the control range (0.17, 0.07 and 0.16 ng/min·kg 7, 14 and 21 days postinjury). Therefore, while excretion of both amines was significantly elevated in the injured group, thermal injury had a more consistent effect on norepinephrine output. Dopamine excretion was highly variable and not significantly elevated at any point following injury.

Thermal injury did not result in a significant change in rectal temperature over the three-week period (Table 4). On occasion, the injured animals appeared to shiver, but such behavior was associated with handling (i.e., tube feeding, weighing, etc.) and would disappear as soon as the animal was left alone.

DISCUSSION

Average oxygen consumption of all uninjured animals was within the normal range for goats studied under comparable experimental conditions (11, 12). Therefore, the observed difference in control values between the two groups (Table 2) was considered a function of normal variation.

Injured animals became hypermetabolic as early as eight days postinjury and remained so for the next two weeks. The 20% increase in $\dot{V}_{O_2}$ of animals studied eight to ten days postinjury was about half that observed in the group at three weeks postburn. Since this difference was the result of lower control levels in the latter group, rather than any difference in $\dot{V}_{O_2}$ at one and three weeks postinjury, it is doubtful that the metabolism of the injured animal continued to increase for three weeks. Alternatively, the lower control $\dot{V}_{O_2}$ values may more accurately represent resting aerobic metabolism and thereby make the 40% increase in $\dot{V}_{O_2}$ of this group a better estimate of the actual energy cost of injury. In addition, the general activity level of these animals decreased slightly following injury, so whether the 20 or 40% figure is chosen, it should be considered a minimal estimate of actual postburn

11. Heisey SR, Adams T, Hofman W, Riegle G: Thermally induced respiratory responses of the unanesthetized goat. Resp Physiol 11:145, 1971.

12. Jessen C: Interaction of air temperature and core temperatures in thermoregulation of the goat. J Physiol (London) 264:585, 1977.

hypermetabolism. Even if the 40% estimate is selected, it is only about half that anticipated in a burned human with the same size injury (13).

Herndon et al (6) found about the same increase in $\dot{V}_{O_2}$ of rats and guinea pigs following 50% total body surface burns. Rats with smaller injuries (burns of a size comparable to that in the goat model) have a more limited metabolic response and most, if not all, of this extra metabolism can be eliminated by increasing ambient temperature (4,5,6). That the energy cost of thermal injury appears to vary with body size (from rat to man) may eventually provide some insight into the basis of burn hypermetabolism.

Under these experimental conditions, the injured goat failed to demonstrate a measurable increase in rectal temperature (Table 4). Considering the variability in control values observed in this and other studies (1*,14,15),, subtle changes in core temperatures following injury could easily go unnoticed. Consequently, before a more definitive statement can be made regarding the thermoregulatory response of this animal, changes in experimental design are necessary. A large animal environmental chamber is currently under development at this laboratory to address this issue.

All animals in this study were housed in an ambient environment well within the thermoneutral zone for an uninjured goat (16), and injured animals did not appear to be cold. But, since goats may develop a degree of non-shivering thermogenesis (14) which triggers metabolic and neuroendocrine responses comparable to those observed in the injured animals, the thermoregulatory contribution to postburn hypermetabolism must be clarified in this particular model.

13. Wilmore DW, Long JM, Mason AD, Jr., Skreen RW, Pruitt BA, Jr.: Catecholamines: Mediator of the hypermetabolic response to thermal injury. Ann Surg 180:653, 1974.

14. Andersson B: Central nervous and hormonal interaction in temperature regulation of the goat. In: JD Hardy, AP Gagge, JAJ Stolwijk (Eds), Physiological and Behavioral Temperature Regulation. Springfield: Charles C Thomas Publisher, 1970, p 634.

15. Jessen C, Clough DP: Evaluation of hypothalamic thermosensitivity by feedback signals. Pflügers Arch 345:43, 1973.

16. Bligh J, Cottle WH, Maskrey M: Influence of ambient temperature on the thermoregulatory responses to 5 hydroxytryptamine, noradrenaline and acetylcholine injected into the lateral cerebral ventricles of sheep, goats and rabbits. J Physiol (London) 212:377, 1971.

Norepinephrine excretion of the uninjured animals was comparable to that reported in other normal control goats (17). Epinephrine output, on the other hand, was twice the reported normal value. Similar shifts in the epinephrine-to-norepinephrine ratio have been observed in baboons and rhesus monkeys studied in primate chairs and is considered a function of confinement (18).

The three- to fourfold increase in urinary excretion of epinephrine and norepinephrine of the injured animals was of the order of magnitude reported in four patients with comparable size burns and studied at the same time postinjury (19). The failure of one animal to increase epinephrine output was unexplained but suggests that factors other than the burn itself may influence postinjury adrenal medullary activity in the goat.

In the injured goat model, dopamine excretion was highly variable and not consistently above control levels at any time postinjury. Dopamine turnover is markedly accelerated in the burn patient where a major portion is rapidly incorporated into norepinephrine (20). Presumably, the rapid rate of dopamine turnover made it impossible to demonstrate a rise in urinary excretion in the injured goat.

The relationship between increased catecholamine excretion and oxygen consumption was described in burn patients as early as 1967 by Harrison et al (21). Since the calorigenic potential of catecholamines had already been established, they concluded that increased sympathoadrenal activity was responsible for the elevation in metabolic rate of burned patients. This hypothesis was later confirmed by Wilmore and collaborators (13) by first demonstrating a significant relationship between metabolic rate and urinary catecholamine excretion and then reducing the hypermetabolism by adrenergic blockade. The results of this study, while obtained in different groups of animals, indicate that the increase in aerobic metabolism of the goat was associated with a simultaneous increase in sympathoadrenal activity. The direct cause and effect relationship must be established in the goat, but the combined neuroendocrine and metabolic responses of this animal to thermal injury strongly suggest that it is a suitable model for further research.

17. Gale CC: Neuroendocrine aspects of thermoregulation. Ann Res Physiol 35:391, 1973.

18. Gale CC, Jobin M, Proppe DW, Notter D, Fox H: Endocrine thermoregulatory responses to local hypothalamic cooling in unanesthetized baboons. Am J Physiol 219:193, 1970.

19. Goodall McC, Stone C, Haynes BW, Jr: Urinary output of adrenaline and noradrenaline in severe thermal burns. Ann Surg 145:479, 1957.

20. Goodall, McC, Alton H: Dopamine (3-Hydroxytegramine) replacement and metabolism in sympathetic nerve and adrenal medullary depletions after prolonged thermal injury. J Clin Invest 48:1761, 1969.

21. Harrison TA, Seaton JF, Feller I: Relationship of increased oxygen consumption to catecholamine excretion in thermal burns.

SUMMARY

A 25% total body surface burn increased oxygen consumption of the goat by 20 to 40%. This hypermetabolism was apparent as early as 8 to 10 days postinjury and remained evident 19 to 21 days postburn. Associated with these changes in aerobic metabolism was a three- to fourfold increase in urinary epinephrine and norepinephrine excretion which again was evident one week postinjury and persisted for at least three weeks following injury. Dopamine excretion was highly variable but not significantly affected by injury. While the thermoregulatory adjustment of this large animal model remains uncertain, changes in oxygen consumption and urinary catecholamine excretion in the goat are very much like the human response to thermal injury.

TABLE 1. EFFECT OF THERMAL INJURY ON BODY WEIGHT*

GROUP	DAYS POSTINJURY			
	-5 to 0	7 to 10	13 to 15	19 to 21
SHAM BURN I	26.4 ± 2.1	26.2 ± 2.2	-	-
BURN I	28.3 ± 1.0	28.6 ± 1.0	-	-
SHAM BURN II	33.1 ± 0.6	33.6 ± 1.3	34.5 ± 1.9	35.4 ± 1.6
BURN II	29.6 ± 1.2	29.2 ± 0.5	29.0 ± 0.5	28.0 ± 0.9
BURN III	35.3 ± 1.0	33.9 ± 2.2	33.8 ± 2.2	34.6 ± 2.4

*Body weight in kilograms; mean ± SEM

TABLE 2. EFFECT OF THERMAL INJURY ON OXYGEN CONSUMPTION*

GROUP	DAYS POSTINJURY		
	-3 to 0	8 to 10	19 to 21
SHAM BURN I	5.49 ± 0.45	5.54 ± 0.44	-
BURN I	5.57 ± 0.23	6.66 ± 0.31 **	-
BURN II	4.58 ± 0.17	-	6.30 ± 0.07 †

*Oxygen consumption in ml O_2/min per kilogram body weight; mean ± SEM

**$p < 0.05$, † $p < 0.01$, paired t test compared with same group of animals before injury

TABLE 3

EFFECTS OF THERMAL INJURY ON URINARY CATECHOLAMINE EXCRETION*

	DAYS POSTINJURY			
	0	7	14	21
NOREPINEPHRINE				
SHAM BURN II	0.34 ± 0.03	0.36 ± 0.05	0.28 ± 0.06	0.23 ± 0.04
BURN III	0.28 ± 0.03	1.03 ± 0.08**	0.78 ± 0.18**	0.89 ± 0.26**
EPINEPHRINE				
SHAM BURN II	0.14 ± 0.01	0.13 ± 0.02	0.13 ± 0.04	0.13 ± 0.02
BURN III	0.18 ± 0.04	0.53 ± 0.14†	0.35 ± 0.08†	0.41 ± 0.18†
DOPAMINE				
SHAM BURN II	1.32 ± 0.18	1.28 ± 0.20	1.34 ± 0.20	0.93 ± 0.24
BURN III	1.23 ± 0.20	2.36 ± 0.40	1.91 ± 0.38	2.45 ± 0.84

*Excretion rate in nanograms/min per kilogram body weight; mean ± SEM

**$p < 0.01$, †$p < 0.05$, analysis of variance comparing burn and sham burned animals at 7, 14 and 21 days.

TABLE 4. EFFECT OF THERMAL INJURY ON RECTAL TEMPERATURE*

GROUP	DAYS POSTINJURY			
	-5 to 0	7 to 10	13 to 15	19 to 21
SHAM BURN I	39.5 ± 0.2	39.8 ± 0.2	-	-
BURN I	39.4 ± 0.1	39.5 ± 0.2	-	-
SHAM BURN II	39.0 ± 0.1	39.1 ± 0.04	39.0 ± 0.05	39.0 ± 0.05
BURN II	39.6 ± 0.1	39.5 ± 0.04	39.6 ± 0.2	39.8 ± 0.1
BURN III	38.8 ± 0.1	39.2 ± 0.2	39.2 ± 0.1	39.3 ± 0.2

*Expressed in ^{o}C; mean $\pm$ SEM

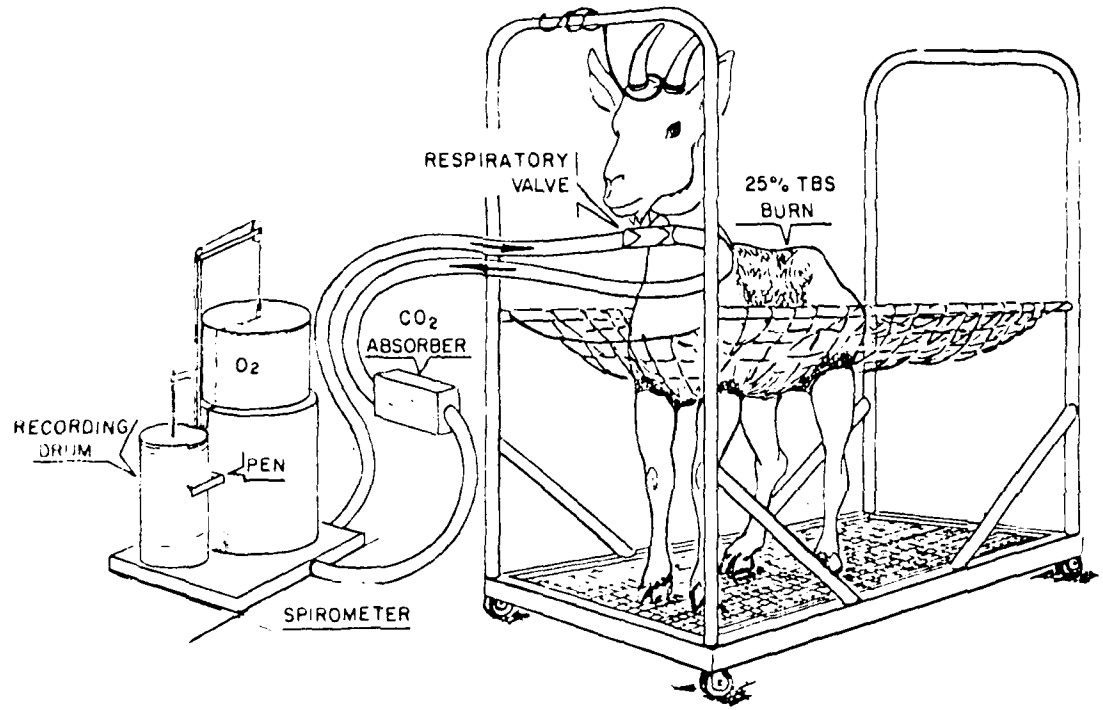

FIGURE 1.

CLOSED CIRCUIT SPIROMETRY IN THE GOAT MODEL

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OG 1842	01 Oct 80	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
01 Oct 79	D. CHANGE	U	U	NA	NL	[X] YES [ ] NO	A. WORK UNIT

10. NO. CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3M161102BS10	BB	304
b. [illegible]	61102A	3S161102BS05	00	092
c. [illegible]	STOG 80-7.2:5			

11. TITLE (Precede with Security Classification Code)

(U) Assessment of L-Triiodothyronine Therapy in Thermally Injured Patients (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 Clinical Medicine 002300 Biochemistry

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
Aug 79	Cont	DA	C. In-House

17. CONTRACT GRANT — Not Applicable

a. DATES/EFFECTIVE — EXPIRATION:
b. NUMBER
c. TYPE — d. AMOUNT
e. KIND OF AWARD — f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 1980	2.0	$ 155
	CURRENT 1981	2.0	$ 159

19. RESPONSIBLE DOD ORGANIZATION

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ADDRESS: Ft Sam Houston, Texas 78234

RESPONSIBLE INDIVIDUAL
NAME: Basil A. Pruitt, Jr., MD, COL, MC
TELEPHONE: 512-221-2720

20. PERFORMING ORGANIZATION

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Surgical Study Branch
ADDRESS: Ft Sam Houston, Texas 78234

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME: Richard A. Becker, M.D.
TELEPHONE: 512-221-4253
SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS
NAME:
NAME: DA

21. GENERAL USE

FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) L-Triiodothyronine; (U) Therapy; (U) Burn Patients; (U) Hypothyroidism

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) To assess the efficacy of L-triiodothyronine (T_3) treatment in thermally injured patients.

24. (U) A prospective, single-blinded, randomized study will be performed. Serum concentrations of T_3 will be maintained within the normal range. Specific endocrine, microbiologic, and pathologic parameters will be monitored.

25. (U) 7910 - 8009. Twenty-eight patients are currently in the protocol and undergoing active study at this time. Our preliminary observations suggest that T_3 levels can be maintained within the normal range in critically injured burn patients without major effect on metabolic rate. Assessment of the marginating leukocyte pool suggests that the marginating pool is present but composed of leukocytes which are immature and of limited metabolic reserve. Collection of urine and plasma specimens for multiple hormonal analysis is underway. The efficacy of T_3 treatment in these patients cannot be assessed until the study is completed and the randomized treatment code is broken.

*Available to contractors upon originator's approval

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 68 AND 1498-1 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

ANNUAL PROGRESS REPORT

PROJECT NO. 3S161102BS05-00, BASIC RESEARCH

REPORT TITLE: A SYNDROME OF SECONDARY OR TERTIARY HYPOTHYROIDISM IN SEPTIC, TERMINALLY ILL BURN PATIENTS

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

1 October 1979 - 30 September 1980

Investigators:

Richard A. Becker, MD
George M. Vaughan, MD, Major, MC
Leonard G. Seraile, MS
Jennifer M. Tucker, SP6
Arthur D. Mason, Jr., MD
Basil A. Pruitt, Jr., MD, Colonel, MC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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Reports Control Symbol MEDDH-288(R)

The argument for euthyroidism in critically ill patients (low T_3 syndrome) has centered on normal serum levels of thyrotropin (TSH) despite depressed serum levels of thyroxine (T_4) and triiodothyronine (T_3). We have previously reported profound suppression of total and free T_4 (FT_4) and total and free T_3 (FT_3) in septic burn patients. In the present study, hormonal data are reported from five septic, terminally ill burn patients (mean burn size 60%) studied on alternate days during the last week of life (20 studies) and compared with similar data from five surviving patients matched for age, extent of burn injury, and postburn day (20 studies). FT_4 and FT_3 were determined by equilibrium dialysis.

	TSH	FT_4	FT_3
		(M±SE)	
normal range	(<6μU/ml)	(1.3-3.9ng/dl)	(230-660pg/dl)
Dying Patients	.6±.2	1.1±.1	85±18
Surv Patients	2.8±.4†	1.9±.1†	247±27†

†p <.01

In two of the dying patients, plasma T_3 was undetectable (<10 ng/dl) within 24 hours of death and in one of these patients plasma T_4 was also undetectable (<.1 μg/dl). Both plasma dopamine and cortisol, known inhibitors of TSH, were significantly higher in the dying patients, $p<.01$. In summary, serum TSH levels were significantly lower in terminally ill burn patients and did not respond to low or undetectable levels of free T_3 or free T_4. These data demonstrate profound suppression of serum T_4, T_3, and TSH levels, consistent with pituitary or hypothalamic failure, and describe a syndrome of secondary or tertiary hypothyroidism in septic, terminally ill burn patients.

PRESENTATIONS/PUBLICATIONS - None

Secondary hypothyroidism
Tertiary hypothyroidism
Septic
Terminally ill
Burn patients

ANNUAL PROGRESS REPORT

PROJECT NO. 3S161102BSO5-00, BASIC RESEARCH

REPORT TITLE: SPLANCHNIC AND RENAL EXCHANGE OF FREE THYROID HORMONES IN CRITICALLY ILL BURN PATIENTS

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

1 October 1979 - 30 September 1980

Investigators:

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

Reports Control Symbol MEDDH-288(R1)

Unclassified

ABSTRACT

PROJECT NO. 3S161102BSO5-00, BASIC RESEARCH

REPORT TITLE: SPLANCHNIC AND RENAL EXCHANGE OF FREE THYROID HORMONES IN CRITICALLY ILL BURN PATIENTS

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

Period cc ed in this report: 1 October 1979 - 30 September 1980

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Reports Control Symbol MEDDH-288(RI)

In the clinically stable burn patient, alterations in peripheral thyroid hormone concentrations consistent with the Euthyroid Sick Syndrome are present. However, in clinically deteriorating or dying burn patients, both total and free thyroid hormone concentrations may be profoundly suppressed or depleted. Splanchnic exchange of free thyroid hormone was measured in 12 critically ill burn patients. In 10 euthyroid patient studies, whether septic or nonseptic, splanchnic release of FT_3 was observed, compared with splanchnic uptake of FT_3 in 4 chemically hypothyroid-septic patient studies, $p < .01$. A positive correlation was observed between splanchnic exchange of FT_3 and arterial FT_4. These data suggest that the splanchnic circulation may have a regulatory role in the peripheral thyroid economy of critically ill burn patients.

Splanchnic exchange
Renal exchange
Free thyroid hormones
Critically ill
Burn patients

SPLANCHNIC AND RENAL EXCHANGE OF FREE THYROID HORMONES IN CRITICALLY ILL BURN PATIENTS

Severely burned patients present many of the clinical manifestations of hyperthyroidism, including hypermetabolism, tachycardia, hyperkinesia, hyperventilation, and weight loss. In clinically stable burn patients, we have recently reported alterations in peripheral thyroid hormone concentrations consistent with the "Euthyroid Sick Syndrome", similar to those reported in a wide variety of stress and disease states (1). In the clinically deteriorating and septic burn patient, however, we have reported significant suppression of both total triiodothyronine (T_3) and free T_3 (FT_3) and free thyroxine (FT_4) concentrations (2). In our laboratory, the hypermetabolism of burn injury has been correlated with increased catecholamine excretion (3). We have recently reported a reciprocal relationship between plasma levels of norepinephrine and epinephrine and T_3 in burn patients with suppressed levels of T_3. This observation is consistent with similar reports in patients with primary thyroidal diseases (4).

The present study was designed to assess the role of the renal and splanchnic circulatory beds in the metabolism of free thyroid hormones. In addition, we report profound suppression or depletion of thyroid hormone concentrations in patients dying from severe burn injury.

MATERIALS AND METHODS

Patients - Serum specimens were obtained from 11 patients with greater than 50% total body surface burns who died during the first seven to ten postburn days.

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1. Cavalieri RR, Rapaport B: Impaired peripheral conversion of thyroxine to triiodothyronine. Ann Rev Med 28:57-65, 1977.

2. Becker RA, Wilmore DW, Goodwin CW, et al: Free T_4, free T_3, and reverse T_3 in critically ill, thermally injured patients. J Trauma 20:713-721, 1980.

3. Wilmore DW, Long JM, Mason AD, et al: Catecholamines: Mediator of the hypermetabolic response to thermal injury. Ann Surg 180:653-669, 1974.

4. Becker RA, Vaughan GM, Goodwin CW, et al: Plasma norepinephrine, epinephrine and thyroid hormone interactions in severely burned patients. Arch Surg 115:439-443, 1980.

Splanchnic and renal exchange of free thyroid hormones were studied prospectively in 12 additional burn patients who had a mean total body surface burn of 57%. Patients were divided into three groups based on presence or absence of sepsis and free thyroid hormone concentrations. Five studies were performed in 5 patients who were chemically euthyroid and nonseptic (EU/NS); 5 studies were performed in 4 patients who were chemically euthyroid and septic (EU/S); and 4 studies were performed in 3 patients who were chemically hypothyroid and septic (HYPO/S). The three groups were compared by the Scheffe technique for a posteriori multiple group comparison.

STUDY DESIGN

Studies were conducted at 0600 after a 6-hour infusion of 0.04 molar nutrient free sodium chloride solution. Following catheterization of the right femoral artery and vein under local anesthesia, the venous catheter was advanced into the left renal vein under fluoroscopic control and renal vein specimens collected. The catheter tip was then advanced into the right hepatic vein, 3 to 4 centimeters from the wedge position. Following a one hour equilibration in an environmental chamber at an ambient temperature of 30°C, simultaneous femoral artery and hepatic vein specimens were obtained for hormone analysis. Splanchnic blood flow was calculated from the proportionality constant for indocyanine green dye disappearance (ICG), ICG hepatic extraction ratio, and the hematocrit. Splanchnic hormone exchange was calculated as the product of the splanchnic blood flow and the AV difference for free thyroid hormone and corrected for body surface area. Metabolic rate was determined by indirect calorimetry using a canopy hood system (3).

ASSAYS

Thyroid hormones were measured by radioimmunoassay using reagents commercially available from Ortho Diagnostics, Raritan, New Jersey. Free hormone concentrations were determined by equilibrium dialysis at the Nichols Institute, San Pedro, California.

RESULTS

Total T_4, T_3 and the free thyroxine index (FTI) were examined in serum specimens obtained from 11 burn patients during the last 4 days of life (Fig. 1). Four days preceding death, the mean hormonal values for these patients were not different from those observed in patients with similar injuries who recovered (Euthyroid Sick Syndrome). However, over the ensuing 4 days, profound suppression of T_4 was observed, with 8 values less than 1.5 µg/dl, and 4 values less than 0.7 µg/dl within 24 hours of death. The FTI was similarly suppressed in these patients. Although recent studies suggest

that the FTI may not be an accurate assessment of free hormone concentration (5), we have not noted significant discrepancies in the percent free hormone, as determined by equilibrium dialysis, in burn patients with normal as compared with suppressed serum levels of T_4. T_3 levels were markedly depressed in these patients as well. Subsequent observations have disclosed 3 additional burn patients with essentially no measurable T_3 in their sera at or near the time of death.

Table 1. Arterial free thyroid hormone concentrations and metabolic rates in critically ill burn patients

(nl range)	FT_4 (1.3-3.8 ng/dl)	FT_3 (230-669 pg/dl) (Mean ± SE)	FrT_3	Metabolic Rate (≈ 30 kcal/hr·m^2)
EU/NS	2.12 ± .30	313 ± 30	125 ± 17	64 ± 3
EU/S	1.86 ± .17	262 ± 28	163 ± 38	69 ± 4
HYPO/S	0.75 ± .12*	222 ± 15*	129 ± 18	66 ± 4

*p <.01

In 14 patient studies of splanchnic and renal exchange of free thyroid hormone, mean arterial FT_4 and FT_3 values were significantly suppressed, p <.01, in the HYPO/S patient group (Table 1). Free reverse T_3 (FrT_3) concentrations were not significantly different between groups. Resting metabolic rates averaged about twice the normal basal level in all patients and were not different between groups. It is of some interest that these metabolic rates appear to be independent of free thyroid hormone concentration.

Among patient groups, no significant AV differences for FT_4, FT_3, or FrT_3 were detected across the renal circulatory bed (Fig. 2). Although a trend toward higher venous concentrations of free hormone is suggested, consistent with the anticipated slight concentrating effect of renal water excretion, none of the mean values were significantly different from zero, nor did the group means differ significantly from one another.

Splanchnic exchange of mean FT_3 differed significantly both between hypothyroid and euthyroid patient groups and from zero for each patient group (Fig. 3). In all euthyroid patients studied, whether septic or non-septic, FT_3 was released from the splanchnic circulation; conversely, FT_3 was taken-up across the splanchnic circulation in all HYPO/S patients studied, p <.01. Splanchnic exchanges of FT_4 and FrT_3 were not different between groups nor were the mean values different from zero. Arterial FT_4 concentrations were significantly correlated with splanchnic exchange of

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5. Woeber KA: Thyroid hormone binding in nonthyroid illness. Clin Res 28:71A, 1980.

FT_3, r=.80, $p < .001$, (Fig. 4). This relationship suggests a substrate threshold for arterial FT_4 ($\approx$1.3 ng/dl), which separates release and uptake of FT_3 by the splanchnic circulation.

DISCUSSION

In ten burn patients studied during the 4 days preceding death, we observed significant depletion of serum concentrations of T_4 and T_3. On the day preceding death, 4 patients were found to have serum T_4 concentrations of less than 0.7 μg/dl. Serum T_3 levels were similarly suppressed. These observations differ from those in burn patients with comparable injuries who survived their wounds. Surviving patients exhibit a similar suppression of thyroid hormone levels during the first week postinjury but are characterized by a gradual return toward the normal range with clinical improvement. The factors which lead to the initial suppression of serum T_4 and T_3 may include the expansion of the albumin space and extensive fluid resuscitation which attend the initial burn injury and recovery period. Those factors which result in further depletion of serum T_4 and T_3 in the dying patient are unknown; however, sepsis may enchance hormonal degradation. We have previously demonstrated a significant decrease in FT_4 and both total and free T_3 in septic patients. In addition, the high cortisol values observed in these patients as well as the high plasma inorganic iodide levels which we have observed in burn patients (6), may inhibit the hypothalamic-pituitary-thyroidal axis.

The extrathyroidal metabolism of thyroid hormone is altered in burn patients. In 10 euthyroid patient studies, whether septic or nonseptic, splanchnic release of FT_3 was observed, suggesting that monodeiodination of FT_4 to FT_3 continues across the splanchnic circulatory bed in these critically ill burn patients. However, in 4 chemically hypothyroid and septic patient studies, FT_3 was taken-up by the splanchnic circulation. Further, a positive correlation was observed between splanchnic exchange of FT_3 and arterial FT_4, consistent with a threshold concentration of arterial FT_4 for splanchnic release or uptake of FT_3. These data suggest that the splanchnic circulation may have a regulatory role in peripheral thyroid economy by varying its rate of release or uptake of FT_3 in response to substrate availability.

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6. Becker RA: Unpublished data.

PRESENTATIONS:

Becker RA: Hepatic and Renal Exchange of Free Thyroid Hormones in Critically Injured Man. Read before VIII International Thyroid Congress, Sydney, Australia, February 1980.

PUBLICATIONS:

Becker RA, Wilmore DW, Goodwin CW, Aulick LH, Mason AD, and Pruitt BA Jr: Hepatic and Renal Exchange of Free Thyroid Hormones in Critically Injured Man. Proceedings of VIII International Thyroid Congress, 1980.

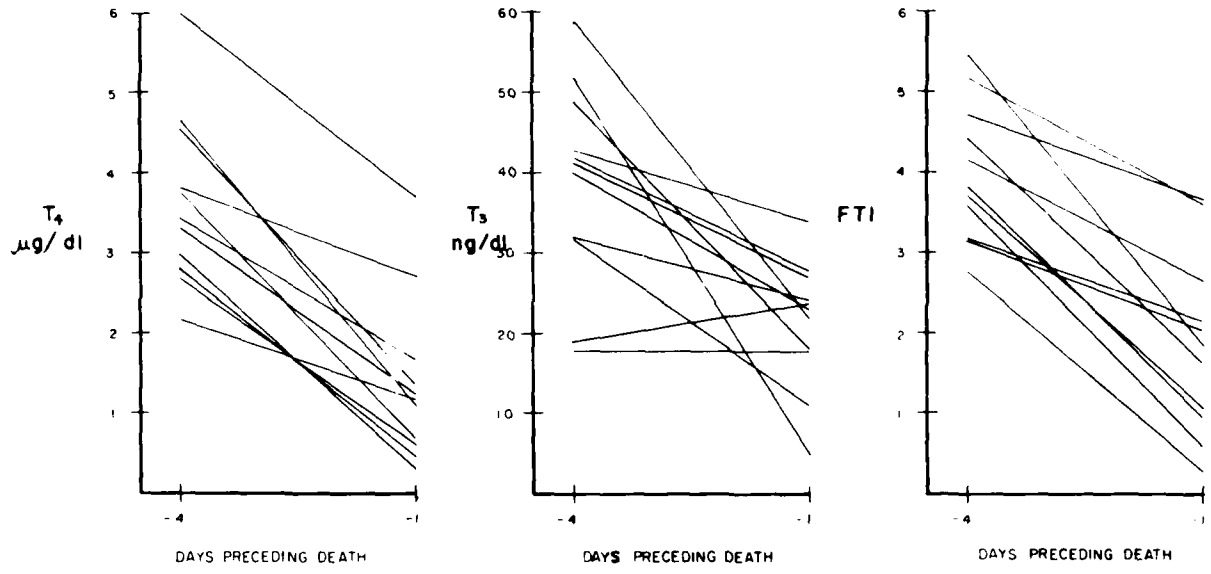

Fig. 1. Serum levels of T_4, T_3, and FTI in 10 burn patients over the 4 days preceding death.

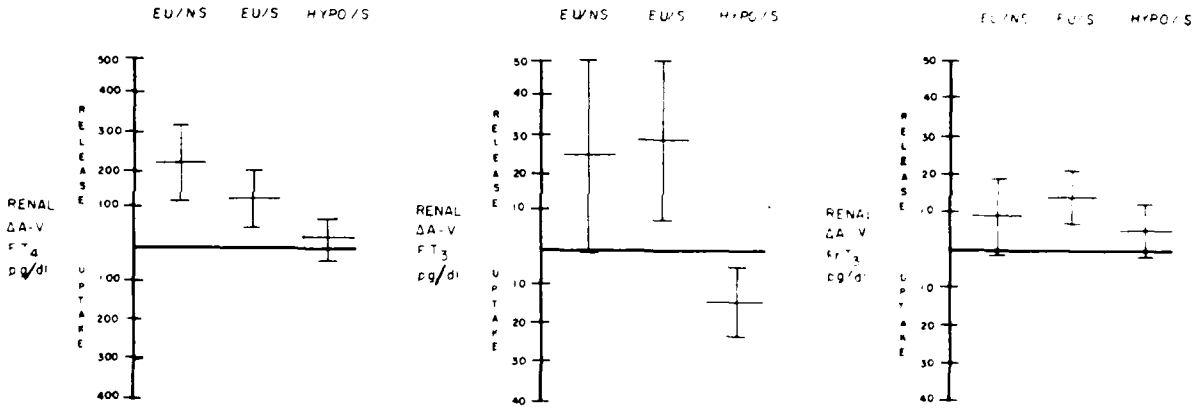

Fig. 2. Renal AV differences (Mean ± SE) expressed as release or uptake for FT_4, FT_3 and FrT_3 in pg/dl for EU/NS, EU/S and HYPO/S patient groups.

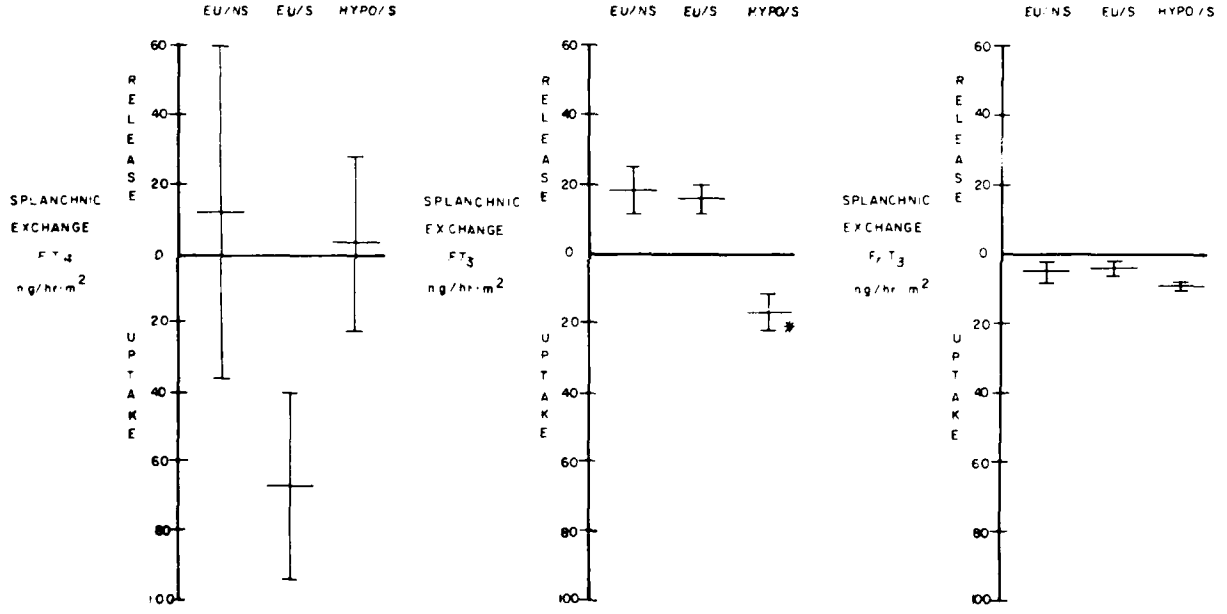

Fig. 3. Splanchnic exchange of FT_4, FT_3 and FrT_3 (Mean ± SE) expressed as release or uptake in ng/hr·m^2 for EU/NS, EU/S and HYPO/S patient groups. *p <.01

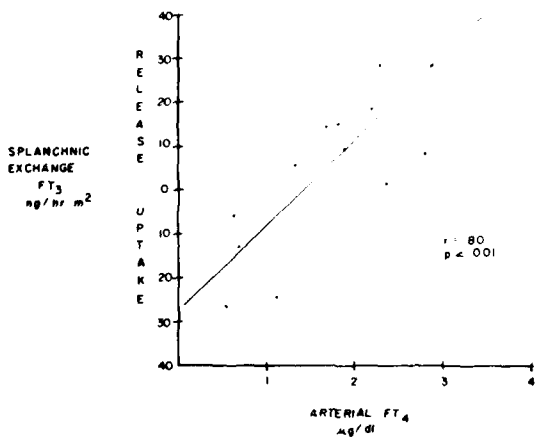

Fig. 4. Linear correlation of splanchnic exchange of FT3, ng/hr·m^2, and arterial FT_4, pg/dl.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0G 6976	79 10 01	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	8a. SPECIFIC DATA CONTRACTOR ACCESS	9. LEVEL OF SUM
79 10 01	D. CHANGE	U	U	NA	NL	[X] YES [ ] NO	A. WORK UNIT

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	089
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) Use of a Laminar Flow Isolator to Control Infection in Burned Troops (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
77 09	Cont	DA	C. In-House

17. CONTRACT/GRANT: Not Applicable
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER:
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 80	.3	11
	CURRENT 81	.5	25

19. RESPONSIBLE DOD ORGANIZATION
NAME: US Army Institute of Surgical Research
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TELEPHONE: 512-221-2720
SOCIAL SECURITY ACCOUNT NUMBER
ASSOCIATE INVESTIGATORS
NAME:
NAME: DA

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Burn injury; (U) Infection; (U) Laminar Flow; (U) Humans (U) Wound Colonization

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) It has been well known in recent years that the development of infection has been the most common cause of death in burned soldiers. As the vast majority of these cases result from invasive infection of the burn wound, methods of reducing burn wound contamination would be expected to result in improved survival. In addition, studies have shown that cross-contamination colonization causes more invasive burn wound infections than auto-contamination colonization. These facts generated interest in the use of laminar air flow isolator units as part of burn care.

24. (U) The Sci-Med Company of Minneapolis, Minnesota, was contracted to develop a Laminar air flow unit to meet certain specifications. Following temporary installation and initial patient trials, necessary modifications were undertaken and the unit was redesigned and replaced. Comparison of burn wound colonization between laminar flow and conventionally treated patients is now in progress.

25. (U) 7910 - 8009. The previous microbial flora count techniques led us to investigate the accuracy and flexibility of air sampling devices and an improved method of accurate sampling for bacterial cross contamination was derived.

*Available to contractors upon originator's approval

DD FORM 1 MAR 66 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 65 AND 1498 1 1 MAR 65 (FOR ARMY USE) ARE OBSOLETE

ANNUAL PROGRESS REPORT

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: USE OF A LAMINAR FLOW ISOLATOR TO CONTROL INFECTION IN BURNED TROOPS

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

1 October 1979 - 30 September 1980

Investigators:

William F. McManus, M.D., Lieutenant Colonel, MC
Robert B. Lindberg, Ph.D.
Arthur D. Mason, Jr., M.D.

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: USE OF A LAMINAR FLOW ISOLATOR TO CONTROL INFECTION IN BURNED TROOPS

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

Period covered in this report: 1 October 1979 - 30 September 1980

Investigators: William F. McManus, M.D., Lieutenant Colonel, MC
Robert B. Lindberg, Ph.D.
Arthur D. Mason, Jr., M.D.

Reports Control Symbol MEDDH-288(R1)

The effectiveness of laminar flow isolation to protect the burn patient from bacterial colonization is currently being evaluated. The original laminar flow isolator unit was found to be unsatisfactory and a new laminar flow isolator was designed and installed to replace the original unit. Installation was completed in September 1980. In addition, improved microbial flora count techniques were developed to permit accurate air sampling for bacterial cross contamination. Patient care protocols for the nursing service and in-service training in the use of the new laminar flow isolator have been accomplished. Comparison of burn wound colonization between laminar flow and conventionally treated patients is now in progress.

Burn injury
Infection
Laminar flow
Humans
Wound colonization

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0G 6977	79 10 01	DD DR&E/AR1630

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
79 10 01	H. TERM	U	U	NA	NL	[X] YES [ ] NO	A. WORK UNIT

10. NO. CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	090
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)

(U) Measurement of Pulmonary Tissue Volume in Thermally Injured Soldiers (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
77 04	Cont	DA	C. In-House

17. CONTRACT/GRANT: Not Applicable

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR — PRECEDING	80	.4	10
FISCAL YEAR — CURRENT	81	0	0

19. RESPONSIBLE DOD ORGANIZATION

NAME: US Army Institute of Surgical Research

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SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS

NAME:

NAME: DA

21. GENERAL USE

FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Burn Injury; (U) Pulmonary Function Tests; (U) Plasma Oncotic Pressure; (U) Pulmonary Extravascular Water; (U) Resuscitation; (U) Humans

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) To evaluate the significance of pulmonary extravascular lung water changes in burned soldiers and assess the effectiveness of conventional therapy.

24. (U) With the disappearance of an indicator soluble gas during a rebreathing maneuver, pulmonary extravascular water will be determined and correlated with changes in arterial blood gas tension and body weight. Concomitantly, respiratory response to CO_2 was determined by standard rebreathing techniques.

25. (U) 7910 - 8009. Eight-non-infected thermally injured patients were studied, their mean age was 29 years and mean burn size 37%. CO_2 response curves are expressed in L/min. torr. and were measured on postburn days 1, 3, 5, 7 and 10. The mean values on those days were: 0.834, 1.413, 1.682, 2.275, and 2.800. Since these patients chronically maintain a state of moderate hypocapnia ventilation appeared to be in excess of that required to eliminate the augmented quantities produced during postburn catabolism. Further studies have confirmed the indifference of lung tissue volume ratio to intravascular colloid oncotic pressure during resuscitation.

*Available to contractors upon originator's approval

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 65 AND 1498-1 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

ANNUAL PROGRESS REPORT

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY
INDEPENDENT RESEARCH

REPORT TITLE: MEASUREMENT OF PULMONARY TISSUE VOLUME IN THERMALLY INJURED SOLDIERS - THE EFFECT OF CRYSTALLOID AND COLLOID RESUSCITATION ON LUNG WATER FOLLOWING THERMAL INJURY

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

1 October 1979 - 30 September 1980

Investigators:

Cleon W. Goodwin, Jr., MD
Victor Lam, MD
Diane Martin, SP5

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: MEASUREMENT OF PULMONARY TISSUE VOLUME IN THERMALLY INJURED SOLDIERS - THE EFFECT OF CRYSTALLOID AND COLLOID RESUSCITATION ON LUNG WATER FOLLOWING THERMAL INJURY

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

Period covered in this report: 1 October 1979 - 30 September 1980

Investigators: Cleon W. Goodwin, Jr., MD
Victor Lam, MD
Diane Martin, SP5

Reports Control Symbol MEDDH-288(R1)

The optimal resuscitation fluid for restoration of hemodynamic stability following major acute injury remains controversial, and numerous reports support the superior efficacy of either crystalloid or colloid solution (1, 2, 3, 4, 5) Following large thermal injury and subsequent resuscitation, pulmonary extravascular lung water increases are resuscitation progresses. (6, 7) Earlier studies from this Institute have demonstrated

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1. Lowe RJ, Moss GS, Jelik J, et al: Crystalloid vs. Colloid in The Etiology of Pulmonary Failure After Tauma - A Randomized Trial in Man in Critical Care Medicine 7: 107 - 112, 1979.

2. Shoemaker WC, and Hauser CJ: Critique of Crystalloid vs. Colloid Therapy in Shock and Shock Lung. Critical Care Medicine 7: 117 - 124, 1979.

3. Skillman JJ: The Role of Albumin and Oncotically Active Fluids in Shock. Critical Care Medicine 4: 55 - 61, 1976.

4. Virgilio RW, Rice CL, Smith DE et al: Crystalloid vs. Colloid Resuscitation: Is One Better? A Randomized Clinical Study. Surgery 85: 129 - 139, 1979.

5. Virgilio RW, Smith DE, and Zarins CK: Critical Care Medicine 7: 98 - 106, 1979.

6. Morgan A, Knight D and O'Connor N: Lung Water Changes After Thermal Burns: An Observational Study. Ann of Surg 187: 288 - 293, 1978.

7. Lam V, Goodwin CW Jr, Treat RC et al: Does Pulmonary Extravascular Water Vary With Colloid Oncotic Pressure After Burn Injury? American Review of Respiratory Diseases 118: 139, 1979.

that pulmonary extravascular lung water increases steadily over the first three days following burn and that lung water was not related to the measured plasma oncotic pressure. Although most studies have demonstrated that hemodynamic stabilization and resuscitation with colloid containing solutions results in a smaller administered fluid volume than that with crystalloid solutions, the effect of these volume differences on lung water is unknown. The current study was designed to access the differential effects of colloid and crystalloid resuscitation on the post-injury increases in lung water and to extend the period of measurement past the point of lung water stabilization.

METHODS

STUDY DESIGN

Two groups of 12 patients each were matched for burn size, age, and lack of complicating conditions such as inhalation injury and myoglobinuria (Table 1). Each patient in the colloid group was resuscitated with a solution composed of 2.5% albumin in lactated Ringer's solution. Each patient in the crystalloid group received lactated Ringer's solution alone. Fluid was administered to all patients so as to affect a urine output of 30 to 50 cc per hour and stabilization of vital signs.

LUNG WATER MEASUREMENT

Pulmonary extravascular lung water measurements were performed in the Institute of Surgical Research Pulmonary Function Laboratory using the rebreathing technique of Cander and Forster as modified by Petrini. (8, 9, 10) A bag-in-box with an 16 inch aluminum pillow rebreathing bag was connected with large bole tubing to an Ohio 843 Data Acquisition Dry Spirometer for a volume signal output. The initial bag volume is adjusted with a test gas mixture of 1.5% DME, 7% helium, 30% oxygen, and balance nitrogen. Following closure of the valve to the rebreathing bag, the patient performs a maximal rebreathing maneuver for five breaths.

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8. Cander L and Forster RE: Determination of Pulmonary Parenchymal Tissue Volume and Pulmonary Capillary Blood Flow in Man. Journal of Applied Physiology 14: 541 - 551, 1959.

9. Petrini MF, Peterson BT and Hyde RW: Lung Tissue Volume and Blood Flow by Rebreathing: Theory. Journal of Applied Physiology 44: 795 - 802, 1978.

10. Peterson BT, Petrini MF, and Hyde RW et al: Pulmonary Tissue Volume in Dogs During Pulmonary Edema. Journal of Applied Physiology 44: 782 - 794, 1978.

The change in concentrations of the test gases is measured with a modified Perkin-Elmer Medical Mass Spectrometer. All signal traces are printed by a rapid response, photographic script chart recorder for offline analysis. The signal tracing is digitized, plotted, and the appropriate calculations for lung water are computed by a BASIC program or the Hewlitt-Packard 9830A Minicomputer. Lung water measured by this method represents the total tissue volume of the pulmonary parenychma. Since the structural elements of the lungs are presumed to remain constant during the seven day study, any change in lung tissue volume is assumed to reflect changes in fluid content of the lung. To compare tissue volumes among multiple patients, lung water is normalized to the measured alveolar volume of each patient.

RESULTS

Lung water (normalized to alveolar volume) progressively increased during the first three postburn days. Over the subsequent four days, a lung water appeared to stabilize at a constant plateau value (Figure 1). There appears to be no clear separation between the lung waters of the crystalloid patients (filled circles) and that of the colloid patients (filled squares). The asterisks represent coincident values of multiple patients. When evaluated as separate treatment groups, there is no statistical difference in the lung water between the crystalloid and colloid resuscitated groups: $Y = .00511X + .13162$ for crystalloid group and $Y = .00862X + .11433$ for the colloid group when the seven day study is analyzed as a linear function. Multiple regression analysis of the two groups suggest that in addition to other clinical variables the inclusion of colloid may affect lung water, but the number of data are insufficient to attach any statistical or physiological significance to this observation.

DISCUSSION

The inclusion of colloid into a crystalloid resuscitation did not affect the measured lung water in these two groups of closely matched patients. However, a smaller volume of fluid was required by patients resuscitated with a colloid formula to maintain the desired hemodynamic response. Since the patients resuscitated with a colloid formula received a smaller volume, it might have been expected that patients in this group would have had less lung water than did the crystalloid resuscitated group. If the two resuscitation groups had been administered equal volumes of fluid, as commonly occurs when inexperienced physicians rely solely on a formula based calculation, it is quite possible that the colloid treated group may have had increased quantitites of lung water. Plasma albumin leaks out of the microcirculation during the resuscitation phase of thermal injury, and this increased tissue accumulation of protein may sufficiently increase the interstitial oncotic pressure

to cause increase flow of water into tissue. (11) Even in the intact circulation, the colloid osmotic gradient between the plasma and interstitium disappears within four hours of colloid infusion, and the effect of colloid on pulmonary extravascular lung water is at best transient. (12)

Although intravascular filling pressures may be normal, burned patients requiring large fluid volumes for resuscitation (greater than 12 to 15 liters over the first 24 hours postburn) occasionally require small doses of albumin during resuscitation to maintain urine output. With this exception, plasma losses of protein should not be replaced until the second postburn day, when capillary integrity is restored. The administration of large amounts of albumin during the first 24 hours postburn does not beneficially affect lung water and may delay the mobilization of tissue water after the resuscitation.

---

11. Goodwin CW Jr, Long JW, Mason AD Jr et al: Paradoxical Effect of Hyperoncotic Albumin in Acutely Burned Children. Journal of Trauma 21: 1981, in press.

12. Demling RH, Will JA, and Perea A: The Effect of Albumin Infusion on Pulmonary Microvascular Fluid and Protein Transport. Journal of Surgical Research 27: 321 - 326, 1979.

Table 1. PATIENT CHARACTERISTICS

	COLLOID	CRYSTALLOID
Patients	12	12
TBS % (Range)	50 (26 to 79)	46 (19 to 48)
AGE Years (Range)	26 (17 to 40)	28 (19 to 42)
RESUSCITATION ml/kg/% burn (± SD)	2.68 ± 1.18	3.62 ±1.24

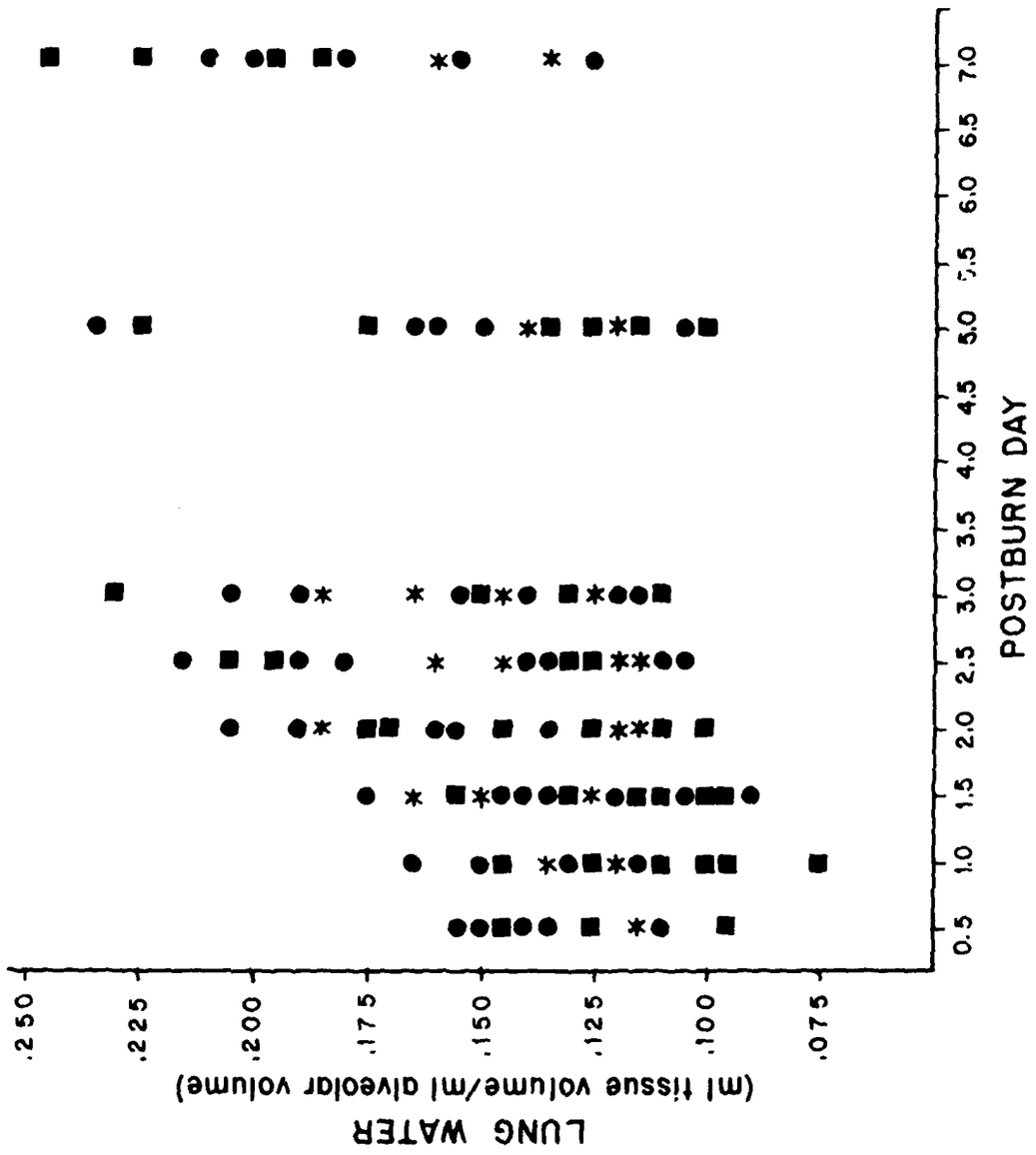
LUNG WATER
(ml tissue volume/ml alveolar volume)
.250
.225
.200
.175
.150
.125
.100
.075
POSTBURN DAY
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0

FIGURE 1.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION* DA 0G 6952	2. DATE OF SUMMARY* 79 10 01	REPORT CONTROL SYMBOL DD DR&E(AR)636
3. DATE PREV SUM'RY 79 10 01	4. KIND OF SUMMARY H. TERM	5. SUMMARY SCTY* U	6. WORK SECURITY* U	7. REGRADING* NA	8a. DISP'N INSTR'N NL	8b. SPECIFIC DATA-CONTRACTOR ACCESS ☒ YES ☐ NO	9. LEVEL OF SUM A. WORK UNIT

10. NO. CODES*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	082
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)* (U) Laboratory Investigation of The Mechanisms of Acquired Leukocyte Dysfunction Following Thermal Injury (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS* 003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
75 12	Cont	DA	C. In-House

17. CONTRACT/GRANT Not Applicable

a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER*
c. TYPE d. AMOUNT:
e. KIND OF AWARD f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 80	1.0	42
	CURRENT 81	0	0

19. RESPONSIBLE DOD ORGANIZATION

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PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic institution)
NAME* Albert T. McManus, MAJ, MSC
TELEPHONE 512-221-3411
SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS
NAME: Arthur D. Mason, Jr., M.D.
NAME DA

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Rat Model; (U) Burns; (U) Leukocytes; (U) Glucose Oxidation; (U) Latex Phagocytosis; (U) Stress Hormones; (U) Cyclic neucleotides

23. TECHNICAL OBJECTIVE,* 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) Efforts will be made to establish one or more metabolic basis for acquired leukocyte dysfunction following thermal injury. Establishment of specific nutritional or environmental effects may allow for corrective management.

24. (U) Initial efforts will be to measure glucose metabolism in normal and burned patients' leukocytes. Purified and washed granulocyte populations will be examined for oxidization of Carbon 14 labeled glucose. Hexose monophosphate shunt and glycolysis activity will be estimated by release of 14_{CO2} from respectively 1-^{14}C glucose. Measurements will be made on resting and latex particle (=0.8 u) stimulated cells. Leukocyte function will be examined in the burned rat. The effect of burn rat serum on leukocyte glucose metabolism and cyclic neucleotide levels will be examined. Establishment of an animal model of burn associated leukocyte dysfunction will allow examination of corrective procedures in vivo.

25. (U) 7910 - 8009. Sera from burned rats were found to inhibit normal rat peripheral neutrophil adherence to nylon fiber (50 mg). Serum taken from 3 day and 9 day 60 burned rats significantly decreased adherence. Burn serum taken at the above times also significantly depressed chemotaxis of purified rat peripheral neutrophils. Sixty percent burned animals were examined for their responses to I.V. injection of the chemotaxic tri-peptide N-formal-L-methinonyl-L-leucyl-L-phenylalanine (F-met-leu-phe). This tripetide showed a marked neutropenia inducing capacity for normal rats at doses of 5 and 50 nano moles. Burned animals appear to be refractory to secondary inflammatory stimulation since administration of F-met-leu-phe at a 5 nano mole dose elicited little response on the part of burned animals 9 days post-injury.

*Available to contractors upon originator's approval

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ANNUAL PROGRESS REPORT

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: LABORATORY INVESTIGATION OF THE MECHANISMS OF ACQUIRED LEUKOCYTE DYSFUNCTION FOLLOWING THERMAL INJURY IN BURNED SOLDIERS

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

1 October 1979 - 30 September 1980

Investigators:

Albert T. McManus, Ph.D., Major, MSC
Arthur D. Mason, Jr., M.D.

Reports Control Symbol MEDDH 288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: LABORATORY INVESTIGATION OF THE MECHANISMS OF ACQUIRED LEUKOCYTE DYSFUNCTION FOLLOWING THERMAL INJURY IN BURNED SOLDIERS

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

Period covered in this report: 1 October 1979 - 30 September 1980

Investigators: Albert T. McManus, Ph.D., Major, MSC
Arthur D. Mason, Jr., M.D.

Reports Control Symbol MEDDH-288(R1)

Sera from burned rats were found to inhibit normal rat peripheral neutrophil adherence to nylon fiber (50 mg). Serum taken from 3-day and 9-day 60% burned rats significantly decreased adherence. Burn serum taken at the above times also significantly depressed chemotaxis of purified rat peripheral neutrophils. Sixty per cent burned animals were examined for their responses to intravenous injection of the chemotaxic tripeptide N-formyl-L-methionyl-L-leucyl-L-phenylalanine (F-met-leu-phe). This tripeptide showed a marked neutropenia-inducing capacity for normal rats at doses of 5 and 50 nanomoles. Burned animals appear to be refractory to secondary inflammatory stimulation, since administration of F-met-leu-phe at a 5-nanomole dose elicited little response on the part of burned animals 9 days post-injury.

Rat model
Burns
Leukocytes
Glucose oxidation
Latex phagocytosis
Stress hormones
Cyclic nucleotides

LABORATORY INVESTIGATION OF THE MECHANISMS OF ACQUIRED LEUKOCYTE DYSFUNCTION FOLLOWING THERMAL INJURY IN BURNED SOLDIERS

We have previously reported that the 60% burned rat displays altered inflammatory responses to intraperitoneal injection of several irritants (1). This altered capacity could not be explained by decreased numbers of circulating neutrophils. In fact, burned animals had a significantly elevated total peripheral neutrophil count. The neutrophilia displayed in burned rats was coincident with a decreased marginated neutrophil pool. When tested *in vitro*, neutrophils in whole blood taken from burned rats showed marked reductions in the surface adherence to nylon fiber.

In this report, the effect of burned rat serum on *in vitro* adherence and chemotaxis of purified normal rat circulating neutrophils is presented. The burned rat was also examined for its ability to respond *in vivo* to injection of the chemotactic tripeptide N-formyl-methionyl-L-leucyl-L-phenylaline (F-met-leu-phe). This stimulus is known to cause rapid neutropenia in the rabbit and rat (2,3).

METHODS AND MATERIALS

Rat Burn Model

Male Sprague-Dawley rats (340-360 g) were anesthetized and scalded (60% total body surface area) as previously described (1).

Isolation of Rat Neutrophils from Peripheral Blood

Initial attempts to isolate rat leukocytes by dextran sedimentation techniques used for human and other species were unsuccessful. It was therefore necessary to devise an alternate method for isolation of rat neutrophils. Rat red blood cells were found to sediment when whole blood was mixed with gelatin. A solution of 3% gelatin (USP), 0.7% NaCl and 0.2% $CaCl_2$-$2H_2O$ was found to be optimal for separation of red blood cells and leukocytes.

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1. McManus AT, Mason AD Jr: Laboratory investigation of the mechanisms of acquired leukocyte dysfunction following thermal injury in burned soldiers. USAISR Annual Progress Report FY 1979, Fort Sam Houston, TX, pp 297-318.

2. O'Flaherty JT, Showell HJ, Ward PA: Neutropenia induced by systemic infusion of chemotactic factors. J Immunol 118:1586-1589, 1977.

3. Gilbertsen RB, Carter GW, Quinn DJ: Effect of F-met-leu-phe and zymosan-activated serum on rat neutrophils in vivo. J Reticuloendothel Soc 27:485-494, 1980.

For isolation, 2 volumes of heparinized blood were mixed with 1 volume of gelatin solution and allowed to settle at 1 x g for 30-40 min. Following sedimentation, the supernatant was centrifuged at 150 x 6 for 10 min and the pellet exposed to 10 ml of 0.87% NH_4Cl until red cell lysis had progressed to the stage where the suspension was transparent ($\cong$ 10 min). The tube was then centrifuged for 10 min at 150 x g. Following centrifugation, the pellet was suspended in Hank's balanced salt solution (HBSS) and layered onto Ficoll-Paque (Pharmacia, Piscataway, New Jersey) at a cell suspension to Ficoll-Paque ratio of 4:1. This preparation was then centrifuged at room temperature for 40 min at 400 x g. Following centrifugation, the pellet was recovered and resuspended in HBSS and the cell preparation placed in an ice bath. Cell counts and differential counts were then performed. The cell suspensions were kept in ice for 30 min before any further procedures were conducted.

Effect of Burn Serum on Normal Rat Neutrophil Adherence

Normal rat peripheral neutrophils were prepared for each experiment from five rats using the procedure described above. One ml volumes of pooled PMN (10^7/ml) were mixed with an equal volume of normal, 3-day or 9-day postburn serum. The serum-cell mixtures were incubated at 37°C for 30 min, then diluted 1:4 with HBSS and assayed for adherence on the 50 mg nylon fiber columns (4). Control experiments were conducted in which diluted sera were passed over the column before the cells were added. This was to test the possibility that protein differences in the sera might have different charge blocking effects on the nylon fibers and thus alter neutrophil adherence.

Effect of Serum from Burned Rats on the Chemotaxis of Neutrophils from Normal Rats

Normal rat neutrophils were prepared by the procedure previously described. The chemotactic effect of casein (MCB Chemicals, Norwood, Ohio) on neutrophils from normal rats was examined following incubation of the neutrophils with normal rat serum, serum collected 3 days postburn or serum collected 9 days postburn. The cell-serum incubations were the same as outlined for neutrophil adherence. A modified Boyden chamber was used to measure chemotaxis (5). Millipore filters (3 μ) were used to separate the lower and upper wells of the chamber. Casein (5 mg/ml in HBSS) was added to the lower chamber until the filter was wet. The upper chamber was next loaded with 0.5 ml of

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4. MacGregor RR, Spagnuolo PJ, Lentnek AL: Inhibition of granulocyte adherence by ethanol, prednisone and aspirin, measured with an assay system. N Engl J Med 291:642-646, 1974.

5. Warden GD, Mason AD Jr, Pruitt BA Jr: Evaluation of leukocyte chemotaxis in vitro in thermally injured patients. J Clin Invest 54: 1001-1004, 1974.

the serum-cell mixtures and incubated at 37°C for 1 hour. Control chambers, to determine the random migration of neutrophils, contained HBSS in the lower chamber. Following incubation, the filters were removed from the chambers and stained with hematoxalin and eosin, cleared with xylene, mounted on microscope slides and covered with coverslips. Cell migrations were measured by the leading front assay, which measures the depth of cell migration into the millipore filter (6). The filters were examined using a 50X oil immersion objective by focusing into the filter until one or two cell nuclei were in focus. The fine focus micrometer reading on the microscope was recorded, and the focus was then moved until the top surface of the filter was in focus. The focal distance between the front edge and top of the filter was read directly from the micrometer. Multiple measurements per filter were made, and chemotaxis was determined by subtracting the random movement distances of control chambers, without casein, from the chemotaxis observed in the casein-containing chambers.

Effect of N-formyl-L-methionyl-L-leucyl-L-phenylalanine (F-met-leu-phe) on the Levels of Circulating Neutrophils

The sensitivity of circulating neutrophils to intravenously administered F-met-leu-phe was examined in normal and 9-day burned rats. Animals were examined for neutropenia following intravenous injection of the chemotactic tripeptide. The aortic cannulation, penile injection and cell counts were as described above. Animals were examined following the injection of 50, 5 or 0.5 nanomoles of saline suspended tripeptide (Bachem, Torrance, California). A saline-sham injection group was included as a treatment control. A neutropenia index was determined at 1 min and 3 min post-injection by the formula (2):

$$\text{N.I.} = \frac{\text{PMN count at 1 min + PMN count at 3 min}}{\text{2 X PMN count, pretreatment}}$$

RESULTS

Examination of Possible in vitro Effects of Burned Rat Serum on Normal Neutrophil Function

The effect on adherence of incubation of isolated normal rat neutrophils with serum from burned rats is presented in Table 1. Serum from either 3-day or 9-day postburn rats decreased the neutrophil adherence to nylon fibers. The effect of serum from burned rats on the adherence of normal neutrophils is similar to the effect of serum taken

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6. Zigmond SH, Hirsch JG: Leukocyte locomotion and chemotaxis: New methods for evaluation and demonstration of a cell-derived chemotactic factor. J Exp Med 137:387-410, 1973.

Table 1. Effect of Burned Rat Serum* on Normal Rat Neutrophil Adherence to Nylon Fiber

	% Adherence		
	Unburned rat serum	3-day postburn serum	9-day postburn serum
Expt. 1	70.6	21.3	36.0
	82.5	31.1	49.0
	62.5	36.4	44.2
	69.9	8.2	62.0
Expt. 2	62.0	13.4	0
	45.0	33.6	21.2
	39.0	35.0	26.8
	76.0	42.0	40.0
	62.1	51.0	11.9
	62.0	51.0	44.5
Expt. 3	44.0	33.0	37.9
	64.9	25.8	47.8
	67.4	53.0	55.0
$\bar{X}$ ± S.E.	62.14 ± 4.1	33.43 ± 3.72†	36.6 ± 4.3†

* Unburned rat serum used as control.
† $P < 0.01$.

from humans injected with anti-inflammatory agents on normal neutrophil adherence (7). Control experiments included nylon fiber columns precoated with normal or burn sera prior to the passage of neutrophils. No difference in adherence was seen between these sera. This confirms an earlier report that nylon fiber adherence is not the result of activated plasma proteins (8).

The effect on chemotaxis of normal granulocytes following preincubation with 3-day burn sera is presented in Table 2. The effect of 9-day burn sera on chemotaxis is presented in Table 3. In both cases, burn sera depressed the chemotaxis of normal granulocytes. A similar inhibition of normal chemotaxis has previously been reported using burn sera from humans (9). This is the first report of burn serum associated chemotactic depression in an animal model.

The in vivo Effects of the Chemotactic Tripeptide F-met-leu-phe on Cellular Responses in Burned Rats

The tripeptide F-met-leu-phe has been reported to mimic activated serum complement when injected intravenously into animals. Normal animals respond with a rapid, transient neutropenia (2,3). Three concentrations of the tripeptide were studied in the rat model.

Data are summarized in Figure 1. A neutropenia index of 100% occurs when the ratio at 1 min and 3 min equals one when compared to the preinjection values, i.e., no neutropenia is present. The burned and unburned animals showed little change following the injection of 0.5 nM of the tripeptide. At the high dose of 50 nM, both control and burned animals showed strong response. However, at 5 nM a difference between groups occurred, with the burned animal being less responsive to the tripeptide ($P < 0.02$). This observation indicates that the 60% TBS burned rats are less responsive to chemotactic stimulation than normal rats. These data are compatible with the observed depression in peritoneal exudate responses in burned rats subjected to various inflammatory agents.

DISCUSSION

Humoral factors affecting the adherence of normal neutrophils have been reported in the blood of humans undergoing induced

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7. MacGregor RR: The effect of anti-inflammatory agents and inflammation on granulocytes adherence: Evidence for regulation of plasma factors. Am J Med 61:597-607, 1976.

8. McGillen J, Phair J: Polymorphonuclear leukocyte adherence to nylon: Effect of oral corticosteroids. Infect Immun 26:542-546, 1979.

9. Warden GD, Mason AD Jr, Pruitt BA Jr: Suppression of leukocyte chemotaxis in vitro by chemotherapeutic agents used in the management of thermal injuries. Ann Surg 181:363-369, 1975.

Table 2. Effect of 3-Day Postburn Rat Serum* on Normal Rat Neutrophil Chemotaxis

	Control unburned rat serum	Burned rat serum
Experiment 1	55†	40
	92	50
	85	44
	80	62
	81	78
	82	60
	85	58
	98	63
	108	61
Experiment 2	90	50
	99	68
	90	44
	85	65
	72	67
	97	70
	105	61
	90	62
	88	57
$\bar{X}$ ± S.E.	87.3 ± 3.87	58.8 ± 2.58§

* Normal rat serum used as control.
† Data are presented as distance migrated in microns.
§ $P < 0.01$.

Table 3. Effect of 9-Day Postburn Rat Serum* on Normal Rat Neutrophil Chemotaxis

Control unburned rat serum		Burned rat serum	
Experiment 1	Experiment 2	Experiment 1	Experiment 2
83†	85	54	75
60	75	30	70
54	60	57	45
55	75	45	64
50	80	90	47
67	77	83	70
65	65	50	64
70	64	60	57
50	52	34	38
110	71	42	74
64	61	35	40
54	51	67	82
$\bar{X}$ = 66.58 ± 2.80		$\bar{X}$ = 57.2 ± 3.38§	

* Normal rat serum used as control.
† Data are presented as distance migrated in microns.
§ $P < 0.04$.

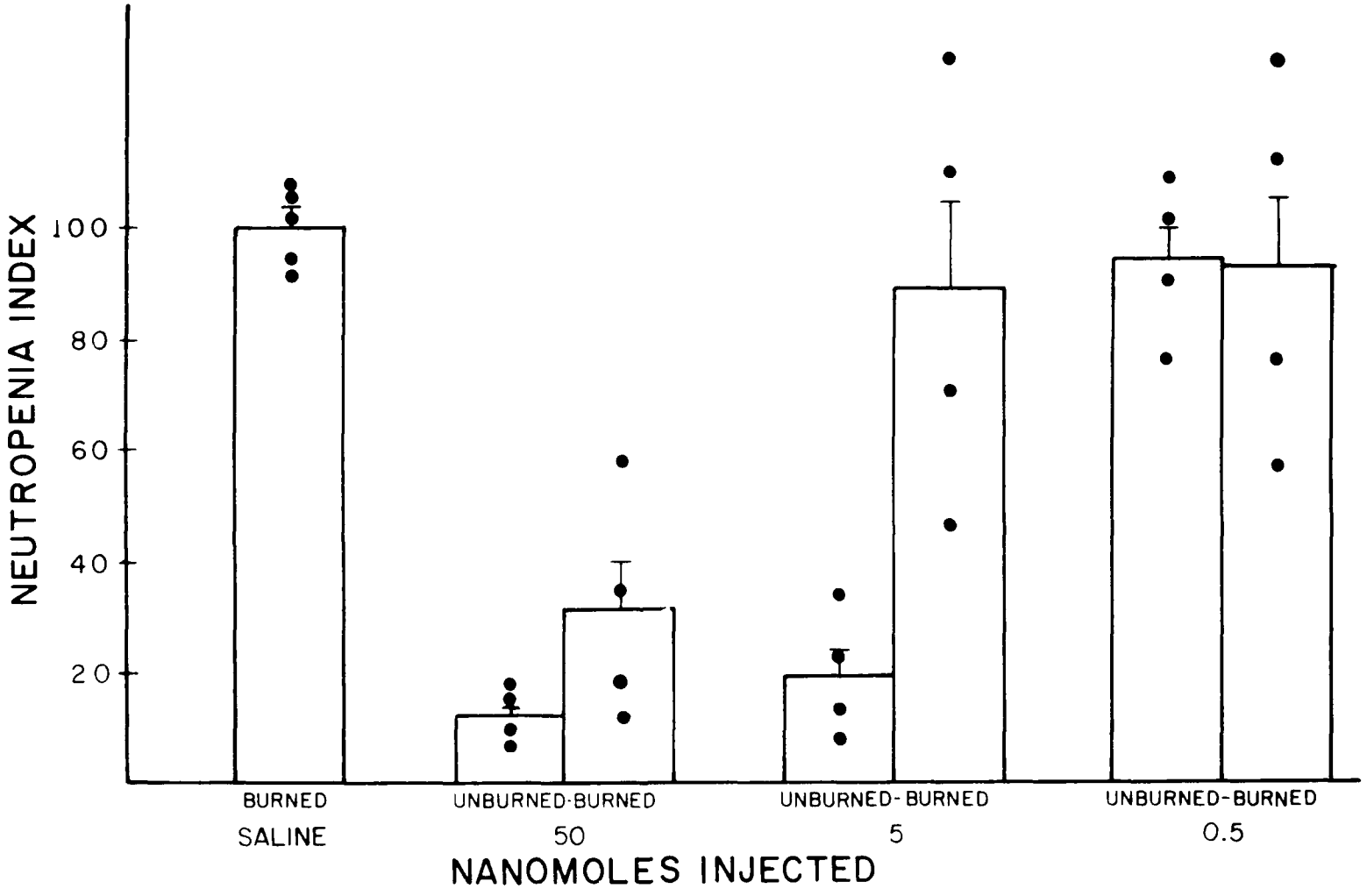

Fig. 1. Relative effect of F-met-leu-phe injection on circulating neutrophil counts. Data are presented as the ratio (neutropenia index) of the 1-min plus the 3-min count to the preinjection count times 2. Data are presented as histograms plus one standard error. Burned animals were less responsive to injection of 5.0 nanomoles F-met-leu-phe than control animals ($P < 0.02$).

neutrocytosis or neutropenia (10,11). These undefined factors may modify normal neutrophil behavior towards nylon fiber by either increasing or decreasing their adherence. The increased or decreased adherence is dependent upon whether the plasma was from a neutropenic or a neutrocytotic donor. When the serum from the burned rat was examined for its effects on normal rat neutrophils, it was observed that burn serum decreased adherence to nylon fiber. These findings agree with the decreased adherence noted with neutrophils in whole blood from burned animals, and also with the fact that animals 9 days postburn have neutrocytosis and a decreased marginated pool. The fact that burned serum affects the function of normal neutrophils also suggests a role for humoral factors in the neutrocytosis and the decreased marginated pool.

Neutrophil adherence and chemotactic activity are related functions. There is an obvious requirement for neutrophils to become attached to a solid surface before they can accomplish movement. A decrease in the negative surface charges on cells is associated with increased adherence and hence their ability to adhere to solid surfaces (12,13). Although not reported, such decreases in the negative charges on cell surfaces would be expected to reduce the ionic adherence to nylon's normally positive charge. The fact that stimuli such as activated complement (C_{5a}) and F-methionyl-L-leucyl-L-phenylalanine (F-met-leu-phe) cause neutropenia and also decrease negative charges suggests the increased nylon fiber adherence caused by these agents is not a surface charge dependent event. Data are not available for agents that cause neutrocytosis. However, it would be most interesting to know if such agents increase the cell surface negative charges while decreasing adherence. The association of decreased cell surface charge and chemoattractive activity may simply reflect the binding of cationic proteins to negative charges as cells degranulate. Such binding would associate degranulation and decreased surface charge in an entirely coincidental manner. Another possibility is that the reported decreased charge is a measurement artifact. That is, because the surface charge measurements are based on electrophoretic mobility, any change in cell shape could alter cell movement in the electrophoretic field. Such altered movement could then be erroneously interpreted as

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10. Lentnek AL, Schreiber AD, MacGregor RR: The induction of augmented granulocyte adherence by inflammation. J Clin Invest 57: 1098-1103, 1976.

11. MacGregor RR: Granulocyte adherence changes induced by hemodialysis, endotoxin, epinephrine and glucocorticoids. Ann Intern Med 86:35-39, 1977.

12. Gallin JI: Degranulating stimuli decrease the negative surface charge and decrease the adhesiveness of human neutrophils. J Clin Invest 65:298-306, 1980.

13. Smith CW, Hollers JC: Motility and adhesiveness in human neutrophils: Redistribution of chemotactic factor-induced adhesion sites. J Clin Invest 65:804-812, 1980.

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US ARMY INSTITUTE OF SURGICAL RESEARCH ANNUAL RESEARCH PROGRESS--ETC(U)
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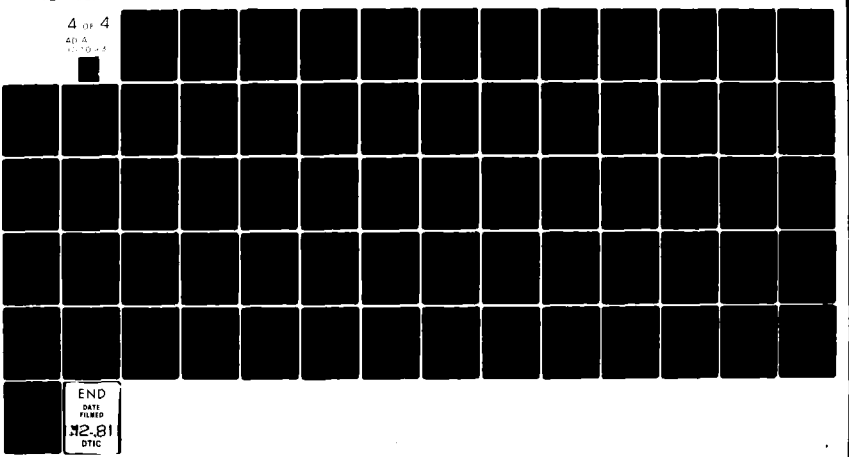

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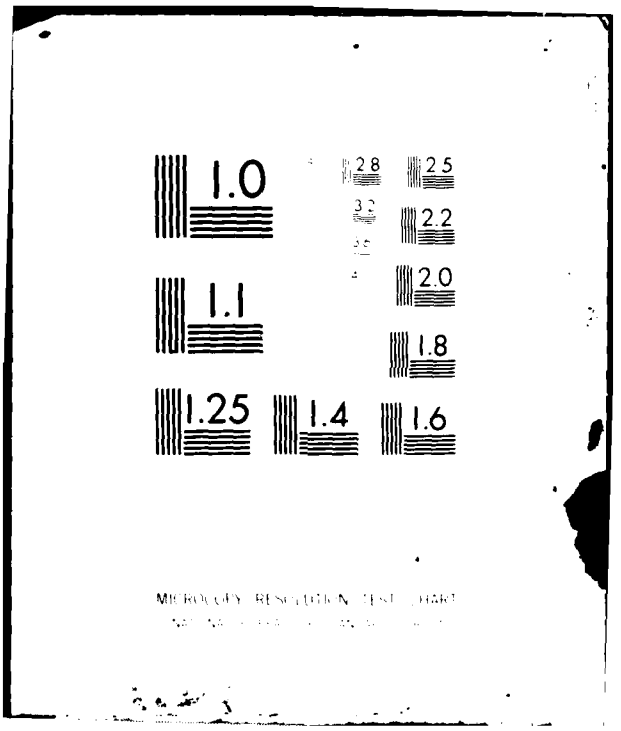
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an alteration in cell surface charge. In fact, the exposure of normal neutrophils to activated complement has been shown to markedly alter the cell surfaces. At 20 seconds post-exposure, the examination of cells by scanning electron microscopy showed a marked ruffling and pseudopod formation (14). Such ruffling would be anticipated to alter the electrophoretic mobility of cells.

Following the recognition of a chemoattractive gradient and cell adherence, the directed movement of neutrophils toward the gradient source (chemotaxis) is considered to be a major defensive function of neutrophils. Following the establishment that burn serum has a negative effect on neutrophil adherence, the effect of burn serum on in vitro chemotaxis was examined. Burn serum was shown to have a depressing effect on the ability of normal rat neutrophils to migrate toward casein. This observation adds strength to the hypothesis that granulocyte dysfunction in this rat model of burn injury is in part the result of alterations in the plasma environment which results in a depression of neutrophil responsiveness. Humoral inhibitors of normal neutrophil chemotaxis have been reported after serious burn injury and following major surgical trauma (9,15,16). Serum inhibitors of normal neutrophil chemotaxis have also been reported in patients with nontrauma-associated leukocytosis (17). The alterations in neutrophil adherence reported by Van Epps are very similar to those reported as a consequence of neutrocytotic plasma.

These investigations have established that rats with 60% TBS burns demonstrate neutrophil functions consistent with a state of depressed inflammatory responsiveness. This conclusion is supported by the failure of burned rats to muster normal levels of inflammatory peritoneal exudate cells. In addition, burned rats were found to have elevated levels of circulating neutrophils with a markedly reduced marginating pool. Rat neutrophils in whole blood were found to have decreased in vitro adherence to nylon fibers, which is consistent with the hypothesis that the observed decrease in the marginated pool was the result of decreased adherence of neutrophils to endothelium. The burned rat

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14. Craddock PR, Hammerschmidt D, White JG, Dalmasso AP, Jacob HS: Complement (C5a)-induced granulocyte aggregation in vitro: A possible mechanisms of complement-mediated leukostasis and leukopenia. J Clin Invest 60:260-264, 1977.

15. Altman LC, Furukawa CT, Klebanoff SJ: Depressed mononuclear leukocyte chemotaxis in thermally injured patients. J Immunol 119: 119-205, 1977.

16. Christou NV, Meakins JL: Neutrophil function in surgical patients: Two inhibitors of granulocyte chemotaxis associated with sepsis. J Surg Res 26:355-364, 1979.

17. Van Epps DE, Palmer DL, Williams RC Jr: Characterization of serum inhibitors of neutrophil chemotaxis associated with anergy. J Immunol 113:189-200, 1974.

was next examined for its ability to respond in vivo to an injection of the chemotactic tripeptide, F-met-leu-phe. The normal response is very similar to the acute neutropenia that is associated with complement activation, filtration leukophoresis and hemodialysis (11,18).

Dose response comparisons of the tripeptide in normal and burned rats showed the burned rats to be significantly less responsive. This finding again supports an anti-inflammatory state in burned rats. A higher dose of the chemoattractant was required to cause margination, which is a primary requirement for tissue mobilization of neutrophils. These data also support the conclusion that the decrease in peritoneal exudate cells was not the result of some failure to generate a chemotactic stimulus, since burned rats would be hyporesponsive to a signal that would cause a normal rat to respond.

The intent of these investigations was to examine an animal model of burn trauma for neutrophil alterations that could explain the increased susceptibility to infection associated with burn injury. The 60% burned, 350 g rat has been found to have several neutrophil functional defects that would indicate these animals are at increased risk of infection. This is in fact true when these animals are intentionally inoculated with Pseudomonas aeruginosa. It must be noted, however, that without inoculation the 60% burned, 350 g rat has a high probability of survival to healing. That is, in spite of the large wounds inflicted on these animals and the observed depression in their inflammatory response, these animals generally resist infection. Since the animals in this study were not infected when the neutrophil data were obtained, it can be concluded that the observed neutrophil responses are the usual response to thermal injury. That is, in this size rat, neutrocytosis, elevated stress hormone levels, and decreased inflammatory responses are a natural adaptation to thermal injury and consistent with survival.

It is tempting to speculate that the observed neutrophil alterations are by evolutionary design a requirement for survival. Although the purpose for such alterations is not immediately obvious, it seems reasonable that the time-consuming process of wound healing would be aided if, after an appropriate time course, further inflammatory accumulation could be inhibited. Such inhibition could decrease the healing time and offer a selective advantage to the injured host. With large wounds, the most efficient mechanism for such inhibition would be, as in the case of these studies, by humoral factors.

The concept of adaptive depression of neutrophil function also implies a balance between healing and response to opportunistic infection.

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18. Fehr J, Jacob HS: In vitro granulocyte adherence and in vivo margination: Two associated complement-dependent functions: Studies based on the acute neutropenia of filtration leukophoresis. J Exp Med 146:641-652, 1977.

It is most probable that the wounds of the animals in this study were colonized by microorganisms, yet there were few infections unless the host-parasite balance was intentionally altered.

The limits of the proposed adaptation mechanism are unknown; however, we have observed in preliminary experiments that young animals (140-160 g) and older animals (> 550 g) given 60% injuries have a high incidence of spontaneous fatal sepsis. It is intended that these observations be extended in an attempt to develop a burn model in which the animals spontaneously develop a fatal sepsis. Such a model would be useful to examine therapies perfected in the noninfected 350 g, 60% burn model, with a goal of eventually improving the survival rate following thermal injury.

PUBLICATIONS

McManus AT: Examination of neutrophil function in a rat model of decreased host resistance following burn trauma. In press, Reviews of Infectious Disease.

PRESENTATIONS:

McManus AT: Examination of neutrophil function in a rat model of decreased host resistance following burn trauma. Symposium on Pseudomonas aeruginosa Infections, Walter Reed Army Medical Center, Washington, DC, 6 December 1979.

McManus AT: Investigation of altered neutrophil functions in a rat model of burn trauma. To be presented at 17th Annual National Meeting of the Reticuloendothelial Society, Tampa, Florida, 2-5 December 1980.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0G 6978	79 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8A. OBS'N INSTR'N	8B. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
79 10 01	D. CHANGE	U	U	NA	NL	[X] YES [ ] NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	078
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) Monitoring and Modification of The Metabolic and Physiologic Alterations Associated With Thermal Injury in Burned Soldiers (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
78 03	Cont	DA	C. In-House

17. CONTRACT/GRANT: Not Applicable

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
PRECEDING FISCAL YEAR	80	.6	20
CURRENT FISCAL YEAR	81	.7	30

19. RESPONSIBLE DOD ORGANIZATION

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ADDRESS: Fort Sam Houston, Texas 78234

RESPONSIBLE INDIVIDUAL

NAME: Basil A. Pruitt, Jr., COL, MC

TELEPHONE: 512-221-2720

20. PERFORMING ORGANIZATION

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C, Biochemistry Branch

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PRINCIPAL INVESTIGATOR (If more than one U.S. Academic Institution)

NAME: Michael C. Powanda, PhD, MAJ, MSC

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME:

NAME:

21. GENERAL USE

DA

22. KEYWORDS (Precede EACH with Security Classification Code): (U) Pathogenesis; (U) Evaluation of Therapy; (U) Patient Profile; (U) Plasma; (U) Urine; (U) Enzymes; (U) Proteins; (U) Metabolites; (U) Rats

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) To develop a profile of plasma/urine constitutents which accurately reflect the severity of the thermal trauma, the presence of infection, and the healing process so as to allow objective assessment of the efficacy of therapeutic measures and to further elucidate the pathophysiology of thermal injury with the ultimate goal of lessening morbidity and mortality due to severe thermal trauma as well as hastening convalescence.

24. (U) Animal and clinical studies will be run concomitantly when feasible. Animal studies, which can be rigorously controlled, will be used to test hypotheses and to expand upon the findings from patient studies. The rat burn model developed by Walker and Mason, suitably modified, will be the primary animal model employed. Initial patient studies will focus on patients who according to age and burn size, are deemed to have a 40-60% chance of survival.

25. (U) 7910 - 8009. (1) There now appear to be three substances detectable in perchloric acid (PCA) filtrates of whole blood which act as early indicators of systemic infection in burned rats. One factor absorbs light at 398 nm and seems to be associated with some cellular components of blood other than mononuclear cells. The other two substances are fluorescent and are detectable in PCA filtrates of plasma as well as blood. All three factors may be proteins of 10,000 molecular weight or greater. Since these indicators are found in rats infected with Proteus mirabilis as well as with Pseudomonas aerugenosa but not in the microorganisms themselves, it is likely these factors are host derived. Characterization and identification of these substances is underway. A clinical evaluation of the usefulness of these indices of infection will begin in the first quarter of FY 81. (2) A compilation of alterations in specific plasma acute-phase proteins following thermal injury is in progress. Preliminary data indicate that some of these proteins achieve levels after severe (60%) burns which, considering their known activities, could compromise the patient's resistance to infection.

*Available to contractors upon collaborator approval.

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ANNUAL PROGRESS REPORT

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: MONITORING AND MODIFICATION OF THE METABOLIC AND PHYSIOLOGIC ALTERATIONS ASSOCIATED WITH THERMAL INJURY

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

1 October 1979 - 30 September 1980

Investigators:

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Calvin R. Kennedy, B.S.
Haywood E. Murray
Ysidro Villarreal, B.S.
Harrel L. Walker, M.S.

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: MONITORING AND MODIFICATIONS OF THE METABOLIC AND PHYSIOLOGIC ALTERATIONS ASSOCIATED WITH THERMAL INJURY

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

Period covered in this report: 1 October 1979 - 30 September 1980

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Ysidro Villarreal, B.S.
Harrel L. Walker, M.S.

Reports Control Symbol MEDDH-288(R1)

Severe thermal injury is often complicated by infection. Moreover, the injury itself renders the early detection of infection more difficult. Rapid early detection of infection would thus aid in the treatment of severely burned patients. Perchloric acid filtrates of whole blood from burned-infected rats contain three substances which appear to be early indicators of infection in the thermally injured animal. These factors are only slightly affected by the extent of injury. These factors do not appear to be microorganism specific in that they are found in rats infected with Proteus mirabilis as well as with Pseudomonas aeruginosa. One factor absorbs light at 398 nanometers (nm) and seems to be associated with some cellular component of blood. The other two substances are fluorescent, one λex 280 nm λem 340 nm, the other λex 355 nm λem 420 nm, and are detectable in perchloric acid filtrates of plasma as well as of whole blood. All factors are retained by filters with a 25,000 dalton pore size. All factors are precipitable from perchloric acid filtrates by phosphotungstic acid, suggesting that they may be proteins. The 355/420 factor increases upon oxidation, while both the 280/340 substance and the 380 nm material decrease.

Severe thermal injury imposes metabolic demands on patients such that two to three times the normal caloric intake is often required to maintain severely burned individuals. Enteral administration of nutrients is less likely to engender septic complications than intravenous administration. To further reduce the possibility of complications, the smallest size feeding tube is recommended, preferably without the use of a pump. We therefore tested in vitro a variety of nutrient

formulations from different manufacturers to ascertain whether sufficient calories could be administered by gravity feed alone through tubing of various diameters. We found that Ensure^{R}, Vivonex^{R} (standard and HN), Amin-aid^{R} and precision isotonic solutions could all provide at least 100 calories/hr as full-strength solutions through a #6 French tube. Magnacal^{R} and Compleat B^{R} needed to be given as half-strength solutions to provide 100 calories/hr, but this entailed an additional 100 ml/hr fluid load. A parabolic relationship between the viscosities of these solutions and flow rates was obtained when all the data were used, while if the data for Compleat B were eliminated a linear relationship existed.

Thermal injury
Infection
Patients
Rats
Indices of infection
Nutrient solutions
Viscosity
Flow rate

MONITORING AND MODIFICATION OF THE METABOLIC AND PHYSIOLOGIC ALTERATIONS ASSOCIATED WITH THERMAL INJURY

DETECTION OF POTENTIAL BIOCHEMICAL INDICATORS OF INFECTION IN THE BURNED RAT

Severe extensive thermal injury is often complicated by the development of infection (1). Severe thermal injury also complicates the detection of infection by altering the patient's febrile and leukocyte response (2), and wound colonization can be mistaken for systemic infection (1), since wound manipulation can of itself induce transient bacteremia (3). A simple, rapid early indicator of infection that requires small amounts of blood (< 5 ml) and that does not respond appreciably to the extent of injury would significantly enhance care of the burn patient.

In the course of studies to determine if a metabolic profile could be identified which would discriminate between burned and burned-infected rats (4), we discovered that the native or background fluorescence of perchloric acid filtrates of whole blood from burned-infected rats was greater than that from either control or burned-noninfected rats. Lloyd et al., who developed the analytic techniques we were using (5), had also noted that samples from very seriously ill patients possessed enhanced background fluorescence. We therefore pursued our observation and now describe the existence of three seemingly disparate substances which appear to be early indicators of infection in the injured host.

MATERIALS AND METHODS

Male albino rats (180-200 gm) were used in all studies (Holtzman Co., Madison, Wisconsin). A 30% total body surface full-thickness burn of the dorsum was achieved by immersing anesthetized, shaved rats, which had been placed in a mold to define the extent of injury, in

1. Lowbury EJL: Wits versus genes: The continuing battle against infection. J Trauma 19:33-45, 1979.

2. MacMillan BG: Infections following burn injury. Surg Clin North Am 60:186-196, 1980.

3. Sasaki TM, Welch GW, Herndon DN, Kaplan JZ, Lindberg RB, Pruitt BA Jr: Burn wound manipulation-induced bacteremia. J Trauma 19: 46-48, 1979.

4. Powanda MC, Dubois J, Villarreal Y, Kennedy CR, Mason AD Jr: Whole blood and plasma amino acid and lipid alterations in burned and burned infected rats. Fed Proc 39:889, 1980.

5. Lloyd B, Burrin J, Smythe P, Alberti KGMM: Enzymatic fluorometric continuous-flow assays for blood, glucose, lactate, pyruvate, alanaline, glycerol, and 3-hydroxybutyrate. Clin Chem 24:1724-1729, 1978.

boiling water for 10 seconds (6). No resuscitation was carried out. A 60% burn was achieved by immersing the ventral surface, as well, for 2 seconds. Those rats with 60% burns were resuscitated with 20 ml normal saline injected intraperitoneally. Pseudomonas infection was induced by placing 1 ml of a 16-hr broth culture on the burned dorsum of the rat within 1 hr after burning, followed by swabbing to distribute the organisms over the surface. A clinical isolate of P. aeruginosa was used, strain 12-4-4. and the culture was adjusted to yield 10^8 organisms/ml. A clinical isolate of Proteus was also used, this time adjusted to 10^4 organisms/ml, so as to extend the time to death in this rapidly progressing disease. The animals in this case were infected 6 hr postburn. Blood samples from burned and burned-infected rats were cultured in trypticase soy broth to assess the presence of bacteria.

At the times required in each of the studies, the rats were anesthetized by the intraperitoneal injection of 0.5-1 mg of sodium pentobarbital/25 gm body weight; the body cavities were opened and blood was taken from the hepatic vein.

Detection of the putative biochemical indicators of infection was accomplished by mixing 1 ml of heparinized whole blood (10-20 units of heparin/ml) with 4 ml of chilled 0.8 M perchloric acid (PCA) in a 17 X 100 mm polypropylene tube. The mixture was allowed to stand for 10 min and then spun in a refrigerated centrifuge (Sorvall RC-3) for 10 min at 2,200 *g*. The filtrates were poured into 12 X 75 mm polypropylene tubes and then spun at 48,000 *g* for 20 min (Sorval RC 2-B), decanted into a second 12 X 75 mm tube and spun again at 48,000 *g* for 20 min. Light absorption was measured using a Gilford 240 spectrophotometer with 0.5 M PCA as a blank, or a Beckman ratio recording spectrophotometer. Fluorescence was measured with an Aminco-Bowman spectrophotofluorometer. The fluorometer was standardized by using a commercially available tetraphenylbutadiene standard.

RESULTS

In the course of studies of metabolism in burned and burned-infected rats, it became apparent that perchloric acid filtrates of whole blood from burned-infected rats had a greater background fluorescence (λex 355 nm, λem 420 nm) than did those from burned or control rats. Filtrates from burned-infected, but not from burned-noninfected rats also displayed a broad band of absorbing material, with a peak from 394-402 nm when scanned in a dual beam spectrophotometer; 398 nm was chosen to assay this factor.

Fig. 1 depicts a longitudinal study of the change in absorbance and fluorescence following injury ± infection. Within 3 days of injury, there was a slight, but transient, increase in optical density in

6. Walker HL, Mason AD Jr: A standard animal burn. J Trauma 8: 1049-1951, 1968.

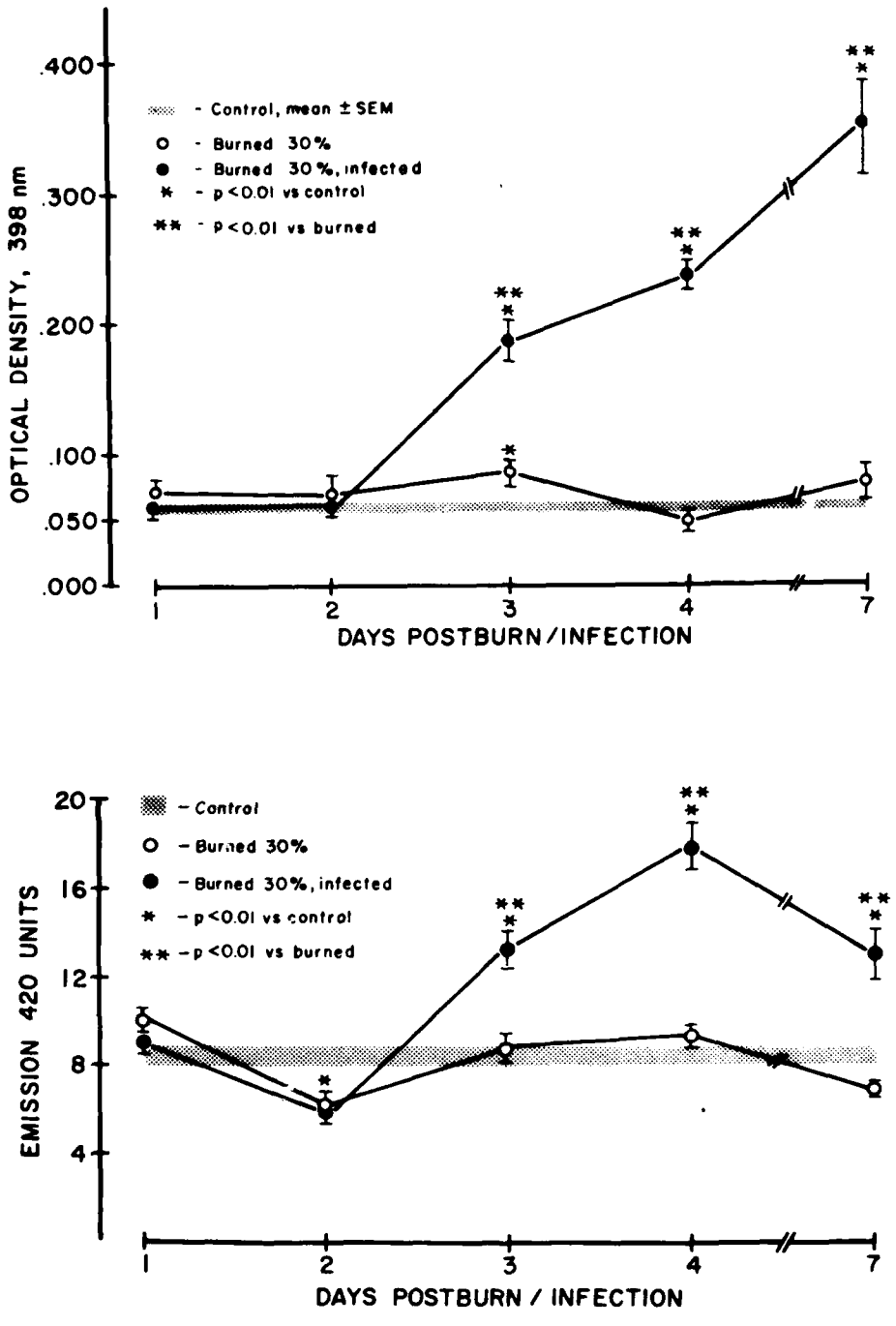


Fig. 1. Light absorption at 398 nm and fluorescence λex 350 nm λem 420 nm of perchloric acid filtrates of whole blood. Mean ± SEM, n = 6. Analysis of variance, least significant difference was used to assess statistical significance.

samples from burned-noninfected rats. Samples from burned-infected rats displayed a significant increase in absorbance versus both control and burned-noninfected rats on day 3 and additional increases in optical density thereafter, so that by day 7 there was a sixfold difference in OD at 398 nm between samples from burned-infected and from either control or burned-noninfected rats. Perchloric acid filtrates from both burned-noninfected and burned-infected rats showed a modest decrease in fluorescence on day 2; samples from burned-noninfected rats displayed no subsequent change, but those from burned-infected animals contained significantly heightened flourescence on days 3 through 7. Pseudomonas bacteremia was detectable in 2/6, 5/6 and 6/6 burned-infected rats on days 3, 4 and 7 respectively. None of the 30% burned-noninfected rats had positive blood cultures for Pseudomonas.

Table 1 indicates that the extent of injury produces no significant change in either absorbance at 398 nm or emission at 420 nm; however, infection overlaid on injury elicits a four- to sixfold increase in OD 398 and a threefold enhancement of fluorescence. These data also indicate that the 398 nm absorbing material appears to be cell associated, while the fluorescence factor can be detected in PCA filtrates of either plasma or whole blood.

A closer examination of the fluorescence scans of PCA filtrates from control, burned-noninfected and burned-infected rats indicates that in addition to the 355/420 factor, there also appears to be a 280/340 substance which increases with infection. Thus in subsequent studies, this substance was also measured. It became apparent that emission at 420 nm increased, while OD at 398 nm decreased if PCA-treated samples were allowed to stand for a few days at 4° C. This suggested that an oxidative process might have been occurring. Hydrogen peroxide was found to maximize the 355/420 reading but to decrease absorbance at 398 nm and the 280/340 fluorescence (Table 2). The assay was then modified so that following readings at 398 nm and 280/340, 0.2 ml of 30% hydrogen peroxide was added, and 1 hr later the 355/420 measurement was made. If whole blood samples were stored at 4° C and PCA added just prior to analyses, the indicators appeared to be stable for at least 3 days (Table 3).

Preliminary evidence indicates that when PCA filtrates from burned-infected rats were centrifuged through an Amicon Centriflo filter (F25), all of the 398 nm material was retained while 93% of the 280/340 and 66% of the 355/420 material was retained (Table 4). All factors were completely removed from PCA filtrates by the addition of 5% phosphotungstic acid in 2N HCl (Table 5).

Alterations in these putative biochemical indicators of infection are not limited to Pseudomonas aeruginosa infection. Proteus mirabilis infection in burned rats also induces changes in optical density at 398 and in fluorescence (Table 6). There is by the second postburn, postseeding day a doubling in the OD 398 and by 3 days a three- to fourfold

Table 1. Light Absorption (398 nm) and Emission (420 nm) of Perchloric Acid Filtrates 4 Days Postburn/Infection

	Absorption (OD)		Emission (units)	
	Whole blood	Plasma	Whole blood	Plasma
Control	0.063 ± 0.007	0.014 ± 0.004	2.4 ± 0.6	5.9 ± 0.9
Burned 30%	0.061 ± 0.007	0.008 ± 0.003	2.0 ± 0.4	5.1 ± 0.5
Burned 30%, infected	0.240 ± 0.032[a,b]	0.010 ± 0.003	6.1 ± 0.8[a,b]	19.8 ± 2.8[a,b]
Burned 60%	0.072 ± 0.009	0.005 ± 0.002	4.3 ± 0.9	6.7 ± 0.9
Burned 60%, infected	0.356 ± 0.018[a,c]	0.012 ± 0.004	12.3 ± 1.4[a,c]	23.3 ± 2.7[a,c]

Mean ± SEM, n = 8. Infection was accomplished by swabbing 1 ml of a *P. aeruginosa* culture containing 10^8 bacteria on the dorsal surface within 1 hr of scalding.

a = $p < 0.01$ vs control)
b = $p < 0.01$ vs 30% burned) by Scheffé.
c = $p < 0.01$ vs 60% burned)

Table 2. Effect of Time, Oxidation and Reduction on Detection of Biochemical Indicators of Infection

	Time of analysis (hr)								
	Untreated samples			MSH-treated samples			H_2O_2-treated samples		
	2	4	24	2	4	24	2	4	24
OD 398	.509 ±.032	.515 ±.023	.428 ±.022	.506 ±.030	.323 ±.034	.403 ±.057	.562 ±.028	.090 ±.007	.115 ±.001
355/420	22.6 ± 2.1	42.8 ± 2.4	88.2 ± 6.5	23.4 ± 2.2	82.3 ± 7.7	125.3 ±10.8	23.3 ± 2.2	300.0 ±21.4	479.2 ±36.6
280/340	2600 ± 225	1001 ± 91	818 ± 75	2500 ± 182	827 ± 57	605 ± 41	2292 ± 229	347 ± 45	237 ± 30

MSH (mercaptoethanol), H_2O_2 were added after 2-hr reading.

Blood was taken from burned-infected rats at 6 days; 5 rats were bled out using heparin (10-20 units/ml).

Table 3. Effect of Storage of Whole Blood Samples at 4° C

		Time of precipitation with 0.8 M perchloric acid (hr)				
		0	4	24	48	72
OD 398	Control	.065 ±.003	.043 ±.003	.030 ±.005	.033 ±.005	.034 ±.005
	Burned	.059 ±.006	.030 ±.008	.023 ±.005	.018 ±.006	.035 ±.003
	Burned infected	.340 ±.042	.376 ±.024	.406 ±.025	.326 ±.030	.489 ±.031
280/140	Control	307 ± 5	303 ± 7	347 ± 25	250 ± 7	324 ± 46
	Burned	478 ± 23	355 ± 9	363 ± 5	295 ± 25	438 ± 36
	Burned infected	1614 ± 93	1023 ± 53	1350 ± 49	1220 ± 57	1388 ± 48
355/420	Control	30.6 ± 0.8	26.8 ± 1.4	36.1 ± 1.8	35.3 ± 1.5	35.2 ± 3.7
	Burned	34.8 ± 2.3	25.6 ± 2.1	35.3 ± 1.8	34.8 ± 2.0	40.8 ± 4.4
	Burned infected	214 ± 11	139 ± 5	173 7	164 ± 8	198 ± 13

Eight rats per group were bled using heparin at 6 days postburn ± infection; 1 ml aliquots of blood were stored at 4° C and precipitated with PCA at the times noted.

Table 4. Effect of Filtration on Detectability of Indicators of Infection

OD @ 398 nm		λex 280 λem 340		λex 355 λem 420	
Initial reading	Filtrate reading	Initial reading	Filtrate reading	Initial reading	Filtrate reading
.359	.000	1700	117	147	50
±.018		± 78	± 3	± 5	±3

One ml of whole blood from burned-infected rats was precipitated with PCA, the absorbance and fluorescence of the supernatant measured; the supernatant was then passed through an Amicon Centriflo filter (F 25) and the absorbance and fluorescence of the filtrate measured; n = 8; mean ± SEM.

Table 5. Effect of Phosphotungstic Acid (PTA) Treatment on Detectability of Indicators

OD @ 398 nm		λex 280 λem 340		λex 355 λem 420	
Initial reading	After PTA	Initial reading	After PTA	Initial reading	After PTA
.361	.006	1619	0	146	0
±.023	±.002	± 77		± 5	

The absorbance and fluorescence of PCA filtrates was measured, then 1 ml PTA (5% in 2 N HCl) was added, the filtrates centrifuged and absorbance and fluorescence again measured; n = 8, mean ± SEM.

Table 6. Alterations in Biochemical Indicators during Proteus Infection in Burned Rats

		Time (hr) postburn/infection		
		24/18 n = 6	48/42 n = 6	72/66 n = 3
OD 398 nm	Controls	.061 ± .005	.040 ± .007	.040 ± .007
	Burned	.061 ± .006	.064 ± .009	.025 ± .003
	Burned, infected	.079 ± .001	.116 ± .014	.123 ± .026
280/340	Controls	218 ± 5	245 ± 10	277 ± 38
	Burned	350 ± 23	510 ± 29	380 ± 6
	Burned, infected	423 ± 22	1317 ± 86	1180 ± 180
355/420	Controls	19.2 ± 0.9	11.2 ± 1.2	19.7 ± 2.8
	Burned	20.3 ± 1.7	22.7 ± 3.1	13.0 ± 2.5
	Burned, infected	24.5 ± 1.5	62.0 ± 4.0	69.6 ± 22.7

increase in optical density. As regards fluorescence, there is a two- to threefold increase in 280/340 at 2 days and a similar increase at 3 days. In regard to the 355/420 factor, there is a two- to threefold increase in it on day 2 and a three- to fourfold increase on day 3. Bacteremia was detectable in 2/6 infected rats on day 2 and 3/3 on day 3. Thus to some degree the increase in biochemical indicators precedes sepsis in this model as well.

The results of analysis of samples of microorganisms for the presence of these indicators suggest that little or no 398 nm absorbing material or 280/340 fluorescent material is associated with either P. aeruginosa or P. mirabilis (Table 7). Pseudomonas does exhibit considerable 355/420 fluorescence, but it does not increase upon the addition of hydrogen peroxide, and most of it readily passes through an Amicon Centriflo filter.

Table 7. Analysis of Microorganisms for Presence of Indicators of Infection

	OD @ 398 nm	280/340	355/420	355/420 (H_2O_2)*	355/420 (filtrate)†
Pseudomonas	.003	0.5	94	70	54
aeruginosa	.000	0	155	140	120
12-4-4	.000	0.5	160	120	105
Proteus	.005	< 0	< 0	7	--
mirabilis	.003	< 0	< 0	8	--
	.004	< 0	< 0	7.5	--

Three different cultures of each microorganism (approximately 1 X 10^9 microorganisms/ml) were tested; 1 ml of culture was mixed with 4 ml of 0.8 M PCA. The original culture medium was used as control.

* = value after addition of H_2O_2.

† = amount of H_2O_2 treated material which passed through an Amicon Centriflo filter.

DISCUSSION

The observation of increased native fluorescence in perchloric acid filtrates of whole blood from burned-infected rats has led us to detect and describe three substances or sets of substances which can be rapidly analyzed (2-3 hr processing and analysis time), and appear to be sensitive, early indicators of infection in the burned rat. The origin of these indicators, whether host or microorganism derived, has not been ascertained. However, perchloric acid filtrates of cultures of Pseudomonas and Proteus (approximately 1 X 10^9 microorganisms/ml) exhibit negligible absorption at 398 nm (< 0.003 OD units) and fluorescence 280/340 (< 1 unit), suggesting that neither of these factors originates with the microorganism. In regard to fluorescence 355/420, Proteus exhibited minor amounts which were, however retained by a Centriflo filter. Pseudomonas cultures, on the other hand, possessed considerable fluorescence 355/420, but unlike that found in PCA filtrate from infected animals this did not increase with peroxide addition; and 70% to 85% of it passed through a Centriflo filter. This suggests that though Pseudomonas could give rise to some of the 355/420 material, there would have to be some change in its form such as would occur with aggregation or binding to a large molecule acting as a carrier to account for the material being retained by molecular sieves when found in blood samples from burned-infected animals.

Whatever the nature of the three factors, they do not appear to be low molecular weight substances but do appear to be distinct one from another. The 398 nm material is found only in whole blood, not plasma, and thus is not equivalent to either the 280/340 or the 355/420 substances which are detectable in both whole blood and plasma. The 355/420 substance increases upon oxidation, while the 280/340 material decreases; though this could be interpreted merely as a shift in molecular configuration resulting in new spectral characteristics, the 280/340 factor is 95% retained by a Centriflo filter while a third of the 355/420 material passes through, which is circumstantial evidence of non-equivalence.

Whatever the ultimate identities of these factors, it seems clear that they respond primarily to the presence of infection and are not significantly affected by extent of injury. The analyses require 2 to 3 hours to perform, including processing time, and no special handling of the blood sample is required. The increases in these three indicators appear to parallel the development of systemic sepsis in this model. The initial increase in these three factors in many cases preceded the onset of bacteremia. Unlike the substances described by Baker et al. (7), these factors confirm the presence of infection and monitor its progression rather than predict ensuing sepsis; they are thus akin to early acute-phase reactants.

7. Baker CC, Trunkey DD, Baker WJ: A simple method of predicting severe sepsis in burn patients. Am J Surg 139:513-517, 1980.

PART II. EVALUATION OF FLOW RATES OF NUTRIENT SOLUTIONS

METHODS AND MATERIALS

A Brookfield synchro-lectric viscometer, model RVT, with an ultra-low viscosity adapter (Brookfield Engineering Laboratories, Stoughton, Massachusetts), was used to measure the viscosities of the nutrient solutions. Brookfield viscosity standards (9.4-98 centipoises) were used to calibrate the instrument. To measure flow rates, samples of each size tubing were attached directly or by means of a needle adapter to a 10 cc disposable pipette and allowed to hang vertically. The tubing and pipette were filled from the bottom with various nutrient solutions by means of a syringe. The time for 9 ml of each nutrient solution to flow through the system was measured.

Calculations and curve fitting were accomplished using a Hewlett-Packard 9815A calculator.

RESULTS AND DISCUSSION

The nutrient solutions tested generally had viscosities of 7 centipoises (cps) or less except for Compleat B and Magnacal with 84.5 and 42.9 cps respectively (Table 8). Plots of viscosity versus flow rates through enteral feeding tubes of various diamaters presented as parabolic relationships even when one-half strength solutions were studied (Fig. 2). If the data for Compleat B were eliminated from the calculations, a linear relationship between viscosity and flow rate obtained. The flow behavior of Compleat B thus appeared anomalous and may be due to the tendency for the components of the suspension to rapidly settle even with frequent mixing.

Using the #6 French Duotubes, flow rates of 100 ml/hr could not be achieved by gravity feed alone for full-strength Magnacal and Compleat B solutions. One hundred cal/hr could be provided by these preparations if half-strength solutions of either preparation was used and if the patient could tolerate the extra 100 ml/hr fluid load. All the other formulations tested should be able to provide adequate nutritional input/hr even when delivered through a #6 French tube and without resorting to positive pressure from a pump.

ABSTRACTS

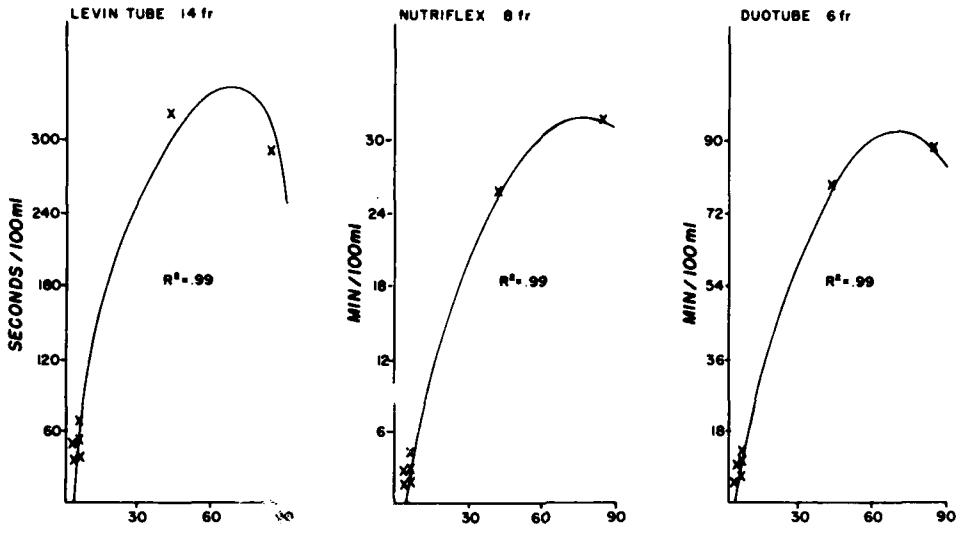
Powanda MC, Dubois J, Villarreal Y, Kennedy CR, Mason AD Jr: Whole blood and plasma amino acid and lipid alterations in burned and burned-infected rats. Fed Proc 39:889, 1980.

Powanda MC, Dubois J, Villarreal Y, Walker HL: Chemical indices of infection in the compromised host. Clin Res 28:377A, 1980.

Table 8. Viscosity and Flow Rates of Nutrient Solution through Various Tubing

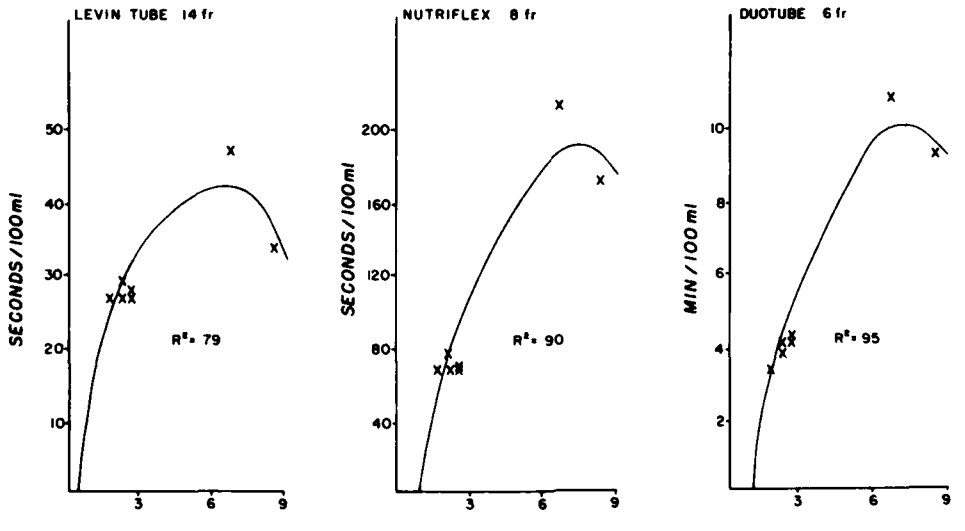
	Strength	Viscosity (cps)	Levin #14 Fr (sec/100 ml)	Nutriflex #8 Fr (min/100 ml)	Duotube #6 Fr (min/100 ml)
Compleat B	1	83.7, 85.4	297, 286, 292	32.7, 31.2, 31.2	88.7, 89.2, 87.2
	½	8.4, 8.3	34, 33, 34	2.9, 2.8, 2.8	9.5, 9.3, 9.1
Magnacal	1	42.9, 42.9	303, 322, 337	25.6, 28.2, 26.0	79.4, 79.5, 79.2
	½	6.5, 6.6	47, 48, 47	3.5, 3.7, 3.6	10.9, 11.0, 10.8
Ensure	1	6.5, 6.5	50, 52, 51	2.8, 2.8, 2.8	10.6, 10.8, 10.7
	½	2.5, 2.6	27, 27, 27	1.2, 1.2, 1.2	4.2, 4.3, 4.2
Vivonex Std	1	5.9, 5.8	40, 36, 38	1.7, 1.7, 1.7	6.6, 6.6, 6.6
	½	2.6, 2.6	28, 28, 29	1.1, 1.1, 1.1	4.0, 4.1, 4.1
Amin-aid	1	5.4, 5.4	68, 69, 68	4.1, 4.1, 4.1	13.2, 13.3, 13.2
	½	2.6, 1.8	29, 29, 29	1.4, 1.3, 1.3	4.1, 4.1, 4.1
Vivonex HN	1	3.7, 3.7	36, 36, 36	1.9, 1.9, 1.9	5.8, 5.8, 5.9
	½	1.6, 1.7	27, 28, 26	1.1, 1.2, 1.1	3.3, 3.3, 3.3
Precision isotonic	1	3.7, 3.6	50, 49, 49	2.8, 2.8, 2.8	9.6, 9.7, 9.6
	½	2.2, 2.2	27, 28, 27	1.2, 1.1, 1.1	3.7, 3.7, 3.7

FULL STRENGTH NUTRIENT SOLUTIONS



VISCOSITY OF SOLUTIONS IN CENTIPOISES

HALF-STRENGTH NUTRIENT SOLUTIONS



VISCOSITY OF SOLUTIONS IN CENTIPOISES

Fig. 2

PUBLICATIONS

Powanda MC, Bostian KA, Dinterman RE, Fee WG, Fowler JP, Hauer EC, White JD: Phagocytosis and the metabolic sequelae of infection. J Reticuloendothel Soc 27:67-82, 1980.

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Wilmore DW, Goodwin CW, Aulick LH, Powanda MC, Mason AD Jr, Pruitt BA Jr: Effect of injury and infection in visceral metabolism and circulation. Ann Surg 192:491-504, 1980.

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Powanda MC: Systemic alterations in metal metabolism during inflammation as part of an integrated response to inflammation. In Trace Elements in the Pathogenesis and Treatment of inflammatory Disorders. K.D. Rainsford, K. Brune and M.W. Whitehouse (Eds), Agents and Actions, Basel, Switzerland, in press.

PRESENTATIONS

Powanda MC: Whole blood and plasma amino acid and lipid alterations in burned and burned-infected rats. 64th Annual Meeting, Federation of American Societies for Experimental Biology, 15 April 1980.

Powanda MC: Biochemical indices of infection in burned rats. Twelfth Annual Meeting, American Burn Association, San Antonio, Texas, 29 March 1980.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0G 6980	79 01 01	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	8b. SPECIFIC DATA CONTRACTOR ACCESS	9. LEVEL OF SUM
79 10 01	D. CHANGE	U	U	NA	NL	[X] YES [ ] NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	075
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) Micromethod For Assessment of Serum Opsonic Capacity in The Burned Patient (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
79 06	Cont	DA	C. In-House

17. CONTRACT/GRANT: Not Applicable
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER:
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 80	.6	17
	CURRENT 81	.6	20

19. RESPONSIBLE DOD ORGANIZATION
NAME: US Army Institute of Surgical Research
ADDRESS: Fort Sam Houston, Texas 78234
RESPONSIBLE INDIVIDUAL
NAME: Basil A. Pruitt, Jr., COL, MC
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20. PERFORMING ORGANIZATION
NAME: US Army Institute of Surgical Research Clinical Division
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PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic institution)
NAME: Robert C. Allen, CPT, MC
TELEPHONE: 512-221-4311
SOCIAL SECURITY ACCOUNT NUMBER:
ASSOCIATE INVESTIGATORS
NAME: Basil A. Pruitt, Jr., MD, COL, MC
NAME: DA

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code): (U) PMN Leukocytes; (U) Chemiluminescence; (U) Opsonization; (U) Immunoglobulins; (U) Complement; (U) Burn Injury

23. TECHNICAL OBJECTIVE. 24. APPROACH. 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) The nonspecific opsonic capacity of sera from patients following burn injury will be compared to normal control sera. Qualification of opsonic capacity will be based upon the rate and magnitude of oxidative microbicidal activation as measured by amplified chemiluminescence using a set number of functional polymorphonuclear leukocytes (PMN) challanged with a set concentration of either zymosan or bacteria (Staphylococcus aureus or Pseudomonas aeruginosa). By holding zymosan and PMN leukocyte number constant, chemiluminescent activity will reflect the opsonic activity of sera.

24. (U) These functional measurements will be correlated with immunologic data, such as serum complement and immunoglobulin, quantified by immunoelectrophoretic and immunodiffusion techniques.

25. (U) 7910 - 8009. Chemiluminesence of leukocyte function in 720 blood specimens from 35 burn patients, and 80 blood specimens from 4 controls has been performed using the alternative pathway titration method described. These sera are presently being titrated for classical pathway complement activity. With the exception of mortality, the opsonic titrations were conducted in a "blind" manner; that is, the patient's clinical status was unknown at the time of testing. The complete collection of opsonic data will be added to the laboratory data accumulated by Becker et als. for correlative evaluations.

(Applicable to contractors only; grantee's summary)

DD FORM 1498 1 MAR 68 — PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 68 AND 1498-1 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

ANNUAL PROGRESS REPORT

PROJECT NO. 3A161101A91C, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: MICROMETHOD FOR ASSESSMENT OF SERUM OPSONIC CAPACITY IN THE BURNED PATIENT

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

1 October 1979 - 30 September 1980

Investigators:

Robert C. Allen, M.D., Ph.D., Captain, MC
Basil A. Pruitt, Jr., M.D., Colonel, MC
Richard A. Becker, M.D.

Reports Control Symbol MEDDH-288(RI)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A161101A91C, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: MICROMETHOD FOR ASSESSMENT OF SERUM OPSONIC CAPACITY IN THE BURNED PATIENT

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

Period covered in this report: 1 October 1979 - 30 September 1980

Investigators: Robert C. Allen, M.D., Ph. D., Captain, MC
Basil A. Pruitt, Jr., M.D., Colonel, M.C.
Richard A. Becker, M.D.

Reports Control Symbol MEDDH-288(R1)

A new method is presented for evaluation of both classical and alternative complement activities. This functional method is based upon the use of chemilumigenic probes for the detection of the oxidative activity associated with activation of polymorphonuclear leukocyte microbicidal metabolism. Examples of application of the methods to patient studies are presented. Chemiluminesence evaluation of leukocyte function in 720 blood specimens from 35 burn patients, and 80 blood specimens from 4 controls has been performed using the alternative pathway titration method described. These sera are presently being titrated for classical pathway complement activity. With the exception of mortality, the opsonic titrations were conducted in a "blind" manner; that is, the patient's clinical status was unknown at the time of testing. The complete collection of opsonic data will be added to the laboratory data accumulated by Becker et als. for correlative evaluations.

Polymorphonuclear Leukocytes
Chemiluminescence
Opsonization
Immunoglobulins
Complement
Burn Injury

MICROMETHOD FOR ASSESSMENT OF SERUM OPSONIC CAPACITY IN THE BURNED PATIENT

Infection continues to be a major problem in management of thermal injury patients, and inspite of an expanding arsenal of antibiotics, septic complications are a major cause of mortality. The opportunistic nature of the infecting microbe implies a defect in the humoral-phagocyte axis of host immune defense. This implication has experimental support.

The humoral component of host immunity in essence serves as an information system; that is, it identifies the infecting microbe. Under certain conditions the humoral system may directly effect microbicidal action, or it may act by directing a phagocyte effector of microbicidal activity. Humoral immunity is a composite of several different systems, each composed of numerous different proteins. The specific immunoglobulin such as IgG and IgM, the classical and alternative pathways of complement, and the acute phase reactants all belong to the humoral immune system.

The polymorphonuclear neutrophil (PMN) leukocyte is the predominant phagocyte of acute immune defense. The PMN leukocyte is a highly specialized effector of microbicidal action, and is controlled by the products of humoral activation. In response to a gradient of humoral-generated chemoattractants, the PMN leukocytes are able to migrate directly to the site of infection. Contact between the phagocyte and the immune-labeled microbe results in recognition, via membrane receptor sites, and phagocytosis. The microbicidal action that follows requires expenditure of metabolic energy in reactions ultimately effecting oxidative damage to the microbe. This oxidative microbicidal action has been demonstrated to yield electronically excited products, and the relaxation of the high energy states by photon emission results in detectable luminescence.

One of the major difficulties in management of the immunocompromised patient is the lack of reliable, objective laboratory tests for assessment of humoral-phagocyte function. The titration of complement, or "true" CH_{50}, was one of the few laboratory methods for estimating an humoral immune function. However, this procedure is expensive, consumes a large amount of time, and requires a highly trained technician. In most hospitals the $C'H_{50}$ titration method has been replaced by the inferior $C'H_{100}$ hemolysis plates; however, results are reported in so-called "$C'H_{50}$ units".

The individual proteins that comprise the various systems of humoral immunity can be quantified by antigen-immunologic techniques such as radial immunodiffusion, immunoelectrophoresis, and laser nephelometry. These tests are also expensive and time consuming, but they are relatively dependable. Unfortunately, they only measure the antigenic presence of the molecules and not its function. Furthermore, only one molecular component of one system can be measured per test.

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The ideal laboratory test for evaluating the humoral component of the humoral-phagocyte axis should: (1) accurately assess the function of a humoral system or subsystem, (2) require a very small blood sample, (3) provide results on the day of sampling, (4) be compatible with future automation, (5) be inexpensive, and (6) be dependable. Preliminary results indicate that the PMN leukocyte-chemilumigenic probe technique described meets these criteria.

MATERIALS AND METHODS

After obtaining informed consent, whole blood was collected from burn patients and controls. The blood specimens were collected throughout the course of hospitalization, and therefore, the values provide "linear" information that can be correlated with clinical status. Approximately 5 ml of whole blood was drawn per person per sample. The blood was allowed to clot at room temperature, the serum removed, and 0.5 ml aliquots placed in individual containers. The sera were then stored at -70°C until tested. Eighty individual specimens were obtained from 4 control volunteers, and 720 specimens were obtained from 35 burn patients.

PMN leukocytes were obtained from the whole blood of healthy volunteers. The leukocyte-rich plasma was separated from erythrocytes by dextran sedimentation. After hypotonic lysis of remaining erythrocytes (0.2% saline for 15 sec.), and two additional washes in Dulbecco's phosphate buffered saline, total and differential counts were obtained, and the volume was adjusted to yield 1000 PMN leukocytes/μl. The desired quantity of PMN leukocytes (25,000) was added to each vial containing 1.95 ml of barbital (veronal) buffered saline with Ca^{+}, Mg^{++}, albumin (0.1% w/v) and glucose (0.1% w/v). Each vial also contained one nanomole of luminol (5-amino-2, 3-dihyro-1, 4-phthalazinedione) as a chemilumigenic substrate probe. Serum was titrated over the range of dilutions from 1:50 to 1:800; that is 40 μl to 2.5 μl of serum was added per vial.

Activation of alternative pathway complement was effected by addition of 20 μl of zymosan (2.5 μg/μl) at time zero. The classical pathway was activated by addition of IgM-coated Shigella sonnei phase I (formalin treated) at time zero. The bacteria to PMN leukocyte ratio was 100 to 1.

Chemiluminescence (CL) was quantified at room temperature (23°C) using the single photon counting capacity of a Beckman LS-150 scintillation counter equipped with EMI 9829A (Bialkali spectral response) photomultiplier tubes. The counter was operated in the out-of-coincidence mode using the tritium channel settings. The photon counter was calibrated with a known blue light emitter and the photon conversion factor was calculated to be 14. Multiplication of the raw counts by this photon conversion factor converts the value to photons. The CL from unstimulated PMN leukocyte suspensions was monitored for 3 cycles (7 min/cycle) prior to addition of immune stimulant.

RESULTS

The opsonification of a particulate antigen defines the complex information link that exists between the humoral immune system and the microbicidal effector phagocyte. Identification of antigenic material may be specific, or it may involve a more general or nonspecific mechanism. An overview of the humoral-phagocyte interactions to be discussed is presented in Figure 1.

Alternative Pathway of Complement

A general mechanism for the recognition of foreign molecular components in the absence of previous immune exposure is provided via the alternative pathway of complement activation. The sequence of molecular interaction responsible for activation is complex and not completely understood. However, with regard to the phagocyte, the most important aspect of alternative pathway activation is the generation of an enzyme, C3 activator, responsible for the hydrolytic cleavage of C3 to yield C3b. Immune labeling involves the binding of C3b to the microbe or particulate material. As depicted in Figure 1, PMN leukocytes have membrane receptors for the recognition of C3b- and Fc-labeled material. Contact between a C3b-labeled particle and the C3b receptors of the PMN results in phagocytosis of the particle and activation of microbicidal oxidation resulting in CL.

The plots of CL intensity and integral CL versus time presented in Figure 2 describe the effect of serum titration on the kinetics and magnitude of the PMN leukocyte response. Zymosan, a boiled proteolytically digested preparation of yeast cells, was used as the particle to be phagocytosed, and is a known activator of the alternative pathway of complement. Each vial contained an equivalent quantity of zymosan. The differences in the curves of CL therefore reflect the functional opsonic activity of the serum based on the quantity employed.

Complement activation requires the participation of numerous serum components acting in a concerted fashion. The complexity of this interaction is responsible for the sigmoidal nature of the relationship between hemolytic complement activity and the volume of complement-containing serum used. This sigmoidal relationship also exists between the quantity of complement employed and the integral of CL obtained.

Figure 3 is a plot of the maximum intensity against the quantity of serum added per vial (2.0 ml volume). The ordinate values are given as log (CL max (sample)/CL max (normal control)-CL max (sample)). The ordinate value of zero defines the quantity of serum that will yield 50% of the activation of the PMN leukocyte preparation. This value is therefore an opsonic 50 for alternative pathyway complement.

Figures 4, 5, and 6 describe the application of the above techniques to a clinical situation. The three patients described were studied throughout the course of hospitalization to within two days of death. The ordinates of the figures represent the serum opsonic 50 values for alternative pathway complement expressed as percent of control. The axis describes the day of sample acquisition.

Classical Pathway of Complement

Estimation of classical pathway function employed an analogous approach. The major difference was the stimulant employed. Shigella sonnei phase I possesses an unusual carbohydrate in its lipopolysaccharide (LPS) and is not susceptible to the action of alternative pathway complement. Therefore, antibody was prepared against this LPS in rabbits; the IgM and IgG fractions were separated by Sepharose chromatography, and the IgM fraction was used for coating the S. sonnei I (formalin treated). No phagocytosis or CL was observed from PMN leukocytes upon addition of S. sonnei labeled with IgM. However, the further addition of serum complement was sufficient for activation, phagocytosis, and CL. S. sonnei without IgM, and IgM without S. sonnei, did not stimulate the PMN leukocytes in the presence of complement. Figure 7 is the plot of CL intensity and integral CL against time. IgM-labeled S. sonnei were added at time zero. The various curves represent the same serum titration effect as previously described for alternative pathway study presented in Figure 2, with Figure 8 representing the plot of data analogous to Figure 3 for alternative pathway. Note that in Figure 8 the activity never crosses the zero point on the ordinate. The ordinate values were calculated from the data depicted in Figure 3, and therefore, the values presented in Figure 8 represent activity relative to alternative pathway stimulation of the PMNL. As such the curve does not represent a true "opsonic 50" as in the case of Figure 3.

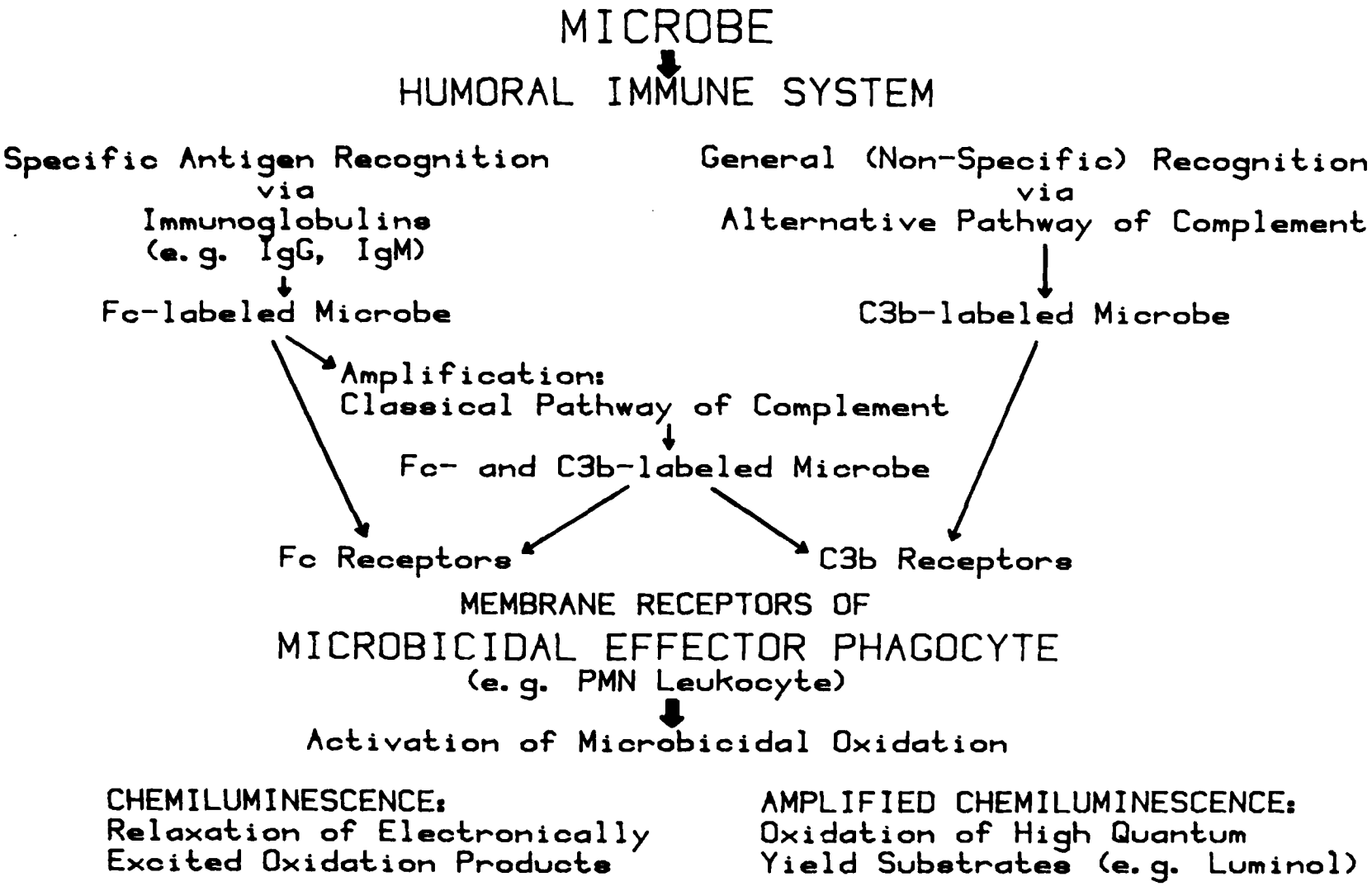
PUBLICATIONS

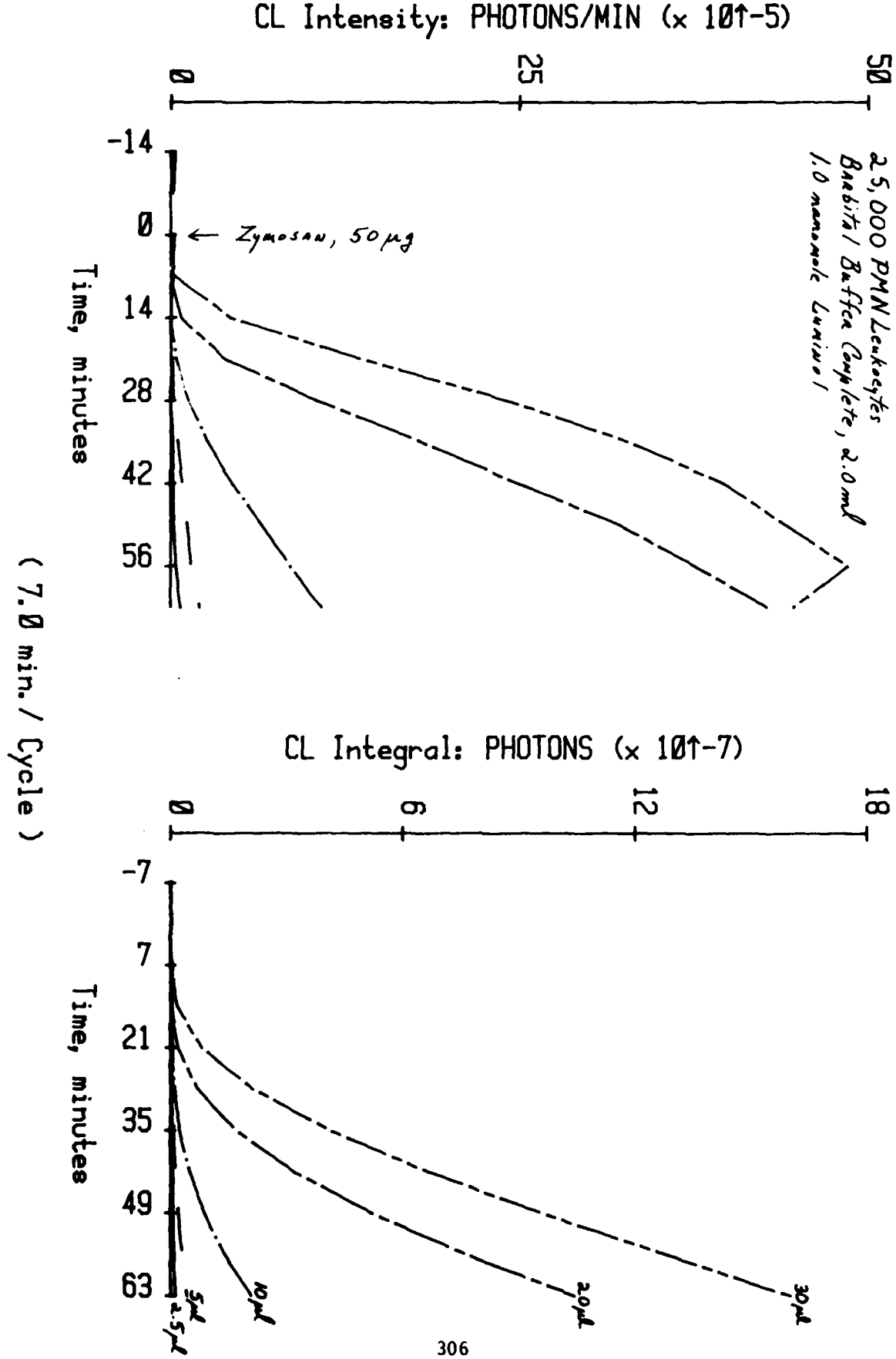
Allen, R. C. (1980) Chemiluminescence: An Approach to the Study of the Humoral-Phagocyte Axis in Host Defense Against Infection. In "Liquid Scintillation Counting Recent Applications and Developments Vol. II". (C. T. Peng, D. L. Horrocks, and E. L. Alpen, Eds.) Academic Press, pp 377-393.

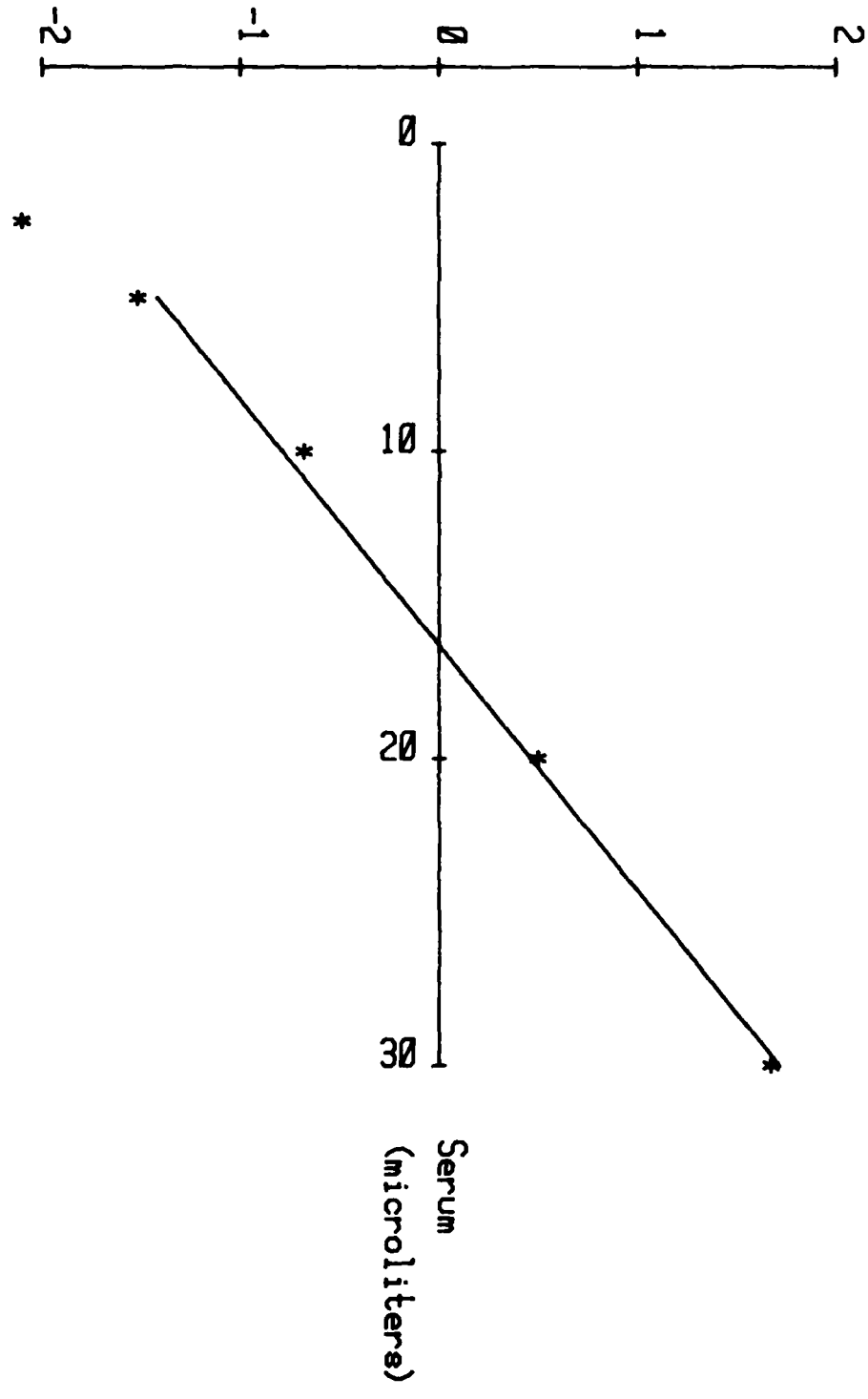
PRESENTATIONS

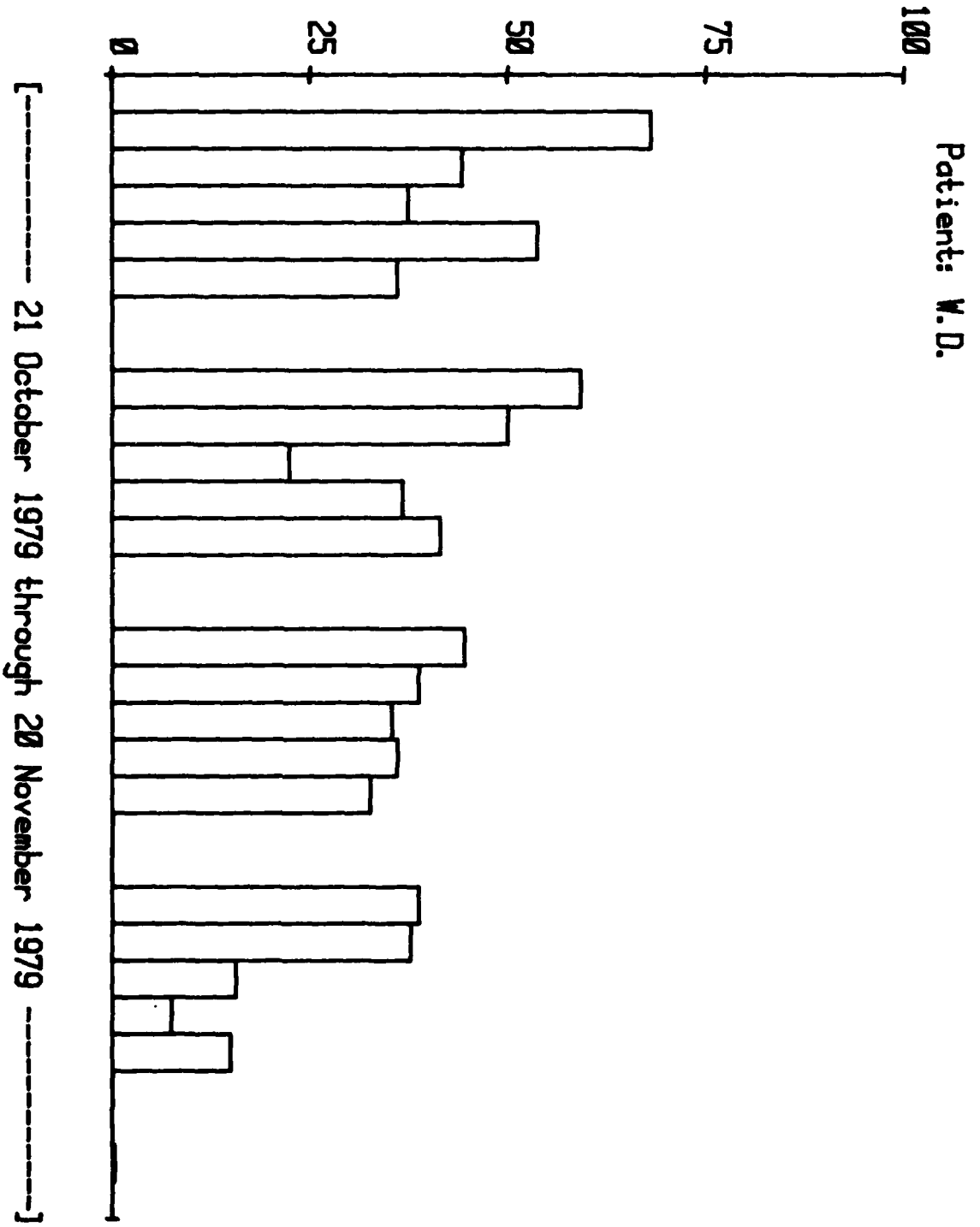
Allen, R. C. (1980) Lucigenic Chemiluminescence and the Study of PMN Redox Metabolism, Symposium on Phagocyte Chemiluminescence, American Society for Photobiology.

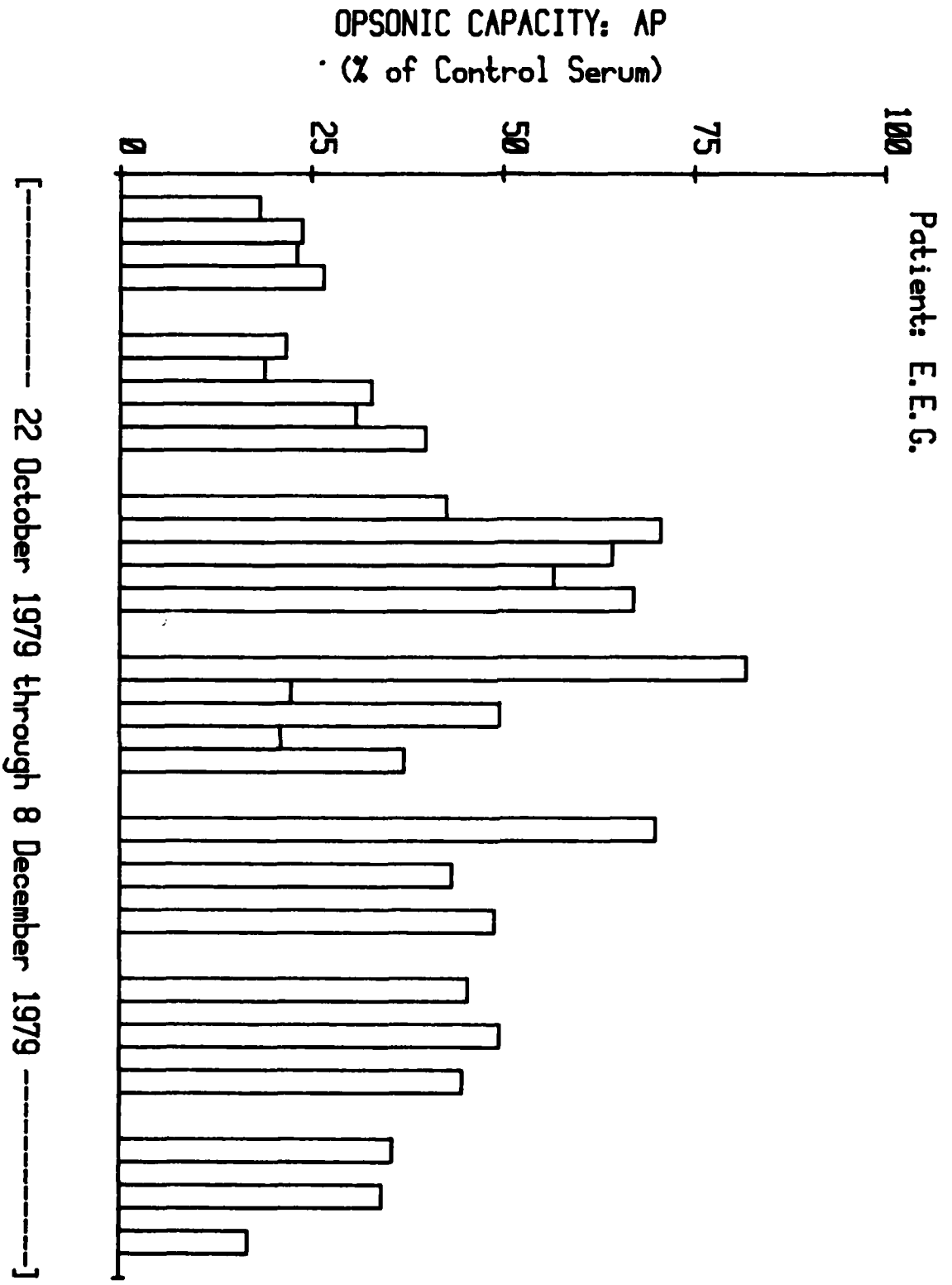
Figure #1.

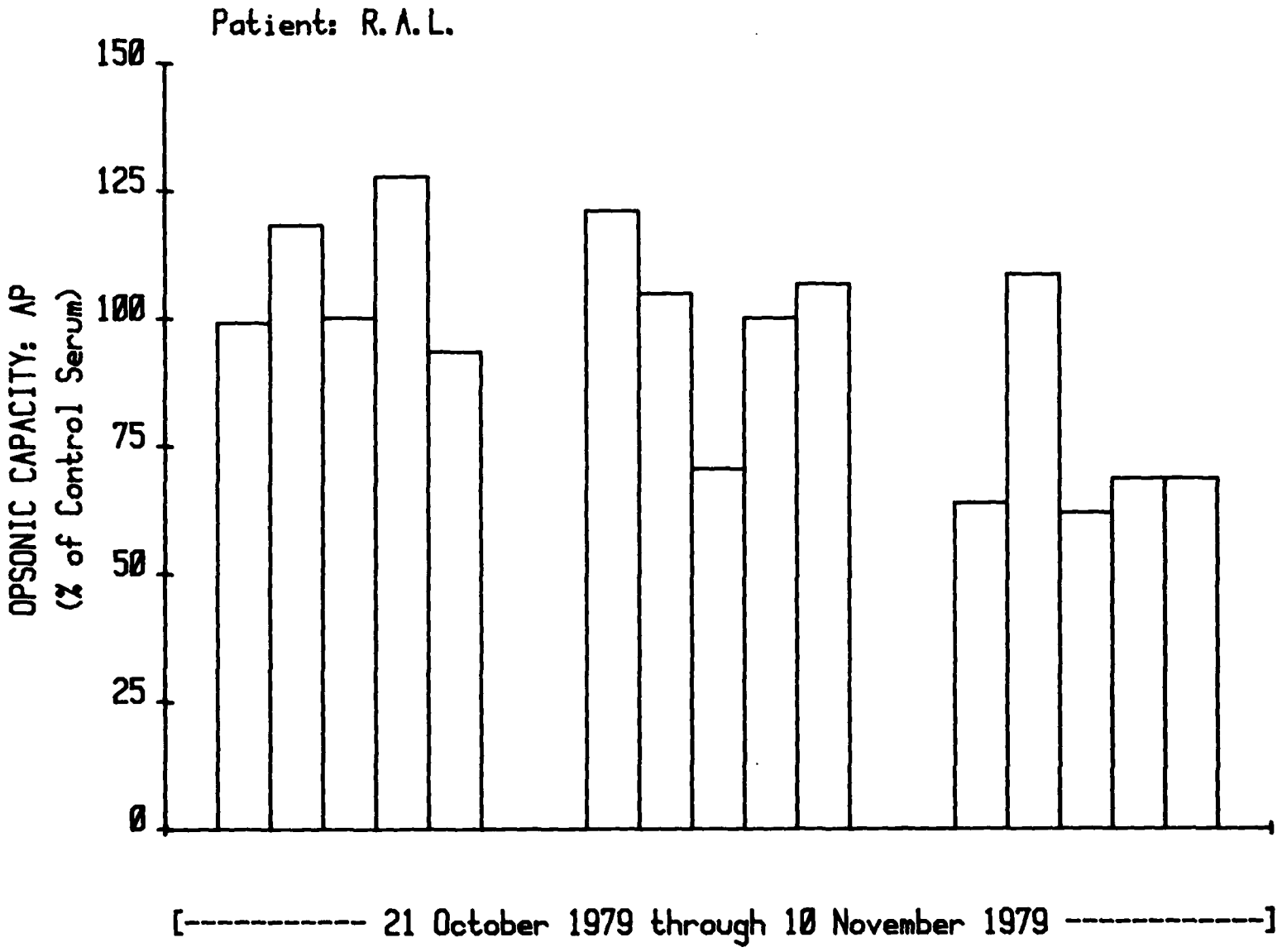

MICROBE
HUMORAL IMMUNE SYSTEM
Specific Antigen Recognition via Immunoglobulins (e.g. IgG, IgM)
General (Non-Specific) Recognition via Alternative Pathway of Complement
Fc-labeled Microbe
C3b-labeled Microbe
Amplification: Classical Pathway of Complement
Fc- and C3b-labeled Microbe
Fc Receptors
C3b Receptors
MEMBRANE RECEPTORS OF
MICROBICIDAL EFFECTOR PHAGOCYTE
(e.g. PMN Leukocyte)
Activation of Microbicidal Oxidation
CHEMILUMINESCENCE: Relaxation of Electronically Excited Oxidation Products
AMPLIFIED CHEMILUMINESCENCE: Oxidation of High Quantum Yield Substrates (e.g. Luminol)

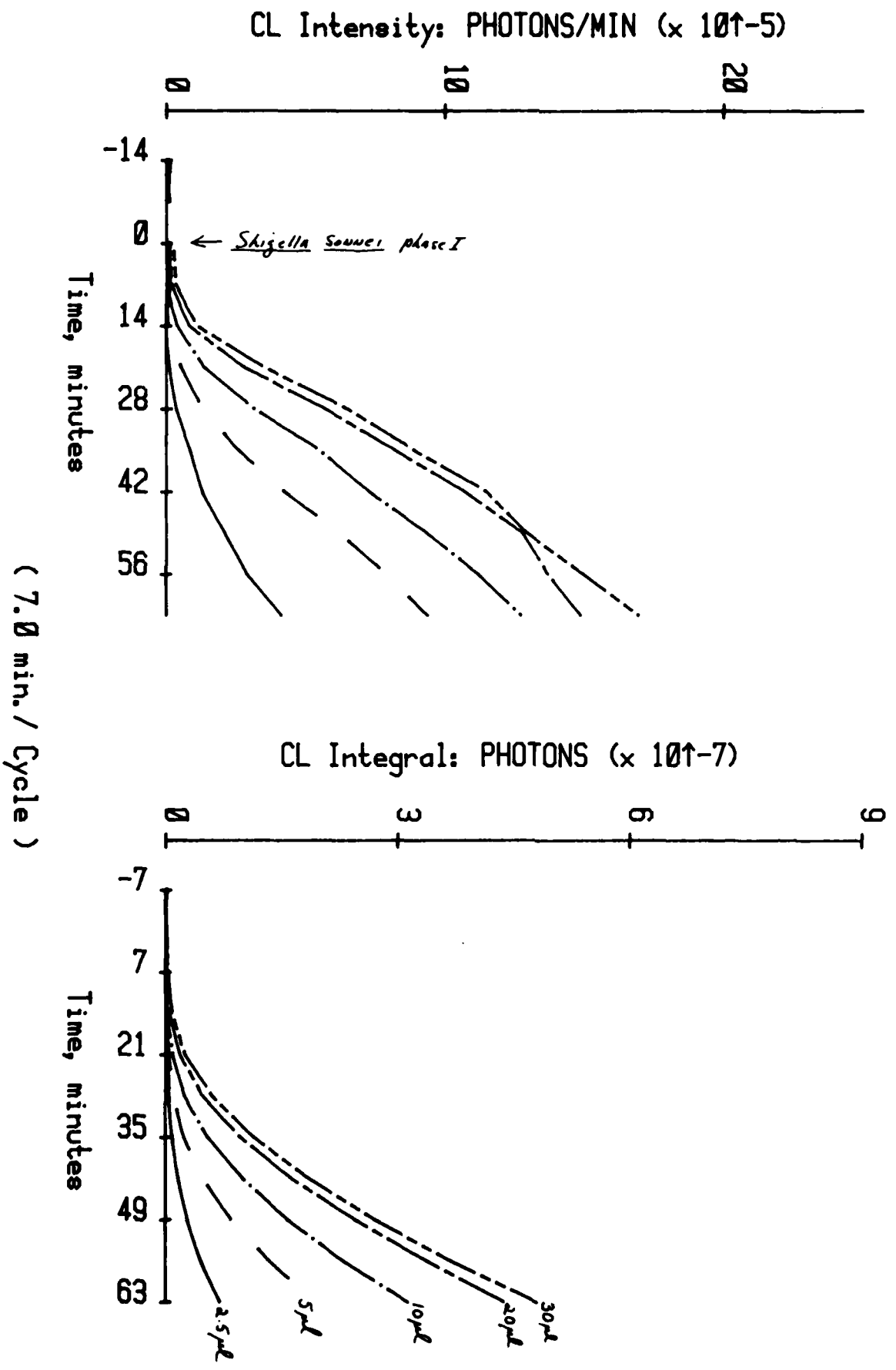

CL Intensity: PHOTONS/MIN (x 10↑-5)
0
25
50
-14
0
14
28
42
56
Time, minutes
← Zymosan, 50 μg
2.5,000 PMN Leukocytes
Barbital Buffer Complete, 2.0 ml
1.0 mmole Luminol
CL Integral: PHOTONS (x 10↑-7)
0
6
12
18
-7
7
21
35
49
63
Time, minutes
5 μl
2.5 μl
10 μl
20 μl
30 μl
(7.0 min. / Cycle)

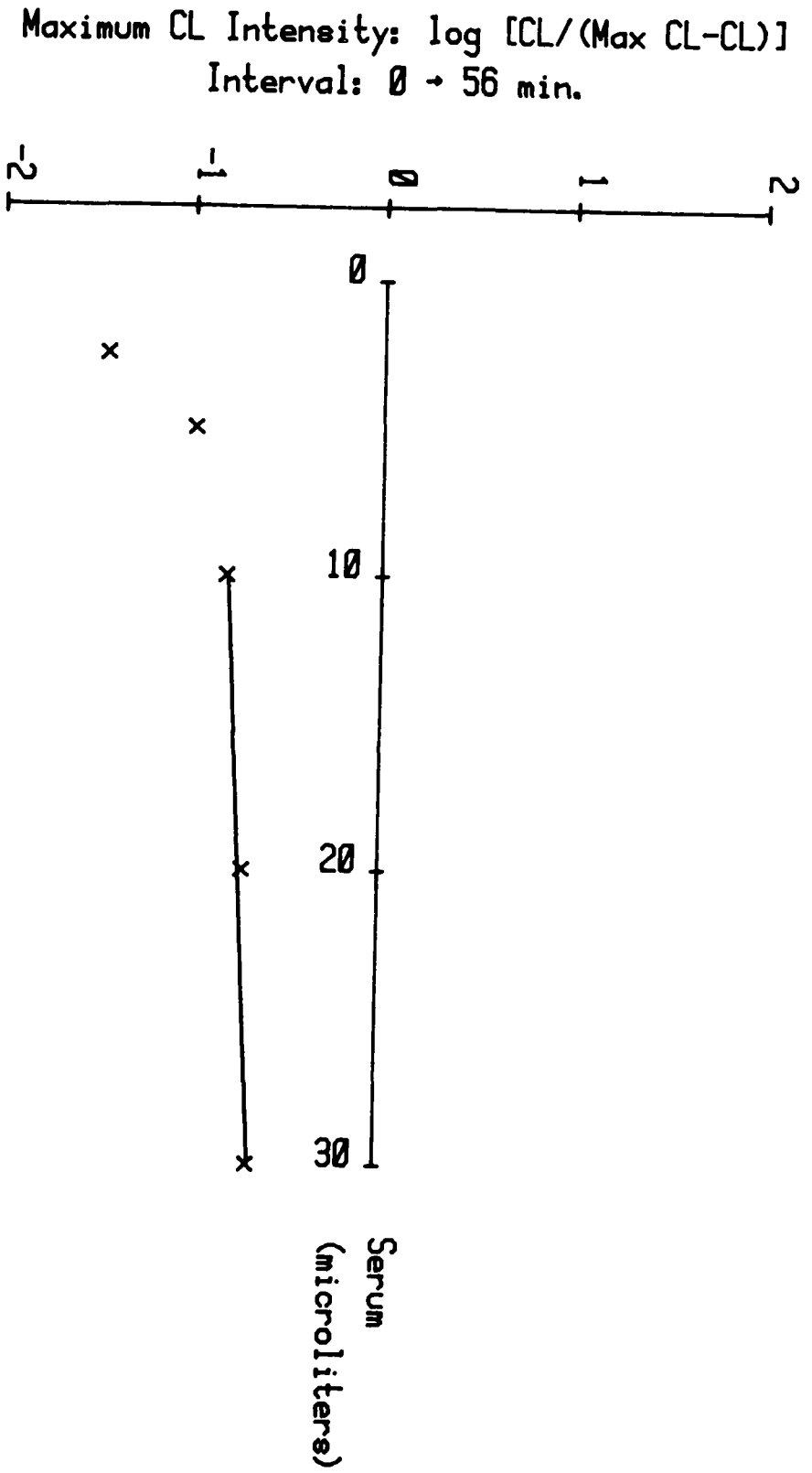

Maximum CL Intensity: log [CL/(Max CL-CL)]
Interval: 0 → 56 min.
-2
-1
0
1
2
0
10
20
30
Serum
(microliters)


OPSONIC CAPACITY: AP
(% of Control Serum)
0
25
50
75
100
Patient: W.D.
[---------- 21 October 1979 through 20 November 1979 ----------]


OPSONIC CAPACITY: AP
(% of Control Serum)
0
25
50
75
100
Patient: E.E.G.
[---------- 22 October 1979 through 8 December 1979 ----------]


Patient: R. A. L.
OPSONIC CAPACITY: AP
(% of Control Serum)
150
125
100
75
50
25
0
[---------- 21 October 1979 through 10 November 1979 ----------]


CL Intensity: PHOTONS/MIN (x 10↑-5)
0
10
20
-14
0
14
28
42
56
Time, minutes
← Shigella Sonnei phase I
(7.0 min. / Cycle)
CL Integral: PHOTONS (x 10↑-7)
0
3
6
9
-7
7
21
35
49
63
Time, minutes
2.5 µl
5 µl
10 µl
20 µl
30 µl


Maximum CL Intensity: log [CL/(Max CL-CL)]
Interval: 0 → 56 min.
-2
-1
0
1
2
0
10
20
30
Serum
(microliters)

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0G 6979	79 10 01	DD DR&E AR(6)30

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
79 10 01	D. CHANGE	U	U	NA	NL	[X] YES [ ] NO	A. WORK UNIT

10. NO. CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	085
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) Mitochondrial Oxidative Function in The Burn Wound and The Effect of Resuscitation in Burned Soldiers (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
78 09	Cont	DA	C. In-House

17. CONTRACT/GRANT: Not Applicable
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER:
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 80	.7	21
	CURRENT 81	.7	23

19. RESPONSIBLE DOD ORGANIZATION
NAME: US Army Institute of Surgical Research
ADDRESS: Fort Sam Houston, Texas 78234
RESPONSIBLE INDIVIDUAL
NAME: Basil A. Pruitt, Jr., COL, MC
TELEPHONE:

20. PERFORMING ORGANIZATION
NAME: US Army Institute of Surgical Research
Surgical Study Branch
ADDRESS: Fort Sam Houston, Texas 78234
PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME: Cleon W. Goodwin, LTC, MC
TELEPHONE: 512-221-3411
SOCIAL SECURITY ACCOUNT NUMBER
ASSOCIATE INVESTIGATORS
NAME:
NAME: DA

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Metabolism; (U) ATP; (U) Oxygen; (U) Cytochromes; (U) Goats

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) To define pathologic alterations in cellular function produced by thermal injury and to assess efficacy of treatment in reversing the process in burned soldiers.
24. (U) Initial studies will utilize the burned rat as a model for human burns. Following specific interval of time, sample of granulation tissue from burned areas will be sampled for separation of its subcellular components. Mitochondrial oxidative phosphorylation will be assayed by appropriate techniques, oxygen uptake by polarographic electrode, cytochrome content and activity by the double beam dual wave length spectrophotometry, and calcium transport by its reaction with murexide. After these baseline data are obtained, the effects of various resuscitation formulae in improving tissue perfusion will be assessed by changes in cellular functions described above.
25. (U) 7910 - 8009. After entraining two groups of 8 rats each on a 14-hour-on, 8 hour-off light-dark cycle, the effect of circadian rhythm on mitochondrial function was assessed. The respiratory control ratios (RCRs), a measure of mitochondrial integrity, were not significantly different between group A (no light exposure) and group B (8 hour light exposure): RCR 5.90 for group A, and 6.23 for group B, $P > 0.05$ by student t-test for paired data. Likewise, the state 3 ("activated") rates of mitochondrial oxidation were not significantly different: (A) 44.4 nmoles/min/mg protein vs. 48.2 nmoles/min/mg protein. These preliminary data imply that this animal model may be utilized in serial studies without concern for the normal circadian fluctuations of metabolically active hormones.

*Available to contractors upon originator's approval

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 68 AND 1498-1 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

ANNUAL PROGRESS REPORT

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: MITOCHONDRIAL OXIDATIVE FUNCTION IN THE BURN WOUND AND THE EFFECT OF RESUSCITATION IN BURNED SOLDIERS

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

1 October 1979 - 30 September 1980

Investigators:

Cleon W. Goodwin, M.D.
Arthur D. Mason, Jr., M.D.
Joseph Whitson, SP4

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: MITOCHONDRIAL OXIDATIVE FUNCTION IN THE BURN WOUND AND THE EFFECT OF RESUSCITATION IN BURNED SOLDIERS

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

Period covered in this report: 1 October 1979 - 30 September 1980

Investigators: Cleon W. Goodwin, M.D.
Arthur D. Mason, Jr., M.D.

Reports Control Symbol MEDDH-288(R1)

Biophysical laboratories investigating the metabolism of subcellular organelles, including mitochondria, routinely conduct such studies without regard to the possible effects of circadian rhythms. Such an approach is probably legitimate when using the isolated fractions of a single animal as its own control when describing specific reaction pathways. However, when studying the effects of longterm environmental or physiological stresses on subcellular function, this approach may yield misinterpreteted results. Numerous reports have described in laboratory animals diurinal variations in plasma and tissue hormones and enzymes. (1, 2) Further, these daily rhythms are affected by feeding schedules, and continuous feeding may eliminate the circadium rhythms altogether. (3, 4, 5) When planning the

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1. Kinson GA and Lieu Chung-Ching: Diurinal Variation in Plasma Testosterone of the Male Laboratory Rat. Hormone and Metabolism Research 5: 233-234, 1973.

2. Perlow MJ, Festoff B, Gordon EK et al: Daily Fluctuation in the Concentration of cAMP in the Conscience Primate Brain. Brain Research 126: 391 - 396, 1977.

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4. Morimoto Y, Arisue K, and Yamamura Y. Relationship Between Circadian Rhythm of Food Intake and That of Corticosterone and Affect of Food Restriction on Circadian Adrenocortical Rhythm in the Rat. Neuroendocrinology 23: 212 - 222, 1977.

5. Rusak, B, Neural Mechanisms for Entrainment and Generation of Mammalian Circadian Rhythms. Federation Proceeding 38: 2589 - 2595, 1979.

experimental design for the study of postburn hypermetabolism, the influence of time of study on mitochondrial biochemical reactions must be known if many separate studies are planned throughout the course of a working day. If such an affect is found, then all studies using animal models must utilize isolation procedures which are standardized for time of sampling and method of feeding.

METHODS

ANIMAL PREPARATION

Male Holzman rats (475 to 500 grams body weight) were placed in single cages and allowed to acclimate for two weeks in a light tight room. During this stabilization, the rats were entrained to light on a 0600 to 2000 hours on - 2000 to 0600 hours off cycle. Temperature was controlled to $27 \pm 2^{o}C$. Animals were fed a standard laboratory chow diet which was maintained in excess in all cages until 16 hours before sacrifice. On the morning of study, animals in Group A were sacrificed at 0555 hours, after approximately 10 hours of darkness, and animals from Group B were sacrificed at 1355 hours, after approximately eight hours of light.

ISOLATION OF MITOCHONDRIA

Following removal, liver (approximately 5 grams) was placed in a 0^{o} C. medium and chopped into small pieces to facilitate rapid cooling. Mitochondria were isolated in a medium consisting of 0.225 M mannitol, 0.075 M sucrose, 100 μM EGTA, and a final pH of 7.4. The mitochondria were gently homogenized by a motor-driven Teflon pestle in a glass homogenizer. The resulting suspension was centrifuged at 600 X g to remove residue, and the mitochondria were washed four times and recovered at 8000 X g. Washed mitochondria were suspended in an EGTA-free medium at 20-30 mg protein per ml.

MITOCHONDRIAL ASSAYS

All measurements were carried out in a medium containing 0.225 M mannitol, 0.075 M sucrose, 15 mM TRIS, 10 mM KH_2PO_4, and a final pH of 7.4. Oxygen uptake was measured polarographically with a Clark O_2 electrode in mitochondria respiring in State 4 (excess substrate) and State 3 (excess substrate and ADP). (6) Respiratory control ratios

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6. Chance B, Williams GR: The Respiratory Chain and Oxidative Phosphorylation. Adv Enzymol 17: 65 - 134, 1956.

(RCR) were calculated as the ratio of State 3 to State 4 rates. ADP/O ratios were calculated from the measured O_2 consumption (O_2 capacity of medium 240 nanomoles/ml) with 500 μM ADP as the phosphate acceptor. Protein concentrations of the mitochondrial samples were determined by a modification of the biuret reation. (7)

STATISTICS

The data were analyzed by paired t-tests. A probability of less than 0.05 was used to judge significant differences between treatment groups.

RESULTS

Two groups of eight rats each were entrained on a 14 hour on, 10 hour off light-dark cycle, and the effects of circadian rhythm on mitochondrial function were assessed. The respiratory control ratios (RCRs), a measure of mitochondrial integrity, were not significantly different between Group A (no light exposure) and Group B (10 hours light exposure): RCR was 5.90 for Group A and, 6.23 for Group B, $P > 0.05$ by Student's t-tests for paired data. Likewise the State 3 (activated) rates of mitochondrial oxidation were not significantly different: State 3 rates for Group A were 44.4 + 10.2 nanomoles per minute per ml protein (+ SD), while that for Group B was 48.2 + 12.3 nanomoles/min/ml of protein.

DISCUSSION

Although periodicities in the sleep-waking or fasting - eating cycles may synchronize the pituitary - adrenal systems circadian rhythm and thus influence various metabolically active hormones, these affects do not appear to influence the biochemical reactions studied in this series of experiments. This allows current studies to be designed so that animals may be studied at various points during the day. This allows the accumulation of more numerous data points over a shorter period of time than would be possible if only one point could be obtained at a specific time of day. These investigations are now being expanded to look at a wider variety of Kreb's-cycle substrates and to assay possible circadian affects on mitochondrial cytochrome concentrations and activity and on ion transport.

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7. Gornall AG, Bardawill CJ, David MM: Determination of Serum Proteins by Means of the Biuret Reaction. J Biol Chem 177: 751 - 766, 1949.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0G 6981	79 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA: CONTRACTOR ACCESS	9. LEVEL OF SUM
79 10 01	H. TERM	U	U	NA	NL	[X] YES [ ] NO	A. WORK UNIT

10. NO. CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	076
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Procede with Security Classification Code): (U) Distribution and Control of Peripheral Blood Flow Following Extensive Leg Surface Injury in Burned Soldiers (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
79 03	Cont	DA	C. In-House

17. CONTRACT/GRANT: Not Applicable
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER:
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING: 80	0	0
FISCAL YEAR CURRENT:		

19. RESPONSIBLE DOD ORGANIZATION
NAME: US Army Institute of Surgical Research
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20. PERFORMING ORGANIZATION
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TELEPHONE: 512-221-5712
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME:
NAME: DA

21. GENERAL USE: FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code): (U) Vasomotor Control; (U) Granulation tissue; (U) Arteriovenous shunts; (U) Nutrient Blood Flow

23. TECHNICAL OBJECTIVE. 24. APPROACH. 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) To establish vascular dimensions in large surface granulating wound and determine extent of functional innervation of the neovasculature.

24. (U) A large animal model has recently been established in order to study, in greater detail, the anatomical and physiological basis for wound directed peripheral blood flow. The wound is created in one hindlimb of a 20-40 kg goat by surgically removing the skin from that leg from groin to hock. Over the next week the wound develops into a rich bed of granulation tissue and blood flow to that limb increased to twice that of the contralateral, uninjured extremity. Peripheral glucose uptake and lactate production increased in the injured leg, comparable to changes previously observed in burn patients, suggesting similar local chemical environments are present in both granulating wounds. The current study is designed to partition these total leg blood flow measurements into wound, skin, and muscle compartments through the entrapment of radiolabelled microspheres. Through the use of different size spheres, one can also describe the vascular dimensions in the wound (capillaries vs A-V shunts). Once peripheral flow distribution and vascular dimensions are known, various features of vasomotor control will be evaluated. Of primary concern is degree of neurogenic control but this model is also ideally constructed for additional studies in local chemical influences on wound perfusion.

25. (U) 7910 - 8009. This project, as originally described in paragraph 24, was designed to extend work in progress on a large animal model. The primary goal was to understand better the degree of neurogenic vasomotor control of granulation tissue. Subsequent studies in the model answered many more questions than originally anticipated. This data has recently been published (Aulick, L.H., et al Ann Surg 191:249, 1980). Further work with radiolabelled microspheres is now considered of limited value and, therefore, this project is terminated.

*Available to contractors upon originator's approval

DD FORM 1 MAR 66 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65 AND 1498-1, 1 MAR 66 (FOR ARMY USE) ARE OBSOLETE

FINAL REPORT

PROJECT NO. 3A161101A91C-00, IN-HOUSE INDEPENDENT RESEARCH

REPORT TITLE: DISTRIBUTION AND CONTROL OF PERIPHERAL BLOOD FLOW FOLLOWING EXTENSIVE LEG SURFACE INJURY IN BURNED SOLDIERS

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

1 October 1979 - 30 September 1980

Investigators:

L. Howard Aulick, Ph.D., LTC, MSC
Douglas W. Wilmore, M.D.

Reports Control Symbol MEDDH-288(R1)

Unclassified

ABSTRACT

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: DISTRIBUTION AND CONTROL OF PERIPHERAL BLOOD FLOW FOLLOWING EXTENSIVE LEG SURFACE INJURY IN BURNED SOLDIERS

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

Period covered in this report: 1 October 1979 - 30 September 1980

Investigators: L. Howard Aulick, Ph.D., LTC, MSC
Douglas W. Wilmore, M.D.

Reports Control Symbol MEDDH-288(R1)

In an effort to evaluate reflex vasoconstrictor control of the burn wound, two water filled, venous occlusion plethysmographs were constructed and forearm blood flow (FBF) was measured bilaterally in patients with unilateral forearm burns. Reflex vasoconstriction was achieved by a combination of environmental cooling and ice consumption. This cold stress produced a symmetrical 60% decrease in FBF of normal control subjects. Two patients have been studied to date. For technical reasons, blood flow measurements were only performed on well-healed wounds. In the first patient, FBF fell from 4.75 to 2.46 ml/min·100ml forearm volume in the uninjured limb and from 10.18 to 8.03 in the contralateral injured limb. In the other patient, FBF decreased from 3.59 to 2.54 in the control limb versus 6.24 to 5.26 in the injured limb. These results indicate that reflex vasoconstriction occurred in both fully-healed forearm burns. Whether vasomotor nerve regeneration and vascular reinnervation were complete in these wounds remains doubtful, however, since the relative drop in FBF was always less in the wound. Freund, et al,[1] utilizing strain gauge plethysmography on a small portion of the forearm have recently reported that one, fully healed, second degree burn demonstrated reflex vasoconstriction while

1. Freund PE, Brengelmann GL, Rowell LB, Engrav L, Heimbach D: Cutaneous vascular responses in healed grafted burns. Fed Proceed 39:268, 1980.

Reflex vasomotor control
Wound blood flow
Plethysmography

another well-healed, full thickness injury did not. Since our studies measured blood flow in limbs containing mixed second and third degree burns, some of the relative difference in vasoconstriction between burned and unburned arms may reflect the vasomotor deficit of the full thickness component. Other work in animals has shown that α-adrenergic receptors are absent in granulation tissue and reflex vasoconstriction of the highly vascular, open wound is markedly depressed (2). Taken together, these animal and clinical studies suggest that some of the elevation in blood flow to the burn wound may be explained by a reduction in neurogenic vasoconstrictor tone.

This study has been completed, and no further work is planned.

2. Aulick LH, Baze WB, McLeod CG, Wilmore DW: Control of blood flow in a large surface wound. Ann Surg 191: 249-258, 1980.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OG 5028	80 08 19	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	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
80-08-01	D. CHANGE	U	U	NA	NL	[X] YES [ ] NO	A. WORK UNIT

10. NO./CODES.	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	087
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Procode with Security Classification Code)
(U) Role of Lipid Metabolism in Burn Injury (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS
003500 Clinical Medicine and 012900 Physiology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
80 02	Cont	DA	C. In-House

17. CONTRACT GRANT: Not Applicable
a. DATES/EFFECTIVE: EXPIRATION
b. NUMBER:
c. TYPE: d. AMOUNT
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR — PRECEDING	80	.3	9
FISCAL YEAR — CURRENT	81	.8	35

19. RESPONSIBLE DOD ORGANIZATION
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NAME: Arthur D. Mason, Jr., M.D. DA

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Procode EACH with Security Classification Code) (U) Lipid Metabolism; (U) Fatty Acid Oxidation; (U) Mass Spectroscopy; (U) Burn Injury; (U) Mitochondrial; (U) Gluconeogenesis

23. TECHNICAL OBJECTIVE. 24. APPROACH. 25. PROGRESS (Furnish individual paragraphs identified by number. Procode text of each with Security Classification Code.)

23. (U) To evaluate the changes in lipid metabolism following thermal injury and to assess the effectiveness of conventional nutritional support in the presence of these alterations.

24. (U) Using gas chromatography - mass spectrometry, the major metabolic pathways of lipid metabolism will be delineated by tracer dilution of stable non-radioactive labeled triacylglycerol intermediates.

25. (U) 8002 - 8009. Experiments are in progress to develop an isolated adipocyte preparation. This system will be used to study the effects of burn injury on fat metabolism at the cellular level. The initial studies are designed to determine the responsiveness of adipocytes from normal and burned organisms to various lipolytic hormones. For this purpose a sensitive glycerol assay has been employed and plans are being developed for assaying and identifying the various lipid components consumed and produced by the cell. This latter procedure, as well as those designed for metabolic tracer experiments using stable isotopes, await the delivery of instrumentation and supplies.

*Available to contractors upon originator's approval

ANNUAL PROGRESS REPORT

PROJECT NO. 3A161101A91C-00, INHOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: THE ROLE OF LIPID METABOLISM IN BURN INJURY: I. THE EFFECT OF EPINEPHRINE ON ADIPOCYTE FUNCTION

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

1 October 1979 - 30 September 1980

Investigators:

David R. Strome, Ph.D., Captain, MSC
Cleon W. Goodwin, Jr., M.D.
Arthur D. Mason, Jr., M.D.

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A161101A91C-00, INHOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: THE ROLE OF LIPID METABOLISM IN BURN INJURY: I. THE EFFECT OF EPINEPHRINE ON *ADIPOCYTE FUNCTION*

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

Period covered in this report: 1 October 1979 - 30 September 1980

Investigators: David R. Strome, Ph.D., Captain, MSC
Cleon W. Goodwin, Jr., M.D.
Arthur D. Mason, Jr., M.D.

Reports Control Symbol MEDDH-288(R1)

The role of epinephrine in producing the increased lipid metabolism observed in hypermetabolic burn patients was investigated in this preliminary report. Adipocytes were isolated from the epididymal fat pads of both unburned rats and rats which had been subjected to 60% body surface area burn by scalding. These isolations were made over the course of the first 20 postburn days. The isolated cells were incubated for 60 minutes in the presence and absence of epinephrine (10^{-5}M), and glycerol production was measured. Since glycerol is not reutilized by adipose tissue, it provides quantitative evidence of triglyceride breakdown. The following results were observed: (1) in the absence of epinephrine, glycerol production was similar in both groups of rats, and (2) the response of glycerol production to epinephrine stimulation was smaller in the burned animals than in the unburned controls. It would appear, therefore, that even though glycerol production is higher in adipocytes from the burned animal in which epinephrine is present than in adipocytes from unburned animals where epinephrine is absent, it is not due to an increased responsiveness of the tissue to the hormone.

Adipocytes
Glycerol
Epinephrine

THE ROLE OF LIPID METABOLISM IN BURN INJURY:
I. THE EFFECT OF EPINEPHRINE ON ADIPOCYTE FUNCTION

Hypermetabolism in the thermally injured patient is characterized in part by the increased metabolism of body fat (1,2,3). This state is reflected in elevated serum fatty acids (3) and triglycerides (primarily very low density lipoproteins) (4), increased clearance rate of intravenous fat emulsions from plasma (5), increased glycerol turnover (6), depletion of body fat stores (2), and frequently, in this and other severe trauma, essential fatty acid deficiencies (7,8,9,10). The mechanisms governing this alteration in energy flow have not been clarified.

1. Wilmore DW, et al: Influence of the burn wound on local and systemic responses to injury. Ann Surg 186:444-458, 1977.

2. Milstein W, and Coalson E: Depot fat depletion following thermal trauma. Am J Physiol 193:75-78, 1958.

3. Birke A, Carlson A, and Liljedahl SO: Lipid metabolism and trauma. III. Plasma lipids and lipoproteins in burns. Acta Med Scand 178:337-350, 1965

4. Coombes EJ, at al: Lipoprotein changes after burn injury in man. J Trauma 20:971-975, 1980.

5. Wilmore DW, et al: Clinical evaluation of a 10% intravenous fat emulsion for parenteral nutrition in thermally injured patients. Ann Surg 178:503-513, 1973.

6. Carpentier YA, et al: Effects of hypercaloric glucose infusion on lipid metabolism in injury and sepsis. J Trauma 19:649-654, 1979.

7. Elwyn DH: Nutritional requirements of adult surgical patients. Critical Care Med 8:9-20, 1980.

8. O'Neill JA, Jr, Caldwell MD, and Meng HC: Essential fatty acid deficiency in surgical patients. Ann Surg 185:535-542, 1977.

9. Reiss E, Pearson E, and Artz CP: The metabolic response to burns. J Clin Invest 35:62-77, 1956.

10. Helcamp GM, Jr, et al: Essential fatty acid deficiency in red cells after thermal injury. Correction with intravenous fat therapy. Am J Clin Nutr 26:1331, 1973.

This increase in lipid metabolism in the burned individual is accompanied by an increase in circulating epinephrine (11). Since epinephrine is known to increase triglyceride breakdown and glycerol production in adipose tissue (12,13,14), it could follow that the observed changes in lipid metabolism are normal responses to elevated epinephrine concentrations in plasma. Therefore, it is of primary importance to elucidate the responses of adipose tissue to this hormone in burned and normal individuals. This can be accomplished by measuring the production of glycerol due to triglyceride breakdown in tissue from burned and unburned animals in the presence and absence of epinephrine.

MATERIALS AND METHODS

In these preliminary experiments, male rats were randomly divided into two groups. One group was anesthetized, shaved and subjected to a 60% body surface area burn by scalding. The remaining group was treated in the same manner except they were not injured. Upon recovery, all animals were given free access to food and water.

During the first 20 days postburn, animals from each group were selected at random for study. Following decapitation, the epididymal fat pads were removed and placed in warm Krebs-Ringer bicarbonate buffer (KRB). Adipocytes were isolated from these tissue samples by digestion with collagenase (Worthington), washed, and suspended in KRB containing 4 mg/ml albumin Fraction V (Sigma Chemical Company). Buffer solutions were equilibrated with 5% CO_2: 95% O_2 at all times during the experiment.

The following experimental protocol was used for adipocytes isolated from both burned and unburned animals. Duplicate 5 ml aliquots of the cell suspensions were incubated for 60 minutes at 37°C with gentle shaking. Epinephrine was present in one pair of samples at a final concentration of 10^{-5}M. The second pair contained no hormone and served as controls. At the conclusion of the incubation period, the samples were added to 0.5 ml cold tricholoracetic acid (TCA; 50% W/V) and filtered. A third pair of samples was added to TCA

11. Wilmore DW, et al: Catecholamines: Mediator of the hypermetabolic response to thermal injury. Ann Surg 180:653-669, 1974.
12. Gilbert CH and Galton DJ: The effect of catecholamines and fasting on C-AMP and release of glycerol from human adipose tissue. Horm Metabol Res 6:229-233, 1974.
13. Carlson LA, Liljedahl SO, and Wirsen C: Blood and tissue changes in the dog during and after excessive free fatty acid mobilization. Acta Med Scand 178:81-101, 1965.
14. Steinberg D: Catecholamine stimulation of fat mobilization and its metabolic consequences. Pharmacol Rev 18:217-235, 1966.

immediately upon dispensing to provide pre-incubation values. The filtrates were analyzed for glycerol content by enzymatic spectophotometric assay after TCA extraction with diethyl ether. The difference between glycerol content at 60 minutes and at time zero equalled the glycerol production in $nmoles X hr^{-1} X ml^{-1}$. These values were normalized per 10^6 cells by counting under a microscope 5 µl aliquots of suspension which had been fixed in osmium tetroxide.

RESULTS

Tables 1 and 2 present the glycerol production as a function of postburn day for these initial experiments. Values in Table 1 are from young, growing rats initially in the 160 to 180 gm weight range. Each day is the result of two unburned and three burned rats. In Table 2, the data is from older rats in the 520 to 540 gm range whose weight was more stable. Each day represents one rat from each group.

TABLE 1. Glycerol Production ($nmoles X 10^6 cells^{-1} X hr^{-1}$) With Postburn Day for the Group of Younger Rats.

	Day 6	Day 12	Day 19
Unburned-Control	-83	-372	-250
Unburned-Epinephrine	1708	2863	2497
ΔGlycerol Production	1791	3235	2747
Burned-Control	98	-357	112
Burned-Epinephrine	484	325	1665
ΔGlycerol Production	385	652	1553

Δ Glycerol production is the difference between production in the presence and absence of epinephrine stimulation and is given for both the unburned and burned groups. Each day represents pooled tissue from two unburned and three burned rats.

TABLE 2. Glycerol Production (nmoles X 10^6cells^{-1} X hr^{-1}) With Postburn Day for the Group of Older Rats.

	Day 1	Day 3	Day 7	Day 10	Day 15	Day 17
Unburned-Control	436	-263	620	320	236	74
Unburned-Epinephrine	3064	455	2265	1537	929	655
ΔGlycerol Production	2628	718	1645	1217	693	581
Burned-Control	297	-122	217	-222	-89	338
Burned-Epinephrine	1550	714	575	164	989	705
ΔGlycerol Production	1253	836	358	386	1078	367

ΔGlycerol production is the difference between production in the presence and absence of epinephrine stimulation and is given for both the unburned and burned group. Each day represents one animal from each group.

DISCUSSION

Since these were preliminary experiments, a few technical difficulties were unexpectedly encountered which are correctable but which must be recognized when interpreting the data. It is widely believed that adipose tissue cannot utilize glycerol for triglyceride synthesis (15). Therefore, glycerol production is a representative parameter for triglyceride breakdown, and negative glycerol production (equivalent to glycerol uptake) should not occur. The negative glycerol

15. Vaughan M and Steinberg D: Glyceride biosynthesis, glyceride breakdown and glycogen breakdown in adipose tissue: Mechanisms and regulation. In: Renold and Cahill Handbook of Physiology, Section 5. Washington, D.C.: American Physiological Society, pp 239-252, 1965.

production values observed in these tests evidently arose from the assay procedure and reflect the inherent error in diluting and reading the samples. Also, since few animals were involved in this preliminary study, the data reflect some scatter due to interanimal variation. Neither of these facts, however, can account for the overall results, which provide some unexpected and interesting insight into the effect of thermal injury on adipose tissue function. Both experimental series suggest that basal glycerol production in the absence of epinephrine is similar in adipocytes from both burned and unburned animals. However, the response of adipocytes to pharmacological doses of epinephrine, in terms of glycerol production, is in general lower in the burned animal than the unburned animal. This depressed hormonal response in the burned animal is unexpected, since other published data suggest indirectly that the response should be increased in the burned animal (16). This unanticipated but apparent discrepancy makes further experiments essential.

It is interesting to note that glycerol production in adipocytes from the burned animal in the presence of epinephrine is higher than that seen in adipocytes from unburned animals in the absence of epinephrine. This combination of conditions is the most physiological and is in accord with what would be expected from the standard finding of increased fat metabolism in the burned individual. Furthermore, credence is given to the data in that values for glycerol production in the unburned animal in the presence of epinephrine agree with other published values (17,18,19).

Experiments are in progress to expand this series in order to clarify and confirm the findings.

PUBLICATIONS/PRESENTATIONS: None.

16. Aprille JR, et al: Adenylate cyclase after burn injury: Resistance to desensitization by catecholamines. J Trauma 19:812-818, 1979.

17. Burns TW, et al: Pharmacological characterizations of adrenergic receptors in human adipocytes. J Clin Invest. In Press.

18. Yu BP, Bertrand HA, and Masoro EJ: Nutrition-aging influence of catecholamine-promotid lipolysis. Metabolism 29:438-444, 1980.

19. Bertrand HA, Masoro EJ, and Yu BP: Maintenance of glucagon-promotid lipolysis in adipocytes by food restriction. Endocrinol 107:591-595, 1980.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0G 1841	80 10 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. OBS'N INSTR'N	8b. SPECIFIC DATA CONTRACTOR ACCESS	9. LEVEL OF SUM
79 10 01	D. CHANGE	U	U	NA	NL	☐ YES ☐ NO	A. WORK UNIT

10. NO. CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	080
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code) (U) Assessment of Thyroid Hormone Kinetics in Thermally Injured Patients (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS
003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
79 08	Cont	DA	C. In-House

17. CONTRACT GRANT: Not Applicable
a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER:
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 80	.8	30
	CURRENT 81	.6	27

19. RESPONSIBLE DOD ORGANIZATION
NAME: US Army Institute of Surgical Research
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ASSOCIATE INVESTIGATORS
NAME:
NAME: DA

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Thyroxine; (U) 1-triiodothyronine; (U) 1-reverse-T_3; (U) Kinetics; (U) Burn Patients

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) To assess metabolic clearance rate and production rate of Thyroxine, 1-T_3, and 1-rT_3 in burn patients.

24. (U) 1-T_3 labeled with ^{125}I and 1-T_4 labeled with ^{131}I have been injected intravenously into 6 burn patients and their disappearance from plasma monitored. A single compartmental model was used for analyzing data.

25. (U) 7910 - 8009. Thyroid hormone kinetics were assessed in six patients between the ages of 18 and 45 with burns covering more than 50% of their body surface. Following injection of isotopically labeled T_3 and T_4, the disappearance from the serum of the labeled hormone was followed over the next six days. In burn patients both the clearance rates and production rates for T_4 are significantly greater than those of control subjects or euthyroid sick patients. The half-life of T_4 is much shorter in burn patients than in either of the other two groups. These data describe a high flow state for T_4 which has not been previously observed in any other non-thyroidal critical illness. The half-life of T_3 was significantly shorter in burn patients than in control subjects or euthyroid sick patients. A profound block of T_4 to T_3 conversion is apparent in burn patients, especially in comparison to the euthyroid sick patients. These data are supportive of our earlier observation of a profound T_3 depletion state in critically ill burn patients.

*Available to contractors upon originator's approval

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PROGRESS REPORT

PROJECT NO. 3A161101A91C, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: ALTERED PERIPHERAL THYROID HORMONE KINETICS IN BURN PATIENTS: A HIGH THYROXINE FLOW STATE

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

1 August 1979 - 30 September 1980

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ABSTRACT

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We have recently reported profound depression of both total and free serum concentrations of thyroxine (T_4) and triiodothyronine (T_3) in hypermetabolic burn patients. Kaptein, et al reported an increased metabolic clearance rate for T_4 with a normal production rate for T_4 and a decreased production rate for T_3 to explain a similar suppression of T_4 and T_3 in other patients critically ill with non-thyroidal diseases. We now report the results of pulse tracer studies with labeled T_4 and T_3 in six critically ill burn patients, mean burn size 51%, and two non-burned controls:

	T_4 (μg/dl)		T_3 (ng/dl)	
	Controls	Pts (M±SE)	Controls	Pts
Serum Conc	8.2±1.1	4.3±.8	153±17	50±7
$T_{½}$(d)	4.8±.7	2.2±.5	.91±.1	.52±.06
Ki(d^{-1})	.1496	.3826	.7675	1.3987
MCR(l/d)	1.2±.1	3.8±.8	32.3±4	52.6±11.1
V_D(l)	8.4±1.8	12.5±3.1	42.1±.1	54.3±7.1
PR(μg/d)	98.5±19	144.6±30.5	50.1±11.6	26.7±7.9

The PRT_3/PRT_4 ratio was .51±.01 in controls and .24±.1 in burn patients. Burn patients exhibit significant increases in the clearance rates of both T_4 and T_3, with increased production of T_4 but not T_3 when compared to controls. These data describe a high flow state for T_4 and a profound block of T_4 to T_3 conversion in hypermetabolic burn patients.

Thyroxine
Kinetics
Triiodothyronine
Burn patients

PUBLICATIONS

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