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ANNUAL RESEARCH PROGRESS REPORT, 1 JULY 1973-30 JUNE 1974

Brooke Army Medical Center
Fort Sam Houston, Texas

30 June 1974

U. S. Army
Institute of Surgical Research
Brooke Army Medical Center
Fort Sam Houston, Texas

Research Report

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ANNUAL RESEARCH PROGRESS REPORT

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3A162110A821-00, COMBAT SURGERY

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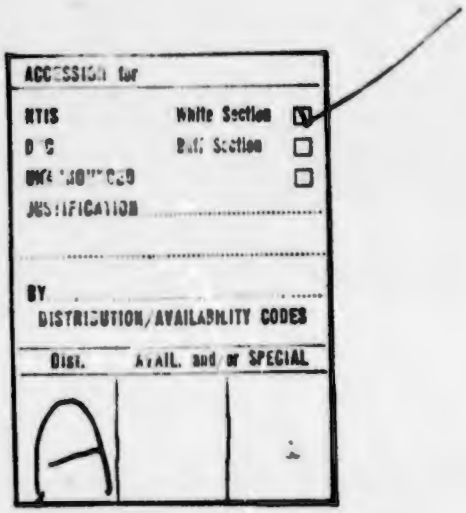

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This report documents the clinical and laboratory activities of the US Army Institute of Surgical Research during fiscal year 1974. These activities include patient care, clinical investigation and laboratory research in the areas of (1) burn injury, (2) acute renal failure, and (3) general trauma. Special emphasis is placed on the clinical management of burned patients and on studies related to prevention and treatment of burn 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

ANNUAL RESEARCH PROGRESS REPORT

30 June 1974

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

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SUBJECT: Annual Research Report FY 1974

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Basil A. Pruitt Jr.

BASIL A. PRUITT, JR, MD
Colonel, MC
Commander & Director

FOREWORD

The cost of the scientific product of this Institute, detailed in this report, is minuscule compared to the entire research budget requested for the Department of Defense for FY 75[1] (only .015% of 9.3 billion dollars). In addition to increasing scientific knowledge, this expenditure results in the saving of lives and diminution of morbidity which defy valuation except in ethical and humanitarian terms. As recently pointed out in an editorial in "Science",[2] biomedical research is ultimately practical, a sound investment and cost effective in a benefit to cost ratio of 20 to 1, a strong argument to advance to arithmetically oriented budgeteers.

The present and prior clinical reports have not only directly improved clinical care, but have established the effectiveness and economy of the "Burn Center" leading to establishment of similar burn care units elsewhere and a general improvement in care of the severely injured. Similarly, our research (particularly the metabolic and gastroenterologic studies this year) has attracted the interests of both the national and international medical communities and attests to the capability of the US Army Medical Research and Development Command and this Institute to produce a scientific product of merit with applicability to all injured patients. Additionally, these studies emphasize again the importance and "multiplier" effect of clinical-laboratory symbiosis in terms of scientific relevance and productivity.

The accomplishments of our Institute in both the clinical and research spheres reported herein testify to the enthusiasm, industry and competence of each of our staff members. It is dedication and excellence of individuals such as these which must be nurtured and recognized to insure the success and viability of medical research within the Army. Conversely, shortsighted quibbling over attempts to make our staff salaries approach those in even the academic community demolishes esprit, makes recruitment impossible, and represents fiscal irresponsibility in terms of the economic facts noted above.

References

1. Editorial, "Clinical and biomedical research is good for the economy". Science 184: 44, 1974.

2. Wade, N., Curry, M.R.: World's largest R&D manager. Science 185: 41-42, 1974.

Basil A. Pruitt, Jr.

BASIL A. PRUITT, JR., M.D.
Colonel, MC
Commander & Director

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c. CONTRIBUTING				

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NAME: US Army Institute of Surgical Research

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RESPONSIBLE INDIVIDUAL

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME:

NAME:

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22. KEYWORDS (Precede EACH with Security Classification Code) (U) Thermal injury; (U) Topical therapy; (U) Autograft; (U) Homograft; (U) Heterograft; (U) Resuscitation; (U) Air evacuation; (U) Mortality

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

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

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

25. (U) 73 01 - 73 12 During 1973, 261 patients were admitted to the Institute. Attention on early diagnosis and treatment of inhalation injury, fiberoptic bronchoscopy as an aid to diagnosis of inhalation injuries, re-evaluation of early fluid resuscitation formulae, and the incidence of acute stress ulceration with the fiberoptic gastroscope are clinical approaches to treatment, currently being assessed. As in the previous year, pulmonary infection with gram-negative bacteria continues to be a frequently observed complication of thermal injury, and intensive investigation of methods to prevent and more adequately treat this complication continues. Principles of management previously developed at this institute remain unchanged. Several new clinical approaches to the treatment of severe thermal injury and its complications have been evaluated and adopted.

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ANNUAL PROGRESS REPORT

PROJECT NO. 3A61102B71R-01, RESEARCH IN BIOMEDICAL SCIENCES

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 - 31 December 1973

Investigators:

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Daryl R. Erickson, MD, Major, MC
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ABSTRACT

PROJECT NO. 3A061102B71R-01, RESEARCH IN BIOMEDICAL SCIENCES

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 1973

Investigators: John L. Hunt, MD, Lieutenant Colonel, MC
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Two hundred and sixty-one patients with thermal injury were admitted to the Clinical Division of the United States Army Institute of Surgical Research during the calendar year 1973. The Institute of Surgical Research's main emphasis has continued on providing optimal clinical care to military personnel and civilians with major thermal injury. In addition, clinical investigation has continued into the physiological, biochemical and bacteriological aspects of thermal injury. The personnel of this unit have also participated in many educational programs both military and civilian. This report summarizes the activity of the Clinical Division of the United States Army Institute of Surgical Research in 1973 and cites the recognizable complications which have contributed to mortality and morbidity in burn patients. Clinical evaluations have been carried out in post burn pulmonary changes, the

metabolic response of the burn patient to his injury, evaluation of intravenous fat emulsion for parenteral nutrition, the study of biological dressings, electrolyte changes in the post burn period, high voltage electric injuries, digital escharotomies, post burn protein metabolism, Laser excision of burns, and Vitamin K activity in thermally injured patients, and gastroesophageal endoscopy. In 1973 196 patients were air evacuated by the ISR team or 74% of all admissions.

Thermal injury
Topical therapy
Autograft
Homograft
Heterograft
Resuscitation
Air evacuation
Mortality

CLINICAL OPERATION, CENTER FOR TREATMENT OF BURNED SOLDIERS

The Clinical Division of the United States Army Institute of Surgical Research continued through the year 1973 to have as its primary objective the provision of clinical care for thermally injured soldiers. The number of admissions declined from 300 patients to 261.

In 1973 the ISR flight team did not make any flights to the US Army Medical Center, Okinawa for burn patients. There was a total of 147 flights during 1973, 143 of these were CONUS flights (within the Continental United States) and 192 patients were evacuated. All patients within a radius of 200 miles of Brooke Army Medical Center requiring air evacuation were transported by helicopter. There were 32 flights for that purpose. There were 4 flights outside of the Continental United States, 3 to Alaska and 1 to Guantanamo Bay, Cuba.

CLINICAL MANAGEMENT

Detailed descriptions of the management of patients with thermal injury as practiced by this institute are found in previous Annual Reports and in numerous scientific publications. Therefore the following remarks are limited to new and current methods of clinical therapy.

Pulmonary complications continued to be the leading cause of morbidity and mortality in thermally injured patients at this institute. Consequently emphasis has been directed toward assessment of pulmonary function in the early post burn period. Post burn hyperventilation unassociated with pulmonary parenchymal disease or other injuries, is generally not associated with hypoxemia. Tachypnea and hyperpnea persist and usually peak early in the second post burn week, following which ventilation gradually returns to ordinary post burn levels (a minute ventilation 2-3 times that of normals).

The ^{133}Xenon lung scan has continued to be a useful diagnostic modality for acute inhalation injury. Although a small number of false positive and false negative scans have been identified, these patients can usually be separated out on clinical grounds. Recently a study of the morphologic changes in the tracheobronchial tree resulting from inhalation injury has been evaluated by fiberoptic bronchoscopy. Large airway chemical tracheobronchitis has been identified as a variant of inhalation injury in the absence of parenchymal change identifiable on ^{133}Xenon scintiphotograms. With fiberoptic bronchoscopy inhalation injury can be divided anatomically into supraglottic and infraglottic lesions. An investigation is being conducted to assess the efficacy of systemic steroids in the treatment of inhalation injury. Routine chest roentgenography, fiberoptic bronchoscopy, ^{133}Xenon lung scan and pulmonary function studies will be used to evaluate the effect of the drug on inhalation injury.

Intravenous fat emulsion for parenteral nutrition has been further evaluated. Intralipid, a 10% soy bean oil emulsion, has been found to cause no disturbance of coagulation indices and no disturbance of total pulmonary diffusion capacity when given in doses of up to 3 grams per kilogram per day. The fat is cleared from the blood in hypermetabolic burn patients faster than in normal man. The emulsion is given through peripheral veins and provides more calories per unit volume than other nutrient solutions.

Human growth hormone was administered to seven patients with burns between 34 and 76% of the total body surface to evaluate its effect on post injury nutrition. Increased nitrogen retention occurred in all but one patient. Mean loss of urinary nitrogen significantly decreased as compared to a control period. The protein sparing effect and other beneficial secondary gains of human growth hormone which enhance recovery of injured patients appear to be dose related, to require adequate nutritional loading, and to be mediated by alterations of carbohydrate metabolism in the presence of augmented insulin production.

Two major fluid and electrolyte disturbances which have been reviewed over the last year in thermally injured patients included hypernatremia and hyponatremia. Hypernatremia indicative of a water deficit was found to be the most common electrolyte disturbance following resuscitation of extensively burned patients. The most common cause of hypernatremia was inadequate replacement of evaporative water loss. Conversely hyponatremia has been identified as the most common electrolyte abnormality in burned children who have seizures and was caused by either excessive administration or too rapid administration of electiolyte free fluids, i.e. 5% dextrose in water. This finding calls for caution in the administration of non-electrolyte fluids in small children.

Arteriography has been evaluated as a clinical tool in the diagnosis of deep muscular damage in electric injuries. Arteriography is of value in confirming arterial patency in limbs without palpable pulses and in determining the level of amputation in a limb with an obviously nonviable portion.

Digital escharotomy was evaluated in patients with burned hands and found to result in a significant salvage of intrinsic muscles in hands with third degree burns. Digital escharotomy is of no value in fingers which are "mummified" by the thermal injury.

Interest has again been re-kindled in the primary excision of burns. A comparison of the laser technique with that of standard electro-surgical excision was carried out over the last year. The carbondioxide laser was employed for excision of six burn patients and the speed of excision and blood loss were comparable to that associated with excision of

comparable areas of burn using the standard electrocautery. A readily available source of fresh cutaneous allografts is paramount if one is to attempt excision of large burns.

Gastroesophageal endoscopy with a fiberoptic endoscope has been performed in patients with thermal injury of greater than 25% total body surface. Endoscopic studies have shown early gastric and duodenal mucosal changes compatible with ischemia followed by hyperemia and this is virtually universal in patients with burns of 40% or more of the total body surface. Duodenal changes are compatible with a "duodenitis" and confirm the mutual susceptibility of both the stomach and duodenum to post burn injury and the similarity of the etiology and pathogenesis of gastric and duodenal ulcers in the burn patient. The early occurrence of these lesions (before 72 hours post burn) suggests that an alteration in the mucosal blood flow is the underlying pathogenic factor. Measurement of lithium flux has been used to evaluate the gastric mucosal barrier in 18 patients with burns of greater than 25% of the total body surface. These studies were correlated with endoscopic examination performed within the first 72 hours post burn. The findings suggest that an increased back diffusion of hydrogen ion is not an etiologic factor in the development of early gastric lesions following thermal injury, but that hydrogen ion back diffusion reflects progression of this disease.

EDUCATION

The Institute of Surgical Research has also been extensively involved in the education of both military and civilian personnel. This responsibility has again been fulfilled through numerous scientific presentations and publications, in-house training and education of house officers, staff physicians, foreign physicians and paramedical personnel.

During the period encompassed by this report, 1 surgical resident from Brooke General Hospital, 3 surgical residents from Fitzsimons, 1 from Travis Air Force Base and 1 from Oakland Naval Hospital participated as active members of the medical staff for 1-3 month periods as part of their surgical training program. In addition, 1 civilian physician from New York and 1 from Zurich, Switzerland received training for an extended period. Eleven reserve officers were given tours and briefings, and nine officers were on active duty training with our staff for periods of 2 to 6 weeks. Seven Army physicians, 1 Air Force physician, 17 civilian physicians were visitors of this unit during 1973. Thirty-two foreign visitors from the following countries: Austria, France, South Africa, Switzerland, Pakistan, Norway, Canada, Germany, Italy, England, Japan, Brazil and Lebanon received briefings on the care of the thermally injured patient and on the overall mission of the Institute of Surgical Research. Approximately 147 civilian and military nurses, students and paramedical personnel visited and were briefed during 1973.

In addition, numerous scientific presentations concerning various aspects of thermal injury were made by members of the Clinical Division at local, state, regional and national meetings as listed at the end of this section.

STATISTICAL RESUME

During the year 1973, 261 thermally injured patients were admitted to the Institute of Surgical Research. Unlike previous years no patients were air evacuated by the ISR Burn Team from the Far East. There were 273 dispositions during 1973 and the subsequent data will be based on those dispositions. The patients ranged in age from 3 months to 85 years with 205 males and 68 females. The average age of the patient was 27 years with an average burn size of 38.5%, a 19.5% average third degree component. The average burn index was 29%. Out of 273 dispositions, 207 had third degree burns (75.8%). Sixty-five patients were less than 15 years of age with an average age of 5 years. The average total burn in this pediatric age group was 30.3% with 15% being third degree. The burn index in children was 22.7%. Of the 65 pediatric patients admitted, 45 had some third degree burn (69.2%). The mortality in the pediatric burn age group was 32.3%. In the group of pediatric burn patients who died, the average age was 4 years and the average burn was 52.4% with 33.3% being third degree. The overall mortality for the year 1973 was 41.4% or 113 patients out of 273 expired, of which 83 were male and 30 were female. The average age of patients who died was 33 years, and in this group the average total burn was 60.3% with 36.2% being third degree and the burn index was 48.2%. The increase in mortality compared to previous years is partly related to the fact that no patients from Southeast Asia have been air evacuated but the number of acute admissions has increased markedly, as has the average total per cent burn increased in the patients admitted to the Institute of Surgical Research. Of the 113 patients who expired 107 or 94.7% had some third degree burn. Autopsies were performed on 92 patients (81.4%) of all deaths. The average post burn day of death was 11.8 days, a drop from 16.4 days in 1972.

Table 1 identifies the source of admission of patients during the calendar year 1973. The majority of the burns were from the Continental United States. Table 2 summarizes the burn etiology in 1973. Table 3 summarizes the effect of age and total body surface burn on mortality.

Table 4 lists the mortality rates in increments of 10% total body surface burn from the years 1970 through 1973. Comparison with 1970 reveals an increase in the mortality for 30-50% total body surface burns. Table 5 presents the survival and mortality data for patients with greater than 30% total body surface burn in the years 1956-1973.

Table 6 shows a comparison of burn mortality rates in the pre-Sulfamylon years 1962-1963 and the cumulative experience since 1965

Table 1. Source of Admission, 1973

Area	A	AD	AF	AFD	N	ND	VAB	Other	TOTAL
1st Army	3	1	1	3	9	0	2	10	29
3rd Army	7	9	2	6	1	5	7	9	46
5th Army	23	22	8	9	4	3	26	56	151
6th Army	1	0	2	2	1	0	3	14	23
Germany	4	3	0	1	1	0	0	0	9
Guam	0	0	2	0	0	0	0	0	2
Viet Nam	0	0	0	0	1	0	0	0	1
Korea	2	1	0	0	0	0	0	0	3
Puerto Rico	0	0	0	0	0	0	0	1	1
Iceland	0	0	1	0	0	0	0	0	1
Spain	0	0	0	0	1	0	0	0	1
Cuba	0	0	0	0	2	0	0	1	3
Alaska	0	0	0	0	0	0	1	2	3
	40	36	16	21	20	8	39	93	273

A - Army
AF - Air Force
D - Dependent
N - Navy, Marine Corps & US Coast Guard
VAB - Veterans Administration Beneficiary
Other: Civilian Emergency (67)
Designee of Secretary of Army (14)
US Public Health Service Beneficiary (10)
Bureau of Employees' Compensation Beneficiary (2)

Table 2. Burn Etiology, 1973 - 273 Dispositions

Causes	Number of Patients	% Disposition	Deaths	% Mortality
Gasoline & Kerosene	80	29.3%	38	48%
Structural Fires	12	4.4%	9	75%
Motor Vehicle Accidents	27	9.9%	12	44%
Aircraft Accidents	5	1.8%	3	60%
Open Flames	28	10.3%	13	46%
Electrical	17	6.2%	4	24%
Hot Liquid	33	12.1%	8	24%
Chemical	4	1.5%	0	0%
Others	27	9.9%	5	10%
Butane, Propane or Natural Gas Exp.	31	11.4%	16	52%
Welding Accidents	9	3.3%	5	56%
TOTAL	273		113	

Table 3. Age, Body Surface Involvement & Mortality, 1973

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	1	2	1	0	1	0	1(1)	0	0	0	6	1	16.7
1-2	4	3	5(2)	0	1(1)	0	1(1)	0	0	0	16	4	25.0
2-3	0	1	1(1)	0	1(1)	2(2)	2(2)	1(1)	0	0	8	7	87.5
3-4	1	0	2(1)	1	1(1)	1(1)	0	0	0	0	6	3	50.0
4-5	1	2	1	1	0	0	0	0	0	0	5	0	0
5-10	1	1	3	0	2(1)	2(1)	1	0	1(1)	0	11	3	27.3
10-15	1	3	5(1)	1	1	1(1)	0	0	0	1(1)	13	3	23.0
15-20	4	3	6	6	4(1)	5(2)	4(3)	6(6)	1(1)	0	39	13	33.3
20-30	12	10	11(1)	5(2)	12(6)	7(2)	3(3)	5(5)	5(5)	2(2)	72	26	36.1
30-40	3	3	3(1)	0	3(3)	4(4)	3(2)	3(3)	0	1(1)	23	14	60.9
40-50	4	5	6	5(1)	4	4(3)	3(3)	1(1)	1(1)	1(1)	34	10	29.4
50-60	2	2	2	1	5(4)	2(2)	0	0	3(3)	3(3)	20	12	60.0
60-70	1	0	0	4(4)	1(1)	2(2)	1(1)	0	0	0	9	8	88.9
70-80	1	0	0	2(2)	2(2)	1(1)	0	0	0	1(1)	7	6	85.8
80-90	1	0	0	0	0	1(1)	1(1)	0	1(1)	0	4	3	75.0
Total	39	35	46	26	38	32	20	16	12	9	273		
Deaths	0	0	7	9	21	22	17	16	12	9		113	
% Mortality	0	0	15.2	34.6	55.3	68.8	85	100	100	100			41.4

Note: Deaths shown in parentheses.

Table 4. Per Cent Body Surface Involvement and Mortality, 1970 - 1973

% Burn	0-10	10-20	20-30	30-40	40-50	50-60	60-70	70-80	80-90	90-100	Total
					(1970)						
No. Burned	45	60	65	60	47	17	13	9	3	2	321
Deaths	0	2	10	9	13	10	13	8	3	2	70
% Mortality	0	3.3	15.4	15	27.7	58.8	100	88.9	100	100	21.8
					(1971)						
No. Burned	50	55	57	39	34	20	12	8	10	6	301
Deaths	0	0	2	7	14	12	11	7	9	6	68
% Mortality	0	0	3.5	17.9	41.2	60	91.7	87.5	90	100	22.6
					(1972)						
No. Burned	47	56	43	42	36	23	22	16	11	5	301
Deaths	1	2	7	13	15	13	21	15	11	5	103
% Mortality	2.1	3.6	16.3	31	41.7	56.5	95.4	93.8	100	100	34.2
					(1973)						
No. Burned	39	35	46	26	38	32	20	16	12	9	273
Deaths	0	0	7	9	21	22	17	16	12	9	113
% Mortality	0	0	15.2	34.6	55.3	68.8	85	100	100	100	41.4

Table 5. Per Cent Burn Versus Survival, 1955-1973

Year	Survivors (burns over 30%)			Deaths		
	No. Cases	Average % Burn		No. Cases	Average % Burn	
		Total	3°		Total	3°
1955	20	39.5	20.3	21	55.6	38.1
1956	22	41.0	17.3	20	57.8	37.8
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

Table 6. Comparison of Burn Mortality Rates, 1962-1963 and 1964-1973

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
1964-73	1525	41	2,7	431	70	16.2	359	108	30.0	219	106	48.4	371	317	85.4

when Sulfamylon has been used. As previously reported the improvement is primarily in that group of burn patients whose injury is in the 30-60% range with little if any change in those patients with less than 30% or greater than 60% injury.

The average hospital stay in 1973 was 35.6 days. When convalescent leave for active duty military personnel was excluded the average hospital stay was 32.5 days. The average post burn admission day to the Institute of Surgical Research was 5. This figure reflects a decrease in the average post burn day of admission from 11.2 days in 1970 to 9 days in 1971 to 7 days in 1972. The decrease in average admission day is because the patient population is originating in the Continental United States and also reflects the rapid aeromedical evacuation carried out by the Institute of Surgical Research.

During the year 1973 2,388 operations were performed on 246 patients, an average of 9 operations per patient. Three hundred and eighty-five anesthetics were performed on 141 patients or 1 anesthetic per patient. A total of 1,990 ward procedures (this mainly consisted of application of physiologic dressings, dressing changes, local venectomy procedures) were performed. Two hundred and forty-five patients were autografted or an average of less than 1 procedure per patient. Ninety patients had 409 allograft applications or an average of 1 per patient.

Porcine cutaneous xenograft was applied to 127 patients 910 times or an average of 3 times per patient. Cadaver allograft was aseptically harvested from 56 patients, a drop from 70 in 1972. The drop in procurement of allograft is reflected in the increased use of porcine cutaneous xenografts as physiologic dressings. Escharotomies were performed on 65 patients or 25% of all dispositions. Twenty-nine patients (10%) required an amputation, 20 of which were major.

Tracheostomy was performed on 49 or 18% of all dispositions. The specific indications for tracheostomy were the need for prolonged ventilatory support, upper airway obstruction, pneumonia and inhalation injury.

One hundred and sixty-nine patients or 62% of all dispositions had at least one blood culture drawn during their hospitalization and 99 patients had a positive culture. For information on further bacteriologic data the reader is referred to the succeeding chapters. Of 273 patients, 173 had intravenous catheters inserted either percutaneously or via cutdown sites. Suppurative thrombophlebitis occurred in 27 patients. In keeping with a high index of suspicion for suppurative thrombophlebitis, 47 or 17% of all cutdowns were explored. An important aspect of burn therapy is wound monitoring and this is done with frequent wound biopsies. Ninety-five patients or 35% of all dispositions in 1973 had at least 1 wound biopsy performed.

A total of 671,648 cc of blood was administered to 152 (56%) of all

dispositions in 1973. An average of 4,418 cc of blood were given to each of the 152 patients.

Topical Sulfamylon acetate was utilized to control the burn wound flora in 254 or 93% of all dispositions. Silver nitrate was used in 32 or 12%, and silver Sulfadiazine was used in 4 patients.

One hundred and seven patients (39%) had some type of associated injury on admission. Five per cent of the patients had a major fracture. Other significant orthopedic problems which developed during the hospital stay included exposed tendon or bone in 6 patients, heterotopic bone in the elbow in 1 patient and osteomyelitis in 4 patients. Acute ophthalmologic injuries associated with the burn population were quite frequent. Fifteen patients had some type of an eye injury. Corneal burns occurred in 15 patients, corneal abrasions in 2, loss of eyesight ultimately occurred in 1 patient and loss of eyelids from a chemical burn occurred in one individual. Another significant problem in the thermally injured patient is chondritis. Nine patients or 3% of all dispositions developed chondritis and chondrectomy was performed on 13 ears. Four other patients lost a portion of an ear due to direct thermal injury.

Gastrointestinal complications were quite prominent in our burn population. Gastrointestinal bleeding of some type occurred in 60 patients or 22% of all dispositions. Gastrointestinal ulcers were diagnosed either clinically, roentgenographically or by endoscopy in 58 patients or 21% of all dispositions. Duodenal or gastric ulcers were the cause of bleeding in 36 patients. Eight patients developed perforated ulcers and 7 patients had exploratory laparotomy for bleeding gastric or duodenal ulcers. Superior mesenteric artery syndrome occurred in two patients, neither of which required surgery. Other major gastrointestinal complications detected by fiberoptic gastroscopy included gastritis in 20 patients, duodenitis in 10 patients, duodenal necrosis in 4 patients, esophagitis in 21 patients and esophageal ulceration with associated hemorrhage in 6 patients. Acute cholecystitis was diagnosed at post mortem examination in 6 patients but in no patient was this the cause of death. Pancreatitis usually of the interstitial type and of mild degree was diagnosed in 40 patients or 15% of all dispositions. Major renal complications in our burn population included some degree of renal failure usually as a terminal event in 40 patients. Hemodialysis was carried out in three patients and peritoneal dialysis in 1 patient.

Cardiac complications played a significant role in patient morbidity and mortality. Nine patients sustained acute myocardial infarction and all died. Infectious myocardial complications included myocardial abscess in 1 patient, bacterial endocarditis in 7 patients, acute pericarditis in 6 patients, and acute pancarditis in two patients. Operative pericardectomy was necessary in 1 patient for constrictive pericarditis.

Pneumonitis has continued to be a significant problem in 1973 as it was in preceding years. Bronchopneumonia was diagnosed in 92 patients (34%) and inhalation injury diagnosed by either clinical criteria, bronchoscopy or ^{133}Xenon lung scan was noted in 57 patients (21%). Pulmonary edema occurred in 70 patients (26%) and atelectasis in 23 patients. Pneumothorax occurred in 8 patients and was either associated with the use of a mechanical ventilator or inaccurate insertion of a subclavian vein cannula in each instance. Pulmonary emboli were detected either clinically or at autopsy in 11 patients. At autopsy 10 patients had pulmonary thrombosis and in 7 of these patients the emboli were septic. Herpetic pharyngeal slough occurred in 3 patients. Tracheostomy was performed in 49 patients (18%) for specific indications such as acute inhalation injury, upper airway obstruction or prolonged ventilator support.

SUMMARY

During calendar year 1973 261 patients were admitted to the U.S. Army Institute of Surgical Research. Previously reported therapeutic modalities developed at the Institute over the past seven years were maintained. In addition newer modalities of clinical therapy were instituted and evaluated and these included fiberoptic endoscopy to evaluate gastrointestinal pathology of the early post burn patient, fiberoptic bronchoscopy to better define the spectrum of acute inhalation injury, more sophisticated pulmonary function measurements and critical evaluation of the metabolic response of the thermally injured patient. As in previous years, the Institute of Surgical Research was involved in the education of many military and civilian personnel, not only at the Unit but in the medical community. This was carried out by multiple presentations, publications and participation at professional meetings, as well as in-house training and education of house officers, staff physicians, foreign physicians and paramedical personnel by the staff of this Institute.

Infection continues to be the most frequent cause of morbidity and the major cause of mortality in thermally injured patients. Pulmonary infection remains the most common septic complication. Gram-negative organisms continue to be the offending agent in the majority of septic complications but Staphylococcus aureus, coagulase positive remains a major pathogen in the burn patient. Although new methods of treatment have been instituted and new antibiotics are continually being introduced, the mortality in thermally injured patients has been altered very little. Consequently intensive clinical and experimental research continues at this unit to develop better methods of prevention and treatment.

Table 7. Causes of Death, 1973

Patient	Age	Sex	% Burn Total	% Burn 3°	PBD Death*	Cause of Death
1	26	M	97	97	2	Inhalation injury; bronchopneumonia (Enterobacter cloacae, E. coli, Staphylococcus)
2	22	M	96	84	1	Pulmonary congestion and edema, multifocal myocardial necroses and subendocardial hemorrhages; cerebral edema
3	46	M	95	94	4	*Cause of death uncertain
4	70	F	95	93	0	*Severe inhalation injury
5	56	F	95	91	2	*Hypovolemic shock
6	12	M	94.5	59	1	Hypovolemic shock; pulmonary congestive atelectasis; pulmonary edema; pulmonary artery thrombosis (suppurative)
7	31	F	92	74	7	Shock lung; septicemia (E. coli, Klebsiella, Providence)
8	58	M	90.5	72.5	4	Acute myocardial infarction; acute pulmonary edema; septicemia (Candida, Providence, Enterobacter)
9	55	M	90	77	3	Inhalation injury; septicemia (Proteus , Klebsiella); disseminated microthromboses; suppurative iliofemoral arteritis and thrombosis with ischemic gangrene right leg
10	47	F	89	87.5	1	*Hypovolemic shock; severe inhalation injury
11	18	M	88	58.5	4	Acute pulmonary edema; acute cerebral edema with uncal herniation
12	21	F	87.5	78.5	8	Disseminated intravascular coagulation; acute respiratory distress syndrome (shock lung), severe
13	55	M	87	49	1	*Hypovolemic shock; respiratory insufficiency
14	57	F	84.5	70	7	*Severe inhalation injury; septicemia (Staphylococcus, Bacillus species); bronchopneumonia, bilateral (Staphylococcus)

*Autopsy not performed

Table 7. Causes of Death, 1973

Patient	Age	Sex	% Burn Total	% Burn 3°	PBD Death	Cause of Death
15	25	M	84	76	11	Bilateral adrenal hemorrhagic necrosis, pulmonary edema, septicemia (Providence)
16	85	F	82.5	70	1	*Hypovolemic shock
17	20	M	82.5	57.5	2	Severe inhalation injury; septicemia (E. coli, Klebsiella)
18	22	M	82	49	6	*Pulmonary embolus; bilateral pneumonia (E. coli)
19	53	F	81	75	5	*Cause of death uncertain
20	5	M	81	62.5	12	Acute bilateral bronchopneumonia (Proteus, Candida, Pseudomonas); wound sepsis (Pseudomonas, E. coli, Proteus, Candida); suppurative thrombophlebitis (Candida)
21	20	M	80	45	4	Severe inhalation injury; pulmonary edema and congestion; hypoxic encephalopathy with diffuse cerebral edema
22	15	M	79	59.5	4	*Acute renal failure secondary to irreversible hypovolemic shock; acute pulmonary edema
23	26	F	78.5	44	7	*Bilateral bronchopneumonia (Staphylococcus, E. coli, Klebsiella, Providence); septicemia (Staphylococcus)
24	25	M	78.5	11	37	Lobar pneumonia (Providence, Bacillus, Pseudomonas); disseminated mycosis kidneys, spleen, lung (Fusarium); septicemia (Providence, E. coli, Klebsiella); disseminated intravascular coagulation
25	15	M	78	42	8	Acute hyaline membrane disease; shock lung (severe); cerebral edema; disseminated intravascular coagulation
26	39	M	77.5	34	13	Invasive mycotic wound infection (Fusarium, Candida); pulmonary edema; cerebral edema
27	20	M	77	60	2	Hypovolemic shock; severe steam inhalation injury

*Autopsy not performed

Table 7. Causes of Death, 1973

Patient	Age	Sex	% Burn Total	% Burn 3°	PBD Depth	Cause of Death
28	40	M	76	58.5	6	Bronchopneumonia (Serratia)
29	24	F	75.5	60	18	Pseudomonas burn wound sepsis; bronchopneumonia (Pseudomonas); septicemia (Pseudomonas)
30	23	M	75	66	4	Respiratory insufficiency (interstitial pneumonitis, pulmonary edema and congestion); cerebral edema; hemoglobinuric acute tubular necrosis
31	32	M	75	49	4	*Cause of death undetermined
32	2	M	74.5	25	25	Pseudomonas burn wound sepsis; septicemia (Pseudomonas, Providence, Staphylococcus); necrotizing bacterial pneumonia (Staphylococcus, Pseudomonas, Providence)
33	16	M	73.5	59	23	Acute necrotizing hemorrhagic pneumonia (Pseudomonas); septicemia (Pseudomonas Providence); invasive mycotic wound infection (Candida, Aspergillus, Fusarium)
34	19	M	72	11	4	Steam inhalation injury; bronchopneumonia, severe (Enterobacter aerogenes), septicemia (Enterobacter aerogenes)
35	37	M	72	11	2	Inhalation injury; acute pulmonary edema, hemorrhagic, severe; distal small bowel infarction
36	16	F	71.5	44.5	5	Inhalation injury with bronchiolitis; adult hyaline membrane disease; disseminated intravascular coagulation; cerebral edema
37	19	M	71	19	4	Marked pulmonary edema; pulmonary thromboemboli, diffuse; interstitial pneumonia; ruptured liver with intraparenchymal and intraperitoneal hemorrhage
38	6/12	F	68.5	67.5	0	Hypovolemic shock
39	17	M	68.5	55	7	Septicemia (Staphylococcus, E. coli); acute pulmonary edema; acute cerebral edema

* Autopsy not performed

Table 7. Causes of Death, 1973

Patient	Age	Sex	% Burn Total	% Burn 3°	PBD Death	Cause of Death
40	2	M	68	53	11	Septicemia (E. coli); invasive mycotic wound infection (Candida, Fusarium, Helminthosporium); shock lung and progressive respiratory insufficiency
41	19	M	68	52	3	Pulmonary emboli, acute, bilateral
42	37	F	67.5	46	5	Invasive bacterial wound infection (Bacillus, Providence); pneumonia (Providence); septicemia (Providence); disseminated intravascular coagulation; acute hemorrhagic pancreatitis
43	21	M	67	45	20	Mycotic (Candida, Fusarium) and bacterial (Staphylococcus, Providence) wound colonization and invasion; septicemia, mycotic (Candida, Fusarium) and bacterial (Staphylococcus); hematogenous pneumonitis, acute (Staphylococcus)
44	25	M	66.5	51	4	Cerebral edema, with bilateral uncal and tonsillar herniation; acute tubular necrosis
45	35	M	66.5	39.5	16	Hemorrhagic pneumonitis, necrotizing (Pseudomonas, Providence, E. coli); septicemia (Klebsiella, E. coli, Providence)
46	27	M	66.5	32.5	9	Acute bacterial pneumonia (Klebsiella, E. coli, Enterobacter aerogenes); acute pulmonary edema
47	1-5/12	F	66	43.5	16	Mycotic (Aspergillus) and bacterial (Klebsiella) invasive wound infection; bronchopneumonia (Klebsiella); septicemia (Klebsiella); disseminated intravascular coagulation
48	19	M	66	25.5	22	Mycotic laryngotracheobronchitis with mycotic abscesses (Candida, Aspergillus); septicemia (Pseudomonas, Providence, non-hemolytic strep); disseminated intravascular coagulation; invasive mycotic wound infection(Candida,Aspergillus)
49	49	M	65	22.5	3	Acute bacterial pneumonia (E. coli, Klebsiella, Providence)
50	2-7/12	M	63	28.5	30	Pseudomonas burn wound sepsis; septicemia (Pseudomonas, Providence); Staphylococcal thrombophlebitis; pancarditis, bacterial (Staphylococcus)

*Autopsy not performed

Table 7. Causes of Death, 1973

Patient	Age	Sex	% Burn Total	% Burn 3°	PBD Death	Cause of Death
51	60	M	62	53	47	Bronchopneumonia, severe; septicemia (Pseudomonas, Enterobacter cloacae)
52	81	F	62	36.5	1	*Severe inhalation injury; hypovolemic shock
53	48	M	61	13	29	Acute bacterial endocarditis (Staphylococcus); pulmonary hemorrhage; pulmonary edema; bronchopneumonia (E. coli, Providence, non-hemolytic strep)
54	44	M	60.5	35	20	Perforated gastric ulcer; acute pulmonary edema; septicemia (Enterobacter cloacae)
55	64	M	59	54	14	Invasive burn wound infection (Fusarium, Aspergillus); septicemia (Staphylococcus, Providence, Candida)
56	3	M	59	50	8	Cerebral infarction, massive with hemorrhage; septicemia (Providence)
57	69	F	59	36.5	1	Hypovolemic shock; severe inhalation injury
58	52	M	58	27.5	11	Inhalation injury; disseminated intravascular coagulation; bronchopneumonia, septicemia (Providence)
59	22	M	57.5	19.5	23	Bronchopneumonia (non-hemolytic strep, Klebsiella, Pseudomonas); erosive tracheobronchitis (herpetic)
60	41	M	57.5	16	5	Inhalation injury; bronchopneumonia (Klebsiella, E. coli); septicemia (Klebsiella); disseminated intravascular coagulation
61	18	M	57.5	14.5	16	Pulmonary infarction (massive); interstitial fibrosis of lungs; cerebral edema
62	38	M	56.5	56.5	11	Bacterial wound invasion (Klebsiella); lobar pneumonia (Klebsiella); septicemia (Klebsiella)
63	2	F	56.5	26	6	Septicemia (Enterobacter cloacae); mycotic burn wound infection (Fusarium, Helminthosporium); respiratory insufficiency

*Autopsy not performed

Table 7. Causes of Death, 1973

Patient	Age	Sex	% Burn Total	% Burn 3°	PBD Death	Cause of Death
64	52	M	56.5	6.5	3	Pulmonary emboli, massive bilateral
65	26	M	56	18	10	Inhalation injury; invasive burn wound infection (Candida); disseminated candidosis; acute myocardial infarction; septicemia (Enterobacter)
66	5	M	55	34	26	Acute bacterial endocarditis (Staphylococcus); mycotic leptomeningitis; cerebritis and hemorrhagic cerebral infarction (septate hyphae); mycotic and bacterial invasive wound infection (Candida, Aspergillus, Providence)
67	19	M	55	30	11	Acute tubular necrosis; bronchopneumonia (Pseudomonas, Providence); septicemia (Providence, Pseudomonas, Enterobacter cloacae)
68	14	F	55	3	11	Bronchopneumonia (Klebsiella); hemorrhagic pulmonary edema; disseminated intravascular coagulation; septicemia (Providence)
69	2-10/12	M	54	15.5	11	Septicemia (Providence, Staphylococcus); bronchopneumonia (Providence, Staph)
70	34	M	53.5	0	6	Severe inhalation injury; bronchopneumonia (Proteus, Providence, Staphylococcus) septicemia (Staphylococcus)
71	30	M	53	21	15	Suppurative thrombophlebitis (staphylococcus); hematogenous pneumonia (staphylococcus); disseminated intravascular coagulation
72	78	M	52	0	1	*Severe inhalation injury
73	47	M	51.5	5.5	9	Cerebral edema and infarction; septicemia (Providence)
74	83	F	51	51	2	Acute congestive heart failure
75	38	M	51	22	35	Bacterial endocarditis; septicemia (Staphylococcus non-hemolytic streptococcus); inhalation injury; bronchopneumonia (Staphylococcus, Providence, Pseudomonas)
76	49	M	50	37.5	22	Septicemia (Providence); bacterial burn wound infection (Providence, non-hemolytic strep)

*Autopsy not performed

Table 7. Causes of Death, 1973

Patient	Age	Sex	% Burn Total	% Burn 3°	PBD Death	Cause of Death
77	50	M	48.5	19.5	7	Mycotic invasive burn wound infection diffuse (Candida, Geotrichum, Fusarium) local invasive phycomycosis left leg
78	24	M	48	40	37	Septicemia (Providence, Pseudomonas); bronchopneumonia (Proteus, Providence, staphylococcus); disseminated intravascular coagulation
79	36	M	47	12	3	Adult hyaline membrane disease, severe; bronchopneumonia (Pseudomonas)
80	29	M	47	0	25	Bronchopneumonia (Bacillus, Pseudomonas, Providence); septicemia (Providence)
81	33	M	47	0	18	Invasive bacterial wound infection (Pseudomonas); pulmonary embolus and infarction; acute tubular necrosis
82	53	M	46	28	7	Bronchopneumonia (E. coli); intracranial hemorrhage, severe; septicemia (E.coli)
83	74	F	46	27	20	Septicemia (Pseudomonas); bronchopneumonia (Pseudomonas)
84	53	M	45	11.5	11	Acute myocardial infarction; pneumonia (Klebsiella, Providence, Pseudomonas)
85	22	F	45	0	17	Septicemia (Pseudomonas); bronchopneumonia (Pseudomonas)
86	8	F	44.5	43.5	44	Disseminated candidiasis with cardiac and renal abscesses
87	21	M	44	14.5	6	Septicemia (Staphylococcus); disseminated intravascular thrombosis; gastric perforation
88	72	M	43	41	17	*Septicemia (Providence)
89	29	M	43	10	1	Massive skull fractures; epidural and subarachnoid hemorrhage; brain necrosis; septicemia and pneumonia (Klebsiella)
90	1-1/12	M	42.5	42.5	15	Pseudomonas burn wound sepsis with ecthyma gangrenosum; bronchopneumonia (Pseudomonas)

*Autopsy not performed

Table 7. Causes of Death, 1973

Patient	Age	Sex	% Burn Total	% Burn 3°	PBD Death	Cause of Death
91	46	M	42.5	41	21	*Pneumonitis (Providence, Klebsiella, Pseudomonas); septicemia (Candida); cerebral vascular accident
92	2-4/12	M	42.5	7	6	*Hypoxic encephalopathy
93	23	M	42.5	7	12	Disseminated intravascular coagulation; acute pulmonary edema; acute cerebral edema
94	32	F	41	16.5	10	Inhalation injury; bronchopneumonia (Pseudomonas); pulmonary thromboembolism and infarction; cerebral edema with uncal and tonsillar herniation
95	19	M	41	10	15	*Inhalation injury; bronchopneumonia (Providence, Pseudomonas, Staphylococcus); septicemia (Staphylococcus, Providence, Pseudomonas)
96	3	F	40	34.5	5	*Bronchopneumonia (E. coli, Providence); septicemia (Providence)
97	56	F	40	25.5	13	Invasive mycotic burn wound infection (Candida); Candidemia; acute bronchiolitis (Klebsiella)
98	64	M	39	26	17	*Inhalation injury; bronchopneumonia (Pseudomonas); septicemia (Pseudomonas)
99	20	M	39	0	4	Inhalation injury
100	24	M	38	10	7	Inhalation injury; bronchopneumonia (Providence, non-hemolytic Streptococcus)
101	76	M	37.5	16.5	2	Acute pulmonary edema; septicemia (Klebsiella)
102	70	F	36.5	11.5	15	*Bronchopneumonia (Staphylococcus Providence, Klebsiella); septicemia (Staphylococcus)
103	47	M	34	10	4	Acute pulmonary edema
104	63	M	31.5	25	21	Septicemia (Providence); disseminated intravascular coagulation
105	62	M	30	26.5	5	Bronchopneumonia (E. coli, Klebsiella)

*Autopsy not performed

Table 7. Causes of Death, 1973

Patient	Age	Sex	% Burn Total	% Burn 3°	PBD Death	Cause of Death
106	66	F	30	9	21	Acute myocardial infarction
107	10	F	29.5	1	16	Pseudomonas burn wound sepsis; disseminated candidiasis
108	28	M	29	2	11	Bronchopneumonia (Providence); disseminated intravascular coagulation
109	2	F	28.5	15.5	30	Bacterial pneumonia (Staphylococcus); Staphylococcal endomyocarditis; septic thromboemboli (Staphylococcus)
110	3	M	26.5	18.5	31	Bacterial endocarditis (Staphylococcus); bronchopneumonia (Staphylococcus); septicemia (Staphylococcus, Providence)
111	1	F	26	13	11	Inhalation injury; bronchopneumonia (E. coli, Staphylococcus); erosive laryngotracheobronchitis (Staphylococcus); cerebral edema with herniation
112	1-1/12	M	25.5	25.5	23	Disseminated Staphylococcus and Providence infection involving brain, heart, lung, kidney and myocardium
113	39	M	23	10	50	Immediate cause of death uncertain.

PRESENTATIONS

Pruitt BA Jr: Role of the Nurse in Burn Care. Registered Nurses Club, Fort Sam Houston, TX 16 Jan 73.

Pruitt BA Jr: Resuscitation and Hemodynamic Changes Following Burn Injury. Sixth Anl Symp. of Military Plastic Surgery, Wash DC 17-19 Jan.

McGranahan BG: Care and Treatment of Burns. 91C students, Brooke Gen Hosp, BAMC, Fort Sam Houston, Texas 24 Jan 73.

Agee RN: The Treatment of Burns. Off Basic Course, MFSS, BAMC, Fort Sam Houston, TX 31 Jan 73.

Long JM III: Current Techniques of Parenteral Hyperalimentation. Annual Mtg of the Brooklyn Surgical Society and St. Albans Naval Hosp staff, N.Y. 1 Feb 73.

Long JM III: Intravenous Hyperalimentation: Rationale and Potential Complications. House staff and students, Downstate Medical Center Kings County Hospital, Brooklyn, N.Y. 2 Feb 73.

Long JM III: Current Management of Acute Thermal Injury. Visiting staff, surgical staff, house staff and students Downstate Medical Center Kings County Hospital, Brooklyn, N.Y. 2 Feb 73.

McGranahan BG: Nursing Care of Burns. Air Force Nurses and corpsmen, Lackland Dispensary, Lackland AFB, TX 14 Feb 73.

Pruitt BA Jr: (1) The Use of Physiologic Dressings; (2) Electrical Burns. Management of the Burned Patient Course, Dept of Surg, Washington Univ & Hartford Burn Unit, Barnes Hosp, St Louis, MO 16-17 Feb 73

Pruitt BA Jr: Current Management of the Severely Burned Patient. Crozer-Chester Medical Center Cont Edu Program, Philadelphia, PA 20 Feb.

Pruitt BA Jr: Fluid Therapy and Fluid Problems in the Burn Patient. Plastic Surg Conf, Univ of Tex Med Sch at San Antonio, San Antonio, TX 21 Feb 73.

Pruitt BA Jr: Current Management of the Burn Patient. Children's Hosp, Columbus, OH 28 Feb 73.

Long JM III: The Current Status of Parenteral Hyperalimentation in the Management of Malnutrition in the Hospitalized Patient. Seminar on Total Intravenous Feeding, Denver, Colorado 1 Mar 73.

The following presentations were given to the Brooke Army Medical Center- Univ of Texas Medical School at San Antonio Symp on Surgical & Orthopaedic Aspects of Trauma, San Antonio, TX 6 Mar 73:

McManus WF: Early Care of the Burn Patient
Warden GD: Fluid Management Following Resuscitation
Agee RN: Topical Therapy of the Burn Wound
Taylor JW: Coverage of the Burn Wound
Long JM III: Pulmonary Complications
Erickson DR: Other Septic Complications
Wilmore DW: Nutritional and Metabolic Considerations

Pruitt BA Jr: (1) Burn Wound Care, Including Topical Chemotherapy and the Use of Physiologic Dressings. (2) Recent Advances in Burn Management. (3) Hemodynamic Changes Following Thermal Injury as Related to Resuscitation. First National Australian Burn Symposium, Melbourne, Australia. 5-13 Mar 73

Pruitt BA Jr: Recent Studies on Fluid Resuscitation, Inhalation Injury, and Wound Care. Staff Conf of the Royal Prince Alexandre Hosp for Children, Sydney, Australia.

McManus WF: Resuscitation and Fluid Balance. 2nd Flame-Free Design Conference, Baton Rouge, LA 8 Mar 73.

Long JM III: The Treatment of Burns. Allied Medical Officers AAMAMO Course USAF Sch of Aerospace Med, Brooks AFB TX 9 Mar 73.

Wilmore DW: Nutrition and Metabolic Considerations in Burn Care. Allied Medical Officers AAMAMO Course USAF Sch of Aerospace Med, Brooks AFB TX 9 Mar 73.

Reardon JC: Burns - General O.T. Treatment. Occupational Therapy Brooke Army Medical Center, Fort Sam Houston, TX 10 Mar 73.

McGranahan BG: Nursing Care of Burns. Flight Nurses and Technicians, Sch of Aerospace Med, Brooks AFB TX 13 Mar 73.

Pruitt BA Jr: Burn Research. Army Scientific Adv Panel, Wash DC 19-20 Mar 73.

McManus WF: Modern Treatment of Burns. Mtg of the Texas State Safety Assn, Houston, TX 27 Mar 73.

The following presentations were made at a Team Symposium on Burns at the Univ of Missouri, Columbia, MO 29-30 Mar 73:
Agee RN: (1) Initial Treatment of the Burn Patient; (2) Burn Complications; (3) Burn Research
Loveless SD: (1) Physical Therapy for the Burn Patient; (2) Occupational Therapy for the Burn Patient
Wooten C Jr: (1) Nursing Implications in the Care of the Burn Patient; (2) Nursing Care of the Burn Patient

Pruitt BA Jr: (1) Non-bacterial Opportunistic Infections; (2) Complications of Intravenous Alimentation; (3) Treatment of Burns ACS Postgraduate Course on Fluids & Electrolytes, New York, NY 2-4 Apr 73.

The following presentations were made to the American Burn Assn Anl Mtg, Dallas, TX 6-7 Apr 73:

Reardon JC: An Axillary Splint for Thermally Injured Patients
Taylor JW: Sensory Stimulation of the Burned Patient
Slogoff S: Subanesthetic Ketamine for Debridement of Thermally Injured Patients
Warden GD: Water Deficit in Hypermetabolic Burn Patients
Bristow B: Provision of Nutritional Care to the Burned Patient
Clayton WH: Use of Inhalation Therapy in Burn Care
Pruitt BA Jr: Invited discussant of 3 papers.

The following presentations were made to the Global Med Course, USAF Sch of Aerospace Med, Brooks AFB, TX 9 Apr 73:

McManus WF: Initial Care and Resuscitation of the Burn Patient
Long JM III: Wound Care; Topical Chemotherapy and Biologic Dressings
Wilmore DW: Post Resuscitative Fluid and Caloric Requirements
McAlhany JC Jr: Complications of Thermal Injury

Warden GD: Resuscitation and Initial Care of Thermally Injured Patients. Emergency Room Staff, Brooke Army Medical Center, Fort Sam Houston, TX 12 Apr 73.

Pruitt BA Jr: Treatment of the Burn Victim. Univ of Tex Med Sch at San Antonio, San Antonio, TX 14 Apr 73.

Long JM III: Treatment of Burns. Academy of Health Sciences Physician's Assistant Program students. USA ISR, BAMC, Fort Sam Houston, TX 18 Apr 73.

Agee RN: Treatment of Burns. Academy of Health Sciences Physician's Assistant Program students, USA ISR, BAMC, Fort Sam Houston, TX 20 Apr 73.

Erickson DR: Treatment of Burns. Academy of Health Sciences Physician's Assistant Program students, USA ISR, BAMC, Fort Sam Houston, TX 25 Apr 73.

Pruitt BA Jr: Discussant of paper entitled "Changing Patterns in Surgical Infection," presented by Dr. WA Altemeier, American Surg Assn Mtg, Los Angeles, CA 25-27 Apr 73

Pruitt BA Jr: Fluid Resuscitation and Hemodynamic Changes in Burn Patient. Staff mtg, Los Angeles County Hosp, Los Angeles, CA 26 Apr 73.

Taylor JW: Treatment of Burns. Academy of Health Sciences Physician's Assistant Program students, USA ISR, BAMC, Fort Sam Houston, TX 27 Apr 73.

Wilmore DW: Clinical Evaluation of a 10% Intravenous Fat Emulsion for Parenteral Nutrition in Thermally Injured Patients. American Surgical Assn Mtg, Los Angeles, CA 27 Apr 73

Pruitt BA Jr: Current Management of Extensively Burned Patients. Staff Mtg, Harbor General Hospital, Torrance, CA 27 Apr 73.

Pruitt BA Jr: Grand Rounds, Surgical Service, Harbor General Hosp, Torrance, CA 28 Apr 73.

Agee RN: Complications and Management of the Burn Patient. Emerg Rm Staff, Brooke Army Medical Center, Fort Sam Houston, TX 3 May 73.

Long JM III: The Current Management of Burn Injury. Texas Medical Assn on Trauma Mtg Dallas, TX 4 May 73.

Pruitt BA Jr: The Organization of the Texas Division of the Amer Trauma Soc Mtg, Chicago, IL 6 May 73.

McManus WF: Care of the Burn Patient. Clinical Pastoral Education for Chaplains Course, Brooke Army Medical Center, Fort Sam Houston, TX 7 May 73

The following presentations were made at the Team Symposium on Burns Arkansas Chapter of the American Physical Therapy Assn Mtg, Little Rock, AR 5-6 May 73:

Erickson DR: The Treatment of Burns
Hall WF: Physical Therapy of the Burn Patient
Reardon JC: Occupational Therapy of the Burn Patient
Wooten C Jr: Nursing Care of the Burn Patient

Erickson DR: Care of the Burn Patient. Allied Medical Officers, Sch of Aerospace Med, Brooks AFB, TX 10 May 73.

Taylor JW: Burns in Pregnancy. San Antonio OB-GYN Society Mtg, San Antonio, TX 16 May 73.

Long JM III: Current Techniques of Total Intravenous Nutrition. Kansas City, MO. 18 May 73

McManus WF: Thermal Injuries. AF Reserve Technician Officers Course Brooks AFB, TX 23 May 73.

McGranahan BG: Nursing Care of the Burn Patient. Paramedical students, Central Service Program, Bexar County Hosp District, San Antonio, TX 25 May 73.

Pruitt BA Jr: Pulmonary Complication of Trauma. Pathobiology of Trauma Program, Univ of Minnesota, Minneapolis, MN 5-7 Jun 73.

Pruitt BA Jr: Current studies on Fluid Replacement in the Extensively Burned Patient. Surg Conf, Univ of Tex Med Sch at San Antonio, San Antonio, TX 8 Jun 73.

McGranahan BG: Nursing Care of the Burn Patient. Air National Guard Nurses and Technicians, Kelly AFB, TX 9 Jun 73.

Pruitt BA Jr: Fluid Resuscitation of the Extensively Burned Patient. Surg Svc, William Beaumont Gen Hosp, El Paso, TX 8-10 Jun 73.

McGranahan BG: Nursing Care of the Burn Patient. LVNs and Instructor from Jourdanton, TX 13 Jun 73.

Pruitt BA Jr: (1) Initial Care of the Extensively Burned Patient; (2) Management of the Burn Wound. Staff Boston City Hospital, Boston, MA 12-13 Jun 73.

Wilmore DW: Use of I.V. Fat Emulsions for Parenteral Nutrition. Walter Reed Army Medical Center, Washington, DC 16 Jun 73.

McGranahan BG: Nursing Care of Burns. Nursing students and staff Brackenridge Hospital, Austin, TX 20 Jun 73

McGranahan BG: Nursing Care of Burns. Clinical Specialist School Brooke Army Medical Center, Fort Sam Houston, TX 21 Jun 73.

Pruitt BA Jr: Early Care of the Extensively Burned Patient. County Medical Society, Shelby, NC 21 Jun 73.

Pruitt BA Jr: Current Management of the Burn Wound. Continuing Education Program, Cleveland Memorial Hospital, Shelby, NC 22 Jun 73.

Pruitt BA Jr: Course Director, American Medical Association. Postgraduate Course on Burn Care, New York, NY 24 Jun 73.

Pruitt BA Jr: Treatment of Burns. Symp. on Management of Shock. Hahnemann Gen Hosp, Philadelphia, PA 24-26 Jun 73.

Wilmore DW: Nutrition in Burns. Nurses Burn Course, University of Texas Southwest Medical School, Dallas, TX 26 Jun 73.

Allen GW: Anesthesia and the Burn Patient: Anesthesia residents Brooke Army Medical Center, Fort Sam Houston, TX 26 Jun 73.

Taylor CH: Care of Burns. Volunteer Firemen, Pipe Creek, TX 12 Jul 73.

McGranahan BG: Nursing Care of Burns. Nursing Students Baptist Memorial Hospital, San Antonio, TX 26 Jul 73.

Taylor, JW: Treatment of Burns. Officers Basic Course, Academy of Health Sciences, Fort Sam Houston, TX 17 Aug 73.

Agee RN: Burns - Initial Care. Students Intensive Care Nursing Course, Brooke Army Medical Center, Fort Sam Houston, TX 22 Aug 73.

McGranahan BG: Nursing Care in Burns. Flight Nurses and Technicians, Brooks AFB TX 28 Aug 73.

McGranahan BG: Nursing Care in Burns. Students Advanced Operating Room Nurses Course, Brooke Army Medical Center, Fort Sam Houston, TX 29 Aug 73.

Hunt JL: Burn Wound Therapy. Students Intensive Care Nursing Course, Brooke Army Medical Center, Fort Sam Houston, TX 31 Aug 73.

Long JM III: Hyperalimentation. Students Intensive Care Nursing Course, Brooke Army Medical Center, Fort Sam Houston, TX 5 Sep 73.

McGranahan BG: Nursing Care and Treatment of Burns. AF Nurses and Corpsmen, Specialized Treatment Center, Lackland AFB, TX 5 Sep 73.

McGranahan BG: Nursing Care and Management of Burns. Enlisted personnel of 4ist Hospital Group, Fort Sam Houston, TX 10 Sep 73.

McAlhany JC Jr: Complications. Students Intensive Care Nursing Course, Brooke Army Medical Center, Fort Sam Houston, TX 12 Sep 73.

McGranahan BG: Nursing Care of Burns. Students Intensive Care Nursing Course, Brooke Army Medical Center, Fort Sam Houston, TX 13 Sep 73.

Warden GD: Management of Thermal Injuries. Officers Basic Course, Academy of Health Sciences, Fort Sam Houston, TX 14 Sep 73.

Long JM III: Management of Acute Burn Injuries. University of Texas Medical School at Houston, Houston, TX 14 Sep 73.

Wilmore DW: Human Growth Hormone and High Caloric Feedings: Modification of the Metabolic Response Following Thermal Injury. Joint meeting of the European Society for Clinical Research and the British Society for Clinical Investigation, Garmish, Germany. 17 Sep 73.

Wilmore DW: Burn Care. University of Padova, Padova, Italy. 21 Sep 73.

Pruitt BA Jr: Discussion of Laser Excision of Burns, Use of Biologic Dressings in the Care of Burn Patients, Early Resuscitation, and Synthetic Skin. Staff Conference, Institute of Plastic and Reconstructive Surgery, University of Padua, Padua, Italy. 21 Sep 73.

Pruitt BA Jr: Control of Burn Wound Infection by Topical Therapy, Results in over 2600 Burn Patients. Twenty-Fifth Congress of the Societe Internationale de Chirurgie, Barcelona, Spain 25 Sep 73.

Long JM III: Surgical Grand Rounds, University of Oklahoma Medical Center, Oklahoma City, OK 6 Oct 73.

The following presentations were made to the Amer Assn for Surgery of Trauma Mtg, Chicago, IL 11 Oct 73:
Hunt JL: Vascular Lesions in Acute Electrical Injuries.
Salisbury RE: Artificial Tendon Design, Application and Results.

Wilmore DW: Stress in Man a Reflection of Metabolic and Organ Changes as Seen in the Injured Patient. Surgical Grand Rounds. University of Wisconsin, Madison, WI 13 Oct 73

Wilmore DW: Hyperglucagonemia Following Thermal Injury: Insulin Glucagon Ratio Following Thermal Injury. American College of Surgeons Mtg, Chicago, IL 16 Oct 73.

Taylor JW: Burns - Fluid and Electrolyte Management, Sepsis. Nursing students, ICU, CCU nurses, Brackenridge Hospital, Austin, TX 16 Oct 73.

McGranahan BG: Nursing Care in Burns. Flight Nurses and Technicians, Sch of Aerospace Med, Brooks AFB, TX 17 Oct 73.

The following presentations were made and meetings attended by Pruitt BA Jr in connection with the ACS Mtg Chicago IL 15-19 Oct 73: (1) Meeting, Surgical Biology III Club; (2) Meeting, ACS Pre- and Post-operative Care Committee; (3) Chairman, ACS Postgrad Course No 5, "Total Care of the Burned Patient"; (4) National Burn Information Exchange Mtg; (5) North American Chapter International Society of Surgery Mtg; (6) Moderator, Contemporary Surgery Symposium, "Clinical Aspects of Burn Care" and (7) Panel Member, ACS Interdisciplinary Panel on, "Management of the Problem, Hypertrophic Scar and Keloid," and presentation, Surgical Approach and Use of Pressure in the Management of Hypertrophic Scar and Keloids"

Wilmore DW: Metabolic Changes in Nutrition in Burn Patients. Post graduate course on burns, American College of Surgeons, Chicago, IL 18 Oct 73.

McGranahan BG, Warden GD, Loveless SD, Shaw AL, Din A, Brownlee JW: (1) Working Together for the Burn Patient; (2) Team Approach to Care of Burn Patients. Current Concepts in Nursing Course, Clinical Concepts Group, San Antonio, TX 18 Oct 73.

Wilmore DW: Post Traumatic Changes in Metabolism and Energy Balance. Dietitians Brooke Army Medical Center, Fort Sam Houston, TX 24 Oct 73.

Levine NS: Reversal of Intraperitoneal Pseudomonas Mortality in Rats by Surgical Excision and Grafting. American Society of Plastic and Reconstructive Surgeons mtg, Hollywood, FL 24 Oct 73.

Wilmore DW: Parenteral Nutrition. Dietitians Brooke Army Medical Center Fort Sam Houston, TX 25 Oct 73.

Wilmore DW: Endocrine Changes Following Trauma. Discussion at Surgical Grand Rounds, University of Texas at Houston, TX 29 Oct 73.

Pruitt, BA Jr: Surgical Infections and Burns. Symp. Pseudomonas Infections, Walter Reed Army Institute of Research, WRAMC, Wash DC 29 Oct.

McGranahan BG: Burn Care. Lions Clubs of San Antonio, San Antonio, TX 31 Oct 73.

Wilmore DW: Glucose Regulation Following Operation and Injury. Medical College of Wisconsin and Surgical Grand Rounds VA Hospital, Milwaukee, WI 5 Nov 73.

Orcutt TW: Current Initial Management of Burn Patients. Officers Basic Course, Academy of Health Sciences, Fort Sam Houston, TX 9 Nov 73.

Taylor JW: Burns Then and Now. Assn of Operating Room Technicians, Regional mtg, San Antonio, TX 10 Nov 73.

Wilmore DW: Treatment of Burns. Panel Discussion, Southern Surgical Society. 11 Nov 73.

Pruitt BA Jr: Panel moderator "What's New in Burns" symp. on Burns. Presentation: "Sepsis in Burns" Southern Medical Assn Mtg San Antonio, TX 14 Nov 73.

Wilmore DW: Weight Changes Following Burn Injury. Nursing Staff, USA ISR Brooke Army Medical Center, Fort Sam Houston, TX 14 Nov 73.

Taylor JW: Burns. Explorers, Boy Scouts of America, San Antonio, TX 14 Nov 73.

The following presentations were made at the Southern Medical Assn mtg, San Antonio, TX 14 Nov 73:
Hunt JL: Complications of Thermal Injury
Long JM III: Nutrition in Burns.

Bluemle ML: Role of the Research Nurse. Nursing students, Incarnate Word College, San Antonio, TX 19 Nov 73.

McGranahan BG: Nursing Care of Burn Patients. Students 91C Course, Brooke Army Medical Center, Fort Sam Houston, TX 21 Nov 73.

Orcutt TW: Current Trends in Burn Therapy and Prevention. Armed Forces Communications & Electronics Assn, San Antonio, TX 29 Nov 73.

Hunt JL: Asepsis and Antisepsis in Wound Care. Burn Symposium for Nurses and other Health Professionals. University of Southern California School of Medicine, Postgraduate Division, Los Angeles, CA 3 Dec 73.

Long JM III: Triage and Management of Mass Burn Casualties. AF Medical Officers, Medical Aspects of Advanced Warfare Course, Brooks AFB TX 4 Dec 73.

McGranahan BG: Nursing Care of Burn Patients. Flight Nurses and Technicians, Sch of Aerospace Med, Brooks AFB TX 5 Dec 73.

Pruitt BA Jr: (1) Use of Biological Dressings; (2) Use of Sulfamylon Burn Cream. Paramedical Burn Care Panel. Symposium on the Treatment of Burns, Columbia Presbyterian Medical Center, Medical College of Virginia, Richmond, VA 7 Dec 73.

Wilmore DW: Metabolic Effects and Demands of Trauma. Postgraduate Course, Wayne State University, Detroit, MI 8 Dec 73.

Wilmore DW: Energy and Metabolic Changes Following Thermal Trauma. Grand Rounds, Albert Einstein University Sch of Med, Dept of Surg, N.Y. 11 Dec 73.

Bluemle ML: (1) The Health Nurse Clinician as Research Nurse; (2) Nurse Clinician Role Development - Group Discussion. Health, Education and Welfare Mtg, Wayne State University, Detroit, MI 14 Dec 73.

PUBLICATIONS

Reckler JM, Flemma RJ, Pruitt BA Jr: Costal chondritis: An unusual complication in the burned patient. J Trauma 13:76-80, 1973.

Silverstein P, Ruzicka FJ, Helmkamp GM Jr, Lincoln RA Jr, Mason AD Jr: In vitro evaluation of enzymatic debridement of burn wound eschar. Surgery 73:15-22, 1973.

Silverstein P, Peterson HD: Treatment of eyelid deformities due to burns. Plast Reconstr Surg 51:38-43, 1973.

Spitzer MF, Ritchey C, Glennon JM, Villarreal Y, Mason AD Jr: A rapid method of preparing food for sodium and potassium analyses. J Amer Diet Assn 62:44-46, 1973.

Rogers PW, Kurtzman NA, Bunn SM Jr, White MG: Familial benign essential hematuria. Arch Intern Med 131:257-262, 1973.

Salisbury RE, Palm L: Dynamic splinting for dorsal burns of the hand. Plast Reconstr Surg 51:226-228, 1973.

Curreri PW, Bruck HM, Lindberg RB, Mason AD Jr, Pruitt BA Jr: Providencia stuartii sepsis: A new challenge in the treatment of thermal injury. Ann Surg 177:133-138, 1973.

Kurtzman NA, White MG, Rogers PW: The effect of potassium and extracellular volume on renal bicarbonate reabsorption. Metabolism 22:481,492, 1973.

Newsome TW, Eurenius K: Suppression of granulocyte and platelet production by Pseudomonas burn wound infection. SG&O 136:375-379, 1973.

Moylan JA Jr, Mason AD Jr, Rogers PW, Walker HL: Postburn shock: A critical evaluation of resuscitation. J Trauma 13:354-358, 1973.

Helmkamp GM, Blackwell JP, Wilmore DW: Eruthrocyte sodium transport and membrane adenosine triphosphatase in patients with thermal injury. Clinica Chimica Acta 47:5-12, 1973.

Warden GD, Wilmore DW, Rogers PW, Mason AD Jr, Pruitt BA Jr: Hypernatremic state in hypermetabolic burn patients. Arch Surg 106:420-427, 1973.

Moylan JA Jr, Pruitt BA Jr: Aeromedical transportation. JAMA 224: 1271-1273, 1973.

Salisbury RE, Wilmore DW, Silverstein P, Pruitt BA Jr: Biological dressings for skin graft donor sites. Arch Surg 106:705-706, 1973.

McManus WF, Eurenius K, Pruitt BA Jr: Disseminated intravascular coagulation in burned patients. J Trauma 13:416-422, 1973.

Morris AH, Spitzer KW: Lung function in convalescent burn patients. Am Rev Respir Dis 108:989-993, 1973.

Salisbury RE, Hunt JL, Warden GD, Pruitt BA Jr: Management of electrical burns of the upper extremity. Plast Reconstr Surg 51:648-652, 1973.

Pruitt BA Jr, Foley FD: The use of biopsies in burn patient care. Surgery 73:887-897, 1973.

Warden GD, Wilmore DW, Pruitt BA Jr: Central venous thrombosis: A hazard of medical progress. J Trauma 13:620-626, 1973.

Langlinais PC, McKeel DW Jr: Massive accumulation of alveolar lattice formations and lamellar bodies in experimental phycomycotic pneumonitis. Texas Rep Biol Med 31:203-213, No 2, 1973.

Asch MJ, Feldman RJ, Walker HL, Foley FD, Popp RL, Mason AD Jr, Pruitt BA Jr: Systemic and pulmonary hemodynamic changes accompanying thermal injury. Ann Surg 178:218-221, 1973.

Newsome TW, Mason AD Jr, Pruitt BA Jr: Weight loss following thermal injury. Ann Surg 178-215-217, 1973.

Rogers PW, Kurtzman NA: Renal failure, uncontrollable thirst, and hyperreninemia. JAMA 225:1236-1238, 1973.

Eurenius K, Brouse RO: Granulocyte kinetics after thermal injury. Amer J Clin Path 60:337-342, 1973.

Newsome TW, Asch MJ, McGuigan JE, Pruitt BA Jr: Gastrin levels following thermal injury. Arch Surg 107:622-624, 1973.

Slogoff S, Allen GW, Warden GD, McManus WF: Tracheoesophageal fistula following prolonged tracheal intubation in a thermally injured patient. Anesthesiology 39:453-455, 1973.

Spitzer KW, Morris AH, Arnhold H: A valve assembly for studying pulmonary function in trauma patients. J Thorac & Cardiovasc Surg 60:607-612, 1973.

Salisbury RE, Loveless S, Silverstein P, Wilmore DW, Moylan JA Jr, Pruitt BA Jr: Postburn edema of the upper extremity: Evaluation of present treatment. J Trauma 13:857-862, 1973.

Wilmore DW, Moylan JA Jr, Helmkamp GM, Pruitt BA Jr: Clinical evaluation of a 10% intravenous fat emulsion for parenteral nutrition in thermally injured patients. Ann Surg 178:503-513, 1973.

Wessels JV, Allen GW, Slogoff S: Effect of nitrous oxide on ketamine anesthesia. Anesthesiology 39:382-386, 1973.

Helmkamp GM Jr, Wilmore DW, Johnson AA, Pruitt BA Jr: Essential fatty acid deficiency in red cells after thermal injury: Correction with intravenous fat therapy. Amer J Clin Nutr 26:1331-1338, 1973.

Pruitt BA Jr: Recent advances in burn treatment, pp 638-644; and Overview, pp 644-648. In, Readings in Modern Surgery (eds, RH Egdahl, JA Mannick). Grune & Stratton, New York, 1973.

Hughes JR, Cayaff JJ, Pruitt BA Jr, Boswick JA Jr, McManus WF, Bruck HM, Borges J: Seizures following thermal burns of the skin. Dis of Nervous System 34:347-353, 1973.

MOTION PICTURES

The following motion picture was shown at the American College of Surgeons Ani Conv, Chicago, Ill., 15-19 Oct 73 and at the American Soc of Plastic and Reconstructive Surgeons Mtg, Hollywood, Fla., 21-26 Oct 73:

"Management of Upper Extremity Burns"

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0A 6983	74 07 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8A. DOD'D INSTR'N	8B. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
73 07 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	61102A	3A161102B71R	01	168
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) Clinical Operation, Surgical Study Branch For Treatment of Injured Soldiers (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
62 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 74	1.3	34
	CURRENT 75	1.0	36

19. RESPONSIBLE DOD ORGANIZATION
NAME: US Army Institute of Surgical Research
ADDRESS: Ft Sam Houston, Tx 78234
RESPONSIBLE INDIVIDUAL
NAME: Basil A Pruitt, Jr, COL, MC
TELEPHONE: 512-221-2720

20. PERFORMING ORGANIZATION
NAME: US Army Institute of Surgical Research
ADDRESS: Surgical Study Branch
Ft Sam Houston, Tx 78234
PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic participation)
NAME: Roger E Salisbury, MAJ, MC
TELEPHONE: 512-221-5712
SOCIAL SECURITY ACCOUNT NUMBER
ASSOCIATE INVESTIGATORS
NAME: Albert J Czaja, MAJ, MC
NAME: Basil A Pruitt, Jr, COL, MC DA

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede each with Security Classification Code): (U) Trauma, (U) Combat casualties; (U) Laboratory animals; (U) Thermally injured soldiers; (U) Wound healing; (U) Gastrointestinal pathology

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

23. (U) Clinical and laboratory investigations pertaining to severe physical trauma which has been sustained by soldiers in the field.

24. (U) Planned clinical and laboratory studies relating to acute and chronic injury. Studies conducted by this branch have included both clinical studies involving patients on the ward and laboratory involving animal models.

25. (U) 73 07 - 74 06 This year the Surgical Study Branch was involved in better defining the relationship between thermal injury, wound healing, and infection. Investigations were also performed on the development of an artificial skin and artificial tendon prostheses for thermally injured soldiers. Clinical investigations have centered on improving the function of the thermally injured hand. In addition, ongoing investigation of gastrointestinal and liver function has aided understanding of the pathophysiologic dysfunction of these organs which occur following injury. In addition, ward officer coverage and gastroenterologic coverage were provided by members of this branch.

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Available in computerized form, withdrawer's approval

ABSTRACT

PROJECT NO. 3A161102B71R-01, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: CLINICAL OPERATION, SURGICAL STUDY BRANCH FOR TREATMENT OF INJURED SOLDIERS

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

Period covered in this report: 1 January 1973 - 31 December 1973

Investigators: Roger E. Salisbury, MD, Major, MC
Albert J. Czaja, MD, Major, MC
Basil A. Pruitt, Jr., MD, Colonel, MC

Reports Control Symbol MEDDH-288(RI)

The Surgical Study Branch has continued to render clinical care to burn and trauma patients admitted to the Institute from all three branches of the Armed Forces, in addition to veterans and civilian emergencies.

In addition to the clinical care of the seriously injured, the members of this branch have been concerned with problems relating to wound healing, rehabilitation of the thermally injured hand, and alterations in function of the gastrointestinal tract and liver. Both branch members have participated actively in various teaching programs, both on a local, national, and international basis.

Research projects include evaluation of digital escharotomy in salvaging thermally injured fingers, the effect of infection on the rate of wound healing in the thermally injured, a comparison of the efficacy of the various topical antibiotics in the prevention of burn wound sepsis, and the development of an artificial tendon for use in severely injured fingers. In addition, stress ulcers have been studied extensively by endoscopy, biopsy of the gastric mucosa, measurement of acid secretion and back diffusion from the stomachs of seriously injured soldiers. Liver function and bilirubin conjugation studies have been determined to reflect hepatic alteration following trauma.

Trauma
Post-traumatic gastrointestinal function
Combat casualty
Wound healing

CLINICAL OPERATION, SURGICAL STUDY BRANCH FOR TREATMENT OF INJURED SOLDIERS

The three major activities of the Surgical Study Branch are: 1) primary delivery of medical and surgical care to acutely burned soldiers admitted to this institute; 2) clinical and laboratory research in problems related to the care and rehabilitation of burn patients; 3) the education of medical and paramedical personnel in the care of the seriously injured.

The delivery of medical care is the prime purpose of this branch with emphasis placed on the application of knowledge gained from clinical research integrated into management of the seriously injured patient. The branch chief serves as ward officer and medical officer for Ward 13B, and all branch members provide consultation and care in the areas of hand surgery, gastrointestinal function, and liver function. Techniques and modalities developed in this unit are currently applied to the care delivered to seriously injured patients who remain hospitalized until all wounds are healed. At time of discharge, the patients are referred to their local physicians or specialty centers for attention to specific reconstructive and rehabilitation programs or for return to duty.

Clinical and laboratory research may be placed in the following categories: 1) the study of various treatment modalities that might maximize return of function in those patients with thermal injury of the upper extremity; 2) elucidation of factors affecting wound healing post-thermal injury; 3) development of an artificial tendon for use in soldiers with severe hand injuries; 4) description of the evolution of stress ulcers of the gastric mucosa in burn patients and the interrelationship of the observed changes with such factors as gastric mucosal blood flow, back diffusion, gastric acid production, bowel reflux, and mucus production; 5) description of the liver dysfunction which occurs following injury and specific relationionship of alterations in bilirubin conjugation to the neuroendocrine response which characterizes the catabolic phase of trauma.

Finally all branch members participate in teaching activities by discussing methods of care, research findings and techniques with others, both locally and nationally. This has aided understanding of the changes in wound healing and organ dysfunction which occur following thermal injury and added to scientific interchange in these areas of study.

PUBLICATIONS AND/OR PRESENTATIONS

See report of Clinical Division, USAISR

ABSTRACT

PROJECT NO. 3A161102B71R-01, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: ANESTHESIOLOGY

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

Period covered in this report: 1 January 1973 - 31 December 1973

Investigators: Stephen Slogoff, MD, Major, MC
Gary W. Allen, MD, Major, MC
Allister K. Morris, MD, Major, MC

Reports Control Symbol MEDDH-288(R1)

In 1973, 141 of 273 patients whose disposition was completed at the United States Army Institute of Surgical Research were given 377 anesthetics at this institute. Of the anesthetics given, 40.3% were halothane, with or without nitrous oxide in oxygen, 33.5% were ketamine, with the remainder consisting of nitrous oxide, barbiturate, Ethrane with nitrous oxide and oxygen, and regional blocks. Of those patients receiving anesthesia at the Institute of Surgical Research, the mean number of anesthetics per patient was 2.67. One intraoperative complication occurred during the year and will be discussed in detail in the text. No intraoperative deaths occurred. During this year, subanesthetic ketamine for debridement or dressing change in the Hubbard tank became accepted practice, and approximately 100 patients received 400 exposures.

ANESTHESIOLOGY

The following is a description of current anesthetic practices and techniques at the US Army Institute of Surgical Research. Pertinent statistical data are included in this report.

PREOPERATIVE PREPARATION

Patients for elective surgery are held NPO after midnight. This usually involves a fasting period of some 8-14 hours. Infants and children through age four are permitted clear liquids until 0400 hours. Using this regimen, we have had no vomiting or aspiration of stomach contents on induction in patients for elective surgery. Seriously ill or dehydrated patients are given intravenous fluid preoperatively including Ringer's lactate and 5% dextrose in Ringer's lactate or saline solution. Solutions designed for pediatric use are given to infants and children.

HEMODYNAMIC AND RESPIRATORY ASSESSMENT

All acutely ill patients have arterial blood gas determinations made daily until their status improves at which time the frequency of determinations is decreased. By knowing these values preoperatively in all seriously ill patients, we are able to adjust our anesthetic techniques accordingly. Patients who are hypoxemic and require ventilatory assistance are transported to and from the operating room with the administration of 100% oxygen, given by positive pressure, utilizing either a Jackson Rees modification of the Ayre's T-piece (Smith, R, Anesthesia for Infants and Children, St. Louis, C. Mosby Co., 1968)[1] or a Bird respirator. Once in the operating room, patients requiring ventilatory assistance may be ventilated manually or with an Air Shield anesthetic ventilator. Circulatory status is assessed by hematocrit, serum electrolytes and osmolality, and urine output, in addition to direct or indirect measurements of blood pressure. Central venous pressure measurements are taken on seriously ill patients.

PREMEDICATION

In general, no narcotics, barbiturates, or anorectics are given preoperatively to adult patients. Rather, atropine, 0.01 mg/kg, is given intravenously 10 minutes prior to induction of general anesthesia. Patients receiving regional anesthesia (regional nerve blocks, spinal, and epidural anesthesia) do receive premedication consisting of a barbiturate, anticholinergic, and occasionally a narcotic (morphine or Demerol®) or anorectic (Valium®). Pediatric patients generally receive

a narcotic plus an anticholinergic agent preoperatively in order to allay anxiety and induce a state of quiescence (Smith R, Anesthesia for Infants and Children, St. Louis, C. Mosby Co., 1968).[1]

TYPES OF ANESTHESIA

A. GENERAL ANESTHESIA

1. Halothane with or without nitrous oxide in oxygen

Approximately 40% (40.3) of the anesthetics at our institution are performed with this combination of agents due to ease of administration, tranquil induction and emergence, relative lack of long lasting cardiovascular depression, and nonflammability. We have to date observed no cases of halothane hepatotoxicity. Since the incidence of this complication is approximately one in 10,000 patients, this seems to be an acceptable risk when it is weighed against the great advantages of the use of this agent in the burn patient (Klatskin G, Kimberg DV, New Eng J Med 280:512-522, 1969).[2] Thiopental (2 to 4.5 mg/kg) or ketamine, intravenously (2 mg/kg) are used in about half of these patients for induction of general anesthesia with no deleterious effects observed. The remainder are induced with inhalation technique. Using this form of anesthesia, we have not observed any significant incidents of prolonged emergence or postoperative grogginess, even in patients who receive thiopental or ketamine inductions, provided that the last incremental dose of the intravenous agent was given more than 30 minutes before the end of the case.

2. Nitrous oxide-relaxant

This technique is often used in very seriously ill patients for laparotomies and other major procedures (amputations, etc.) due to its relative lack of cardiovascular depression. Since the technique requires controlled respiration, the trachea is intubated. Relaxants employed include pancuronium, d-tubocurarine, and gallamine, all nondepolarizing relaxants. The latter has been shown not to raise serum potassium in burn patients (Carr J, Kitchings OE, Garfield JM, et al, Unpublished data presented at annual meeting of the American Society of Anesthesiologists, San Francisco, California, October 1969).[3] Succinylcholine is rarely used except for acute emergencies due to its tendency to cause severe rises in potassium from about postburn day 15 through postburn day 90 (Schaner PJ, Brown RL, Kirksey TD, Gunther RC, Ritchey CR, Gronert GA, Anesth Analg (Cleveland) 48:764-770, 1969).[4]

3. Enflurane (Ethrane®) with nitrous oxide in oxygen

Enflurane is a newly available, nonflammable, inhalation anesthetic agent which has the chemical structure of a halogenated ethyl-methyl ether. The action of this agent is very similar to halothane in uptake, onset of action, and emergence time. In extensive clinical trials prior to release, and six months of clinical usage, the drug has demonstrated no propensity for hepatic toxicity. The only adverse reaction associated with this drug not seen with other halogenated agents is a tendency to produce twitching or involuntary motor activity associated with seizure activity on electroencephalogram at very deep levels of anesthesia, which is exacerbated by hypocapnea. This reaction is rarely seen in clinical concentrations of the anesthetic as long as excessive hyperventilation is not used. No sequelae have been observed from this complication after emergence from anesthesia. During the year 1973, 10 patients at the Institute of Surgical Research received enflurane with nitrous oxide for their anesthesia. No complications were observed. No seizure-like activity was seen. The agent is an extremely weak analgesic, and probably has limited usefulness in the armamentarium of the anesthetist.

4. Ketamine

Ketamine is an intravenous "dissociative" general anesthetic which has been available for clinical use for approximately four years. Approximately one-third (33.5%) of our anesthetics in the operating room are now administered with this agent. Since cardiovascular reflexes and tone are well preserved and a patent airway with good ventilation is usually maintained, even in the lateral and prone positions, this anesthetic has permitted numerous operations to be carried out without the use of an artificial airway. These factors plus the tremendous versatility of the agent for somatic procedures have significantly improved anesthetic management for the thermally injured patient.

5. Subanesthetic ketamine

We have demonstrated in a clinical study (Slogoff S, Allen GW, Wessels, JV, Cheney DH, In Press, Anesth Analg [Cleveland])[5] that ketamine in subanesthetic doses can be extremely useful for the relief of the pain and anxiety associated with debridement and dressing change in the Hubbard tank. Currently, this technique is being used widely at the Institute of Surgical Research. Approximately

100 patients received 400 administrations of intramuscular subanesthetic ketamine for debridement during 1973. Its extreme usefulness and freedom from complications has been continuously demonstrated.

B. REGIONAL ANESTHESIA

Our criteria for regional anesthesia are that a candidate for a nerve block must not be septic, must have a normal mental status, and must not have burns or local infection at or immediately adjacent to the site of the proposed nerve block. By following these guidelines for selection of patients, we have had no complications with regional anesthesia and no incidence of infection or sepsis after nerve blocking was noted.

MONITORING TECHNIQUES

Below is an outline of our current monitoring techniques for patients under anesthesia:

A. CIRCULATION

1. Precordial and/or esophageal stethoscope.

2. Pulse monitoring by (a) one finger over pulse; (b) optical pulse sensor placed on finger.

3. Blood pressure cuff (when feasible to apply).

4. Central venous pressure (CVP) assessment.

5. EKG (major cases and seriously ill patients).

6. Sponge weighing; major cases.

7. Special measurements of urine output during surgery.

B. RESPIRATION

1. Counting of respiratory rate.

2. Observation of chest and rebreathing bag.

3. Auscultation of chest.

4. Determination of tidal volume by Drager respirometer in anesthesia circuit.

5. Periodic assessment of blood gases intraoperatively when indicated.

C. TEMPERATURE

1. Rectal or esophageal thermistor probe; routine for cases lasting more than 45 minutes and in all children.

It should be noted that the K-thermia heating-cooling blanket has proved to be of significant value in maintaining body temperature when large areas of the body are exposed. In addition, it can help to lower body temperature rapidly and safely when a febrile episode occurs intraoperatively. Ambient temperature in the operating room is maintained at 71-72° F., and this has been shown to be of benefit in minimizing heat loss. Difficulty in maintaining the temperature of most children and some adults is still a problem and techniques and devices to overcome this are being evaluated at the present time.

COMPLICATIONS

Case No. 1

Significant Cardiac Arrhythmia During Anesthesia

This 54 year old white male was involved in a tractor accident in which he sustained a fracture of the pelvis and acid burns to the left leg. He was admitted to the Institute of Surgical Research for debridement and grafting of the burn injury. His past history was relevant in that there had been episodes of cardiac arrhythmia since 1968, the nature of which was unknown. Prior to admission, and throughout his hospital stay, he was treated with Inderal,® 10 mg q.i.d., and digoxin, 0.25 mg once a day. No other symptoms referable to the cardiovascular system were elicited. A left herniorrhaphy in 1969 and right herniorrhaphy in 1972 were performed under spinal anesthesia without complication. On the 27th day postinjury, he was brought to the operating room for excision of the burn wound, which was carried out under spinal anesthesia without complication. Fourteen days later, he was returned to the operating room for further debridement, which was to be carried out under general anesthesia. Electrocardiographic monitoring was instituted prior to induction, which showed no arrhythmia.

Blood pressure and pulse rate were within the normal range. Anesthesia was induced with halothane and oxygen, utilizing spontaneous ventilation, and, approximately five minutes after induction, multifocal premature ventricular contractions developed which completely replaced conducted sinus rhythm. Fifty mg of lidocaine Hcl were given intravenously as a bolus with no clinical response. Since no hypotension occurred during the arrhythmia, and the rate of contraction of the ventricles was acceptable, it was decided to continue the surgery, which was expected to be of short duration, with careful monitoring. The arrhythmia persisted during the entire anesthetic, which lasted approximately 20 minutes, and for approximately 10 minutes after discontinuation of the anesthetic. At no time did hypotension occur, and the patient demonstrated no sequelae postoperatively. Two weeks later, the patient was brought to the operating room for grafting, which was performed under spinal anesthesia without complication. The patient was discharged approximately two months postinjury to his family physician. No further episodes of cardiac arrhythmia were noted during his hospital stay.

Comment. Cardiac arrhythmias are a not uncommon occurrence during anesthesia. In this particular case, there were no predisposing intraoperative factors other than induction of anesthesia for the arrhythmia. The patient's history was somewhat nebulous, but apparently was real, for cardiac arrhythmia. His ability to tolerate spinal anesthesia has been demonstrated, and the complication during general anesthesia was carefully explained to him for future reference. The mechanism of this complication is unclear.

REFERENCES

1. Smith R: Anesthesia for Infants and Children, St. Louis, C. Mosby Co., 1968.

2. Klatskin G, Kimberg DV: Recent hepatitis attributable to halothane sensitization in an anesthetist. New Eng J Med 280:515-522, 1969.

3. Carr J, Kitchings OE, Garfield JM, et al: Unpublished data presented at Annual Meeting of the American Society of Anesthesiologists, San Francisco, California, October 1969.

4. Schaner PJ, Brown RL, Kirksey TD, Gunther RC, Ritchey CR, Gronert GA: Succinylcholine induced hyperkalemia in burned patients, Anesth Analg (Cleveland) 48:764-770, 1969.

5. Slogoff S, Allen GW, Wessels JV, Cheney DH: Clinical experience with subanesthetic ketamine. In Press, Anesth Analg (Cleveland).

PUBLICATIONS

Wessels JV, Allen GW, Slogoff S: The effect of nitrous oxide on ketamine anesthesia. Anesthesiology 39:382-387, 1973.

Slogoff S, Allen GW, Warden GD, McManus WF: Tracheoesophageal fistula following prolonged tracheal intubation in a thermally injured patient. Anesthesiology 39:453-455, 1973.

Slogoff S, Allen GW, Wessels JV, Cheney DH: Clinical experience with subanesthetic ketamine. In Press, Anesth Analg (Cleveland).

Cheney DH, Slogoff S, Allen GW: Ketamine-induced stress ulcers in the rat. Anesthesiology 40:531-535, 1974.

Slogoff S, Allen GW: The role of the baroreceptors in the cardiovascular action of ketamine. In Press, Anesth Analg.

PRESENTATIONS: None

Table 1. Overall Patient Data, USAISR (1965-1973)

	A	B	C	D	E	F	G
	No. Patients	No. Patients Anesthetized (ISR Only)	B/A X 100	Total Anesthetics (ISR Only)	D/A	D/B	Average Per Cent Burn
1965	174	107	61.5	495	2.84	4.63	33
1966	311	181	58.2	713	2.29	3.94	30
1967	389	239	61.4	670	1.72	2.80	28
1968	389	259	66.6	794	2.04	3.07	30
1969	294	189	64.3	601	2.04	3.18	36
1970	321	198	61.7	497	1.55	2.51	30
1971	301	179	59.5	475	1.58	2.65	31
1972	301	183	60.8	575	1.91	3.14	34
1973	273	141	51.6	377	1.38	2.67	38.5

Table 2. Nature of Surgery, USAISR

Procedure	1971		1972		1973	
	No.	%	No.	%	No.	%
Debridement and/or Homograft	74	15.5	113	19.7	81	21:5
Autograft	252	52.9	295	51.3	198	52.6
Orthopedics	62	13.0	51	8.9	30	8.0
Ear (Chondrectomy)	19	4:0	18	3.1	10	2.6
Eye and Lid	18	3.8	4	0.7	7	1.8
Intra-abdominal	8	1.7	45	7.8	8	2.1
Tracheostomy & Bronchoscopy	22	4.6	38	6.6	25	6.6
Other	21	4.4	11	1.9	18	4.8
Total	476		575		377	

Table 3. Techniques of Anesthesia, USAISR - 1973

Technique	Number	Per Cent
Halothane	152	40.3
Ethrane	10	2.6
N_2O, O_2	68	18.0
Ketamine	126	33.5
Ketamine alone 103		
Ketamine with N_2O 23		
Barbiturate	10	2.6
Other	8	2.2
Total General Anesthetics	374	99.2
Local Infiltration & Nerve Block	3	0.8
Total Local Anesthetics	3	0.8
All Anesthetics	377	100

Table 4. General Anesthesia Induction Agents, USAISR - 1973

Agent	No. of Inductions	Per Cent of Total
IV Barbiturate	83	22.2
IV Ketamine	101	27.0
IM Ketamine	69	18.4
IV Other	11	3.0
Inhalation	110	29.4
Total	37.4	100

Table 5. Type of Airway During General Anesthesia, USAISR - 1973

	No. of Anesthetics	% of Total No. of General Anesthetics
Mask	116	31.0
Endotracheal Tube		
Oral	107	28.6
Nasal	21	5.6
Tracheotomy	27	7.2
Natural Airway	103	27.6

Table 6. Use of Muscle Relaxants, USAISR - 1973

Total General Anesthetics	No. of Anesthetics Where Muscle Relaxants Used	dT-Curarine	Gallamine	Pancuronium	Succinylcholine
374	44	2	11	31	0
% of Total Gen. Anesth.	11.8	0.5	2.9	8.3	0

	No. of Anesthetics	% of Total General Anesthetics
Muscle Relaxant Used	44	11.8
Used for Intubation	19	5.1
Used for Relaxation	33	8.8

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0A 6956	74 07 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
73 07 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	61102A	3A161102B71R	01	141
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) Clinical Operation, Metabolic Branch, Renal Section, For Treatment of Soldiers With Renal Failure (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
52 07	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 74	1.1	27
	CURRENT 75	.8	29

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

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

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

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME: Richard H Merrill, MAJ, MC
TELEPHONE: 512-221-4307
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: Philip W Rogers, LTC, MC
NAME: DA

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code): (U) Renal failure; (U) Hemodialysis; (U) Soldiers; (U) Peritoneal dialysis

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

23. (U) To provide diagnostic support and consultation for the thermally injured patient and to initiate treatment as is necessary to include both peritoneal and hemodialysis. In addition, the renal section is involved in clinical research activities designed to improve patient care and our understanding of renal problems in the thermally injured patient. The renal section actively supports the clinical and research endeavors of the Nephrology Service at Brooke Army Medical Center in an effort to improve patient care to the soldier and his dependents.

24. (U) The renal section provides consultation to the physician involved in direct patient care to the thermally injured patient. Renal function assessed by various techniques and therapeutic interventions in the form of hemodialysis and peritoneal dialysis are available. In addition, the renal section is involved in several clinical and laboratory research protocols.

25. (U) 73 01 - 73 12 A total of 19 patients underwent hemodialysis in the renal unit for a total of 70 patient treatments. The renal section supervised one of peritoneal dialysis during the same period. Eight patients recovered and 11 patients expired. Members of the renal section have adapted a rapid bedside clotting test for the use in heparinization during dialysis. In addition, the unit is developing a new approach for acute hemodialysis; this work will be presented sometime in the future. Work is underway at present to produce a film showing our technique for venous to venous acute hemodialysis to be shown at various medical meetings around the country. Several other areas of clinical investigation are also underway.

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*Available to contractors upon originator's approval

ABSTRACT

PROJECT NO. 3A161102B71R-01, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: CLINICAL OPERATION, METABOLIC BRANCH, RENAL SECTION, FOR TREATMENT OF SOLDIERS WITH RENAL FAILURE

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

Period covered in this report: 1 July 1973 - 30 June 1974

Investigators: Richard H. Merrill, MD, Major, MC
Philip W. Rogers, MD, Major, MC

Reports Control Symbol MEDDH-228(R1)

A total of 19 patients underwent hemodialysis in the Renal Unit for a total of 70 patient treatments during the period 1 January 1973 - 31 December 1973. The Renal Section supervised one peritoneal dialysis during the same period. Eight patients recovered and 11 patients expired. Members of the renal section have adapted a rapid bedside clotting test for use in heparinization during dialysis. This work was presented at the Southeastern Dialysis and Transplant Association Meeting in Little Rock, Arkansas in August 1973 and has been published in the Journal of Dialysis and Transplantation. In addition, the unit is developing a new approach for acute hemodialysis. Work is underway at present to produce a film showing our technique for venous to venous acute hemodialysis to be shown at various medical meetings around the country. Several other areas of clinical investigation are also underway.

Renal failure
Hemodialysis
Soldiers
Peritoneal dialysis

CLINICAL OPERATION, METABOLIC BRANCH, RENAL SECTION, FOR TREATMENT OF SOLDIERS WITH RENAL FAILURE

The renal section is composed of the chief of the section, a Nephrologist, Medical Corps, and two enlisted technicians, including an NCOIC, and is physically located on Ward 13B. The unit encompasses a one-bed acute dialysis unit and two hemodialysis machines, one a porportioning unit and the other a portable system for use in instances where the patient cannot be moved to the Hemodialysis Unit. The primary mission of the Renal Section is to support the operation of the Clinical Division of the Burn Unit, providing both consultation for patients with renal and metabolic problems and hemodialysis in cases of renal failure. A secondary mission of the unit has been to support the Nephrology Service of Brooke Army Medical Center. The USAISR Hemodialysis Unit now provides backup support when necessary and assists in treatment of cases of acute renal failure occurring at Brooke Army Medical Center. The USAISR Nephrology Staff continues to participate actively in the hospital Nephrology Training Program. The chief of the Metabolic Branch directs the BAMC Nephrology Service and the chief of the Renal Section directly supervises the Brooke Army Medical Center Hemodialysis Unit.

Several patients were dialyzed using the Seldinger technique for femoral vein catheterization, in conjunction with the unipuncture-machine, which allows dialysis with one venipuncture. Dialyzers used routinely include the Travenol 145, the Travenol 202, the Extracorporeal EX-23 and EX-P, and the Travenol UF. 64 and the Cordis-Dow Kidney.

In addition to the dialysis support provided to the hospital and the unit, several pilot studies have been initiated. A new technique has been introduced into the Hemodialysis Unit for controlling blood anticoagulation during dialysis. The results of this innovation were reported at the Southeastern Dialysis and Transplant Meeting and have been published. In addition, a videotape has been produced showing our technique of unipuncture dialysis via femoral catheter and has been submitted as a display as well as presentation at a scientific meeting. Other projects underway include measurement of residual blood volume in coils, evaluation of urinary sediment in thermally injured patients, and use of high-dose heparin prime in hemodialysis.

PRESENTATIONS:

Howard L. Baer, Richard H. Merrill. Bason Test. Southeastern Dialysis and Transplantation Meeting, Little Rock, Arkansas, 10-13 August 1973.

PUBLICATIONS:

Howard L. Baer, Richard H. Merrill. Dialysis and Transplantation: 3, No. 3, 10, Apr, May 1974.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0D 6951	74 07 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRAD	8A. DISP'N INSTR'N	8B. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
73 07 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	61102A	3A161102B71R	01	084
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)

(U) Detection of Endotoxin in Burned Soldiers with Sepsis (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
71 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 74	.4	10
FISCAL YEAR CURRENT 75	.4	11

19. RESPONSIBLE DOD ORGANIZATION

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

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TELEPHONE: 512-221-2018

SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS

NAME: Virginia C English, MS

NAME: Basil A Pruitt, Jr, COL, MC DA

21. GENERAL USE

FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Endotoxin; (U) Sepsis; (U) Assay; (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 feasibility and accuracy of the Limulus blood coagulation test in the detection of endotoxin in the blood of burned soldiers.

24. (U) Burn patients 5 years old and above, with 30% or greater burn area, in whom a clinical suspicion of endotoxemia or septic shock exists, are admitted to the study. Seven ml of heparinized blood are drawn, the plasma is separated by centrifugation and tested for the presence of endotoxin using the Limulus amaebocyte lysate reaction. Plasma is extracted with glacial acetic acid then neutralized to pH 5.2 plus or minus 0.05 and the supernatant fluid is tested for endotoxin. Serial observations are maintained when possible to broaden the base for interpretation of results.

25. (U) 73 07 - 74 06 Patients with plausible gram-negative sepsis have been shown to have demonstrable endotoxin in plasma in approximately 28% of cases. This degree of positive reaction does not make the in vitro detection of endotoxin a useful clinical test in its present form; since the reaction can detect 0.1 monogram of endotoxin per ml, it does not appear that the defect is one of sensitivity. Current efforts are directed toward improved extraction technics, inclusion of platelets in the plasma for extraction, and prolonged vigorous chloroform extraction are both under assessment. Detection of endotoxin in the liver of patients dying with endotoxemia has been successfully established, and this assay will aid in validating the diagnosis of endotoxic sepsis and shock. This assay will be pursued further.

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*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

ABSTRACT

PROJECT NO. 3A161102B71R-01, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: DETECTION OF ENDOTOXIN IN BURNED SOLDIERS WITH SEPSIS

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

Period covered in this report: 1 July 1973 - 30 June 1974

Investigators: Robert B. Lindberg, PhD
Virginia C. English, MA
Arthur D. Mason, Jr., MD
Basil A. Pruitt, Jr., MD, Colonel, MC

Reports Control Symbol MEDDH-288(R1)

Endotoxin (ET) can be detected in plasma by extracting plasma with acetic acid, neutralizing, and testing dilutions of the supernatant fluid with Limulus amoebocyte lysate. The reaction has been positive, with amounts of ET of 0.003 to 0.038 µg/ml, in approximately 30% of burn patients with sepsis and septicemia. A significant part of the population with gram-negative bacteremia fails to show ET present; studies are presently under way to eliminate the possibility that binding of ET to platelets, or failure of the current extraction procedure to recover protein-bound ET, may resolve this discrepancy. Tests of liver tissue from 52 patients dying with severe burns revealed ET present in amounts ranging from 0.0006 µg/gm to 1.3 µg/gm; 35 patients had ET at 0.004 µg/gm or greater, with a mean value of 0.124 µg/gm. This amount is well above that which could be accounted for by the ET contributed by bacteria in the liver. Seventy-eight percent of patients dying with severe burns had ET in the liver; the inference that endotoxemia is a major factor in fatal sepsis is reinforced by this finding.

Endotoxin
Sepsis
Assay
Humans

72<

DETECTION OF ENDOTOXIN IN BURNED SOLDIERS WITH SEPSIS

With the advent of a relatively simple laboratory procedure for detecting *in vitro* the presence of endotoxin in body fluids and tissue, a marked increase in interest in endotoxemia and the phenomenon of "endotoxic shock" occurred. The original communication of Levin (Levin J, Bang FB. Bull. Johns Hopkins Hosp. 115:265, 1964)[1], regarding the *Limulus* amoebocyte lysate gelation reaction with endotoxin led to studies which ultimately permitted the quantitation of the reaction by Reinhold (Reinhold RB, Fine J. Proc Soc Exper Biol Med 132:599, 1969)[2]. His pH adjustment technic for releasing protein-bound endotoxin in plasma and tissue has been used to study burn patients with and without bacteremia, and with varying degrees of tissue invasion. The results in this and in other laboratories undertaking similar studies have been equivocal. Obviously, a direct, positive correlation between bacteremia and a positive lysate test, to connote endotoxemia, would be useful and enlightening. What has actually happened is that in a significant number of cases, gram-negative bacteremia results in endotoxemia as evinced by the lysate reaction, but in a larger proportion of cases, endotoxin is not demonstrable. This phenomenon may be literally correct, or it may reflect failure to detect endotoxin due to unidentified technical reasons. This report is a progress note on application of this reaction to samples from severely burned patients in 1973.

METHODS

The technic of Reinhold and Fine (2), as described in a previous report (Lindberg RB, English VC, Pruitt BA, Jr., Mason AD, Jr. USA Inst Surg Res Ann. Rpt. FY 1973, Sec. 6)[3] was used. Since no differences could be demonstrated in plasmas chilled and those held at room temperature, the samples were cooled until processed. Plasma was separated at 1500 rpm. Recent questions regarding the possible sequestration of endotoxin in platelets led to tests of platelet-rich and platelet-poor plasma. This variable is still under study. Adjustment of the plasma-extract to pH 6.25 to 6.30 with a micro-electrode system was carried out in some series to evaluate possible sequestration of the endotoxin at varying pH.

The *Limulus* lysate used was from the stock collected in 1971 by Lindberg and Inge (Lindberg RB, Inge WW, Pruitt BA, Jr., Mason AD, Jr. USA Inst Surg Res Ann. Rpt. FY 1972, Sec. 55)[4]. This material, representing over 20 pooled collections separately titrated and stored, has been held since April 1972 at -70°C; each lot is titrated when thawed, prior to use. The experience of this laboratory will add to the body of knowledge regarding the retrievability of this material. Stability after thawing has been variable, and the lysate is thawed only one vial at a time, to minimize differences in successive tubes.

Negative results have, in some instances, been obtained with undiluted plasma or tissue extracts, when the same extracts, diluted, gave positive reactions for endotoxin. The nature of this inhibitory factor is not known, but since whole strength extract alone might fail to reveal the presence of endotoxin, the routine test has been modified to include testing dilutions of the extract. In most instances, this procedure becomes the quantitative test; dilutions up to 1:64 or 1:128 are tested. The inhibition occurring in undiluted lysate has in most instances been seen only with relatively high concentrations of endotoxin in blood or tissue.

RESULTS

Demonstration of Endotoxin in Patients with Bacteremia or Clinical Sepsis. Although endotoxin can be detected in fractions of monograms by the amoebocyte lysate gel reaction, efforts to detect endotoxin in the plasma of patients who were recognized as septic or with positive blood cultures have not been as effective as the delicacy of the reaction with endotoxin would lead one to expect. This may not be an actual discrepancy, since it is only a presumption that "endotoxic shock" always denotes the presence of circulating endotoxin. In any event, the results of tests on 25 patients in 1973, as summarized in Table 1, show demonstrable endotoxin in the plasma of 7 patients out of 20 who had positive blood cultures plus one who, although regarded as septic, did not yield a positive blood culture. The four patients listed as "sepsis unconclusive" gave negative reactions, but since the subsequent course of these patients did not validate the initial presumption of sepsis, their inclusion in the total tested is equivocal. With their exclusion, 7 out of 21, or 33% of septicemic or septic patients had endotoxin in the blood stream. This rate of positive reactors among patients with gram-negative septicemia was slightly higher than that found with a comparable group of patients in 1972, when the positive reactor rate was 28%.

The amount of endotoxin detected in blood of patients with endotoxemia and the consistency of the reaction are shown in Table 2. Although in our system the occurrence of a gram-negative bacteremia did not of itself assure a positive endotoxin reaction. This group of six patients with bacteremia each had at least one organism of the Enterobacteriaceas recovered from blood. The two patients with Staphylococcus aureus recovered each had coliform organisms recovered as well. The correlation of gram-negative bacteremia with endotoxemia was complete.

The amounts of endotoxin recovered were relatively high. With 15 samples of plasma positive for endotoxin, the mean level was 0.025 µg/ml. The sensitivity threshold of the reacting system was less than 0.001µg/ml, but for the sake of illustration, presuming that a valid positive reaction could be obtained with 0.001 µg/ml, then the mean level of endotoxin percent was at least 25 times the threshold detection level. Two patients had more than 0.038 µg/ml of endotoxin, but the test was not calibrated to

Table 1. Endotoxin in Plasma of Patients with Bacteremia and/or Signs of Sepsis

	Patients
Blood culture negative, sepsis inconclusive Endotoxin reaction negative	4
Blood culture positive, Endotoxin negative	14
Blood culture positive, Endotoxin positive	6
Blood culture negative, Endotoxin positive	1
Total patients with Endotoxemia	7
Percent of bacteremia/sepsis cases with positive reactions	28%

Table 2. Patients with Positive Plasma *Limulus* Lysate Reactions: Correlation with Bacteremia

Patient No.	Blood Culture Results	*Limulus* Lysate Reaction	Endotoxin mcg/ml
17	Klebsiella; Candida	Positive (5)	0.038 0.003 0.012 0.012 0.012
48	S.aureus; E.coli; Ps.aeruginosa	Positive (2)	0.038 or> 0.013
22	E. coli	Positive (2)	0.038 0.028
55	Negative	Positive (2)	0.015 0.015
23	E. coli	Positive (1)	0.038 or >
39	Ent. hafniae; S.aureus; E. coli; Prov. stuartii; Ps. aeruginosa	Positive (2)	0.01 0.038
24	Klebsiella pneumoniae	Positive (1)	0.07

detect more than this amount per ml.

There were 18 patients, 14 of whom had positive blood cultures, in whom gram-negative sepsis was presumed to be a clinical possibility. None of these patients exhibited endotoxin in the blood stream. The results of these tests are summarized in Table 3.

Four of these patients had negative blood cultures; there was no basis except clinical impression for considering them in the category of endotoxic shock. Three more yielded only Staph aureus in blood culture. Gram positive cocci do not contain endotoxin, nor does staphylococcal bacteremia prompt appearance of endotoxin in the blood stream. The remaining 10 patients had at least one episode of gram-negative bacteremia, although in three of these the organism was Ps aeruginosa. While endotoxin can be recovered from pseudomonads, this genus is not as potent a source of endotoxin as are the Enterobacteriaceae. When gram-negative bacteremias are compared, there were six patients with gram-negative bacteremia and no endotoxin demonstrated. Thus approximately 38% of patients with gram-negative bacteremia were positive for endotoxin by the amoebocyte lysate test.

Endotoxin in Liver Samples of Burn Patients at Autopsy. It has been reported that circulating endotoxin is rapidly removed from the blood and sequestered in the liver, where its subsequent destruction is effected macrophage action. In view of the considerable number of patients in whom no circulating endotoxin was demonstrable, despite the presence of clinical sepsis and gram-negative bacteremia, a study of samples of liver collected at autopsy was made. One gram samples were homogenized in pyrogen-free Ten Broeck grinders, the homogenate taken up in pyrogen-free distilled water, gently centrifuged, and the supernate extracted in the same manner that was used for plasma. There were 52 patients whose livers were thus assayed at autopsy. Of these, 41 (78%) exhibited endotoxin. A summary of antemortem results as related to liver endotoxin assay is given in Table 4.

A bacterial count was made on each sample. The mean bacterial level was 3.0×10^3. Thus an average of 3000 bacteria was present when the tissue was extracted. This number may permit a positive endotoxin reaction when the organisms belong to the Enterobacteriaceae, but the reaction would be minimal. It would usually indicate 0.001 µg/ml or less. As will be shown below, all but one reacting sample had an endotoxin level well above this level.

Two of the positive liver samples yielded only gram-positive cocci, and two more were sterile on culture. This left 37 in which gram-negative bacteria were recovered, either alone or in mixture with gram-positive cocci. Most of the autopsied patients had not had plasma endotoxin levels assayed, but eight had been so tested. Out of these, three patients had positive reacting plasma; five were negative. There was no difference in the

Table 3. Patients with Negative Plasma Limulus Lysate Reaction: Clinically Possible Sepsis

Blood Culture	Endotoxin	No. of Patients
Negative	Negative	4
S. aureus	Negative	3
S. aureus; E. coli	Negative	1
Ent. cloacae; Candida sp..	Negative	1
Staph.epidermidis; Ent. cloacae	Negative	1
Klebsiella pneumoniae	Negative	1
Candida sp; Ps aeruginosa; Corynebacterium sp.	Negative	1
Ps. aeruginosa	Negative	2
Staph. aureus; S. epidermidis; Prov. stuartii; Ps.aeruginosa	Negative	1
Candida sp.; Prov. stuartii	Negative	1
Staph. aureus; Prov.stuartii; Ps.aeruginosa; Strep non-hemo.	Negative	1
Total patients:		18
Staph only		4
Gram-negative bacilli only		3
Staph plus Gram-negative bacilli		4
Gram-negative bacilli + Candida sp.		3

Table 4

SURVEY OF PATIENTS FROM WHOM AUTOPSY LIVER SPECIMENS YIELDED DETECTABLE ENDOTOXIN

NUMBER OF PATIENTS: 41 AVERAGE COUNT: 3.0×10^3

ORGANISMS FOUND:

Gram Positive:	2	Gram Positive & Negative:	18
Gram Negative:	19	Negative:	2

ANTEMORTEM PLASMA LIMULUS LYSATE REACTIONS:		ANTEMORTEM BLOOD CULTURE REACTIONS:	
Reactors:	3	BC Positive:	3
		BC Negative:	0
		BC Not Done:	0
Nonreactors:	5	BC Positive:	5
		BC Negative:	0
		BC Not Done:	0
Plasma Limulus test not done:	33	BC Positive:	21
		BC Negative:	6
		BC Not Done:	6

antemortem blood culture results. All eight patients had positive blood cultures. Among the 33 patients with livers positive for endotoxin in whom no antemortem test was done on plasma, 21 had bacteremia, six had no positive blood culture and six had no blood cultures drawn. In patients on whom antemortem plasma levels of endotoxin were not available, one can conclude that the sequestration of endotoxin occurred whether or not blood cultures were positive. The presence of bacteremia was not a prerequisite for appearance of endotoxin in the liver, but by its nature, sepsis is most often associated with bacteremia. The proportion of non-bacteremic reactors will always be small.

The amount of endotoxin which can be recovered from liver is a critical factor in assigning significance to its presence in that organ. Obviously if the amount reflected only the bacteria present in the liver, it would have no provable significance in relation to endotoxemia as a factor in sepsis. If, however, the amount is much greater than could be accounted for by the number of bacteria in the liver at autopsy, then the presence of endotoxin is more plausibly related to potential antemortem endotoxemia.

The amounts of endotoxin and the number of times a given amount appeared are summarized in Table 5. Out of 41 liver samples, two were suspect as being contaminated with feces. Of the remaining 39, thirty-five had levels from 0.004 μg/ml to 1.3 μg/ml. We have not been able to obtain positive Limulus lysate reactions with fewer than 1000 organisms per ml. At that concentration, the quantitative reading was at the lower limit of detectability, or 0.0006 μg/ml. A quanitity of endotoxin which gave a value of 0.002 μg/ml would represent 3 to 4 times the estimated amount recoverable from 10^3 gram-negative bacteria per ml (or gram). Thus, even if the 0.002 μg/ml levels were considered to be directly due to the bacteria present, a level of 0.004 μg/ml would be six and two-thirds times more than could be ascribed to the bacterial present in the liver sample. Thus 35 out of the 41 patients with positive endotoxin reactions exhibited significant amounts of endotoxin in the liver.

The source of this endotoxin in the liver can only be speculative at this point, but since most if not all of the patients were septic for varying periods of time prior to death, the liver plausibly represents a major site for removal of circulating endotoxin from the blood and its ultimate degradation and elimination. Three out of eight patients on whom antemortem tests for presence of endotoxin were conducted had positive tests for endotoxin in the plasma, with a mean level of 0.02 μg/ml. This positive reaction rate of 37% is consistent with the accumulating data which shows that about one-third of patients with gram-negative bacteremia have demonstrable endotoxin in the blood. This value is obviously related to the technics with which the plasma is extracted and with the criteria of a positive reaction.

There were 11 patients in whose livers no endotoxin was found. The average bacterial count for these livers was 3.5×10^2, in contrast

Table 5

ENDOTOXIN LEVELS OF AUTOPSY LIVER SPECIMENS

mcg ET/gm Tissue:	0.0006	0.002	0.004	0.005	0.01
Number of reacting specimens:	1	3	1	5	9

mcg ET/gm Tissue:	0.02	0.04	0.08	0.32	0.64
Number of reacting specimens:	6	3	4	3	3

mcg ET/gm Tissue:	1.3	2.56*	20.48*
Number of reacting specimens:	1	1	1

*Appearance and odor consistent with fecal material - possible bowel content contaminant

to a mean level of 3×10^3 for the 41 patients in whose livers endotoxin was demonstrated. Table 6 summarizes the data from patients with negative endotoxin tests on liver samples. There were none in which pure cultures of gram-positive organisms were found. Six had only coliform bacteria, four more yielded gram-positive cocci (Staph aureus or epidermides) and gram-negative bacilli, and one liver contained no recoverable bacteria. Only one of these patients had had an antemortem plasma assay for endotoxin. Among the 10 with no antemortem observations, two had had positive blood cultures; in 5, no blood cultures were recorded.

It appears that liver samples per se do not yield endotoxin on extraction. The bacterial content of these samples was not significantly lower than that of the samples from which positive reactions were obtained. The antemortem history of these patients, however, suggests that they had had a course less likely to be regarded as indicating sepsis. Five of them had not had blood cultures drawn; four out of the six on whom blood cultures were taken had no growth reported. More detailed scrutiny of the record of these patients is merited, to detect differences in the course of events between these patients and those in whom endotoxin was found post-mortem.

Integrity of the Limulus lysate stock. Inherent in studies using the Limulus amoebocyte lysate gelation test is an understanding of the status of the lysate itself. Is it unaltered in its reactivity, so that successive tests will be comparable and so that assays can be relied upon to yield meaningful quanititative data? When the lysate was collected by Lindberg, et al in 1971 (Lindberg RB, Inge WW, Pruitt BA, Jr., Mason AD, Jr. USA Inst Surg Res Ann. Rpt. FY 1972, Sec. 55)[4], it was stored at 4°C, in line with recommendations by such experienced workers as Levin and Fine. Throughout 1972, this material showed no overt signs of deterioration in reactivity with endotoxin, but the risk of ultimate contamination of of the storage vials (from fungi growing up through the threaded mouth of the vial) led to a change to storage at -70°C. Several lots of lysate were compared in behavior between the reactions occurring with the unfrozen and the frozen and thawed lysate. Table 7 shows these comparisons.

Successive samples from five different lots are compared. Lot V-1, held at 4°C, showed identical reactions in two successive samples. Lot V-2a Nr.2 exhibited a slight but definite decrease in reactivity during the period from April through August, when the samples were stored at 4°C. This reaction, although it left an effective lysate, added urgency to the change to storage at -70°C. Lot X-1 was compared in four samples, two of which were held at 4°C, while two were frozen at -70°C. The potency of reaction was identical in all of these samples. Lot X-2a Nr.1 included one sample held at 4°C, and two at -70°C. The latter samples showed a higher level of reactivity than the samples stored at 4°C. Lot W-1, with samples at 4°C and -70°C compared, was identical in each sample.

Table 6

SURVEY OF PATIENTS FROM WHOM AUTOPSY LIVER SPECIMENS YIELDED NO DETECTABLE ENDOTOXIN

NUMBER OF PATIENTS: 11 AVERAGE COUNT: 3.5×10^2

ORGANISMS FOUND:

Gram Positive:	None	Gram Positive & Negative:	4
Gram Negative:	6	Negative:	1

ANTEMORTEM PLASMA LIMULUS LYSATE REACTIONS:		ANTEMORTEM BLOOD CULTURE REACTIONS:	
Reactors:	None	BC Positive:	0
		BC Negative:	0
		BC Not Done:	0
Nonreactors:	1	BC Positive:	0
		BC Negative:	1
		BC Not Done	0
Plasma Limulus test not done:	10	BC Positive:	2
		BC Negative:	3
		BC Not Done	5

Table 7

THE INTEGRITY OF LIMULUS LYSATE STOCK

Lysate	Month	Degree of reaction at ET concentration in mcg: .01	.005	.0025	.00125	.0006	.0003	.00015
V-1*	Jan.	4	4	3	2	2	1	*
	Feb.	4	4	3	2	2	1	0
V-2aM2*	Apr.	4	4	4	4	3	2	1
	May	4	4	4	3	3	2	1
	Aug.	4	4	3	3	2	1	0
X-1	Feb.*	4	4	4	4	3	2	1
	Mar.*	4	4	4	4	3	2	1
	Sept**	4	4	4	4	3	2	1
	Dec.**	4	4	4	4	3	2	1
X-2aM1	May*	3	3	3	3	2	2	0
	Sept**	4	4	3	2	1	0	0
	Dec.**	4	4	4	4	3	2	1
W-1	Apr.*	4	4	4	3	2	1	0
	Aug**	4	4	4	3	2	1	0

LYSATE:
*Stored in refrigerator at 4°C.
**Stored at -70°C. & thawed before use.

0 = No reaction 1 = Positive but weak, unstable clot
2 = slightly more stable clot than 1+
3 = Tight clot, but slightly disrupted on tipping tube
4 = Firm clot forms in <1 hour; does not disrupt on tipping tube.

All lysates assayed by using E. coli O111:B4 lipopoly-saccharide (Difco) as endotoxin source

The conclusion that storage at -70°C is a valid and indeed a preferred method for storing amoebocyte lysate was substantiated. Subsequent experience indicates without exception that the deep-freeze environment is, and will henceforth be, the preferred storage condition.

DISCUSSION

The concept of endotoxemia as an essential component in "endotoxic shock" was the basis for extensive trials of the Limulus amoebocyte lysate reaction on plasma of patients with gram-negative sepsis and bacteremia, either gram-negative or gram-positive in origin. The lack of positive reactions in the plasma of patients with Staphylococcal bacteremia affirms the concept that gram-positive infections do not give rise to endotoxemia. In this study, the pH adjustment technic of Reinhold (2) was used to extract endotoxin from plasma. With this procedure, only about one-third of the patients with gram-negative bacteremia yielded positive reactions for endotoxin in plasma. It is possible that other extraction technics, including prolonged shaking with chloroform, may uncover positive reactors not revealed by the procedure which was used, and this possibility is being explored. Das, et al (Das J, Schwartz AA, Falkman J. Surgery 74:235-240, 1973)[5] suggested that separation of plasma with special precautions to collect the platelet-rich fraction would uncover more endotoxin than would be found in platelet-poor plasma. This too is to be explored. However, repeated samples under a variety of conditions still yield about a third of patients with positive endotoxin reactions in plasma. It is distinctly possible that this situation is real and reflects a situation in the septic patient in which only part of those patients with gram-negative bacteremia exhibit endotoxin in the plasma. It is implicit in this concept that rapid clearing of endotoxin from the blood may occur even in the very ill patient.

It was in view of these findings that observation of liver tissue from patients dying with severe burn injury was made. The number of patients with endotoxin demonstrable in liver was strikingly different from that observed with plasma. Seventy-eight percent of patients autopsied exhibited endotoxin in the liver, and the mean value of 0.124 µg/gm was far above the amount that could have been accounted for by the bacterial content of the liver samples. In the 22% of patients who did not give a positive reaction the bacterial content of liver samples was only slightly below that found among reactors. Only one-third of these patients when cultured had bacteremia, and their history did not typically reflect a probable gram-negative sepsis.

It was apparent that, in terms of correlation with a history of sepsis, the liver endotoxin content was far more meaningful than antemortem plasma containing endotoxin. The demonstration of endotoxin must be correlated in detail with the clinical and autopsy data. It appears at this point that this approach offers an added criterion for assessing cause of death. Presence of substantial amounts of endotoxin in the

liver may add significance to a clinical observation of antemortem shock, and its absence may be associated with the absence of shock. These correlations have not yet been made, but are projected.

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PRESENTATIONS and/or PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
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73 07 01	D. CHANGE	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

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(U) Antibiotic Sensitivity of Current Military Burn Patient Flora (44)

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22. KEYWORDS (Provide EACH with Security Classification Code) (U) Burn Wound Flora; (U) Antibiotic sensitivity; (U) Pseudomonas; (U) Providencia; (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) On-going monitoring of new antibiotics and of established antibiotics is essential if the problem of sepsis in severely traumatized patients is to be effectively controlled. The constant change of dominant nosocomial flora and emergence of resistant strains requires a continuing re-determination of optimal antibiotic on the basis of in vitro testing.

24. (U) Tube-dilution determination of minimum exhibiting concentration (MIC) is used on appropriate isolates. Emphasis is placed on strains causing sepsis.

25. (U) 73 07 - 74 06 Totally resistant strains of Providencia stuartii were recognized against all established antibiotics appropriate for gram-negative bacilli A new aminoglycoside, designated BB-K8, showed promise in treatment of Providencia infections; 52.6% of strains assessed were inhibited by 12.5µg or less per ml. Pseudomonas aeruginosa isolates were more sensitive than in 1972; over 80% of strains responded to colistin, Gentamicin or Carbonicillin. A new penicillin derivative, BRL-2285, was found to show promising activity against Pseudomonas aeruginosa, and was more potent than carbenicillin. Staphylococcus aureus type 84 remained predominant but a marked reversal of sensitivity occurred; methicillin-sensitivity was, for the first time in 5 years, increased from a low of 18% in 1972 to 70% of strains tested in 1973. Minocin and Clindamycin were both active against a significant proportion of Staphylococcus strains.

*Apollo No to contractors upon originator's approval

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ABSTRACT

PROJECT NO. 3A161102B71R-01, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: 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 July 1973 - 30 June 1974

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A major change in antibiotic sensitivity of major pathogenic species of bacteria in the burn wards occurred during 1973. Most conspicuous was the almost tota' disappearance of methicillin-resistant, and cross-resistant strains of Staphylococcus aureus, which was replaced by a series of strains relatively sensitive to antibiotic. Phage type did not change during this shift. Pseudomonas aeruginosa was primarily sensitive to gentamycin, colymycin and carbenicillin; with each of these, a shift toward increased sensitivity occurred. Minocin, tested for the first time, was effective against one-third of the strains. Klebsiella pneumoniae was most susceptible to kantrex, minocin, keflin, and gentamycin; sensitivity to colymycin decreased. No change in the totally refractory behavior of Providencia stuartii appeared; thus for the past three years there has been no antibiotic to use in sepsis due to this species. An experimental antibiotic, BB-K8 (an aminoglycoside) inhibited half of Providencia strains tested; this was the first indication of a drug that could reach this refractory species. Both BB-K8 and BRI-2288 (a semi-synthetic penicillin) were highly active against Ps aeruginosa. Despite in vitro effectiveness, clinical response of infection treated with these antibiotics was frequently less than encouraging.

Burn wound flora Providencia Antibiotic sensitivity

Pseudomonas Humans

ANTIBIOTIC SENSITIVITY OF CURRENT MILITARY BURN PATIENT FLORA

Antibiotic sensitivity of microbial strains recovered from burn patients summarized on an annual basis, serves as a useful procedure for detecting changes in the burn wound flora. These occur as a result of long term shifts in colonizing and invading strains; as applied in this Institute, these data offer a perspective on the complex shifting of nosocomial hospital flora in this extremely susceptible patient group. The species of major importance include staphylococci, members of the Enterobacteriaceae and Pseudomonas aeruginosa. Emergence of resistant flora, which occurs with distressing frequency, is best detected by periodic summation of behavior of strains recovered from patients with sepsis or other significant invasive involvement (Finland M. Ann Int Med 76: 1009, 1972[1]). Such samplings ensure that the antibiotic spectrum observed does indeed involve not only the important species but those strains of presumably greatest pathogenicity. Mechanisms of acquisition of antimicrobial resistance have been elaborated in the recent past, but this information does not aid significantly in preventing or controlling the emergence of resistant strains. Precise current information on antibiotic resistance remains essential for use of effective antibiotic therapy.

Technics and Source of Strains. Procedures for preparing antibiotic stock solutions were described in the Annual Research Progress Report, Section 7, 1973 (Lindberg RB, et al. USA Institute Surg Res Ann Res Rpt FY 73, Sec 7[2]). A tube dilution technic for determining Minimum Inhibitory Concentration (MIC) has been used in a standardized procedure, to make annual increments of information comparable with earlier accretions. Dilutions of antibiotic in distilled water were prepared in concentrations from 50 ug/ml down to 1.5 ug/ml, so that the addition of an equal volume of double strength nutrient broth would result in a standard nutrient environment. Inoculum was a 4 to 6 hour culture diluted to 10^{-2} or 10^{-3} in double strength broth to give a concentration of approximately 2×10^4 organisms per ml. The final inoculum strength sought was 1×10^4; naturally there was an unavoidable variation from this ideal concentration in successive tests. Minimum Lethal Concentration (MLC) was determined by plating out tubes showing negative end points at the 18 hour reading interval.

Sources of strains tested and the proportion of each species recovered from blood, sputum, wound or other sources are set down in Table 1. There were 322 strains tested. The largest proportion were from blood cultures. As has long been the case, sepsis was a major factor in morbidity and mortality in burn patients. Among gram negative bacilli, Providencia stuartii strains were the species most often recovered from blood culture. The other two species of greatest numerical import were Staphylococcus aureus and Ps aeruginosa. A relatively large number of pseudomonads tested were collected from the respiratory tract. Staphylococci, aside from blood stream isolates, were more often involved in wound infections than were other species. Klebsiella pneumoniae, ever ubiquitous in burn patients, was the fourth most important species numerically.

Table 1. Sources and Major Species Tested for MIC of Antibiotics, ISR, 1973

Species	No. Tested	Sources and % of all Strains Tested			
		Blood	Lung (or Sputum)	Wound*	Other**
Staphylococcus aureus	109	68.7	7.3	12.8	11.0
Staphylococcus epidermidis	20	85.0	10.0	0	5.0
Strep. non-hemolytic	11	81.8	18.1	0	0
Pseudomonas aeruginosa	51	62.7	23.5	7.8	5.8
Klebsiella pneumoniae	25	44.0	40.0	8.0	8.0
Entero. cloacae	15	86.6	6.6	0	6.6
Escherichia coli	11	45.4	45.4	9.0	0
Proteus mirabilis	8	75.0	12.5	12.5	0
Providencia stuartii	72	91.6	5.5	1.3	1.3
Total tested	322				

* Primarily biopsy cultures

** IV cannula tips, urine, ear, eye

90<

With this species a relatively high proportion of strains tested were recovered from sputum. The remaining species tested were recovered in small numbers although, since they were recovered from the blood, the imputation of a pathogenic role exists.

A standard battery of antibiotics has been set up for routine testing, with the two major categories of organisms being gram positive cocci and gram negative aerobic bacilli. Individual antibiotics are reviewed periodically; when a given antibiotic ceases to be effective against at least a fourth of the strains toward which it is directed, consideration is given to replacing it in the routine test battery with a more effective agent. At the end of 1972, tetracycline had so decreased in effectiveness that it was virtually useless as a therapeutic agnt for these patients. It was removed from the routine test battery and replaced by minocin. Although minocin is an analogue of tetracycline, its in vitro behavior has shown far more promise of effectiveness. Clindamycin was also included in the battery of agents directed toward staphylococci, since experience of the past three years had shown a striking rise in the incidence of antibiotic resistant staphylococci in burn patients at this Institute, and new effective agents must be made available.

The antibiotic sets used for testing gram positive and gram negative organisms during 1973 are shown in Table 2. Other antibiotics, e.g., Penicillin G, are used in specific circumstances as indicated, but are not included in the study since their use was limited.

Results of Sensitivity Tests. Sensitivity of organisms tested is presented in terms of cumulative sensitivity; each increment of antibiotic suppresses all strains inhibited by less than that amount. Although the complete antibiogram for each species is shown, it has also been expedient to assign an arbitrary inhibitory level below which organisms will be regarded as sensitive. For gram positive cocci, organisms susceptible to 6.25 ug/ml or less are regarded as sensitive; above that level, they are resistant. For gram negative aerobic bacilli, the upper limit is 12.5 ug/ml (Finland M. Ann Int Med 76: 1009, 1972)[1].

Staphylococcus aureus. Sensitivity of 109 strains of Staphylococcus aureus tested in 1973 are summarized in Table 3. The methicillin group of penicillin derivatives were of particular interest because of the recent appearance of methicillin-resistant strains of Staph aureus (Lindberg RB, et al. USA Institute Surg Res Ann Res Rpt FY 73, Sec 7)[2]. The most effective antibiotic in the methicillin group was prostaphlin, with 69.7 % of all strains regarded as sensitive. Even the least effective penicillin, staphcillin, inhibited 50% of strains tested. The most effective antibiotic in vitro was minocin, with 84% of strains inhibited by 6.5 ug/ml. Lincocin and clindamycin with effective suppression of 44% and 40% of strains tested respectively, were the least effective agents, but even so, this represented a significant degree of activity. Gentamycin and keflin were each relatively effective. Complete cross-resistance was relatively rare, and of course is not revealed in the tabulation shown. There were, however, 5 strains out of 109 that were compietely resistant to all antibiotics tested. They were recovered from diffeent patients, not in an epidemic sequence.

Table 2. Antibiotics Used in Routine Testing of Microorganisms, ISR, 1974

	Antibiotic	Gram Positive cocci	Gram Negative bacilli
U	Nafcillin (unipen)	X	
Ps	Oxacillin (prostaphlin)	X	
Sc	Methicillin (staphcillin)	X	
L	Lincocin (lincomycin)	X	
Cl	Clindamycin	X	
M	Minocin	X	X
G	Gentamycin (garamycin)	X	X
Kf	Keflin (cephalathin)	X	X
K	Kantrex (kanamycin)		X
Amp	Ampicillin		X
Co	Colymycin (colistimethate sulfate)		X
Cb	Carbenicillin (geopen)*		X

* Tested only against Pseudomonas aeruginosa

Table 3. Cumulative Inhibitory Levels for 109 Strains of Staph aureus, ISR, 1973

Antibiotic ug/ml	Antibiotic and % of Strains Inhibited at Each Level							
	L	Ps	Sc	U	M	Cl	G	Kf
> 25	100	100	100	100	100	100	100	100
25	66.0	92.6	90.5	84.4	90.6	58.3	91.5	93.2
12.5	56.6	85.3	77.3	74.3	88.7	44.4	81.1	87.5
6.25	44.3	69.7	50.0	62.3	84.1	40.7	67.9	72.1
3.12	25.4	55.0	19.8	46.7	69.1	27.7	33.9	55.7
1.56	8.4	43.1	0	28.4	34.5	25.0	7.5	43.2
0.78	5.6	22.0	0	14.6	10.2	15.7	5.6	28.8
< 0.78	5.6	22.0	0	14.6	9.3	13.8	5.6	26.9
Total Tested	106	109	106	109	107	108	106	107

Number of patients from whom strains were collected = 49

The results of tests on this population of staphylococci were in striking contrast to those seen in 1972, when the culmination of years of increasing antibiotic resistance in this species was recorded. In that year, 50 out of 117 strains were completely cross resistant to all antibiotics tested. This progression of increasing resistance over a four year period, with an abrupt reversal of this trend in 1973, is summarized in Table 4. The increase in methicillin-resistance, which started in 1969, reached its peak in 1972. Lincocin, gentamycin and keflin had all lost effectiveness until 1973 when the staphylococcus population reversed its susceptibility patterns.

Susceptibility at the 6.25 ug/ml level is presented annually since 1967. Antibiotics added to routine testing during that time wegentamycin (1969), keflin (1971) and minocin and clindamycin (1973). Tetracycline and kanamycin which fell steadily to a negligible level of effectiveness were dropped in 1972. The rise in sensitivity in 1973 occurred with all antibiotics, although it was of especial interest with regard to the penicillin-derived antibiotics. Lincocin, to which resistance had increased steadily since 1968, was again effective in 1973, against 44% of strains tested. Gentamycin also rose in adequacy to a level which had not been seen since routine testing of the drug was begun in 1969. The 1972 projection of minocin as a promising antibiotic has been affirmed as far as in vitro effectiveness is concerned.

Despite the increased activity of antibiotics in vitro, there remained a significant clinical problem of staphylococcal sepsis in the burned population. Seven out of 10 staphylococcus strains tested were recovered from the blood stream. Blood stream isolates did not differ from other strains in their overall susceptibility to antibiotics.

The chronologic sequence in which sensitivity to antibiotic increased in the Staph aureus population was assessed by breaking down the population tested to bimonthly increments. Table 5 shows the result of this closer scrutiny. At the beginning of 1973, previously used antibiotics were all low in effectiveness. The methicillin group of antibiotics rose strikingly in activity after May, 1973. This was also the case with gentamycin and lincocin. Sensitivity to keflin showed a striking rise at an even earlier time. Mnocin and clindamycin, both new antibiotics, reflected the same pattern of increase as 1973 progressed. The change appeared to be a fundamentally different level resistance in the whole population, even though antibiotics were in use during this period of change.

Staph Epidermidis. The incidence of coagulase-negative non-pigmented staphylococci in burn patients increased in 1973; 20 strains were tested, of which 17 were recovered from blood culture. Table 6 presents the sensitivity of these isolants. The strains were heterogeneous in their antibiogram pattern; half to three-fourths of the isolates were sensitive to the methicillin group of antibiotics and to lincocin. All but one were sensitive to minocin, with keflin the next most effective antibiotic, and 3 out of 4 were inhibited by gentamycin.

Pseudomonas aeruginosa. Despite continued successful suppression of pseudomonas burn wound sepsis by topical Sulfamylon, this species continues

Table 4. The Emergence of Sensitive Strains of Staph aureus in a Burn Ward Previously Populated with Resistant Staphylococci

Month 1973	Antibiotic and % of Strains Sensitive							
	G	M	L	Ps	Sc	U	Cl	Kf
February - March	28.5	63	9	36	19	45	27	57
April - May	45	70	16	55	25	57	31	81
June - July	77	77	66	87	55	77	88	70
August	92	100	54	68	45	48	16	60
September - October	76	100	53	100	84	71	42	95
November - December	94	94	72	94	88	94	72	94

Table 5. Antibiotic Sensitivity of Staph aureus: ISR, 1967-1973

Antibiotic	Year and % Inhibited by 6.25 ug/ml						
	1967	1968	1969	1970	1971	1972	1973
Lincocin	89.4	64.7	48.5	29.8	28.4	26.0	44.3
Prostaphlin	94.0	80.0	33.0	22.4	20.1	18.8	69.7
Methicillin	61.1	84.6	25.7	18.0	15.5	13.1	50.0
Nafcillin	94.4	90.0	41.0	33.9	33.0	26.0	62.3
Gentamycin	-	-	52.0	32.0	50.0	35.0	67.9
Keflin	-	-	-	-	56.4	22.6	72.1
Minocin	-	-	-	-	-	-	84.1
Clindamycin	-	-	-	-	-	-	40.7

Table 6. Staph epidermidis: Cumulative Sensitivity for 20 Strains from Septic Burn Patients, ISR, 1973

Antibiotic ug/ml	Antibiotic and % of Strains Inhibited at Each Level							
	L	Ps	Sc	U	M	Cl	C	Kf
> 25	100	100	100	100	100	100	100	100
25	70	85	90	90	100	84.2	93.3	100
12.5	65	80	85	70	100	63.1	77.7	94.4
6.25	45	70	50	70	95	63.1	72.2	83.3
3.12	45	55	30	55	95	42.1	72.2	72.2
1.56	30	45	5	30	75	26.3	61.1	61.1
0.78	20	35	0	15	60	15.7	44.4	27.7
< 0.78	15	30	0	15	50	15.7	38.8	27.7
Number Tested	20	20	20	20	20	20	18	18

>27<

to play a prominent role in the cause of sepsis in severely burned patients. Blood stream isolates accounted for 62.7% of strains tested with another 23% recovered from sputum. The spectrum of sensitivity exhibited by 51 strains is shown in Table 7. Of the seven antibiotics tested against these, three were virtually ineffective. Colistin, gentamycin and carbenicillin were effective agents against this pseudomonas population. Although colistin and gentamycin were equally effective at the 12.5 ug/ml level, gentamycin was more effective in lower concentrations; at the 3.12 ug/ml level, twice as many strains were inhibited by gentamycin, as were inhibited by colistin. The significant level of carbenicillin inhibition was, of course, higher, but more than half of the strains were inhibited at 78 ug/ml and four-fifths of them at 156 ug/ml. Colistin, the aminoglycosides and carbenicillin were still the most promising drugs for treatment of pseudomonas infections.

The pattern of antibiotic sensitivity of Ps aeruginosa over the past five years is shown in Table 8. Tetracycline was dropped in 1970; since it had become minimally effective. In its place, minocin has shown a promising level of effectiveness, with 31% of the strains inhibited at 12.5 ug/ml. Both colistin and gentamycin were effective against Ps aeruginosa at levels showing little change from 1969 through 1972. In 1973 an increase of 16% in strains sensitive to colistin occurred, while 18% more strains were sensitive to gentamycin. It is noteworthy that over a five year period, no consistent emergence of resistant forms occurred with these two antibiotics. This is contrary to the widespread assumption that continued use of a given antibiotic will result in increase in the proportion of resistant strains encountered. This, of course, appears to have been the case with tetracycline, kantrex and keflin over the past six years. Carbenicillin, it was assumed, would incite emergence of a constantly rising number of resistant variants, but this has not been the case. The initial experience showed a drop from one-half down to one-third of the strains being sensitive. Again, in 1973, this ratio changed abruptly to 80% sensitivity. Control of pseudomonas burn wound sespsis is still a critical objective of burn therapy, and antibiotics effective against this species in vitro are of major importance.

Klebsiella pneumoniae. Klebsiella sp. have not been routinely speciated, but classification of autopsy isolates to species level has shown presence only of K. pneumoniae. No other Klebsiella species has been recovered. This is the second most frequently tested species among Enterobacteriaceae. Sensitivity of 29 strains is summarized in Table 9. The organism was relatively sensitive to several antibiotics: In descending order of effectiveness there were minocin, gentamycin, kantrex, keflin and colymycin. Despite a general impression that species of Enterobacteriaceae are generally increasing in antibiotic resistance, there was no significant change in sensitivity levels of strains of Klebsiella during the period 1970-1972. In 1973, there was a marked rise in incidence of sensitive strains, as was pointed out for other species as well. Keifin showed the most dramatic change: sensitive strains increased from 18.6% to 60.8% of those tested, and kantrex sensitivity in a parallel shift, went from 35% to 72% sensitive. Gentamycin was slightly more effective in 1973 than before, but colymycin, in an exception to this trend, dropped from 48.8% to 32% of strains sensitive. There were 20% of strains tested which were completely

Table 7. Pseudomonas aeruginosa: Cumulative Inhibitory Concentrations for 51 Strains, ISR, 1973

Concentration ug/ml	Antibiotic and % of Strains Inhibited						Conc. ug/ml	
	K	M	Amp	Kf	Co	G		Cb
> 25	100	100	100	100	100	100	1250	100
25	14	78.4	0	0	88.2	86.2	1250	91.3
12.5	2	31.3	0	0	86.2	84.3	625	89.1
6.25	0	0	0	0	76.4	74.5	312	89.1
3.12	0	0	0	0	33.3	60.7	156	80.4
1.56	0	0	0	0	5.8	29.4	78	56.5
0.78	0	0	0	0	0	3.9	39	23.9
< 0.78	0	0	0	0	0	1.9	19	0

Table 8. Antibiotic Sensitivity of Pseudomonas aeruginosa ISR, 1969-1973

Antibiotic	Year and % Inhibited at 12.5 ug/ml 1969	1970	1971	1972	1973
Kantrex	12.0	1.5	0	0	2
Keflin	5.4	0	5.8	0	0
Colymycin	61.0	63.4	73.3	70.0	86.2
Gentamycin	75.8	71.6	71.4	66.0	84.3
Ampicillin	-	-	-	-	0
Minocin	-	-	-	-	31.3
Carbenicillin 156 ug/ml	50.0	33.9	30.0	34.6	80.4

Table 9. Klebsiella pneumoniae: Cumulative Sensitivity for 25 Strains, ISR, 1973

Antibiotic Level ug/ml	Antibiotic and % Inhibited K	M	Amp	Kf	Co	G
> 25	100	100	100	100	100	100
25	76	91.6	36	73.9	92	83.3
12.5	72	83.3	8	60.8	32	83.3
6.2	44	70.8	4	39.1	32	83.3
3.1	24	50	[illegible]	13.0	20	75.0
1.5	0	4.1	0	4.3	16	70.8
0.78	0	0	0	0	16	41.6
< 0.78	0	0	0	0	16	37.5
Total tested	25	24	25	23	25	24

Complete cross-resistance present: 4 strains

cross-resistant; in 1972 this figure was 25%.

Proteus mirabilis. There was a marked drop in incidence of Proteus mirabilis in 1973. There were 36 strains in blood cultures in 1971, and 32 in 1972 but only 8 were recovered in 197 . The incidence in recoveries from other sites was not markedly reduced, but Proteus septicemia was only one-fourth as commonly seen as in previous years. Of these 8 strains 6 were sensitive to ampicillin, 5 to keflin and to gentamycin, and 2 to kantrex. None were inhibited by Penicillin G, although this species has been reported as penicillin-sensitive. During the past three years, 75 strains of Proteus mirabilis have been tested. Only three (4%) were sensitive to Penicillin G.

Providencia stuartii. This species played a major causative role in sepsis in burned patients during 1973. Seventy-two strains were tested. Providencia septicemia carried a high mortality risk, and an antibiotic effective against it would be a great boon. Unfortunately, the opposite is too often the case; in 1973, as in 1972, no strains were susceptible to any of the six antibiotics used. This total resistance to available antibiotics is a continuation of the findings of 1972. Table 10 summarizes the status of sensitivity of Prov. stuartii since 1969. At best, in earlier years, the species was relatively resistant to antibiotics, but since 1971, no strains have been sensitive to these or other clinically available antibiotics. Tetracycline was replaced by minocin in 1972, with no inhibition observed. All strains tolerated 25 ug/ml of antibiotic. Two new experimental antibiotics (discussed below) were tested during 1973; results offer encouragement that one of them may be effective in some degree. The role of Prov stuartii as an opportunistic invader is well established, but control with available antibiotics has not been achieved.

Four other species of Enterobacteriaceae were less frequently recovered from burn patients. The results of tests on these strains are summarized in Table 11. Entero cloacae was the most common species, with 15 strains isolated primarily from blood cultures. Minocin and colistin were active against most of these strains; less than half were senstive to kantrex and gentamycin, and ampicillin and keflin were virtually ineffective against these strains. A similar distribution of activity was seen with four strains of E aerogenes tested. There were 11 strains of Escherichia coli tested. Effective antibiotics included minocin, colistin and gentamycin.

Streptococci were relatively uncommon in burn wounds, but 11 strains of non-hemolytic streptococci and 3 of alpha-hemolytic streptococci were tested. Over 80% of these strains were from blood cultures. The results of these tests are summarized in Table 12. It is quite evident that these cocci are essentially resistant to the antibiotics available. Only keflin and nafcillin were active against more than one of the 11 non-hemolytic strains, and this still left most of these strains resistant to all available antibiotics. The increase in Group D non-hemolytic streptococci, as these proved to be, is not reassuring; these are ubiquitous fecal flora, but had not previously exhibited such capacity for invasive behavior. None of the alpha-hemolytic strains were sensitive to the antibiotics tested.

Table 10. Providencia stuartii: Comparison of Sensitivity to 12.5 ug/ml of Antibiotic ISR, 1969 - 1973

Antibiotic	Year and % of Strains Inhibited			
	1969-1970	1971	1972	1973
Gentamycin	23.4	7.3	0	0
Tetracycline	17.4	1.4	0	-
Minocin	-	-	-	0
Kantrex	7.8	1.4	0	0
Ampicillin	-	-	0	0
Keflin	3.7	0	0	0
Colymycin	15.7	0	0	0
Number Tested	144	68	82	72

Table 11. Less Commonly Encountered Species of Enterobacteriaceae in Burns: % of Strains Inhibited by 12.5 ug/ml

Species	No. Tested	Antibiotic and % Inhibited					
		K	M	Amp	Kf	Co	G
Enterobacter cloacae	15	33.3	71.4	20.0	0	86.6	46.6
Enterobacter aerogenes	4	50	100	25	0	100	100
Enterobacter hafniae	2	50	50	50	0	0	50
E. coli	11	27.2	72.7	36.3	30.0	63.6	66.6

Table 12. Non-hemolytic and alpha hemolytic Streptococci:
% of Strains Sensitive to 6.25 ug/ml or less

Organism	No. Tested	Antibiotic and % Sensitive							
		L	Ps	Sc	U	M	Cl	G	Kf
Non-hemolytic streptococci	11	0	9.0	0	18.1	0	9.0	0	27.2
alpha-hemolytic streptococci*	3	0	0	0	0	0	0	0	0

* All 3 strains confirmed as Group D

Two experimental antibiotics were tested for activity against Ps aeruginosa. One of these was also assessed for its activity against Prov stuartii.

BB-K8 is a semi-synthetic aminoglycoside prepared by Bristol Laboratories, who are requesting its assay by laboratories which have access to a significant number of severe infections due to Ps aeruginosa, and in addition to those experiencing infections caused by Prov stuartii. The compound is described as active against many gentamycin resistant strains of E coli, Klebsiella and Providencia sp, as well as Ps aeruginosa.

This material was obtained in November 1973. Results of MIC determinations to date on strains recovered from blood cultures are summarized in Table 13. Twenty strains of Prov stuartii were tested. Over half were inhibited by 12.5 ug/ml of BB-K8. Only two strains tolerated 25 ug/ml. On this basis, the behavior of this drug appears decidedly encouraging with reference to Providencia. It should be added, however, that initial laboratory testing with Prov stuartii gave far less encouraging results. Twenty-three strains were inhibited by 25 ug/ml; the remainder tolerated 25 ug/ml. After two trials with inoculum at 10^5 per ml (which is within a tolerated limit for most antibiotics in MIC assay), a more dilute inoculum was used, adding 10^4 cells from an 18-hour culture. Resistance was still observed but it was less common. It is well known that with some antibiotics, minor variations in concentration of inoculum exert a profound effect on the end point of inhibition. Further, the composition of the test broth in the case of BB-K8 is a significant factor in the end point observed. Thus, the status of in vitro sensitivity of Prov stuartii to BB-K8 is not yet settled, but the antibiotic is virtually alone in offering effective inhibition of this ubiquitous opportunist, and will be further studied.

Antibiotic activity of BB-K8 against 32 strains of Ps aeruginosa, as shown in Table 13, was extremely high. 93.7% of these strains were inhibited by 12.5 ug/ml, and 38.4% of them were inhibited by 3.12 ug/ml. In this activity, BB-K8, if its safety in human trials is established, could become an important part of the armamentarium for control of gram negative sepsis in burn patients.

The other antibiotic tested during this period was a semi-synthetic penicillin, produced by Beecham-Massengill Pharmaceuticals, and designated BRL-2288. The spectrum of this antibiotic is similar to that of carbenicillin. It is bactericidal, as is carbenicillin, and is not strongly bound by serum. This agent was tested against 59 strains of Ps aeruginosa, primarily from cases exhibiting septicemia or pneumonia. The sensitivity response is summarized in Table 14.

The strains were relatively sensitive to BRL-2288. At the 39 ug/ml level, 86.4% were inhibited; at 78 ug/ml, 57 out of 59 strains were sensitive. When a series of 25 strains were tested simultaneously for sensitivity to carbenicillin and to BRL-2288, 20% were sensitive to 19 ug/ml of BRL-2288, and 92% sensitive to 39 ug/ml. In contrast, carbenicillin required 39 ug/ml to inhibit 56% and 78 ug/ml to inhibit 76% of the strains. The results of BRL-2288 sensitivity were very promising in vitro; if the clinical acceptability of this drug is established, it could serve as a valuable addition to the armamentarium of

Table 13. Sensitivity of Providencia stuartii and of Pseudomonas aeruginosa from Burns to BB-K8

Concentration ug/ml	Prov stuartii No. Sensitive	Prov stuartii Cumulative % Inhibited	Ps aeruginosa No. Sensitive	Ps aeruginosa Cumulative % Inhibited
>25	2	100	0	100
25	7	90.0	2	100
12.5	6	55.5	1	93.7
6.25	5	25.0	14	90.6
3.12	0	0	14	38.4
1.56	0	0	1	3.1
0.78	0	0	0	0
No. Tested	20		32	

Table 14. Cumulative Sensitivity of Pseudomonas aeruginosa to BRL-2288, ISR, 1974

Antibiotic Concentration ug/ml	Cumulative No. of Strains Inhibited	Cumulative % of Strains Inhibited
>1250	59	100
1250	58	98.1
156	58	98.1
78	57	96.5
39	51	86.4
19	12	20.3
<19	0	0
Total Tested	59	

anti-pseudomonal drugs.

DISCUSSION

During 1973 several striking changes occurred in the spectrum of antibiotic sensitivity of major burn pathogens recovered from patients in this Institute. There was an overall change in the direction of increased sensitivity to the antibiotics tested. These comprise the essential battery available for therapy.

The most dramatic change occurred in the staphylococcus population. Every antibiotic that had been tested in previous years was more effective in 1973 than it had been in 1972. The latter year was a low point on a descending curve of sensitivity, a process which chronologically began in 1968. Methicillin resistance and complete cross resistance had become an almost overwhelming problem. The current increase in susceptible strains can not be attributed to marked alterations in the use of antibiotics, nor to a change in identity of the predominant phage type which continued as type 84, which has predominated since 1971. The alteration in sensitivity reflects a replacement of resistant by susceptible strains, and was probably a plasmid-mediated phenomenon. Even with this mechanism established, of course, the shift in sensitivity could not be considered as having an adequate explanation; the phenomenon occurred but could be neither manipulated nor controlled.

Sensitivity of Ps aeruginosa strains increased in 1973 to a moderate degree to gentamycin and colymycin, and there was a marked increase in sensitivity to carbenicillin, when compared to the preceding four years. With Klebsiella pneumoniae, there was a marked rise in sensitivity to keflin and kantrex, in comparison with 1972. Other antibiotics did not change in level of effectiveness with this species. Prov stuartii strains, which constituted a major problem in sepsis, remained totally resistant to all six antibiotics routinely used. Since it was a frequent cause of sepsis, an effective antibiotic agent for this species was of special importance.

Two experimental antibiotics, one an aminoglycoside (BB-K8) and the other a semi-synthetic penicillin (BRL-2288) were tested during the latter part of 1973. BB-K8 was effective in vitro against half of Providencia strains tested, and was extremely active against Ps aeruginosa. The activity of BRL-2288 resembled that of carbenicillin, but was more potent. BB-K8 has the attributes of gentamycin and kanamycin, as to potential atotoxicity and nephrotoxicity. Since it is the only antibiotic available which was active against Providencia strains, it is of great interest should it become available for clinical treatment of infections due to this organism. Its activity against Ps aeruginosa adds to its potential usefulness.

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PRESENTATIONS

Lindberg RB: An epidemic outbreak of methicillin-resistant Staphylococcus aureus type 84 in a burn unit. Presented at American Society for Microbiology annual meeting, Miami, Florida, May 9, 1973.

Lindberg RB: Nosocomial infections and antibiotic resistance. Presented at Univ. of Georgia: Extension Service Seminar on Hospital Acquired Infections. Athe, Georgia, November, 1973.

PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OE 5955	74 07 01	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISP'N INSTR'NS	9A. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
73 07 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	61102A	3A161102B71R	01	304
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE: (U) Emergence of Methicillin-Resistant Staphylococcus Aureus Type 84 in Burned Military Personnel (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
69 06	Cont	DA	D. 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 74	.5	11
CURRENT FISCAL YEAR 75	.5	13

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21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede each with Security Classification Code)
(U) Staphylococcus; (U) Burns; (U) Septicemia; (U) Burn infection; (U) Humans

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

23. (U) The observation of increasing rates of sepsis due to methicillin-resistant staphylococci in burned patients prompts investigation of phage types to determine the nature of this epidemic-scale outbreak and to uncover means for its control in burned military personnel.

24. (U) The staph phage typing battery was modified by including WH-1 and D-11 phages. These are WHO recommended phages. The phage type patterns were correlated with antibiotic sensitivity.

25. (U) 73 07 - 74 06 Monotype epidemic of Staphylococcus aureus type 84 persisted for the third year of its course as the vastly predominant type of staphylococcus in the burn ward population. Such predominance is a unique epidemiologic phenomenon; the mechanisms permitting it are of broad potential import in the epidemiology of nosocomial staphylococcal infections. Striking alteration in methicillin sensitivity occurred; after four years of progressive increase in resistant strains (with methicillin sensitivity falling from 85% to 13%) a re-emergence of sensitive strains occurred; 50% of isolates tested in 1973 were sensitive, although the phage type remained unaltered. Genetic mechanisms to explain this alteration are under study.

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*Available to contractors upon originator's approval

DD FORM 1 MAR 69 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

ABSTRACT

PROJECT NO. 3A161102B71R-01, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: EMERGENCE OF METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS TYPE 84 IN BURNED MILITARY PERSONNEL

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

Period covered in this report: 1 July 1973 - 30 June 1974

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Reports Control Symbol MEDDH-288(R1)

The staphylococcal population in the burn wards of the Institute of Surgical Research has been evaluated by phage type, antibiotic sensitivity and by cultural evaluation. The replacement of a heterogeneous flora by a virtually pure monotype epidemic of Staphylococcus aureus occurred in burn patients between 1967 and 1971, at which time only type 84 or nontypable strains were recovered. During this interval a progressive decrease in susceptibility to antibiotics occurred, so that by 1971, semi-synthetic penicillins, the methicillin group antibiotics, were largely ineffective, and other categories of antibiotics nearly so. Cephalothins and finally mincocin were introduced in the assessment regimen in an effort to find an effective agent. In mid-1973, a marked change in sensitivity occurred, evidently spontaneously. A large proportion of Staph aureus strains are now sensitive to antibiotic, including the methicillin group of antibiotics. This major shift has occurred without obvious external cause, and it constitutes a unique development in the biology of this important infective agent. The problem of antibiotic resistance has temporarily been alleviated, but there is as yet no explanation for this phenomenon.

Staphylococcus
Septicemia
Burns
Burn infection
Humans

EMERGENCE OF METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS TYPE 84 IN BURNED MILITARY PERSONNEL

Staphylococcus aureus in burned patients occupied a relatively minor role during the period 1962 through 1968, in terms of severity of illness and of frequency with which it was recovered from patients with infections. Gram negative sepsis, primarily due to Pseudomonas aeruginosa, was the major problem during that period, but after 1968, a sharp recrudescence of staphylococcal infection recurred. While gram negative sepsis remained a major problem increasing rates of staphylococcal bacteremia and of systemic infection were observed. At the same time, the level of antibiotic susceptibility, which had characterized the advent of penicillinase-resistant semi-synthetic penicillins, began to diminish. Increasingly resistant strains of Staph auerus were observed from 1969 onward through 1972.

Through 1966, most staphylococcus phage types fell into Group 1, but from 1967 onward, the incidence of Group 3 and of Group 4 strains rose markedly, and Group 1 strains became rare. The alteration observed in the Institute of Surgical Research burn wards closely resemble the emergence of Group 3 and Group 4 phage types, highly resistant to antibiotics, which occurred in Europe at almost the same time (Bulow P. Ann NY Acad Sci 182:21,1971; Jessen O, Rosendo K, Bulow P. NEJM 281:677, 1969). Staph aureus has been studied as to phage type, antibiotic sensitivity, temporal distribution as an indicator of epidemic patterns, and in terms of invasiveness as reflected in the incidence of staphylococcal sepsis in burn patients. The sequence of events observed in this epidemic situation have been unique, and shed light on basic phenomena of staphylococci in hospital-acquired infections.

OBSERVATIONS

Bacteremia. The presence of Staph aureus on burn wounds is not an index of the clinical seriousness of such colonization. Blood stream invasion is a plausible indicator of severity of involvement, and the incidence of staphylococcal bacteremia has been summarized for time periods in which the infection pattern was distinctive.

Table 1 shows the incidence of Staph aureus bacteremia at different periods in the recent history of the Institute of Surgical Research. Four natural divisions present themselves. In 1963, topical therapy was non-existent, and in 1964 topical Sulfamylon was introduced for the first time. There was a moderate drop in the incidence of staphylococcal sepsis during 1964, but it then decreased dramatically in 1965 through 1968. The spectrum of Sulfamylon activity for staphylococci in vitro is the same as its antipseudomonal activity. During this time, strains were largely sensitive to methicillin and to other antibiotics.

Beginning in 1969, a marked rise in staphylococcal bacteremia occurred. It seems improbable that the simultaneous appearance of staphylococci could have been coincidence. The rates of staphylococcal bacteremia increased strikingly from 1969 through 1972, almost 50% of positive blood cultures were

Table 1. Incidence of Staphylococcus aureus Bacteremia in Burn Patients, 1963-1973

Years	Conditions	Nc. Patients With Positive Blood Cultures	Staph aureus Recovered	Incidence of Staph aureus Bacteremia (% of Positive Blood Cultures)
1963-1964	Prior to full use of topical therapy	114 (57/year)	29	25.4
1965-1968	Optimal use of topical therapy	117 (29/year)	14	11.9
1969-1972	Methicillin-resistant Staph aureus appears (type 84)	322 (80/year)	160	49.6
1973	Methicillin-sensitive type 84 Staph aureus reappears	81	30	37.0

7-2

Staph aureus. This situation showed a significant alteration in 1973, when the proportion of staphylococci among all bacteremias fell to 37%. During 1973, a marked increase in the number of staphylococcus strains sensitive to methicillin type antibiotics, as well as to other antibiotic categories, occurred.

Phage Types of Staph aureus. There has been a continuing increase in the number of staphylococci recovered from burn patients as reflected in the number of strains submitted for phage typing. There were 108 strains submitted for typing in 1968; in 1973 there were 1403 strains typed, the largest number ever logged in one year. During the past 6 years, a progression of phage types has occurred. For the major types recovered the sequence of events that occurred is shown in Figure 1. Each phage type is shown as a per cent of the total strains typed in each year. The proportion of patients harboring each type is shown in relation to the number of strains recovered. Nontypable strains predominated in 1968, and among typable strains, type 84 and the classic Group 1 pattern of 52, 52A, 80, 81 were almost equal in occurrence. After 1968, Group 1 strains never have reappeared in significant numbers. In 1969 type 84 and type 84, 85 were predominant, and nontypable strains made up 20% of all isolates. An unusual episode of a group of strains sharing in some degree phage types 47, 54, 75, 84, 85 made an abrupt appearance in 1970, and over shadowed all other types except the recurring type 84. As a group, various phage type combinations continued to give rise to loss variants against which only phage 84 was active. This resulted in the ultimate development, in 1970, of a virtually pure type 84 as the predominant phage type of the burn ward. This monotype epidemic has persisted in the burn ward with minor changes, since 1970. No major intrusion of another type has occurred. The proportion of nontypable strains has remained virtually unchanged at the 20% level since that time.

In 1973 type 84 strains were still predominant although the total recovered fell to 61.1% of all strains. Table 2 summarizes in more precise detail the current status of types of staphylococci recovered. The occurrence of nontypable strains in patients increased to 58.4% of patients. A small but significant number of type 84, 85 reappeared. This type was absent in 1970, and only 3 strains occurred in 1971. The relatively new type WH-1 occurred in 10.8% of patients, although the total number of strains recovered was small. It was usually found only at time of admission, and appeared to be replaced readily by the predominant type 84.

Phage Types of Staph aureus from Blood Cultures, 1973. Since the question of the importance of staphylococcal sepsis has been looming larger in speculation on infections in burn patients, types recovered from the blood stream were assessed to detect any difference in type distribution from that seen with the staph population as a whole. This would aid in recognition of a uniquely invasive type if such exists. Thirty-five patients yielded Staph aureus in blood culture, and there were 115 isolates recovered. Table 3 summarizes the type distribution of these strains. There were 12 patients who survived and 23 expired; these figures, of course, include patients with blood stream infections with more than one species of organism. There were no significant differences in type distribution between the survivors and the nonsurvivors. Type 84 was

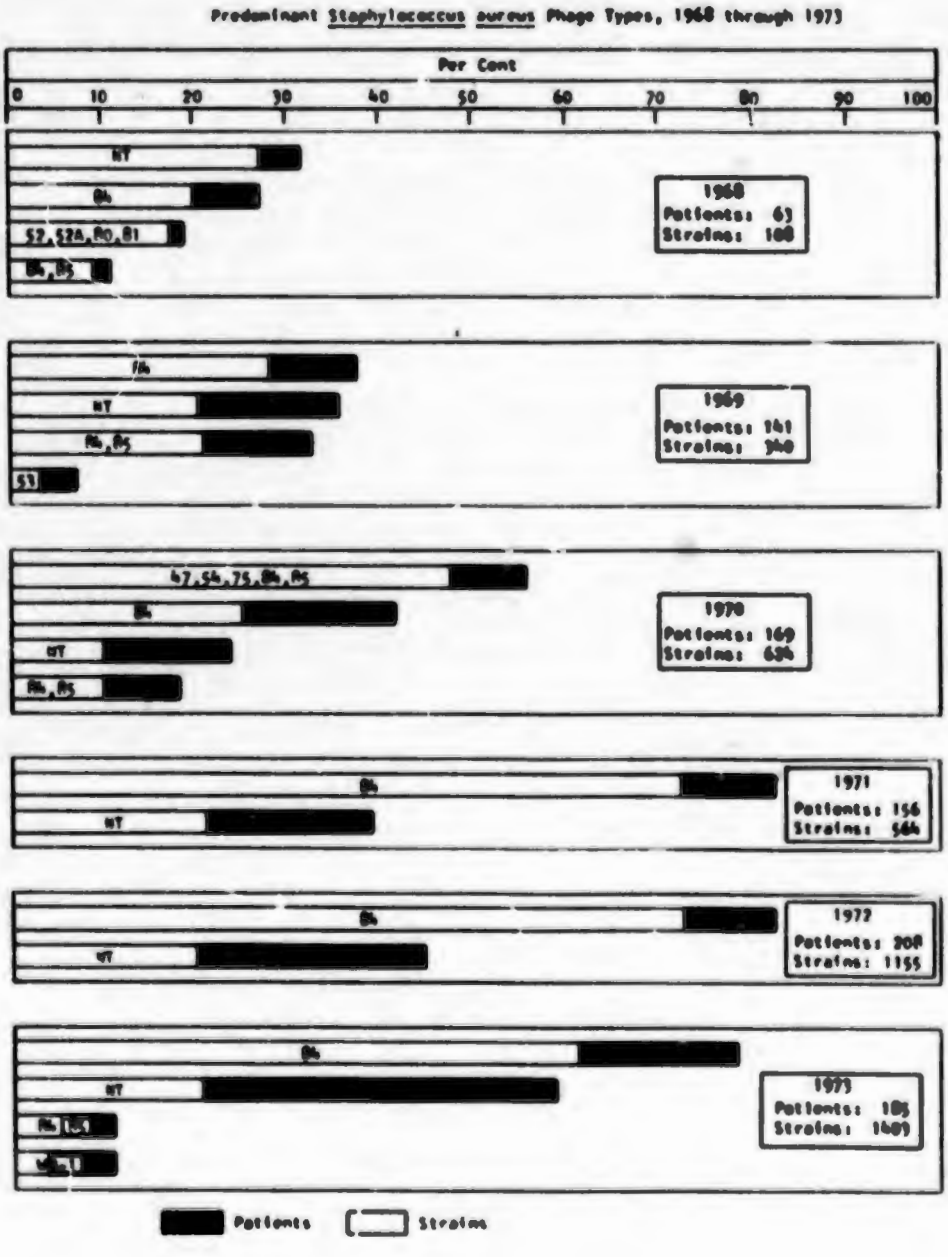
Predominant Staphylococcus aureus Phage Types, 1968 through 1973
Per Cent
0
10
20
30
40
50
60
70
80
90
100
NT
52,52A,80,81
1968
Patients: 63
Strains: 108
53
1969
Patients: 141
Strains: 340
47,54,75,
1970
Patients: 169
Strains: 634
1971
Patients: 156
Strains: 564
1972
Patients: 208
Strains: 1155
1973
Patients: 185
Strains: 1409
Patients
Strains

Figure 1

Table 2. Predominant *Staphylococcus aureus* Phage Types in ISR Burn Patients, 1973

Phage Type	Per cent	
	Patients	Strains
84	77.8	61.1
Non-typeable	58.4	20.5
84,95	10.8	5.2
WH-1	10.8	3.5
29	3.2	2.0

Table 3. Phage Types of Staphylococcus aureus from Blood Stream of ISR Burn Patients, 1973

SURVIVED				
No. of Patients	Phage Type			
	29	84	84,85	NT
	No. of Strains			
7		22		
1			1	
1				1
3		15		4

EXPIRED				
No. of Patients	Phage Type			
	29	84	84,85	NT
	No. of Strains			
1	1			
15		36		
2				5
3		14		3
1			6	1
1		3	3	

7-6

the predominant type among blood stream as well as among wound and sputum isolates. Strains from blood included 82% of type 84. For all sources this proportion was 77.8%.

Phage types among survivors and fatal burns are compared in Table 3. This resume illustrates the nature of mixed phage type populations in these patients.

Phage Type of Staph aureus from Postmortem Lung Tissue of Burn Patients. Pneumonia has become a more and more prominent part of the pathology of burn patients, and a question of epidemiologic concern was the identity of staphylococcus strains found in the lung. A definitive group of cultures were available in those fatal burns in which autopsies were performed and lung samples collected. Sixty-seven strains were collected from lung tissue of 25 patients. Phage types of these strains are shown in Table 4. Eleven of the 25 patients harbored only Staph aureus type 84: 5 more had type 84 plus a nontypable strain. Thus, 64% of these fatalities harbored type 84 in tissues at autopsy. This figure is comparable to the 77.8% of patients harboring type 84 from all sources.

There were more strains other than type 84 in postmortem lung tissue than had been seen in the past three years. One patient harbored type 29; one had 29,52; and one 84,85. Three yielded only nontypable strains. The proportion of nontypable strains is high enough to merit investigation of new phages that might classify some of these strains. Type WH-1, a new international typing phage, was not found in septicemia but occurred in lung tissue of patients.

Antibiotic Sensitivity of Staphylococci, 1969-1974. The demonstration that a single type of Staph aureus has predominated in the Institute of Surgical Research wards for the past three years, and that the typical staphylococcal population has been one which exhibits a single, or at most, three predominant types, prompted a close scrutiny of the antibiotic sensitivity of staphylococci (Lindberg RB, Latta RL, Pruitt BA, Jr, Mason AD, Jr. USA Ann Rpt FY 1973, USA Institute Surg Res[3]). A progressive and steady increase in antibiotic resistance of staphylococci occurred from 1967 through 1972, as shown in a simplified form in Table 5. There was a concomitant increase in staphylococcal sepsis during these years. Kantrex and tetracycline fell to such a low level of effectiveness that they were omitted from the routine testing battery, tetracycline in 972 and Kantrex in 1973. Minocin and later clindamycin were added to the test battery in the hope that they would prove to be more effective antibiosis. Through 1972 the increasingly resistant pattern was especially prominent with the semi-synthetic penicillins. Methicillin itself was the least effective, but the most effective form, nafcillin, still failed to inhibit three-fourths of all strains tested in 1972.

A striking, unique, and thus far unexplained change in sensitivity occurred in 1973. Gentamycin, lincocin, the semi-synthetic penicillins, and keflin, all were in a significant degree more effective than they had been the preceding year.

The natural history of the development of antibiotic resistance and its

Table 4. Phage Types of Staphylococcus aureus from Post Mortem Lung Tissues, 1973

No. of Patients	Phage Type					
	84	NT	84,85	WH-1	29	29,52
	No. of Strains					
11	28					
3		5				
3				9		
5	11	5				
1		1	1			
1					4	
1						3

Table 5. Antibiotic Sensitivity of Staph aureus:
% of Strains Inhibited by 6.25 ug/ml or less

Year	Antibiotic and % Inhibited							
	G	L	Ps	Sc	U	Kf	M	Cl
1967	-	89.4	94.0	61.1	94.4	-	-	-
1968	-	64.7	80.9	84.6	90.0	-	-	-
1969	52.0	48.5	33.0	25.7	41.0	-	-	-
1970	32.0	29.8	22.4	18.0	33.9	-	-	-
1971	56.0	28.4	20.1	15.5	33.0	56.4	-	-
1972	35.6	26.0	18.8	13.1	26.0	22.6	51.5	-
1973	67.9	44.3	69.7	50.0	62.3	72.1	84.1	40.7

Kanamycin: Discontinued 1973: Dropped from 42.8% sensitive in 1968 to 6.0% in 1972.

Tetracycline: Discontinued in 1972: Dropped from 38.4% sensitive in 1968 to 1.9% in 1972.

G - Gentamycin
L - Lincocin
Ps - Prostaphlin (oxacillin)
Sc - Staphcillin (methicillin)
U - Unipen (nafcillin)
Kf - Keflin (cephalothin)
M - Minocin (minocycline)
Cl - Cleocin (clindamycin)

sudden reversal is more precisely shown in graphs of the cumulative sensitivity to selected antibiotics assayed on an annual basis. Figures 2 through 8 show the sequence of events in regard to sensitivity of Staph aureus from 1969 through 1973 and the first six months of 1974. Through 1972, the six antibiotics are summarized annually in one figure. 1969 and 1970 were years when the strains were relatively resistant. At the level of 6.25 ug/ml, between 20% and 38% of strains were sensitive, depending on the antibiotic. In 1971 there was an increase in sensitivity for Prostaphlin, gentamycin and unipen, but at best the pattern was only within an adequate range from the viewpoint of therapy. In 1972, the sensitivity dropped dramatically; there was little likelihood of a given strain being adequately controlled with antibiotics. In 1973, when clindamycin and minocin were charted for the first time, a dramatic reversal of resistance to antibiotics occurred. Even at lower concentrations, a rise in sensitivity was seen, and the striking increase in effectiveness of the semi-synthetic penicillins and of Keflin were especially striking. These curves represent a complete reversal of a trend repeatedly seen in the preceding four years.

Data were available for 50 strains from 16 patients, but this ultimate expression of the reversal from the pattern recorded 18 months previously is clearly shown. These curves represent an extremely sensitive population of staphylococci; 2 of the 3 semi-synthetic penicillins are noteworthy for their extremely high level of effectiveness, while the third, staphcillin, was far more effective than it had been in over five years. Clindamycin was, with Keflin, the most effective non-penicillin compound, while minocin and lincocin, less active at very low dilutions, were still highly effective at 3 ug/ml.

The remarkable fact about this abrupt and striking reversal of antibiotic sensitivity in an epidemic population of staphylococci is that it appears to have occurred de novo. No striking alteration in the routine use of antibiotics was instituted; by and large, they were used when sepsis, pulmonary infection, or some other specific diagnosis indicated their use. Topical therapy has been changed to include use of silver sulfadiazine on many more patients since January, 1974, but the sensitivity shift was well established at least six months earlier. If we were to take the data from 1 July 1973 to 30 June 1974, there would be little change in the profile of Figures 7 and 8, shown for six months of 1974.

CONCLUSIONS

A monotype epidemic of Staph aureus has been present in the Institute of Surgical Research patient population since 1971. It may, in 1973, have shown slight signs of introducing phage types other than type 84, but such change was peripheral. Since 1969, a steady rise in antibiotic resistance to most antistaphylococcal antibiotics has occurred. This was especially marked with methicillin and the semi-synthetic penicillins, but resistant strains to the aminoglycosides and tetracyclines also increased. At its peak, in 1972, this broadly resistant population of staphylococci was amenable to few antibiotics; gentamycin and minocin were the only two which might have affected half of the strains on the basis of in vitro data. Yet, in 1973 an abrupt reversal

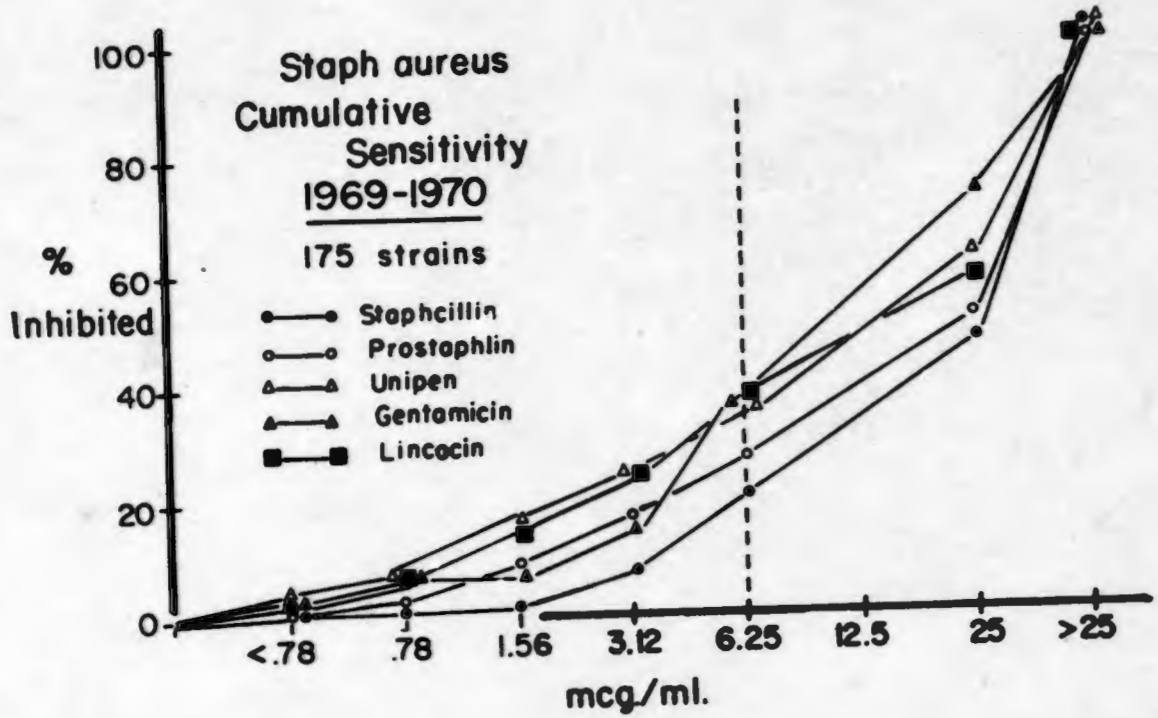
Staph aureus
Cumulative
Sensitivity
1969-1970
175 strains
% Inhibited
100
80
60
40
20
0
Staphcillin
Prostaphlin
Unipen
Gentamicin
Lincocin
<.78
.78
1.56
3.12
6.25
12.5
25
>25
mcg./ml.

Figure 2

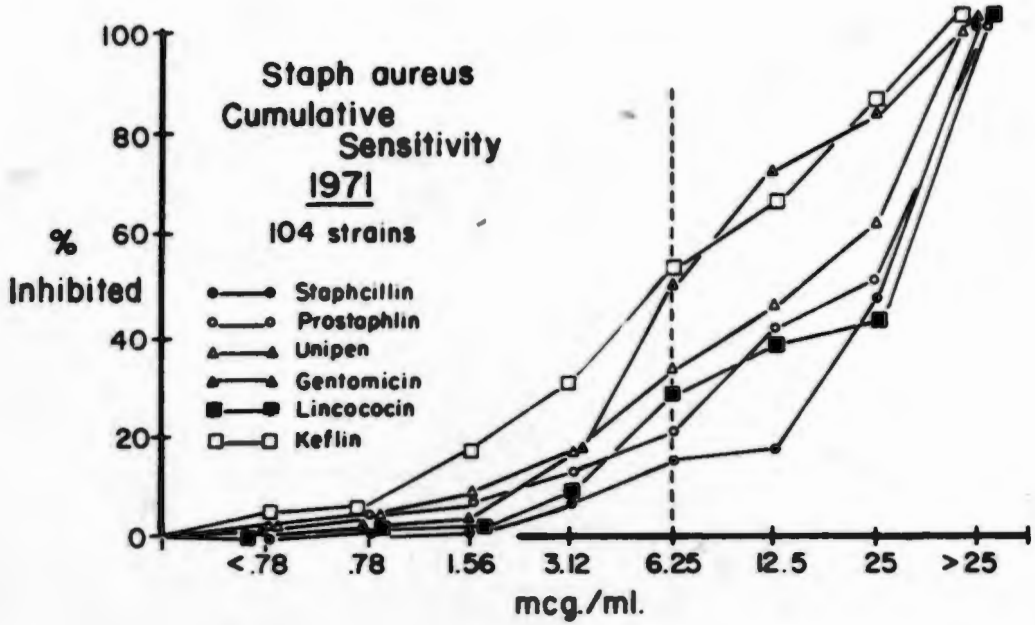
Staph aureus
Cumulative
Sensitivity
1971
104 strains
% Inhibited
100
80
60
40
20
0
Staphcillin
Prostaphlin
Unipen
Gentomicin
Lincococin
Keflin
<.78
.78
1.56
3.12
6.25
12.5
25
>25
mcg./ml.

Figure 3

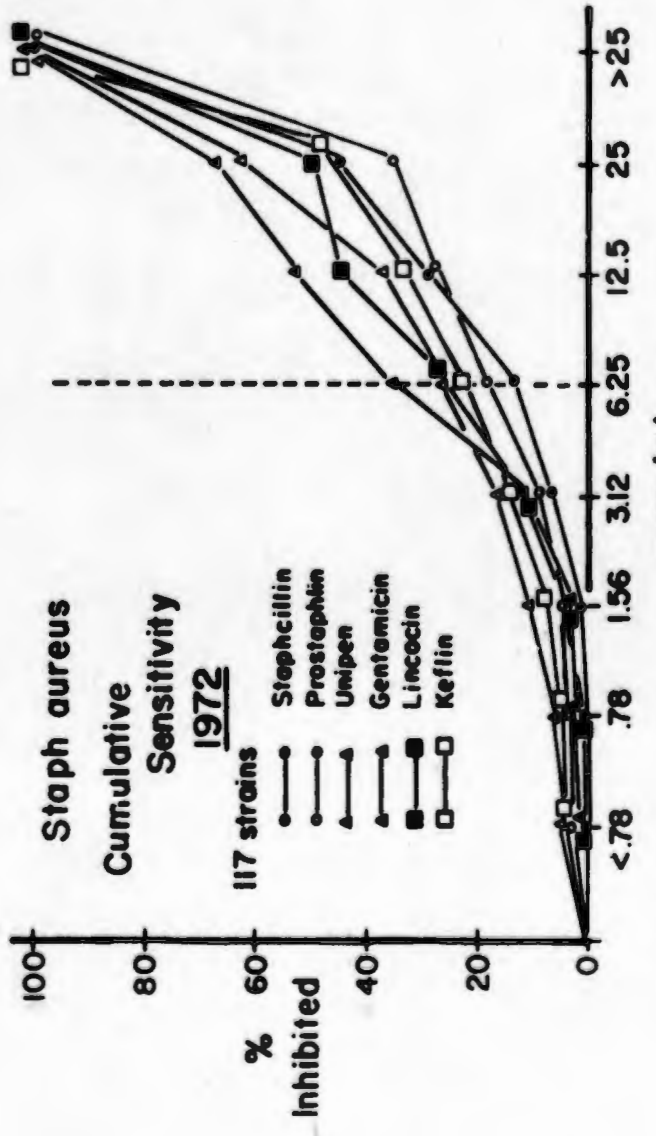
Staph aureus
Cumulative
Sensitivity
1972
117 strains
Staphcillin
Prostaphlin
Unipen
Gentamicin
Lincocin
Keflin
% Inhibited
100
80
60
40
20
0
<.78
.78
1.56
3.12
6.25
12.5
25
>25
mcg./ml.

Figure 4

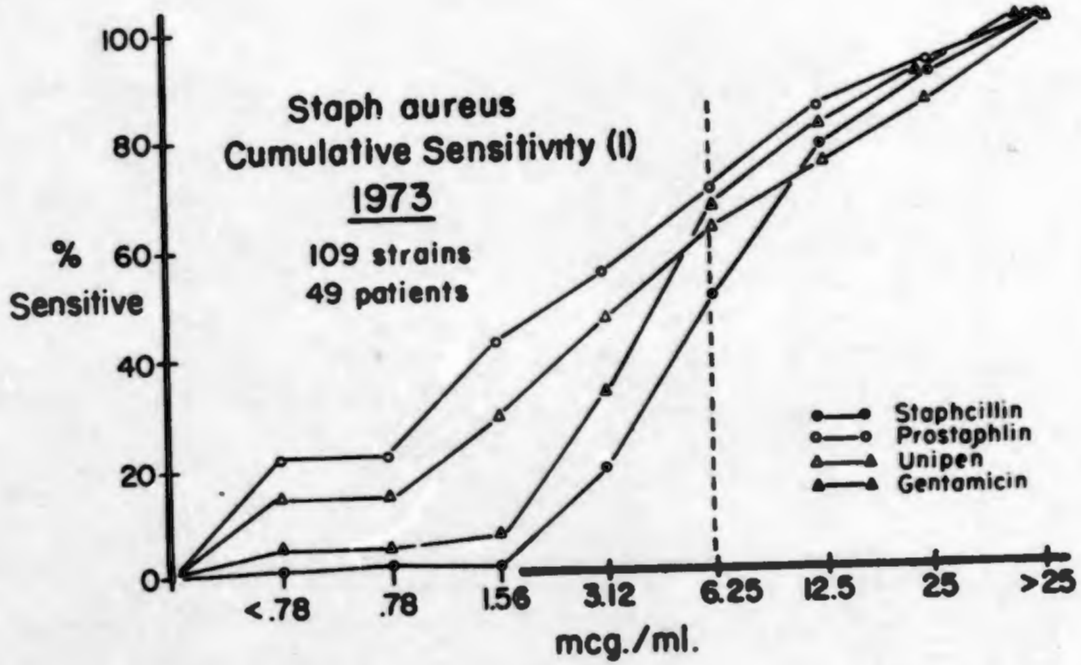
Staph aureus
Cumulative Sensitivity (I)
1973
109 strains
49 patients
% Sensitive
100
80
60
40
20
0
<.78
.78
1.56
3.12
6.25
12.5
25
>25
mcg./ml.
Staphcillin
Prostaphlin
Unipen
Gentamicin

Figure 5

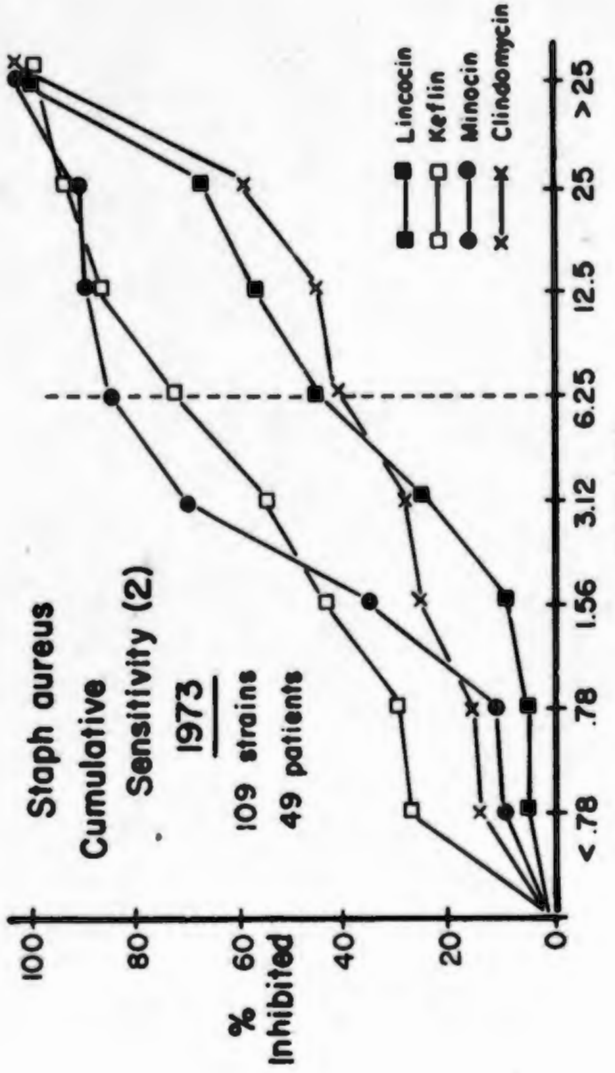
Staph aureus
Cumulative
Sensitivity (2)
1973
109 strains
49 patients
% Inhibited
100
80
60
40
20
0
< .78
.78
1.56
3.12
6.25
12.5
25
> 25
mcg./ml.
Lincocin
Keflin
Minocin
Clindomycin

Figure 6

7-16

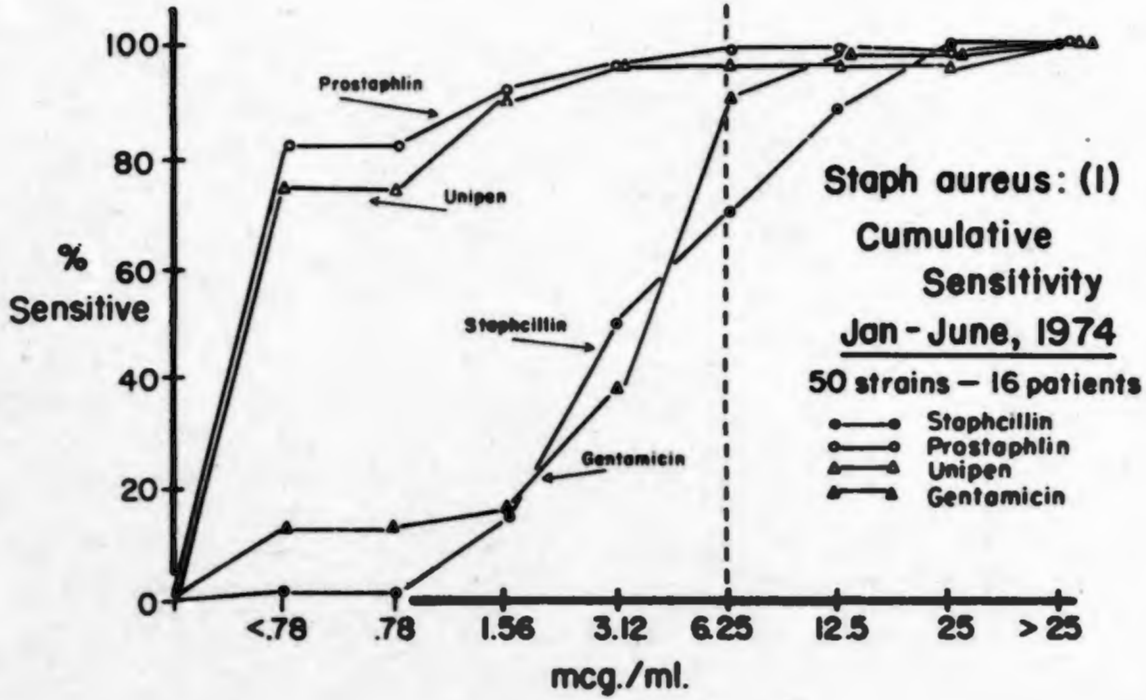
100
80
60
40
20
0
% Sensitive
Prostaphlin
Unipen
Staphcillin
Gentamicin
<.78
.78
1.56
3.12
6.25
12.5
25
>25
mcg./ml.
Staph aureus: (I)
Cumulative
Sensitivity
Jan - June, 1974
50 strains — 16 patients
Staphcillin
Prostaphlin
Unipen
Gentamicin

Figure 7

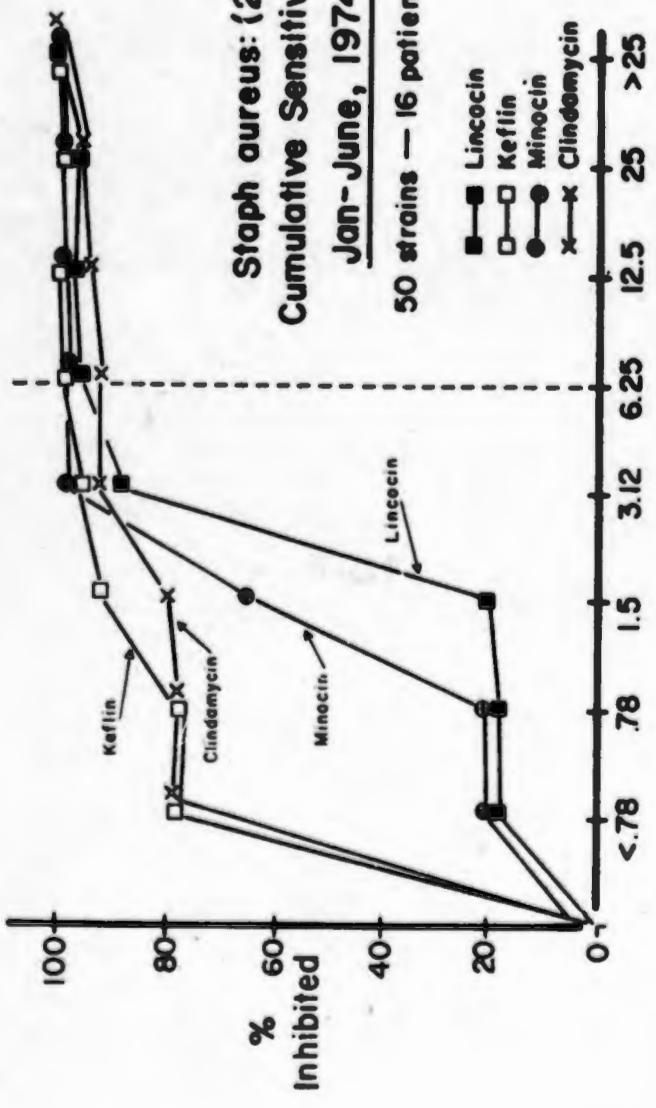
Staph aureus: (2)
Cumulative Sensitivity
Jan-June, 1974
50 strains — 16 patients
Lincocin
Keflin
Minocin
Clindamycin
Lincocin
Keflin
Clindamycin
Minocin
% Inhibited
100
80
60
40
20
0
<.78
.78
1.5
3.12
6.25
12.5
25
>25
mcg./ml.

Figure 8

of this pattern occurred, and the staphylococcal population, still predominantly a type 84 epidemic, became increasingly susceptible to antibiotics. In the first six months of 1974 the strains were shown to be extremely sensitive to methicillin and to other categories of antibiotics. This development is not characteristic of the worldwide current pattern of antibiotic resistance, and presents a new dimension in the biology of antibiotic sensitivity and its persistence in this major pathogen, Staph auerus.

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1. Bulow P. Staphylococci in Danish Hospitals during the last decade. Ann NY Acad Sci 182: 21, 1971.

2. Jessen O, Rosendo K, Bulow P. Changing staphylococci and staphylococcal infections. New Eng J Med 281: 677, 1969.

3. Lindberg RB, Latta RL, Pruitt BA, Jr, Mason AD, Jr. Emergence of methicillin-resistant Staphylococcus aureus type 84 in burn patients. US Army Institute of Surgical Research, Ann Rpt FY 1973, Brooke Army Medical Center, Fort Sam Houston, Texas. Section 8.

PRESENTATIONS

Lindberg RB. "Methicillin-resistant staphylococci in nosocomial infections" presented at annual meeting of American Society for Microbiology, Miami, Fla. 9 May 1973.

PUBLICATIONS

130<

None

8

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6970	74 07 01	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. GROUP INDIC	9. SPECIFIC DATA-CONTRACTOR ACCESS	10. LEVEL OF SUM
73 07 01	D. CHANGE	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

11. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A161102B71R	01	267
b. CONTRIBUTING				
c. CONTRIBUTING				

12. TITLE (U) Sensitivity of Pseudomonas Aeruginosa Recovered from Burned soldiers to Sulfamylon (44)

13. SCIENTIFIC AND TECHNOLOGICAL AREAS
003500 Clinical Medicine

14. START DATE	15. ESTIMATED COMPLETION DATE	16. FUNDING AGENCY	17. PERFORMANCE METHOD
68 07	Cont	DA	C. In-House

18. CONTRACT/GRANT: Not Applicable
a. DATE(S)/EFFECTIVE: EXPIRATION:
b. NUMBER:
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

19. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS ($ in thousands)
FISCAL YEAR PRECEDING 74	.5	13
FISCAL YEAR CURRENT 75	.4	10

20. RESPONSIBLE DOD ORGANIZATION
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RESPONSIBLE INDIVIDUAL
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21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. PERFORMING ORGANIZATION
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Microbiology Branch
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TELEPHONE: 512-221-2018
SOCIAL SECURITY ACCOUNT NUMBER:
ASSOCIATE INVESTIGATORS
NAME: Virginia C English, MA
NAME: DA

23. KEYWORDS (Provide KWIC and Security Classification Code)
(U) Pseudomonas; (U) Burns; (U) Sulfamylon; (U) Topical therapy; (U) Humans

24. TECHNICAL OBJECTIVE, 25. APPROACH, 26. PROGRESS (Furnish individual paragraphs identified by number. Provide text of each with security classification code.)

23. (U) Burns to military or civilian personnel are a major problem in warfare; the control of infection in burns with Sulfamylon has greatly reduced lethal sepsis. Since resistance to chemotherapy of bacterial infection has been a continuing problem, the surveillance of sensitivity to Sulfamylon is a key factor in modern military medicine.

24. (U) Sensitivity to Sulfamylon is determined by an agar plate dilution technic which yields minimum inhibiting concentration data. A major part of Pseudomonas isolates from the entire year is tested.

25. (U) 73 07 - 74 06 On an annual basis, Sulfamylon sensitivity of Pseudomonas aeruginosa strains has fluctuated. The minimum inhibitory concentration rose sharply in 1972, which led to a real concern that resistant strains were becoming more numerous and that the drug might correspondingly become less effective in control of burn wound sepsis. The median level of inhibition of Pseudomonas aeruginosa was 0.316% in 1972. However, in 1973, with no significant alteration in topical therapy, this pattern disappeared and reversion to a more sensitive form of Pseudomonas aeruginosa occurred. The median MIC level for 1973 was 0.111%; this was the lowest median value observed since 1970. This shift represents presence of a larger population of strains sensitive to Sulfamylon than had been recovered in the past 2 years. The sensitivity level, while not as low as it was prior to 1968, is well within the range at which Sulfamylon is an effective agent in topical therapy of burns.

*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.

ABSTRACT

PROJECT NO. 3A161102B71R-01, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: SENSITIVITY OF PSEUDOMONAS AERUGINOSA RECOVERED FROM BURNED SOLDIERS TO SULFAMYLON

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)

Two hundred and eighty-five strains of Pseudomonas aeruginosa from burned patients were tested for sensitivity to Sulfamylon. A lower proportion of relatively resistant strains was observed than had been seen in 1972, and the median sensitivity level for 1973 fell to 0.111% from a high of 0.316% in 1972. Relatively resistant strains were concentrated in two phage types, H-71 and NT4. The former constituted a group of strains distributed over a wide range of sensitivities; the latter were unique in being fitted on a narrow MIC range of 0.312% to 0.156%. No specific rise in wound sepsis or septicemia due to these types was observed; they appeared in the blood stream in proportion to their temporal incidence in the burn population. Thusfar, moderately resistant strains have not presented a therapeutic problem. Their continued monitoring is mandatory if such an eventuality is to be promptly recognized should it occur.

Pseudomonas
Burns
Sulfamylon
Topical therapy
Humans

SENSITIVITY OF PSEUDOMONAS AERUGINOSA RECOVERED FROM BURNED SOLDIERS TO SULFAMYLON

Since the introduction of Sulfamylon burn cream as a prophylactic agent in treatment of burns, the sensitivity of Pseudomonas aeruginosa strains, recovered from burn patients in a ward where Sulfamylon was being used extensively as a treatment regimen, has been monitored closely. In view of the long history of emergence of resistant strains in the presence of an antimicrobial agent, it would be expected that the initial success which attended the use of topical Sulfamylon might be blunted with the passage of time, and if, indeed, in vitro resistance occurred and lessened the effectiveness of the drug in therapy, such a development would call for prompt re-evaluation of the topical therapy regimen. There has been no marked resurgence of Pseudomonas burn wound sepsis in the Institute of Surgical Research burn population for several years, but Pseudomonas colonization occurs almost as a continuous phenomenon, and the organism appears in the blood stream of a significant number of patients each year. These are usually terminally ill patients, and it may well be that were Pseudomonas not at hand, other nosocomial infecting agents would take its place. This evidence of continued invasive pathogenicity of this organism makes its monitoring and control a matter of significance.

METHODS

Sensitivity to sulfonamide compounds and related agents has been found to be most effectively determined by using a Minimum Inhibitory Concentration (MIC) technic with Sulfamylon in agar plates at appropriate dilutions (Lindberg RB, Calvert J, Brame RE, Dent R: USA Surgical Research Unit, BAMC, Ft Sam Houston, Tx. Annual Research Report FY 1965, Section 15)[1]. Concentrations from 2.5% Sulfamylon acetate down to 0.019% are made up in square, ruled 100 mm square plates, with 36 spaces marked off in the rectangle. Since the acetate form is used in the therapeutic application of Sulfamylon, it is also used for in vitro sensitivity testing procedures, the number of colony forming units is a critical factor in reaching a consistent, reliable and reproducible end-point of inhibition. The objective is 1000 cfu per inoculum drop; this is made up by diluting an 18-hour broth culture.

RESULTS

Two hundred and eighty-five strains of Ps aeruginosa were tested in 1973. The number of isolates was only 61% of the number tested the previous year, and reflected a drop in incidence of Pseudomonas on burned patients. A retrospective comparison of the proportion of strains inhibited at each test dilution, observed on an annual basis, is shown in Table 1. Comparisons since 1967 are presented. The annual increments show that there was an increase in the proportion of sensitive strains in 1973, compared to 1972, when a relatively high level of resistance occurred.

Table 1

Inhibiting Concentrations of Sulfamylon for Pseudomonas aeruginosa, 1969-1973

Year	Concentration of Sulfamylon in % and Number Inhibited								
	2.5	1.25	0.625	0.312	0.156	0.078	0.039	0.019	<0.019
1969	0	0	13	179	89	74	28	2	0
% of total (385)	0	0	3.4	46.5	23.1	19.2	7.3	0.5	0
1970	0	0	0	65	83	83	59	6	0
% of total (296)	0	0	0	21.9	28.0	28.0	19.9	2.03	0
1971	0	0	48	41	56	57	65	13	0
% of total (280)	0	0	17.1	14.6	20.0	20.4	23.2	4.7	0
1972	0	29	212	46	88	31	37	15	5
% of total (463)	0	6.3	45.8	9.9	19.1	6.7	7.9	3.2	1.1
1973	0	4	14	85	85	52	32	12	1
% of total (285)	0	1.4	4.9	29.8	29.8	18.3	11.2	4.2	0.4
Total (1709)	0	33	287	416	401	297	221	48	6
% of total	0	1.9	16.8	24.3	23.5	17.4	12.9	2.8	0.4

8-2

Between 1970 and 1972 the resistance level was not as high, but this increased sensitivity came after 1969, during which time there had been a rise in resistance to Sulfamylon. The pattern of sensitivity, observed at annual intervals, shows not a gradual or stepwise increase but a fluctuating pattern in which groups of resistant strains appear, only to be succeeded by more sensitive strains.

A more coherent picture of the sensitivity of Pseudomonas strains to Sulfamylon is shown in Table 2, where the cumulative sensitivity is set down. From a maximum resistance level observed in 1972, there was a reappearance of more sensitive strains; one-third of the strains were inhibited by 0.078% of Sulfamylon in 1973, in contrast to only 19% sensitive at this level in 1972. Over half of the strains were sensitive to 0.156%, and the proportion of sensitive strains approached but did not equal those seen in 1970 and 1971. The changes represent a fluctuation rather than a progressive increase in resistance. When projected graphically, in Figure 1, the sensitivity patterns for the years 1969 - 1973 were essentially consistent, except for the marked shift toward resistance seen in 1972. There were changes in the proportion of strains inhibited in the median range from year to year, but these were not extreme. In 1969 the curve closely paralleled that seen in 1973; in 1970 and 1971, the sensitivity values were again very similar. When it occurred, the shift from a linear response denoted the presence of a homogeneous group of resistant strains that distorted the typical curve. Such a group of strains accounted for the shift to the right seen in 1969 and 1973, and in 1972 the distortion was even more extreme due to the large group of more resistant strains recovered in that year.

Median levels of sensitivity for all the strains collected are shown on an annual basis in Table 3. It was evident that the median value for 1973 was next to the lowest observed over the whole five-year period. The relatively large variations in median range of sensitivity indicate that the response of Ps aeruginosa to Sulfamylon is a naturally fluctuating entity, and not one in which resistance is increasing slowly and progressively due to continuous selection of resistant strains in the presence of the drug.

A scrutiny of the principal phage types of Ps aeruginosa, and of strains typable only with concentrated phage (the "NT" strains), was made since the sensitivity totals suggested that groups of strains varying markedly in resistance to Sulfamylon, were being encountered at intervals (Latta RL, Lindberg RB, Pruitt BA, Jr., Mason AD, Jr. Institute of Surgical Research Annual Research Progress Report FY 1973, Section 12)[2]. In terms of numbers of strains, a conspicuous nosocomial epidemic in the first half of 1973 was due to a strain with phage type A-71, together with two loss-variants designated A-71a and A-71b. Table 4 shows the reactions of these three strains. A-71, with 17 out of 29 isolates requiring 0.312% for inhibition was evidently a relatively resistant strain although this resistance was obviously not complete, since inhibition occurred at 0.156% or less in 42% of A-71 strains. The numbers of A-71a and A-71b strains was small, but the distribution of strains requiring 0.312% for inhibition was

Table 2

Culmulative Sensitivity To Sulfamylon Of *Pseudomonas aeruginosa*
1969-1973

Year	No. of Strains	Concentration* and % of Total Strains Inhibited								
		2.5	1.25	0.625	0.312	0.156	0.078	0.039	0.019	< 0.019
1969	385	100	100	100	96.5	50.0	26.9	7.7	0.5	-
1970	296	100	100	100	100	78.0	49.9	21.9	2.0	-
1971	280	100	100	100	82.9	68.3	48.3	27.9	4.7	-
1972	463	100	100	93.7	48.0	38.0	19.0	12.3	4.3	1.1
1973	285	100	100	98.1	81.3	57.0	33.5	16.1	3.2	0.4

*Concentration in gms. of drug/100 ml. of medium

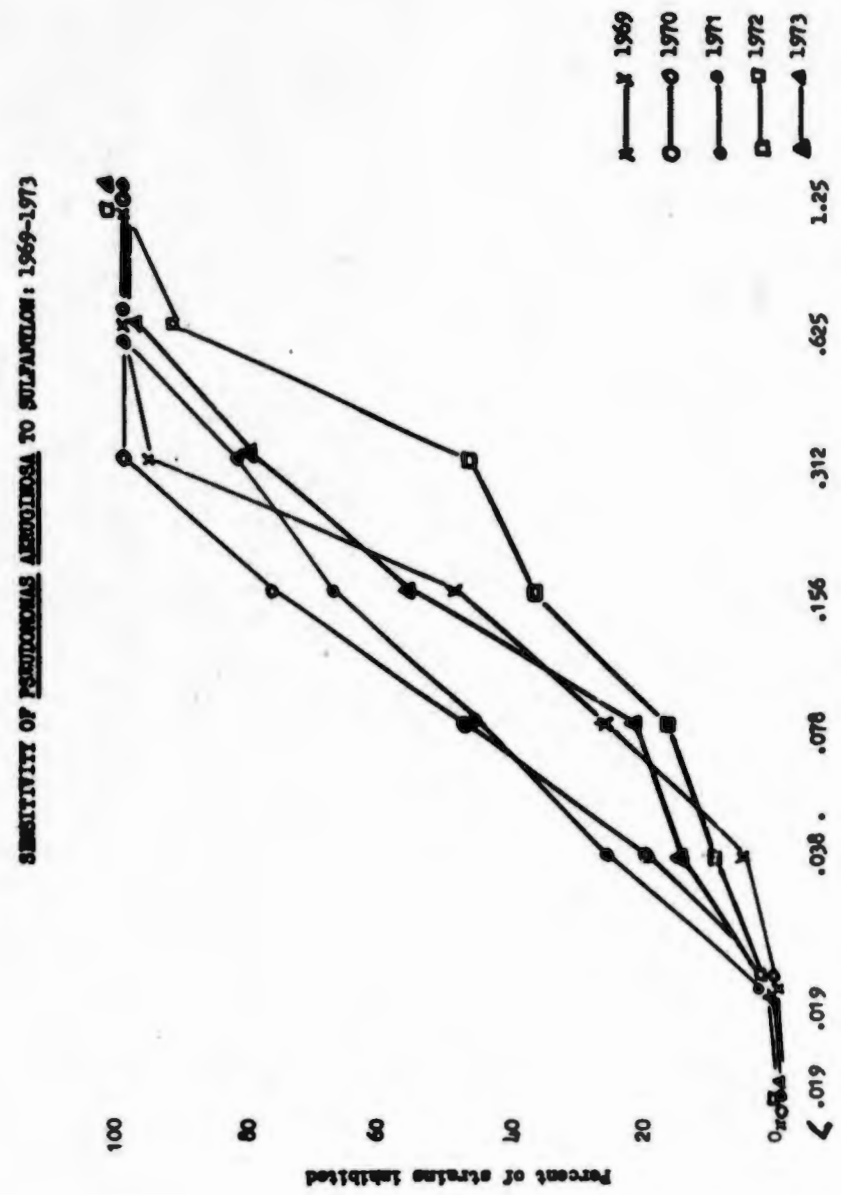
SENSITIVITY OF PSEUDOMONAS AERUGINOSA TO SULFANILAMIDE: 1969-1973
1969
1970
1971
1972
1973
Percent of strains inhibited
100
80
60
40
20
0
<.019
.019
.038
.078
.156
.312
.625
1.25
Concentration in gms/100 ml of medium

Figure 1

Table 3

Median Value of *Pseudomonas aeruginosa* Sensitivity to Sulfamylon

Year	No. of Strains	Median Inhibitory Value (gms.%)
1969	385	0.156
1970	296	0.078
1971	280	0.125
1972	463	0.316
1973	285	0.111
Total of 5 years	1709	0.131

8-6

Table 4

SULFAMYLON SENSITIVITY REACTION OF PREDOMINANT PHAGE TYPES - 1973

	PATIENT NO.	ISOLATES WITH INHIBITING CONCENTRATIONS* AT 1.25	0.625	0.312	0.156	0.078	0.039	0.019
Type A-71	39			3	2			
	44			3				
13 patients	30			2				
	55			1	1	1		
29 isolates	77			1		1		
	6				2			1
	64			4				
	47			1				
	9				1			
	97					1		
	75					1		
Total-each inhibiting strain	103						1	
	70			2				
				17	6	4	1	1
Type A-71 Pattern # 2	44				1			
5 patients, 8 isolates	30			2				
	77					1		
	34			1				
Total-each inhibiting strain	102					2	1	
				3	1	3	1	
Type A-71 pattern # 3	71			1				
	77					2		
5 patients 9 isolates	9							1
	30			2				
Total-each inhibiting strain	95					1		2
				3		3		3
Type A-2	48			1		1		
	177						5	
5 patients 12 isolates	199		2					
	78					1		
Total-each inhibiting strain	86					1		
	34							1
			2	1		3	5	1

*All concentrations in Gms. Sulfamylon/100 ml. medium

the same as that found for type A-71. A different type, M-2 appeared in a small epidemic outbreak later in 1973. Here too a small number of relatively resistant strains were recovered, although two-thirds of the strains were highly sensitive.

When the NT strains were reviewed as shown in Table 5, two distinguishable patterns appeared in large numbers, extending in each instance over a four-month period. One of these, NT4, showed a well defined group possessing slightly heightened resistance, with 52% of isolates requiring 0.312% for inhibition, and all but one of the remainder sensitive to 0.156%. This was a homogeneous pattern, with variation limited to the range of 0.156% to 0.312%. This narrow range was unique in the experience of this laboratory. There has usually been a broader zone over which variation occurred.

Type NT5, which also occurred over a four-month interval, made up a major part of the relatively resistant strains recovered in 1973. Twenty-six percent of these strains required 0.312% up to 1.25% for inhibition. At the same time, 67% were sensitive to 0.156% or less, so that on the whole, this type, if the strains were indeed all part of one epidemic, was not of a resistance level that would be expected to create problems in patients treated with Sulfamylon.

The remaining NT strains were distributed over a wide range of identity patterns. Twenty-one percent of them required 0.312% to 0.625% for inhibition, so that they could be regarded as relatively resistant. No one type occurred, however, in a predominant role.

Thus the predominant types of Ps aeruginosa encountered in 1973 included two, A-71 and NT4, with a group of strains recovered under temporal circumstances that suggested the occurrence of a nosocomial epidemic. However, highly resistant strains were recovered only in small numbers and in no instance could one of these be seen to proliferate selectively because of its drug tolerance.

The incidence of burn wound invasion could not be shown to occur in an extreme degree in the presence of any of these relatively resistant strains. During the periods in which type A-71 or NT4 were predominant, they were observed from all sources, but there was not an unusual incidence of burn wound infection occurring with either of these strains.

DISCUSSION

On an annual basis, populations of Ps aeruginosa in the Institute of Surgical Research patients have fluctuated in terms of the incidence of Sulfamylon-resistant strains. The term "resistance" is relative, since an MIC of 0.625% may, and often does, occur without clinical evidence that the strain in a burn wound is refractory to treatment. Conversely, strains inhibited by 0.312% or less have in some instances

Table 5

SULFAMYLON-SENSITIVITY REACTIONS OF VARIOUS NON-TYPABLE STRAINS

Non-typable Strain	1.25 gms.%	0.625 gms.%	.0312 gms.%	0.156 gms.%	0.078 gms.%	0.039 gms.%	0.019 gms.%
NT-4 21 patients 66 isolates			35	30	1		
NT-5 23 patients 61 isolates	4	5	7	41	4		
Other Non-typables 20 patients 28 isolates		3	3	5	10	6	1
TOTAL: ISOLATES - 155 64 PATIENTS	4	8	45	46	76	6	1

been shown to be refractory to treatment in the experimental burned rat model.

The relatively resistant groups of pseudomonads recovered during 1973 were distributed between principal phage patterns of identity - A-71 and NT4. A-71 strains were distributed over a wide range of inhibitory values; NT4 strains were inhibited in a uniquely narrow range between 0.312% and 0.156%. Such behavior cannot be described as "resistant", but may be considered to indicate moderate tolerance of Sulfamylon. Thus far the behavior of Ps aeruginosa in the presence of therapuetic Sulfamylon levels indicates that the strains remain essentially sensitive to the drug. Continued monitoring is mandatory in view of the unpredictable fluctuations that occur and of the demonstration of certain types that have been refractory to Sulfamylon burn cream ir treatment of experimental burn wound infection in the rat model.

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PRESENTATIONS

Lindberg RB. Gram negative opportunistic infections. Presented at Microbiology Seminar, Mayo Clinic, Rochester, Minn., 20 June 1973.

PUBLICATIONS

Lindberg RB, English VC, Latta RL, Pruitt BA, Jr. Differentiation of Virulence Mechanisms in Pseudomonas aeruginosa Invasive Infections. Fed Proc. 32:704, 1973.

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	8. DISP'N INSTR'NS	9a. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
73 07 01	K. COMPLETION	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	3A161102B71R	01	242
b. CONTRIBUTING				
c. CONTRIBUTING				

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FISCAL YEAR — CURRENT:		

19. RESPONSIBLE DOD ORGANIZATION
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21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede each with Security Classification Code): (U) Microbiology of burns; (U) Burns; (U) Pseudomonas; (U) Providence; (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) Determine qualitative and quantitative burn flora on admission of injured soldiers. Thermal injury results in maximum threat of infection, and military personnel in war and training, incur burn risk which calls for detailed knowledge of infecting agents to aid in therapy.

24. (U) Flora from day one onward sampled with specially devised contact plates, at time of admission. Detailed determinative bacteriology, typing and pathogenesis determinations aid in planning therapy and explaining problems.

25. (U) 73 07 - 74 06 In contrast to the period when most of the ISR patients were brought in from the Far East combat zone, admissions for the past 2 years have been primarily CONUS injuries, and a higher proportion of children have been admitted. This altered experience was reflected in a marked change in bacterial flora on arrival, and the use of culture swabs in sampling also resulted in fewer positive cultures and a smaller range of species observed than was the case with 100 mm agar contact cultures. Predominant flora on arrival included Enterobacter cloacae, Klebsiella pneumoniae, E. Coli, and Staphylococcus aureus. Two nosocomial infecting species of major importance, Staphylococcus aureus Type 84 and Providencia stuartii, were found on incoming patients, in a ratio that was unchanged over the past 2 years. These epidemic strains were obviously available from extraneous sources-they were not endemic epidemic strains of significance only under conditions of local transmission. Each of these species contributed in a major degree to the nosocomial infection problem of the burn ward.

*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.

ABSTRACT

PROJECT NO. 3A161102B71R-01, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: BACTERIAL FLORA ON MILITARY BURN PATIENTS AT TIME OF ADMISSION TO INSTITUTE OF SURGICAL RESEARCH

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

Period covered in report: 1 July 1973 - 30 June 1974

Investigators: Robert B. Lindberg, PhD
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The bacterial flora on 113 burn patients at the time of admission to the Institute of Surgical Research was assessed by swab cultures. Although this technic was again found to be less sensitive than the agar plate contact technic, it reflects the proportion of species brought into the burn ward. Sixty-nine percent of the patients yielded positive cultures. Predominant flora on admission included Staphylococcus aureus as the principal species. Phage types were heterogeneous, but type 84 was recovered in significant numbers. Staphylococcus epidermidis, an endogenous non-pathogen, was far more common than in previous years. The principal gram-negative enteric forms were Klebsiella pneumoniae, Enterobacter cloacae, and Escherichia coli. Pseudomonas aeruginosa and Providencia stuartii were most commonly found at three to five days postburn, and were found on one-eighth of all incoming patients. Continuous re-seeding of the burn ward population by the flora of incoming patients is indicated by these observations, and makes less plausible the assumption that a unique microbial flora is indigenous to this burn ward.

Microbiology of burns
Burns
Pseudomonas
Providence
Humans

BACTERIAL FLORA ON MILITARY BURN PATIENTS AT TIME OF ADMISSION TO THE INSTITUTE OF SURGICAL RESEARCH

Identification of bacterial flora resident on burn injuries constitutes an essential starting point in the study of the role of bacteria in burn wound pathogenesis. While numerous observations indicate that at the moment of injury the burn wound is essentially sterile, the wound surface is an extremely congenial environment for growth of many bacterial species, and it is rare for such wounds to remain sterile for more than 48 hours; far more often they are contaminated within minutes after injury.

The identity of bacteria present on burn wounds at the time of their admission to the burn ward obviously influences subsequent bacterial contamination and infection on these patients. The frequent speculations on the source of infecting species on a burn ward must at the least start with a differentiation between the bacterial flora present on the burn upon arrival at the burn ward, and that component which is subsequently acquired by cross-infection or self infection from the patient's own stool, urine or sputum, to name three common endogenous sources of burn wound seeding.

An example of a bacterial species which has presented a major problem as an infecting agent in burn patients at the Institute is Providencia stuartii, which was observed as an epidemic strain in burn patients before it had been reported from other burn treatment centers. It was suggested that the strain might be unique in having colonized this burn ward, but when as many as 50% of patients on arrival were found to harbor the organism, it was obvious that the epidemic aspect of this infection involved constant re-seeding by arriving patients.

The bacterial flora of arriving patients underwent marked change with the cessation of hostilities in Southeast Asia. The proportion of burn patients arriving soon after injury has been higher and the antecedent care of these patients is undoubtedly quite different from that of patients passing through the patient transfer system from the Far East. The present report is based on culture data from 113 patients examined on arrival at the Insitute burn ward during 1973.

METHODS

Earlier observations made use of contact cultures using 100 mm agar discs placed on the wound surface. This technic recovers a higher percentage of organisms than do other technics for wound surface sampling (Lindberg RB, English V, Moncrief JA, Pruitt BA, Jr. USA Surg Res Unit Ann Rpt FY 1968, BAMC, Ft Sam Houston, Tx. Sec 7)[1]. This technic has not been feasible under current conditions of admissions, and admission cultures have been taken with culture swabs moistened and transported in holding medium. This technic produces a smaller yield of positive cultures than the contact plate technic, but significant information on the incoming flora can still

be obtained.

RESULTS

Incidence of recovery of bacterial species among 113 patients on admission is shown in Table 1. As had been observed in 1972, the swab technic lowered the rate of recovery of bacteria. Only 69% of patients cultured on admission showed bacteria present on the burn; with contact plate technic, the recovery rate was in the range of 98% positive cultures. The high proportion of negative cultures were recorded in the zero to two day postburn group. Incidence of each species among those patients with positive cultures is a more realistic way of showing species incidence. The total of all cultures positive showed that Staphylococcus aureus was the predominant species, with 33% of positive patients carrying this organism. Typing results showed this group of strains to include a variety of phage types, which were usually quickly replaced by the resident phage type 84, after admission to the ward. Staphylococcus epidermidis, present in 29.4% of positive patients, was far more common than had previously been the case. Among coliform bacteria, Klebsiella pneumoniae, Enterobacter cloacae, and Escherichia coli were the most frequently encountered species. Pseudomonas aeruginosa and Prov stuartii, two species that play a large role in sepsis in the burn ward, were recovered each in 12.8% of positive patients. This incidence is similar to that seen in recent years. Control measures for these agents must be used with the realization that re-seeding of the burn ward will occur with new patients admitted.

Comparison of the principal predominant species with earlier data on bacterial flora of patients on arrival at the Institute of Surgical Research is made in Tables 2, 3, and 4. These data include earlier observations made with more sensitive sampling technics. Figures for 1968 serve as a typical year of large numbers of admissions from Viet Nam. The breakdown of time intervals was intended to detect seeding rates in patients soon after injury, and at arbitrarily designated intervals thereafter.

Ps aeruginosa has, in the past two years, been relatively low in incidence on arriving patients through the first 10 postburn days. The higher incidence noted in 1968 and 1970 was a reflection of the extensive seeding that occurred among Viet Nam evacuees. The likelihood of finding Ps aeruginosa on burns two weeks or more after injury is still very high. Widespread use of effective topical therapy may contribute to the recent finding of a lower incidence of Ps aeruginosa on burn wounds in the early postburn period. In 1973, the number of patients admitted later than the 10th postburn day made the incidence figures of less significance.

Klebsiella pneumonisa and Entero cloacae: The initial seeding of burn wounds with K pneumoniae reflected the common experience of endogenous forms seeding the burn wound. The contamination rate was at its highest, based on a significant number of admissions, up to five days. It was lower than had been the case in previous years, after the 6th postburn day.

Table 1. Cultures on 113 Patients on Admission to ISR, 1973: Swab Culture of Burn Surface

Species	% of all Patients Positive	% of Each Species Among Patients Positive
Pseudomonas aeruginosa	8.8	12.8
Klebsiella pneumoniae	19.4	28.2
Enterobacter cloacae	22.1	32.0
Enterobacter aerogenes	7.9	11.5
Escherichia coli	15.9	23.0
Serratia marcessens	1.7	2.5
Citrobacter freundii	3.5	5.1
Proteus mirabilis	3.5	3.5
Providencia stuartii	8.8	12.8
Mima-Herellea group	4.4	6.4
Staphylococcus aureus	23.0	33.3
Staphylococcus epidermidis	20.3	29.4
Streptococcus, non-hemolytic	8.8	12.8

No growth on swab culture: 30.9%

Total patients cultured: 113

Patients with positive cultures: 78

Table 2. Pseudomonas, Klebsiella, and Enterobacter on Burn Wounds at Time of Admission to ISR: 1968 - 1973

Postburn Interval	Species and % of Patients Positive											
	Pseudomonas				Klebsiella				Enterobacter*			
	68	70	72	73	68	70	72	73	68	70	72	73
0 - 2	21	7	9	0	29	21	20	22	40	32	38	41
3 - 5	46	54	5	17	55	54	32	39	48	54	64	35
6 - 10	50	4	16	10	67	30	42	20	37	27	37	14
11 - 20	52	55	48	40	51	40	52	20	26	21	38	3
21+	54	70	38	100	45	44	62	66	18	15	13	6

	No. Patients Cultured at Each Interval			
	1968	1970	1972**	1973**
0 - 2	47	28	34	37
3 - 5	56	11	22	23
6 - 10	97	26	19	10
11 - 20	78	82	21	5
21+	11	27	8	3
Total	289	174	104	78

* 73% of these strains were E. cloacae.
** This figure in 1972 and 1973 represents only patients with positive cultures. Before that time, more sensitive culture procedures recovered bacteria from every patient.

The number of patients admitted after 21 days was too small to permit conclusion to be drawn regarding late incidence of species. A similar pattern was seen with Enterobacter cloacae. The low carrying rate for this species is particularly emphasized because in subsequent observations, the contamination rate for E cloacae, once the patient reached the ward, became very high.

Prov stuartii and E coli: Prov stuartii has been a major offender in causing sepsis in burned patients in the Institute of Surgical Research. Its transmission pattern has been, presumably, patient-to-patient; as shown in Table 3. It has, since 1970, been relatively scarce at 0-2 days postburn, and indeed none were found in 1973 at this time interval. Significant numbers were recovered from 3 to 10 days postburn, although not nearly the magnitude seen in 1968. The incidence of this presumably exotic opportunistic burn infecting agent rose markedly in the second and third postburn weeks.

E coli is one of the most ubiquitous fecal species, and could be expected to be found in a high proportion of contaminated wounds. It was found in approximately one-fifth of patients at 0 to 2 days, then rose markedly up to day five. From the sixth postburn day onward the incidence rate fell through the third week postburn. E coli has, in frequent episodes, shown that it is capable of invading burn wounds, but the drop in incidence in 1973 suggests that such invasive episodes may be decreasing in incidence.

Staphylococci: Staph aureus has assumed an increasingly serious role as an infecting species in burn patients in this Institute. Table 4 shows that incoming patients in the first five postburn days were seeded with Staph aureus to a level higher than was seen several years ago. The rate of colonization by this species dropped in the later postburn period. The carrier rate was sufficient to serve as a source of seeded patients capable of contributing new strains to a burn ward. Thus current emphasis on anti-staphylococcal prophylaxis and therapy cannot offer an escape from the contamination brought in by new patients. The carrier strains included a significant number of phage type 84; thus, although the Institute of Surgical Research wards have entertained a virtual epidemic of type 84 over the last three years, this type also appears as part of the flora of newly-arrived patients.

Staph epidermidis, which is viewed as primarily part of the normal skin flora, exhibited an increased incidence on new arrivals in the first five postburn days. This organism is not regarded as of great importance in the development of burn wound infections and this increased incidence may reflect an absence of interfering flora, including gram-negative fecal bacilli.

DISCUSSION

The role of the bacteria brought by burned patients into a large burn ward

Table 3. Providencia stuartii and Escherichia coli on Burn Wounds at Time of Admission to ISR: 1968 - 1973

Postburn Interval	Species and % of Patients Positive							
	Providencia stuartii				Escherichia coli			
	68	70	72	73	68	70	72	73
0 - 2	21	7	9	0	29	21	20	22
3 - 5	46	54	5	17	55	54	32	39
6 - 10	50	4	16	10	67	30	43	20
11 - 20	52	55	48	40	51	40	52	20
21+	54	70	38	100	45	61	44	66

Table 4. Staphylococcus aureus and Staphylococcus epidermidis on Burn Wounds at Time of Admission to ISR: 1968 - 1973

Postburn Interval	Species and % of Patients Positive							
	Staph aureus				Staph epidermidis			
	68	70	72	73	68	70	72	73
0 - 2	14	21	38	38	48	70	20	35
3 - 5	13	36	22	39	30	27	14	30
6 - 10	24	54	21	20	17	15	5	20
11 - 20	28	54	38	20	30	8	5	0
21+	18	48	62	0	27	22	12	33

is intimately associated with the problem of control of nosocomial infection in burns. With two major epidemic problems, Providencia stuartii and Staph aureus Type 84, were brought in at a rate which, while lower than that seen in earlier years, was quite enough to keep the ward seeded with these species on a renewing basis. Ps aeruginosa, which has remained a recurrent problem in burn sepsis despite effective topical therapy, was brought on at a rate far lower than that seen five years ago, but still frequently enough to offer a new source of strains to keep the burn ward seeded. The incoming patient carrier rate has been low for the past two years, but this does not eliminate the probability that imcoming patients constitute a recurrent source of bacterial seeding in a burn ward.

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PRESENTATIONS AND/OR PUBLICATIONS

None

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(U) Burns; (U) Staph aureus; (U) Providencia stuartii; (U) Sepsis; (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) Soldiers in combat incur thermal injury at a high rate; in its treatment, suppression of invasiv infection is essential for survival and return to duty. Sulfamylon topical has achieved this end, but continued monitoring of wound flora is necessary to recognize new facets of infection as they occur.

24. (U) Contact cultures, biopsies, sputum, blood, urine and autopsy tissue cultures, qualitative and quantitative, are carried out to obtain a detailed chronologic picture of burn wound infection.

25. (U) 73 07 - 74 06 The overall incidence revealed both persistence of some major species and changes in the incidence of others. Staph aureus, predominantly type 84, increased by 43% over the preceding year. A three-fold rise in incidence of non-hemolytic streptococci was also observed. However, a 50% drop in incidence of Providencia stuartii occurred; the major epidemic of this opportunistic fecal form subsided in part, while Enterobacter cloccae and E. coli rose in incidence Total antibiotic resistance in Providencia stuartii still made sepsis with this organism a major problem. Pseudomonas aeruginosa, in micro-epidemics of specific types, presented a unique problem in drug-resistance not previously observed. Gram-negative sepsis continued to present a major threat to survival of the severely burned soldier.

*Available to contractors upon originator's approval

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ABSTRACT

PROJECT NO. 3A161102B71R-01, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: PATHOGENESIS OF BURN WOUND INFECTION: BACTERIAL FLORA OF BURN WOUNDS ON MILITARY PERSONNEL RECEIVING SULFAMYLON TREATMENT

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Period covered in this report: 1 July 1973 - 30 June 1974

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Sepsis as a major cause of morbidity and death continued with no indication of improvement in its control in 1973. Epidemic populations of Staphylococcus aureus, Pseudomonas aeruginosa and Providencia stuartii remained a fixed part of the burn ward population; other species of Enterobacteriaceae, including Escherichia coli and Klebsiella pneumoniae, may have increased in incidence and as incitants of burn wound infection and systemic infection secondary to the burn wound. Staph aureus was most prominent in septicemia and on burn wounds, and the second most common organism in sputum. Providencia stuartii and Pseudomonas were the other major offenders in septicemia; for the first time E coli was prominent in sputum and in burn wounds. Highly resistant strains of gram negative bacilli were a major factor in gram negative sepsis.

Burns
Staph aureus
Providencia stuartii
Sepsis
Humans

PATHOGENESIS OF BURN WOUND INFECTION: BACTERIAL FLORA OF BURN WOUNDS ON MILITARY PERSONNEL RECEIVING SULFAMYLON TREATMENT

It is almost an anomaly that, with years of intensive effort to control infection in burns, with the evolution of a massive armamentarium of new and broad spectrum antibiotics, and with development of three widely used topical agents for control of burn wound infection, that infection should still be the major factor in deaths due to burns, and that control of infection remains a continuing and an incompletely solved problem. Classical burn wound sepsis, as a primary lethal event occurring in an otherwise potentially viable burn patient, has become relatively rare, but late-occurring burn wound sepsis, due to one of several species of Enterobacteriaceae or to Pseudomonas aeruginosa, is seen with disturbing frequency. Burn wounds from which eschar has separated but which are not suitable to accept skin grafts constitute a major threat to survival, and infection in such sites involving a mixed microbial flora or a succession of species remains a frequently insoluble problem. The burn wound flora has not remained constant from year to year. Alterations in incidence of species and the predominant flora observed in various sites of infection can be determined by scrutiny of bacteriologic culture results, on an annual basis. Patterns of colonization are a fundamental aspect of the epidemiologic problems involved in infection control in burns, and this on-going study presents the infection patterns observed in 1973. Observations on post mortem bacteriology are here summarized as well. Not infrequently definitive information on the role of bacterial species in burn deaths can only be determined by autopsy bacteriology.

The topical agent used in treatment of burned patients affects not only the flora on the treated patient, but also the wound flora on all burn patients in the burn wards. The information set forth here presumes that most of the patients, if not all, were treated with Sulfamylon. There were, in 1973, several patients treated with silver nitrate soaks, and an additional modifying factor was the very extensive use of 5% Sulfamylon soaks on burns after the second postburn week (Erickson DL, Hunt JL, Pruitt BA, Jr. USA Institute of Surg Res, BAMC, Ft Sam Houston, Texas Ann Rpt FY 73, Sect 14)[1]. These factors are significant in the milieu which affects survival of bacterial species on a burn. Treatment undoubtedly affects the total burn patient flora, and can account for changes which may be observed. Since infection is due to specific organisms, whose role varies with the presence of an environment that permits their survival, these institutionally modified bacterial populations are of fundamental concern in burn management.

ANTEMORTEM BACTERIOLOGY IN BURNED PATIENTS

Total Cultures. The bacterial flora (plyeasts) cultured in 1973 is summarized in Table 1. The principal sources were the burn wound itself, sputum (or Luken's tube aspiration) and biopsies. Predominant species

Table 1. Bacteriology of Antemortem Burn Patients - 1973

Organism	Source and Number Isolates				Cath. Tip			Total Isolates
	Wound Surfaces	Blood	Lukens Tube	Urine	I.V.	Foley	Biopsy	
Staph coag.pos (1)	541	108	205	33	64	27	135	1113
coag.neg (2)	79	7	66	16	14	15	15	212
Alpha hemolytic strep.	39	1	171	2	-	-	1	214
Beta hemolytic strep.	2	-	10	-	-	-	-	12
Group A hemolytic strep.	1	-	2	-	-	-	-	1
Non-hemolytic strep.	81	14	140	31	7	21	28	322
Diplococcus pneumoniae	-	-	1	-	-	-	-	1
Corynebacterium sp.	67	4	12	3	2	2	16	106
Bacillus sp.	6	3	3	3	2	1	19	37
Pseudomonas sp. (3)	190	30	242	34	9	19	68	592
Mima-Herellea gp.	21	-	15	2	2	2	8	50
Aeromonas sp.	3	-	2	-	-	-	-	5
Klebsiella pneumoniae	148	16	281	53	11	37	32	578
Enterobacter aerogenes	42	3	54	7	3	4	10	127
cloacae	105	11	42	12	11	12	53	266
hafniae	7	2	7	-	-	1	2	19
species	-	-	3	-	-	-	-	3
Serratia marcescens	9	-	9	1	4	1	-	24
Escherichia coli	159	10	257	60	11	45	53	595
Citrobacter sp.	6	-	5	2	1	1	4	19
Proteus mirabilis	43	8	62	30	2	11	14	170
morganii	3	-	-	4	-	-	-	7
Providencia stuartii	236	79	275	77	46	56	104	873
Neisseria sp.	6	-	28	1	-	-	1	36
Candida sp. (4)	73	28	43	39	18	17	44	262
"Escherichia sp." (5)	6	-	12	2	1	2	2	25
Number of Isolates	1873	324	1971	412	208	275	609	5672
Number Patients Cultured	191	150	130	125	163	122	106	
Total Specimens Received	5794							

(1) Staph aureus
(2) Staph. epidermidis in all instances in which definitive identification was made
(3) Essentially Ps aeruginosa; a small number of maltophilia, stutzeri and fluorescens occurred
(4) Most were C albicans, but other species were also found.
(5) These resembled E. coli but did not conform to that species. Less frequently encountered genera, including Levines and Yersinia, may have been involved.

included Staphylococcus aureus, Pseudomonas (primarily Ps aeruginosa), Klebsiella pneumoniae, Escherichia coli and Providencia stuartii. These have been the predominant species from all specimens cultured for the past several years. In 1973 they made up 66.2% of all bacterial isolates. This incidence has been remarkably consistent in successive years but shifts in the occurrence of these five species of opportunisitic pathogens are noteworthy (Lindberg RB, Contreras AA, Smith HOD,Jr, Kirchgessner PM, Pruitt BA, Jr. USA Institute Surg Res, BAMC, Ft Sam Houston Texas Ann Rpt FY 73, Sect 11)[2]. The incidence of these species since 1970 is summarized in Table 2. In 1973 a sharp rise in incidence of Staph aureus occurred, as was the case also with E coli. Pseudomonas and Klebsiella strains have been recovered at a remarkably uniform rate, in terms of total isolates. Providencia stuartii occurred far less frequently in 1973 than in 1972 or 1970; it virtually coincided with the recovery rate of 1971, and the overall picture is that of an important opportunistic species which fluctuates markedly in incidence over extended time periods. This appears to be the case with E. coli as well.

Table 2. Predominant Species Among all Clinical Isolates, 1970-1973

Species	% of all isolates from Clinical Specimens			
	1970	1971	1972	1973
Staph aureus	12.6	15.0	13.8	19.6
Ps aeruginosa	13.6	12.4	13.2	10.4
Klebsiella pneumoniae	11.5	9.7	11.5	10.1
E coli	6.4	11.0	6.1	10.4
Providencia stuartii	21.0	15.2	23.1	15.7
% of all isolates	65.1	63.3	67.7	66.2
Number of isolates	3293	3179	6696	5672

There were 26 bacterial species or groups recovered from burn patients in 1973. Most of these were infrequent in producing invasion of burn wounds, or sepsis from some other site. A major part of the infection problem in the Institute of Surgical Research is concerned with five predominant species.

Bacteriology of the Burn Surface. The burn wound is a uniquely susceptible site for infection, although it is not of necessity a major source of sepsis. Control of invasive wound sepsis is now a well-established part of modern burn management, and this is done primarily by topical therapy, up to the point in the patients' course when debridement and homografting or xenografting are carried out. Surface flora is a major

source of the extensive environmental contamination that usually occurs in burn wards. Burn patients, once seeded, can shed a vast number of bacteria in their immediate environment.

Bacterial species recovered on the burn wound surface, and the proportion of patients colonized are summarized in Table 3. The number of strains isolated included surface cultures and biopsies, since both reflect the burn wound flora. Seventy-four per cent of all admissions were cultured at least once.

Table 3. Predominant Burn Wound Surface Flora in 201 Patients, ISR, 1973

Species	No. of Strains Isolated	No. of Patients Positive on Burn Surface	% of Patients Positive
Staph aureus	676	148	73.6
Staph epidermidis	94	41	20.4
Strep non-hemo*	109	59	29.4
Kleb. pneumoniae	180	67	33.3
Enterobacter cloacae	203	46	22.9
E. coli	212	78	38.8
Proteus mirabilis	57	33	16.4
Prov stuartii	340	79	39.3
Ps aeruginosa	258	70	34.8

* Strep were primarily Group D.

Staph aureus was the most frequently encountered pathogen on burn wounds as it had been in the previous year. The strains were predominantly type 84. This type has been endemic in this burn ward and vastly predominant since 1971. Antibiotic resistance of this epidemic strain was not as high as it had been in 1972, but it was still a major threat as a strain causing serious infection. Seventy-three and six-tenths per cent of patients had this organism on their burn wounds, a level close to the 69.6% incidence recorded in 1972. There were still 26.4% of patients who did not yield staph cultures during their hospital stay. The reason for this absence has not been determined.

Staph epidermidis was found on burns of one-fifth of patients admitted to this Institute. This was a marked increase over the incidence in 1972. While it is not necessarily an indication of a new pathogenic problem,

this increase was the basis for more careful scrutiny of the behavior of Staph epidermidis in the burn wound. The strains were recovered in most instances early in the course of the patients' illness, and in cases where sepsis or pneumonia supervened, the coagulase negative staphylococci tended to disappear.

Non-hemolytic Streptococci occurred on burn wound surfaces at a relatively constant rate, on approximately one-fourth of the patients cultured. These strains were almost entirely Lancefield Group D. They were occasionally the cause of sepsis, and 14 episodes of septicemia were caused by this organism.

Klebsiella pneumoniae was an important part of the burn wound flora numerically, although the proportion of patients harboring it decreased in 1973 from a high level of 43% observed in the previous year. Other Enterobacteriaceae of major importance included Enterobacter cloacae, E. coli, Proteus mirabilis and Providencia stuartii. The proportion of patients from whom these species were recovered did not vary markedly from that noted in 1972; if anything, there was a drop in the percentage of patients with positive burn cultures. This was also the case with Ps aeruginosa; in 1972, 40.2% of patients harbored this species in wounds at some time during their hospital stay. This proportion was 34.8% in 1973. Such variation does not suggest any striking change in the burn wound flora during 1973.

An opportunistic pathogen which is now rare, Group A Streptococci, changed its incidence strikingly in 1973. After several years of extreme rarity, a series of small epidemics occurred among burn patients in 1972. This was the year when routine prophylactic use of penicillin on incoming patients was discontinued. Fifty-six strains were recovered from 31 patients in that year, as shown in Table 4. In 1973, only one strain was recovered from a wound, and two more from bronchial cultures. The cause of the unusual incidence observed in 1972 was, of course, assumed to be the discontinuation of use of routine penicillin G on incoming patients, but the diappearance of the organism in 1973, despite the absence of routine prophylactic penicillin, makes this explanation less likely. The marked variability of behavior of woundinfecting species is underscored by these changes in Group A Streptococci.

Table 4. Incidence of Group A Streptococci on Burn Wounds

Year	Nr. of Strains of Group A Streptococci Recovered
1969	8
1970	2
1971	1
1972	56
1973	3

RESPIRATORY TRACT BACTERIAL FLORA IN BURNS

Pneumonia has become, in the past four years, a major cause of death in burn patients. The disease is one in which various opportunistic invaders may function as the actual infecting agent, but the disease appears more as one in which an opportunistic organism invades a site pre-disposed to its entry by preexisting damage or a poorly functioning defense mechansim. The increasing concern of clinicians with the respiratory aspect of burn injury is reflected in the number of sputum and Luken's tube samples submitted for culture. The number of samples increased from 287 in 1971 to 721 in 1972 and to 847 in 1973. These figures suggest, at the least, a concern for the respiratory problem in burn patients, if not an actual increase in symptoms of pneumonia.

The results of sputum cultures, for the most important species recovered, are shown in Table 5. The comparative figures for proportion of patients positive are set down for 1971 and 1972 as well. There was an actual rise in the incidence of Staph aureus, from 38.5% in 1972 to 56.9% of patients cultured in 1973. At the same time, an increase in staphylococcal pneumonia was noted among patients on the burn wards.

Klebsiella pneumoniae remained the most prominent part of the sputum flora in burn patients, since 60% of patients cultured harbored this species. It was not, however, the princpal offending agent in bacterial pneumonia. This role was occupied by Providencia stuartii, although a smaller proportion of patients harbored this organism than was the case with Klebsiella pneumonia. Providencia actually dropped 16% in incidence in sputum cultures in 1973, but it was the predominant organism in clinical pneumonia.

Table 5. Principal Species of Bacteria Recovered from Respiratory Tract of Burned Patients, 1971-1973

Species	% of Patients Exhibiting Positive Sputum on Culture		
	1971	1972	1973
Staph aureus	43.0	38.5	56.9
Klebsiella pneumonia	45.0	58.8	60.0
Enterobacter cloacae	11.0	27.0	23.8
Escherichia coli	27.2	40.9	53.8
Proteus mirabilis	-	19.0	10.8
Providencia stuartii	33.0	56.5	40.8
Pseudomonas aeruginosa	39.0	38.5	36.2
Patients cultured	94	122	130

Pseudomonas aeruginosa, which continued to play a significant role in lethal pneumonia, remained almost uniquely constant in its rate of appearance in sputa in 1973. It was remarkable that this ubiquitous and effective opportunistic invader displayed, over this three-year period, a maximum variation of only 2.8% in incidence on an annual basis. The presence of Pseudomonas and Providencia together as predominant organisms in a sputum with a bacterial content of 10^5 or greater was one of the most serious prognostic bacteriologic signs. Such patients were among the highest risk category in terms of mortality ascribed to pneumonia.

Enterobacter cloacae and E. coli continued, in 1973, the trend toward increased incidence first seen in 1972. Neither species caused a marked rise in sepsis or septicemia, although a discernible increase in E. coli septicemia was suggested by the cultured results.

SEPTICEMIA AND BACTEREMIA IN BURN PATIENTS

It has been observed with a certain degree of surprise that, despite an ever-increasing armamentarium of antibacterial drugs, both topical and systemic, that sepsis and blood stream invasion remain the major cause of death in burns. Septicemia in a burn patient is today, as it was three decades ago, a grave threat to survival. Blood cultures in this, as in other burn study centers, are collected at the least sign of impending sepsis, and often in a serial fashion in any patient who remains febrile. In 1973, there were 150 patients from whom blood cultures were collected. Eighty-one, or 54.0% of these, exhibited at least one positive culture. This rate of positive cultures was in the same range as the 47% rate observed in 1972. The rate of bacteremia has apparently risen from the incidence of 30% of positive blood cultures which occurred in 1971. There were 1119 sets of two-bottle blood cultures collected, for an average collection of 7.4 cultures per patient. In 1971 the rate had been 7.5; in 1972, many more cultures were collected, and the rate in that year was 11.8% cultures per patient.

Results of blood cultures from 150 patients in 1973 are summarized in Table 6. Predominant species included Staph aureus, Ps aeruginosa, Klebsiella pneumoniae, Providencia stuartii, Candida sp, and non-hemolytic streptococci. Eighty-one patients exhibited at least one positive blood culture. Of these, 42, or 51.8%, yielded only one species of bacterium. The remaining 39 yielded two or more species in successive blood cultures. Among those species which occurred often enough to be numerically important, survival rates for patients with positive blood cultures were not encouraging. Table 6 sets forth the incidence of different bacterial species and the outcome probablity in relation to species of organism invading the blood stream. In particular, the outcome in patients who had Staph aureus septicemia at some stage in their illness was not encouraging. Seventy per cent of these patients died. Among the same group in 1972, the death rate in this group was 49%. This included both patients with multiple species present in successive blood cultures, and those with only a staphylococcus bacteremia. The death rate for the

Table 6. Blood Culture Isolates from 150 Burn Patients, 1973
Relation of Species to Mortality

Species	No. Patients Positive	No. Isolates	% of all Patients Cultured Positive	No. Patients Expired	% of all Patients with Positive Cultures who Expired
Staph.coag.pos.	30	108	20.0	21	70
coag.neg	7	7	4.7	3	42.8
alpha hemol.strep	1	1	0.7	1	-
non-hemol.strep	9	14	6.0	7	77.7
Bacillus sp.	3	3	2.0	2	-
Pseudomonas sp.	18	30	12.0	16	88.8
Kleb.pneumonia	9	16	6.6	7	77.7
Entero.aerogenes	2	3	1.3	1	-
cloacae	6	11	4.0	5	83.3
hafniae	1	2	0.7	1	-
E. coli	5	10	3.3	4	80.0
Prot.mirabilis	3	8	2.0	3	-
Prov. stuartii	30	79	20.0	27	90.0
Candida sp	15	28	10.0	12	80.0

10-8

latter group was much lower. Numerically important species included the gram negative bacilli enumerated above. With these, when all patients with a given species were included, mortality ranged from 77.7% with Klebsiella sp to 90.0% for Providencia stuartii.

The patients with only one species recovered offer a different perspective on septicemia and lethality than is the case with mixed infections. The number of patients with each species is shown in Table 7. The death rates for Providencia and Klebsiella infection were extremely high. Other gram negative species were not numerous enough to offer significant data. Staph aureus was recovered as the sole blood stream invader in 12 patients; 4 died. When recovered as the sole blood stream pathogen, the survival rate was far better than when it was one in a succession of invaders.

Table 7. Bacteremia with Only One Species Recovered, Burn Patients, 1973

Species	Total Patients Positive	Deaths	%
Providencia stuartii	8	6	75.0
Staph aureus	12	4	38.3
Staph epidermidis	3	1	33.3
Klebsiella sp	7	6	85.7
E coli	3	2	66.6
Enterobacter cloacae	1	1	-
Pseudomonas sp	3	2	66.6
alpha hemol.strep	1	1	-
non-hemol.strep	2	1	-
Bacillus sp	1	1	-
Candida sp	1	0	-
Totals	42	25	

Blood culture findings when a succession of two or more species were recovered, or when mixed cultures were recovered in a single specimen, are summarized in Table 8. These are the results observed in 39 patients during 1973. A very heterogeneous flora characterized almost half of the burn patients who developed sepsis. Only in five instances did a combination occur more than once. Two of th[illegible] in which Staph aureus occurred in conjunction with Providenceia [illegible]ne combination and with Pseudomonas and Providencia in the other, each occurred in four patients.

Table 8. Blood Culture Results in Patients with More than One Species Recovered, 1973

Species Recovered	No. Patients
Staph aureus, Prov. stuartii	4
Staph aureus, Pseudomonas, Prov.stuartii	4
Staph aureus, Pseudomonas	2
Staph aureus, Prov.stuartii,Candida	2
Staph aureus, E. coli	1
Staph aureus, Prot.mirabilis	1
Staph aureus, Corynebacterium, Prov.stuartii	1
Staph aureus, Corynebacterium, Prov.stuartii, Staph epidermidis, Candida sp	1
Staph aureus, non-hemolytic strep, Klebsiella sp	1
Staph aureus, Corynebacterium,non-hemolytic strep, Pseudomonas	1
Staph aureus, Pseudomonas, E. coli, Entero.hafniae, Prov.stuartii	1
Prov. stuartii, Candida	2
Prov.stuartii, non-hemolytic strep	1
Prov. stuartii, Pseudomonas	1
Prov. stuartii, Prot.mirabilis, Pseudomonas, non-hemolytic strep	1
Prov.stuartii, Pseudomonas, Entero. aerogenes, Entero. cloacae, Candida	1
Prov. stuartii, Candida, non-hemolytic strep	1
Prov. stuartii, Candida, Staph.epidermidis	1
Prov. stuartii, Pseudomonas, Prot. mirabilis	1
Prov. stuartii, Candida, Entero. cloacae	1
Prov. stuartii, E. coli	1
E. coli, Klebsiella sp, Entero.aerogenes	1
Klebsiella sp, Candida	1
Entero.cloacae, non-hemolytic strep, E.coli,Candida	1
Entero. cloacae, Candida	1
Entero. cloacae, Staph. epidermidis	1
Pseudomonas, non-hemolytic strep, Candida	1
Staph epidermidis, Corynebacterium, Candida	1
Staph epidermidis, Bacillus sp	1
Candida, Bacillus sp	1
Total Patients	39

		% of all Positives
No. of patients with 2 spp recovered	19	23.4
No. of patients with 3 spp recovered	14	17.2
No. of patients with 4 spp recovered	3	3.7
No. of patients with 5 spp recovered	3	3.7
No. of patients with one spp recovered	42	
Total with positive blood cultures	81	51.8

Staphylococci accompanied Pseudomonas in two patients and in two others, occurred with Providencia and Candida. There did not appear to be a uniquely lethal infection sequence. In general, Staph aureus appeared early in the patients course, with gram negative bacilli appearing later.

BIOPSY OF BURN WOUNDS

Diagnostic and prognostic criteria of burn wounds have been assessed in increasing degree by biopsy of wound areas, with species, predominant organism, and total number of bacteria all contributing to the diagnostic effort. There has come to be an increasing emphasis on the count of viable bacteria as a major index of nature of bacterial involvement in the burn wound. The relation of species recovered in biopsy to likelihood of survival may be revealed in some degree by summarizing data on biopsies collected.

Table 9 summarizes the species recovered from biopsies on 106 patients during 1973. This is the largest group of patients to be thus studied in one year in this Institute. In terms of number of patients positive, Staph aureus, Pseudomonas, Klebsiella pneumoniae, Enterobacter cloacae, E. coli and Providencia stuartii were the most important species. Patients with positive biopsies for these species had a relatively high mortality rate: from 50% with Staph aureus to 66% with E. coli. There is, of course, no valid basis for regarding these phenomena as causally related, but these data are consistent with other indices of systemic involvement with invading microorganisms. The remaining species recovered were numerically of less importance, although they could be associated with a mortality rate as high as that seen with the predominant species.

A retrospective consideration of biopsy flora and the mortality rate in patients cultured by this means was assembled by comparing results from 1969 to 1973. Table 10 presents these results. The recovery rate in biopsies varied from year to year but was more noteworthy for the relative constancy of occurrence of several of the major species. Providencia was the most variable of this group of infecting agents; on an annual basis it fluctuated between 56% and 66%. The mortality rate among patients positive for the different species fluctuated in a manner not consistent with the incidence of the organism. The staphylococcus-positive group had the highest death rate in 1973 of any year thus far observed. The death rate associated with Providencia cultures was high, but it had been higher in 1971. Klebsiella was markedly higher in association with mortality than it had been in 1972, as was E. coli, and Proteus mirabilis, although its numerical incidence was low, exhibited an associated death rate far higher than that for any previous year. The comparison suggests a relatively high death rate for the whole burn population in 1973, rather than a specific association with any one species in the series.

PROVIDENCIA STUARTII IN BURN PATIENTS

Table 9. Bacterial Flora of Biopsies on Burn Wounds of 106 Patients, 1973

Species	No. Patients Positive	% of Patients Positive	No. Patients with Positive Cultures who Expired	% of Patients Positive who Expired
Staph aureus	55	51.9	28	50.9
Staph epidermidis	13	12.2	6	46.1
alpha hemolytic strep	1	0.9	1	-
Non-hemolytic strep	17	16.0	9	52.9
Corynebacterium sp	11	10.4	4	36.3
Bacillus sp	10	9.4	4	40.0
Pseudomonas sp	34	32.1	17	50.0
Mima gp	6	5.7	1	-
Klebsiella pneumoniae	18	17.0	10	55.5
Enterobacter aerogenes	7	6.6	3	-
cloacae	21	19.8	13	61.9
hafniae	2	1.9	2	-
E. coli	27	25.5	18	66.6
Citrobacter sp	3	2.8	2	-
Proteus mirabilis	10	9.4	6	60.0
Prov.stuartii	39	36.8	20	51.2
Candida sp	21	24.5	16	61.5
Neisseria sp	1	0.9	1	-
Escherichia sp	2	1.9	2	-

No. of specimens collected: 400
No. of samples per patient (average): 3.7

Table 10. Burn Wound Biopsy Flora, 1969-1974:
Species Incidence and Mortality

Species	% of Patients Positive				% of Patients Positive who Expired			
	69	71	72	73	69	71	72	73
Staph aureus	42	44	41	51.9	22	38	22	50.9
Prov.stuartii	51	40	56	36.8	14	58	36	51.2
Ps.aeruginosa	30	30	32	32.1	39	57	20	50.0
Klebsiella sp	20	17	32	17.0	50	31	19	55.5
E. coli	14	19	27	25.5	47	33	16	66.6
Prot. mirabilis	34	13	14	9.4	38	40	9	60.0

Recent recognition of burn infection due to Providencia stuartii on an epidemic scale have prompted more detailed study of this little-known opportunistic invader. Variations in its frequency of occurrence have been seen, and its natural history as infecting agent in a susceptible population is not known. The severity of these infections in this burn center has exceeded that of outbreaks reported elsewhere (Zawacki, B Personal communication)[3]. Incidence of Prov. stuartii in clinical and autopsy specimens in 1973 is summarized in Table 11. Among clinical specimens, it was slightly less common on the wound surface than in wound biopsies, where 36.8% of all patients, and 26% of all samples were positive. One-fifth of all patients with blood cultures collected had Prov. stuartii recovered. Sputum samples were the most heavily colonized of all clinical specimens, and in burn autopsies, 53.8% of 91 patients harbored the organism in the lung. The highest concentration of all was observed in autopsy burn wound samples; with 59.3% of samples positive. The invasiveness of this opportunist is strongly indicated.

A comparison of the frequency of occurrence of this important burn pathogen since 1969 is shown in Table 12. There has been a fluctuation of frequency of Prov. stuartii in successive increments of burn patients. Septicemia caused by Prov. stuartii was most frequent in 1971, although the highest frequency in sputum biopsy and wound surface occurred in 1972. Although there was a slight drop in frequency of recovery in 1973, there is no reason to regard the problem as controlled, and it remains a major threat to survival in the severely burned patient. Since all strains were completely cross-resistant to antibiotics, this therapeutic approach remained ineffective in 1972.

I.V. CATHETER TIP CULTURES

The policy of regarding indwelling I.V. catheters as a major cause of intravenous infection has made surveillance of this entity an on-going project. In 1973, I.V. catheter tips from 163 patients were cultured. Four hundred seventy-five catheter tips were cultured. The bacterial flora recovered differed qualitatively from that seen on wound surfaces; it suggested a species differential potential for colonization of the indwelling catheter. The species recovered are shown in Table 13, Staph aureus was the most commonly encountered organism; of the other species important in causing sepsis in burns, only Providencia was encountered at a significant rate. Ps aeruginosa, Klebsiella and Enterobacter sp were far less frequently encountered than would be expected with the incidence of these species on the burn wound and in the respiratory tract. Localization of bacteria on the catheter tip is, in view of this information, a presumably selective phenomenon.

DISCUSSION

Sepsis as a complication of burns remains a critical factor in burn wound pathology. The continued elevated mortality seen in 1973 and the higher colonization rates for opportunistic invaders suggest that this

Table 11. Providencia stuartii: Isolates from Clinical and Autopsy Specimens 1973

Source	No. Isolates/ Total Specimens	Per Cent Positive	No. Patients Positive/Total Patients Cultured	Per Cent of Cultured Patients Positive
Burn wound, swab, clinical	236/1216	19.4	65/191	34.0
Biopsy, wound	104/400	26.0	39/106	36.8
Blood Culture	79/1119	7.0	30/150	20.0
Sputum (and Lukens)	275/840	32.7	53/130	40.8
Urine	77/438	17.6	29/125	23.2
Foley Cath. tips	56/187	29.9	38/122	31.1
I.V. Cath. tips	46/475	30.7	27/163	16.6
Autopsy: burn	274/705	38.9	54/91	59.3
Autopsy: lung	163/362	45.0	49/91	53.8

Table 12. Percent of Patients Cultured who Harbored Providencia stuartii

Year	Site of Culture and % Positive			
	Wound	Biopsy	Blood	Sputum
1969	46	51	32.9	54.4
1970	43.7	45.6	14.0	67.7
1971	34.0	40.0	36.5	33.0
1972	49.5	55.4	23.9	56.6
1973	34.0	36.8	20.0	40.8

Table 13. Bacterial Flora of I.V. Catheter Tips from 163 Burn Patients, 1973

Species	No. Patients Positive	% of Patients Positive
Staph aureus	37	22.7
Staph epidermidis	8	
Non-hemolytic strep	13	8.0
Corynebacterium sp	2	
Bacillus sp	2	
Pseudomonas sp	8	4.9
Mima group	2	
Klebsiella pneumoniae	10	6.1
Enterobacter aerogenes	3	
Enterobacter cloacae	9	5.5
Serratia sp	4	
E. coli	10	6.1
Citrobacter sp	1	
Proteus mirabilis	1	
Prov. stuartii	27	16.6
Candida sp	14	8.6
Escherichia sp	1	

problem has indeed become more acute in the past 2 to 3 years. The principal offending species in most sites included Staph aureus which has appeared in several infection sites with increasing frequency in the past 2 years. Elsewhere the biology of this epidemic situation is treated in greater detail. Among gram negative opportunistic invaders Providencia stuartii was still conspicuous but less so than in 1971 and 1972. Klebsiella pneumoniae and E coli were more conspicuous than they had been in 1973, and recurrent small epidemics of Serratia marcessens served as a reminder that this opportunist retains a degree of invasive capability not seen in other enteric bacteria. The concentrations of Providencia stuartii continued to indicate tissue-invading capability of this little understood enteric form. There was a continued indication that Klebsiella pneumoniae has increased in pathogenic potential in the past two years; both in overall incidence and in recovery from patients with sepsis, this species has increased in significance in burn wounds. The high levels of antibiotic resistance still seen in Enterobacteriaceae and especially in Providencia militate against successful control of sepsis due to these forms in burn patients.

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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 SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8A. GP/O HISTR'N	8B. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
73 07 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	61102A	3A161102B71R	01	188
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Procede with Security Classification Code)

(U) Bacteriophage Types of Pseudomonas Aeruginosa Found in Burned Soldiers (44)

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003500 Clinical Medicine

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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)
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	CURRENT 75	.6	14

19. RESPONSIBLE DOD ORGANIZATION

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FOREIGN INTELLIGENCE NOT CONSIDERED

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NAME: DA

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(U) Pseudomonas; (U) Phage typing; (U) Burn wounds; (U) Topical chemotherapy; (U) Humans

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by item no. Precede text of each with DoD Security Classification Code)

23. (U) Pseudomonas aeruginosa has remained a major pathogen in lethal burn infections in spite of the discovery of effective formulations which control Pseudomonas burn wound sepsis. Nosocomial infections in patients with traumatic injuries other than thermal continue to increase. Precise delineation of identity of infecting strains thus continues to be urgently needed, and phage typing as developed in this laboratory, is an effective procedure for monitoring effectiveness of therapy, the recognition of drug resistant forms, and the interpretation of environmental contamination.

24. (U) Classical phage typing procedures, which formerly categorized over 90% of isolates, now fail to differentiate an acceptable proportion of strains. To correct this deficiency, concentrated typing fluids of the original typing set are used to type otherwise non-typable strains. The concentrated fluids are used in a manner comparable to that used for conventional phage typing.

25. (U) 73 07 - 74 06 Approximately half of the 788 strains studied in 1973 required concentrated typing fluid for typing. Among those reacting only with undiluted phage, there were 27 different lytic patterns. Three-fourths of these fell into two lytic patterns which were thus the most frequently encountered types. The most common pattern, NT-5, had been found in 1972 in only 3% of all patients. Six months later it abruptly reappeared and remained in epidemic form through 1973. A unique epidemic outbreak of a form designated type A-71 involved 14% of all patients during a 5-month period. It was noteworthy as a strain entirely refractory to topical treatment in the experimental animal model. Phage loss-variants of this epidemic offered a new opportunity to study virulence mechanisms of Pseudomonas aeruginosa.

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Availability to DDC (Indicate with originator's approval)

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ABSTRACT

PROJECT NO. 3A161102B71R-01, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: BACTERIOPHAGE TYPES OF PSEUDOMONAS AERUGINOSA FOUND IN BURNED SOLDIERS

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

Period covered in this report: 1 July 1973 - 30 June 1974

Investigators: Ruth L Latta, BS
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Reports Control Symbol MEDDH-288(R1)

The continued importance of capability for precise strain recognition of Pseudomonas aeruginosa in burn wards led to further definitive refinement of the Institute of Surgical Research typing system. Strains nontypable by routine test dilution were effectively typed with undiluted phage, in a pattern which retained type designations from previous years. A set of stable phages which were part of the original 18-phage typing set were used effectively. All but 2% of 788 strains recovered from burn ward patients were typed. Predominant types varied widely from those seen in preceding years. A major epidemic of type A-71 presented a test of recognition of a nosocomial infecting strain, complicated by the presence of unique virulence and drug resistance. Recognition of epidemic patterns and tracing of variants of these strains in a hospitalized population was achieved with precision.

Pseudomonas
Phage typing
Burn wound
Topical chemotherapy
Humans

BACTERIOPHAGE TYPES OF PSEUDOMONAS AERUGINOSA FOUND IN BURNED SOLDIERS

Pseudomonas aeruginosa exists in a unique relationship to burn wound infection. Many other species of bacteria, together with yeasts, fungi and viruses can invade burn wounds, but few are able to initiate a progressive invasive infection extending from damaged tissue to adjacent normal tissue, nor are they, as individual species, associated with lethal infection secondary to burn wound colonization. Since strain differences in virulence and invasive potential have been demonstrated for Ps aeruginosa in experimental models, the role of differences in virulence and drug susceptibility are a plausible basis for differences in severity of infection, epidemic spread and lethality in Pseudomonas burn wound infection. To assess these differences in a burn population, recognition and differentiation of strains of the organism are essential. Ps aeruginosa frequently pervades the environment of a colonized burn patient, and such seeding and epidemic spread can only be recognized by precise strain differentiation. Such differentiation can be achieved with great precision by an effective bacteriophage typing system, and this approach has been used in this Institute since 1961. Several modifications, directed toward improving the sensitivity and differentiating capability of the typing system, have been introduced into the typing set. The system is still subject to periodic review and alteration of typing strains or of technical procedure if indicated (Lindberg RB, Latta RL, Brame RE, Moncrief JA. Bact Proc 1964, 81; Latta RL, Lindberg RB, Pruitt BA, Jr. USA Inst Surg Res, BAMC, Ft Sam Houston, TX, Ann Res Rpt FY 70, Sect. 18). The incidence of nontypable strains grew with passage of time, and alterations which would permit strain recognition have had to be made.

MODIFICATION OF PHAGE TYPING SYSTEM

With the increase of strains not typable by the conventional diluted phage procedure, modifications have been made. Review of the large collection of phages which were acquired in deriving the original typing set did not uncover new effective typing strains. The basic technic was then modified, as decribed previously (Latta RL, Lindberg RB, Pruitt BA, Jr, Mason AD, Jr. USA Inst Surg Res, BAMC, Ft Sam Houston, TX, Ann Res Rpt FY 73, Sect. 12). Undiluted typing fluids, in which stable pyocin elements may exist, were tested for lytic ability. With this approach, lysis of a major part of the "nontypable" population could be achieved, and an effective differentiation of strain identities was achieved. At the same time, a search for new typing phages was made among lysogenic isolates of Ps aeruginosa. Five new phages were collected, and tentatively incorporated in the typing schema. These were assigned numbers - #6, #7, #9, #25 and #37 - to distinguish them from earlier strains which were designated by numerical designations only. In 1972, a trial was made in which five phages which had become steadily less active against successive isolates were dropped: these were 2, 7, 352, F10 and M4. This modified set was used for typing isolates through 1972.

However, it has appeared, in light of subsequent experience, that not enough was gained by dropping the original phages to merit this alteration of continuity in type designations. In either event, two levels of sensitivity to phage typing fluid were used in typing Ps aeruginosa strains. The initial typing was with a Routine Test Dilution (equal to or greater than a 1:1000 dilution of phage). Strains not lysing with this procedure were retested with undiluted phage. Phage patterns were set down in the same manner for reactions at either strength, but the pattern was marked with an asterisk to denote an undiluted phage typing identity. When lysis occurred at the RTD, it was set down simply as a listing of the phages in their established order. Phages 2,7,352, F10 and M4 were one incorporated in the typing set, and the five new phages were removed to re-establish the original typing sequence of 18 phages.

The nature of lysis with undiluted phage remains to be clarified. The plaque behavior resembles that of bacteriophage, but this does not account for the singular and extreme loss of titer which occurs with nontypability at the routine test dilution. It has been postulated that the lysis with concentrated fluids is due to stable pyocins which are elaborated during phage preparation. Since many pyocins are labile, these lytic agents represent a fortuitous selection of propagating strains which produce stable, reproducible agents. In any event, as epidemiologic application has shown, the mixed system is entirely effective as a typing method, and its use permits extension of previously collected data on the behavior of Pseudomonas populations in burn wards.

RESULTS OF TYPING WITH DILUTE AND UNDILUTED BACTERIOPHAGE

During 1973, 97 patients harbored Ps aeruginosa, and 788 strains were typed. The colonization rate was questionably higher than is reflected in the number of patients with positive cultures, but the organism was not sought specifically. The predominant phage types occurring in this burn population are summarized in Table 1. The type patterns include the concentrated phage reactions, which are listed with code designations in the NT series, and dilute (RTD) patterns which are listed without asterisk and with the code designations as used in earlier reports on this topic from this laboratory.

There were 54.3% of strains nontypable by RTD in 1973, in contrast to 68% of such strains in 1972. These 428 strains were typed using the full set of 18 Institute of Surgical Research undiluted phages, and 27 different lytic patterns were derived. The major part of the nontypable strains fell into one of two lytic patterns with undiluted phage typing. The remaining types among nontypable strains were smaller in number than were strains typable by RTD. Out of the 428 strains nontypable by RTD, 328, or 76.6%, fell into patterns NT4 or NT5. Thirty-five more strains were pattern NT18, and the remaining 65 strains fell into 24 different lytic patterns. The same system of diluted and undiluted phages used in 1972 detected a pattern NT2, representing reactions (21),44,1214,(68), 109,(119X),F8. This type made up 39% of all strains recovered. In 1973,

Table 1. Predominant Phage Types of 788 Pseudomonas Strains from 97 ISR Burn Ward Patients, 1973

Phage Type Code	Phage Type	Per Cent Each Phage Type	
		Patients	Strains
NT-5	Non-typeable *(21),24,44,1214,(68),119X,(F7)	22.7	19.3
NT-4	Non-typeable *(21),24,31,44,1214,(68)	21.6	22.2
A7;	2,7,21,24,44,68,352,119X,F7,M4	14.4	8.9
A71#2	2,7,21,24, 68, F7,M4	6.2	2.5
A71#3	2,7,21, 68, F7	5.1	1.8
M 2	119X		2.8
M 8	119X,F7	2.1	5.3
NT-18	Non-typeable *1214,M4		4.4
D34	21,68,119X		3.0
A181	2,7,21,68		1.4
A27	2,7,119X,M4		1.3
I 9	68,119X		1.1
G20	44,119X		0.2

* Phage type using undiluted phage

not one strain of NT2 was recovered. Another extensive pattern seen in 1972, NT1, also disappeared completely in 1973.

Table 1 shows the code designation for each type and the detailed phage pattern. Phages with ( ) are those which sin the pattern listed are inconsistent in reaction. They may fail to lyse or may react up to a 3+ lysis level. The most meaningful criterion in distribution is the incidence rate of individual types among patients, since some patients may be the source of a large number of isolates which would distort the incidence pattern.

Type NT5 was the most common strain occurring during 1973. It was found in 22.7% of patients positive for Pseudomonas. It was found in small numbers in 1972; 3% of patients harbored it. It appeared from August to November 1972, then did not reappear until May 1973, and then on only one patient. It quickly spread to become the predominant strain. This prolonged disappearance and reappearance is exactly the behavior seen in previous years with other, unrelated types.

Type NT4 was almost as common as NT5. This was a new type; it had not previously been seen.

Strain A-71, with two sub-types, A-71#2 and A-#3, were three distinct patterns which resembled a type with two loss variants. Type A-71 was seen in three patients in December 1972; it continued to appear in 1973 until June, at which time it disappeared abruptly. It behaved as a recognizable, obviously transmissible strain in the burn ward population, and during its stay was clearly the epidemic type. Type A-71 was found in 14.4% of patients; type A-71#2 and A-71#3 were less common and were found in 6.2% and 5.1% of patients respectively. This strain was relatively tolerant of Sulfamylon, and to antibiotics. Although its stay in the burn population was a period of peak colonization and sepsis due to Pseudomonas, it could not be shown that the strains were treatment refractory in patients.

Phage type 119X, coded M2, occurred in 5% of patients positive for Pseudomonas. The incidence was the same in 1972; this is a type which persists but does not enlarge its foothold.

There were seven more types found each in two patients. One of these, NT18, reacted only with undiluted phage. Interestingly enough, none of these types of low incidence had been observed in 1972, nor did comparison with the 1972 data show that any of the five types occurring in 5% or less of patients were recovered in 1973. The frequently emphasized heterogeneity of the Ps aeruginosa population was reaffirmed in 1973. Whatever else happens to its population, this species continues to appear in a wide range of different phage types.

In Table 1, the total of types listed made up 74.2% of the types as distributed by patient. There were, out of the 788 strains typed, 29

which reacted at the dilute, RTD level, and 24 NT strains, for a total of 53 different types each of which occurred in only one patient. This vast number of individual, different phage types is a valid finding of individual strain identities. Significantly, other so-called "typing" systems, in particular the designation of seven "immuno-types" by Fisher (Fisher MW, Devlin HG, Grabosik FJ. J Bact, to be published)[4] encompass many individual phage types, or for that matter, pyocin types. Thus, the presumably simple typing system using seven sera is seen to be, in reality, a grouping system. While it is useful in some situations, it does not permit even reasonably precise reactions of identity with individual strains. Different strains would thus be grouped incorrectly into a "type" which was really heterogeneous.

The striking change that occurred in the burn ward flora was the greatly increased number of strains typable only with concentrated phage, and a disapparance of phage types that had for years been present in significant numbers. Type 21,68, for example, has been so ubiquitous that it has been regarded as a major phage type throughout the country. Yet it did not appear in 1973 in even two patients. Other major epidemic types in earlier years included H3, M2, and F12. Only M2, among these, was recovered in 1973, and then in only two patients.

Sepsis due to Ps aeruginosa is usually manifest as positive blood cultues. Such septicemia has an extremely high mortality rate. The majority of positive Pseudomonas blood cultures occurred within 48 hours of death, and it is often remarked that Ps aeruginosa appears to have a unique ability to invade the terminally ill patient. During 1972 and 1973, pulmonary complications were a conspicuous feature of seriously or fatally ill patients, and there was an obvious sequential relationship between Pseudomonas infection of the lung and septicemia. Pseudomonas frequently appeared in sputum several days or more before it was found in blood culture. Comparison of strain identities of Ps aeruginosa from lung tissue at autopsy and antemortem blood cultures was made, to confirm the presumed relationship of these two manifestations of sepsis. Phage types of Ps aeruginosa from blood culture and autopsy lung tissue were set down in matching chronologic sequence, as shown in Table 2.

The 43 patients with blood or lung cultures positive for Pseudomona are listed by patient accession number, in chronologic sequence. Each patient is listed in the month in which he had either a positive blood culture or lung tissue at autopsy. Nineteen patients had both positive blood and autopsy lung tissues. Twelve had only positive blood cultures and 12 had only positive lung cultures. The mortality rate for patients with Pseudomonas in blood or lung is extremely high; of the 43 patients listed here, only four (9.3%) survived. The number of cultures listed refers to positive blood cultures; often there were large numbers of negative cultures collected during the patients' course. There were two instances, in patients #142 and #182, in which two different types were recovered from blood cultures. The remaining 29 patients with positive blood cultures each yielded only one type.

Table 2. Phage Types of *Pseudomonas* from Blood Stream and Post Mortem Lung Tissues of ISR Burn Ward Patients, 1973

		SOURCE							
		Blood					Post Mortem Lung Tissue				
Month	Pat. No.	No. of Cultures	Phage Type Code				No. of Cultures	Phage Type Code			
			A71	NT-4	NT-5	Other		A71	NT-4	NT-5	Other
JAN	6	1	1				1	1			
FEB	12	1				NT					
MAR	55	1	1								
	44	3	3								
	38	1				NT					
	39	1	1				4	4			
	35						2				G12
	30						3	A71#2			
APR	64	3	3				4	4			
	77						3	A71 -1 A71#2-1 A71#3-1			
MAY	102						3	A71#2			
	97						4	A71#2			
JUN	98	1			1						
	119	1				D96					
	106	1				M 8					
	109						4				M 8
	121						3				NT-18
JUL	126	1				D34	1				D34
	127	2				NT-18	2				NT-18
	136	2				B13	2				B13
AUG	144	2			2		1			1	
	142	2		1	2		4			4	
	153	2			2		1		1		
	170	1			1						
	165						4			4	
SEP	157	3		3			4		4		
	176	1			1		4			4	
	177	3			3		4			4	
	138	1		1			4		4		
	182	3		1	2		4			4	
	189	1				M2a					
	181						2			1	D34
	192						4			4	
OCT	197	1		1			4		4		
	196	1		1			4		4		
	206	2		2			4		4		
	204	1		1			1		1		
	191	1		1							
	195						3		2	1	
NOV	223	1				C16					
	218	1				D41					
DEC	245	4			4		3		2	1	
	248	1			1						

The principal phage types were A-71, NT4, and NT5. These are listed in Table 2; other strains are listed by their code designation for the phage type of the strain recovered.

SEPTICEMIC STRAINS: PHAGE TYPES RECOVERED

Thirty-two patients yielded 52 strains of Pseudomonas from blood cultures. There were 19 strains of NT5 recovered from a total of 10 patients. NT4 was recovered 12 times from 9 patients. Five patients contributed a total of 9 strains of A-71. Ten other patients yielded 12 strains of 10 other types.

The distribution of these major strains in septicemia is shown in Table 3.

Table 3. Ps aeruginosa Phage Types in Strains Recovered from Blood Culture

Phage Type Code	% of Isolates	
	Patients	Strains
NT5	31.2	36.5
NT4	28.1	23.1
A-71	15.6	17.3
10 Other Types	31.2	23.1

The order of frequency of strains from blood cultures is the same as that for strains from all sources, but the incidence of these types is higher. NT5 strains from blood were 8.5% more frequent in patients than the overall distribution, and NT4 was 6.5% more frequently found. A-71 was found as frequently in all cultures as in blood cultures, but there were 8.4% more strains recovered.

A-71 types were predominant in January, March and April. NT5 appeared once in June, then was very common in August, September and December. NT4 was found in August, September and October. With a total of 13 types recognized among 52 isolates, it is once again affirmed that no one type causes fatal bacteremia.

PHAGE TYPES FROM PULMONARY TISSUE AT AUTOPSY

There were 91 strains of Ps aeruginosa recovered from lung tissues of 30 patients. The distribution pattern is shown in Table 4. The order of frequency is again the same as that for all sources combined. In the

Table 4. Phage Types of Ps aeruginosa in Burned Soldiers

Post Mortem Lung Tissues		
Phage Type Code	Patients	Strains
	Per cent	
NT-5	33.3	30.8
NT-4	30.0	28.6
A71	13.3	10.9
A71#2		12.1
NT-18	6.7	5.5
D34		3.3
A71#3	3.3	1.1
Other types	10.0	8.8

same patient, types from lung and blood stream were the same. There was one exception: patient #153 had a strain of NT5 in the blood, but NT4 was recovered from lung tissue. The lung tissues present a more diverse population than the blood.

The relative frequency of types from all sources, from blood and from lung are compared in Table 5. The sequence of frequency of occurrence was the same for all three major categories of sources. Individual differences in site of recovery of certain types occurred. NT5 strains were most common, but the incidence in blood and lung tissue was higher. NT4 strains were conspicuously more common in blood and lung, especially the latter. Type A-71 was the third most common type, with its distribution relatively the same in all three categories. A-71#2 and A-71#3 were not found in the blood. A-71#2 was relatively common in lung tissue. A-71#3 was found in lung tissue but not in the blood. Strain M2 was found in neither blood nor lung tissues. M8, NT18 and D34 were recovered in numbers too small to make comparisons.

A chronologic sequential charting of phage type distribution during 1973 is presented in the figure. Phage code designations are set down on the left hand margin. At the bottom are shown the number of patients positive in a given month and the number of strains recovered. Totals of each type, in patients positive and total strains are in the right hand column. Each block indicates the number of patients for a given type and the total strains recovered. Comments are made in order of descending types.

Type A27 was seen on only two patients but was second most common in the month where it occurred. A-71 strains were predominant in January, March, April and May. Their disappearance was abrupt in June. A-71 #3 was found in March, April and May, and was the second most common type in April and May. A-71#3 was the least common of the group, but in April was as common as A-71#2.

Phage types A181, D34, G20 and 19 were each found in only two patients and were never predominant in any month.

Type M2, a recurrent part of the flora, was found in five patients and shared the most common type category with A-71 in April.

During the last half of 1973, a major shift in predominance occurred. NT4 was prominent, and was the predominant type in October and November. NT5 strains were present from May onward, and were present from May onward, and were the predominant type in June, August and September. Type NT18 was a relatively minor type, found on only two patients. Other nontypable strains were found in almost every month, but in small numbers. In July and December these NT strains were conspicuous although the whole Pseudomonas population was low.

DISCUSSION

Table 5. A Comparison of the Incidence of the Predominant Phage Types from All Sources, Blood, and Lung Tissues

Phage Type Code	All Sources		Blood		Lung Tissue	
	Patients	Strains	Patients	Strains	Patients	Strains
	Per cent		Per cent		Per cent	
NT-5	22.7	19.3	31.2	36.5	33.3	30.8
NT-4	21.6	22.2	28.1	29.1	30.0	28.6
A71	14.4	8.9	15.6	17.3	13.3	10.9
A71#2	6.2	2.5			13.3	12.1
A71#3	5.1	1.8			3.3	1.1
M 2	5.1	2.8				
M 8	2.1	5.3	3.1	1.9	3.3	4.4
NT-18	2.1	4.4	3.1	3.8	6.7	5.5
D34	2.1	3.0	3.1	1.9	[illegible]	3.3

Figure. Monthly Distribution of Predominant *Pseudomonas* Phage Types in ISR Burn Ward Patients, 1973

Phage Type Code	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total Patients-Strains Each Type
A27											2-10		2-10
A71	2-12	2-3	5-34	3-23	3-6	1-1							14-70
A71#2			2-5	2-6	2-9								6-20
A71#3		1-1	1-7	2-4	1-2								5-14
A181				1-4			1-5	1-2					2-11
B34							1-4		1-20				2-24
C20					1-1	1-1							2-2
I 9		1-5	1-3		1-1								2-9
N 2			1-2	3-10	2-2				1-8				5-22
N 8					1-2	2-15	1-12	1-13					2-42
NT-4				1-2	2-8	1-1		5-30	6-29	9-90	3-7	3-19	21-175
NT-5					1-1	4-20	1-1	9-64	10-46	1-1		2-19	22-152
NT-18						1-5	1-30						2-35
Other NT	1-2	4-15	3-16		3-6		4-4	3-7	2-4	5-5	1-1	5-5	21-65
Total Patients-Strains Each Month	4-15	8-27	12-74	8-69	14-54	12-60	12-75	17-133	17-114	13-89	7-31	8-47	97-798

Most prevalent phage type during the month

2d most prevalent phage type during the month

The total number of Ps aeruginosa strains recovered in 1973 was far lower than in 1972. The type patterns detected, however, showed a distribution very similar to that seen in previous years. With 788 strains as opposed to 1087 collected in 1972, the diversity of patterns seen was somewhat less, but the essential epidemiologic picture was the same.

A new element in the 1973 picture was the occurrence of an acute, fulminating epidemic of Ps aeruginosa of a type, A-71, seen only once before in the history of phage typing this species. The strain's virulence was unique in its severity, and it was treatment-refractory with Sulfamylon in an animal model. It appeared on the basis of clinical operation that burn wound sepsis did not occur with type A-71, despite its extraordinary virulence in the seeded burned rat.

The new system for typing and recording types on the NT basis has been entirely effective and is being used on populations of otherwise refractory strains. The role of Ps aeruginosa in burns will continue to require precise monitoring of species and type identity, if micro-epidemics are to be recognized and controlled.

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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 SUMRY	4. KIND OF SUMMARY	5. SUMRY SCTY	6. WORK SECURITY	7. REGRADING	8. DISP'N INSTR'N	8a. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
73 07 01	D. CHANGE	L	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
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22. KEYWORDS (Precede EACH with Security Classification Code): (U) Burns; (U) Serratia; (U) Bacteriophage; (U) Humans

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

23. (U) Serratia marcessens is one of the Enterobacteriaceae with documented capability for wound invasion. Thermal injury, which is a major threat to military personnel, makes it mandatory that differentiation of such opportunistic pathogens be characterized in detail, as part of the overall program of controlling nosocomial infections.

24. (U) A phage typing set, devised for this purpose, is propogated to yield high titer typing fluids for differentiating strains from wound, lung, biopsy, blood, urine and autopsy tissues.

25. (U) 73 07 - 74 06 Serratia marcessens continued to appear in epidemic outbreaks on the burn ward, between intervals in which the organism was not seen. There were 139 strains recovered from 36 different patients. Only nine different types were differentiated, while 27 strains from 9 patients were untypable by the phage set presently in use. A single type appeared in 17 patients; only one other was found in more than one patient. The infection pattern was thus one of recurrent episodes of a major epidemic strain. This type was the only one recovered from patients at autopsy. The behavior of Serratia marcessens in a burn ward is distinctive among opportunistic invaders, and type differentiation makes it possible to recognize an epidemic episode and to know when it has disappeared or been controlled.

* Available to contractors upon originator's approval

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ABSTRACT

PROJECT NO. 3A161102B71R-01, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: BACTERIOPHAGE TYPES OF SERRATIA MARCESSENS FROM BURN WOUNDS OF MILITARY PERSONNEL

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

Period covered in this report: 1 July 1973 - 30 June 1974

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One hundred thirty-nine strains of Serratia marcessens from patients in the Institute of Surgical Research burn wards were typed by the phage typing system developed in this laboratory. Eight phage types plus non-typable strains were recognized; the vast preponderance of strains fell in type 5,7,9,11,15,18, as had been the case in several years prior to 1972. Although the pattern was that of a monotype epidemic, the episodes were widely separated. Sepsis due to this type of S marcessens was substantiated by antemortem and postmortem cultures, and the validity of phage typing technic for assessing nosocomial infections due to S marcessens was affirmed.

Burns
Serratia
Bacteriophage
Humans

BACTERIOPHAGE TYPES OF SERRATIA MARCESSENS FROM BURN WOUNDS OF MILITARY PERSONNEL

Infection in patients with traumatic injury not only has remained a major cause of morbidity and mortality but, in recent years, has appeared to be increasing in extent and severity as indicated by the frequency of publications on this topic. Members of the genus Enterobacteriaceae have been conspicuous in this increasing infection pattern, and a species of special interest is Serratia marcessens. This organism is a frequent opportunistic infecting agent among burn patients, and in some burn treatment centers has been described as causing epidemics of burn infection and sepsis. In the Institute of Surgical Research, the appearance of S marcessens has been episodic; intermittent positive cultures have, at times, extended to what could be described as a micro-epidemic, and then the strain would disappear. Sepsis due to S marcessens was not observed during 1973 in this Institute, but in some previous years it has played a prominent role. Its potential for causing sepsis in burned patients and the unknown attributes that accompany its presumed epidemic potential prompted development and application of technics for strain characterization. Thus, the epidemic potential of this species can be delineated. A phage typing system was developed and has been applied to strains of S marcessens recovered from burn patients (English VC, Latta RL, Brame RE, Lindberg RB. USA Inst Surg Res, BAMC, Ft Sam Houston, TX. Ann Res Rpt FY 68, Sect. 32)[1]. The typing system and its application have been described in previous reports (English VC, Lindberg RB, Mason AD, Jr, Pruitt BA, Jr. USA Inst Surg Res, BAMC, Ft Sam Houston,TX, Ann Res Rpt FY 73, Sect. 13)[2]. As in previous years, achromogenic strains of Serratia predominated, and recognition of this specie depended on lactose fermentation, formation of gas glucose and by non-fermentation of arabinose and raffinose.

RESULTS OF PHAGE TYPING

One hundred thirty-nine strains of S marcessens were recovered from patients on the burn wards of the Institute of Surgical Research during 1973. There were 24 patients, antemortem and postmortem, from whom strains were recovered. Table 1 presents the phage types recovered. Eighty-one per cent of the isolates could be typed with the system used. As has been observed in some previous years, there was a great predominance of strains of a single type; in this series, type 5,7,9,11,15,18. Seven other types, plus the nontypable group, were recognized. This was an abrupt change from 1972 in which type 11 was predominant.

A more coherent picture of predominant phage types of S marcessens is shown in Table 2, where the predominant types for each year since 1967 are summarized. Obviously, type 5,7,9,11,15,18 has been the predominant type every year except 1972. Seldom, however, has it been as unique in incidence as was the case in 1973. The phage type patterns showed only type 15 as occurring in more than one patient in 1973, except

Table 1. Phage Types of *Serratia marcessens* Recovered from Burn Patients

Phage Type	Number of Patients	Number of Isolates
3,5,7,9,11,15,18	1	1
5	1	1
5,11,15	1	1
5,7,9,11,18	1	2
5,7,9,15,18	1	98
5,7,9,11,15,18	17	1
5,7,11,15,18		
5,9,11,15,18	1	7
15	4	27
Nontypable	9	

Table 2. Predominant Phage Types of Serratia marcessens Annually: 1967-1973

Year	Phage Type	No. Patients Positive	No. Strains Recovered
1973	5,7,9,11,15,18*	17	98
1972	11	19	63
1971	3,5,7,11,15,18	5	11
	5,7,9,11,15,18*	3	7
1970	5,7,9,11,15,18*	10	29
	5,7,9,15,18	8	9
	15	8	13
1969	5,7,9,11,15,18*	10	19
	5,7,9,15,18	4	4
	7,9,15	5	7
	15	5	5
1968	5,7,9,11,15,18*	7	18
	5,7,15	7	7
	11,15	7	16
	15	8	16
1967	3,5,7,9,11,15,18	6	8
	3,5,7,11,15	5	21
	3,5,7,11,15,18	5	7
	5,7,9,11,15,18*	12	21
	11	5	5

* This type among predominant all years except 1972.

for the predominant type. Whatever the meaning of the disappearance of type 5,7,9,11,15,18 after 1971, it obviously was a temporary change, and the predominant type pattern was reestablished in 1973.

Mixed infections occurred in five patients, as summarized in Table 3. In patient 6, it is possible that the two type patterns were related, since only factor 11 separated them. Similarly, the two patterns in patient 176 might be related. Among the others, the nontypable strains were possibly late contaminants, and the sole type 15 seen in patient 35 was probably unrelated to the predominant type. This patient was essentially a monotype infection except for the one strain of type 15.

The chronologic sequence of the appearance of type 5,7,9,11,15,18 in the Institute of Surgical Research burn wards is summarized in Table 4. Admission dates preceded the acquisition of the strain by from 2 to 3 days up to a month; only once, in patient 214, was S marcessens type 5,7,9,11,15,18 found on the wound at time of admission. Episodes occurred in March, August, October and December of 1973 and in January, February and March of 1974. The episode in March occurred in the first week of admission, as did the August infection and that of January 1974. October saw a second week post-admission occurrence, as did November and March episodes. The transmission pattern could be readily traced by the time of isolation of the strain.

The occurrence of Serratia strains in patients offers some clue as to the potential clinical significance of the strain. In Table 5, strains are tabulated according to type and to their presence only in antemortem or only in postmortem specimens. It would appear that extensive colonization with Serratia may still fail to leave evidence that the strain was really invasive. Only one patient with cultures positive for Serratia had a positive blood culture. The postmortem findings were primarily of interest in the lung samples. In two of six patients all lobes of the lungs were invaded. In two autopsies the predominant type was present in the burn wound. The type designation obviously did not differentiate invasive from colonizing strains.

In Table 6 five patients in whom S marcessens was recovered pre- and postmortem are summarized. These patients included three with septicemia, and one with extensive colonization, involvement of venous catheters, and lung involvement. The postmortem findings included extensive lung involvement plus, in two patients, burn wound invasion. The homogeneity of the type involvement was obvious; only in one patient was a nontypable strain recovered.

DISCUSSION

The validity of strain differentiation of S marcessens by phage typing with the Institute of Surgical Research system is substantiated by this application to 139 strains in 1973. A monotype strain generated repeated epidemic episodes, but these were very limited in numbers. The spread

Table 3. Serratia Phage Types in Patients with Multiple Types, ISR, 1973 - Feb 1974

Patient Number	Admission Date	Phage Type	Date Isolated	Source of Culture
176	8-28-73	5,9,11,15,18	9-6-2	R.arm
		5,7,9,11,18	9-13-9	?
212	10-20-73	5,7,11,15,18	10-20-12	I.V.catheter
		Nontypable	10-24-5	R.wrist
6	1-10-74	5,7,9,15,18	1-14-11	I.V. tip
			1-15-8	Lukens
		5,7,9,11,15,18	1-30-2	Lukens
		Nontypable	1-11-12	Wound
			1-14-17	Chest
			1-14-35	Lukens
10	1-13-74	5,7,9,11,15,18	2-15-14	Sputum
			2-16-1	R.Leg
			2-19-2	L.leg
			2-21-5	Lukens
			2-24-2	Lukens
			2-28-16	Blood
			3-1-2	Foley Catheter
		Nontypable	2-26-4	Lukens
			2-28-2	Lukens
35	2-25-74	5,7,9,11,15,18	3-2-2	R. thigh
			3-4-14	R.thigh
			3-4-15	Chest
			3-5-15	I.V. tip
			3-6-5	Chest
			3-7-6	R.brachial vein
			3-7-9	R.saph.vein
			3-9-1	Lukens
			3-9-3	Foley Catheter
			3-10-4	Lukens
			3-11-7	Lukens
			3-12-1 thru 3-12-10	Aut.Tiss., Bld. spleen,LLL,.UL, RLL,RUL,Tiss.# 2 & 3
		15	3-11-8	Foley Catheter

Table 4. DISTRIBUTION OF THE PREDOMINANT SERRATIA MARCESSENS TYPE NUMBER 5,7,9,11,15,18

Patient Number	Admission Date	Date Isolated	Source
	1973		
Dialysis Pt.	- -	P-6	Blood
		P-9	Blood(3 samples)
35	2-27	3-1	5 PM viscera
173	P-25	P-27	Stool
197	9-25	10-4	L.leg
214	10-20	10-20	Wound swab
245	11-25	12-3,12-6	Sputum
256	12-12	3-12(74)	R. thigh
	1974		
10	1-1?	2-15	Sputum
		2-16	R.leg
		2-19	L.leg
		2-21	Lukens
		2-24	Lukens
		2-28	Blood
		3-1	Foley
19	1-25	1-28	Blood
		1-29	Chest;I.V.
		1-30	Lukens;Blood
		1-31	Blood
		2-4	6 PM viscera 4 PM tissues
27	2-9	2-13	2 PM viscera
28	2-9	2-15	Lukens
30	2-11	2-15	L.arm;Lukens
		2-16	Blood
		2-20	6 PM viscera
31	2-14	3-5	Lukens
		3-6	Blood
35	2-25	3-2	R. thigh
		3-4	R.thigh;Chest
		3-5	I.V. tip
		3-6	Chest; Right brachial vein
		3-7	Rt.saph vein
		3-9	Lukens;Foley
		3-10	Lukens
		3-11	Lukens
		3-12	PM blood; 5 PM viscera 2 PM tissues

12-6

Table 5. RECOVERY OF SERRATIA FROM PATIENTS WHO EXPIRED

SERRATIA FOUND IN ANTEMORTEM SPECIMENS - NO SERRATIA FOUND IN POSTMORTEM SPECIMENS				
Patient Number	Admission Date	Culture Date	Source	Phage Type
176	8-28-73	9-6	R.arm	5,9,11,15,18
		9-13	?	5,7,9,11,15,18
241	11-17-73	11-29	vein	5,7,9,11,15,18
245	11-25-73	12-3	Sputum	5,7,9,11,15,18
		12-6	Sputum	5,7,9,11,15,18
197	9-25-73	10-4	L.leg	5,7,9,11,15,18
212	10-20-73	10-20	I.V.	5,7,9,11,15,18
		10-24	wrist	Nontypable
9	1-22-73	2-13	lukens	Nontypable
144	7-28-73	8-10	lukens	Nontypable
		8-11	lukens	Nontypable
44	3-5-73	3-10	lukens	15
		3-11	I.V.	15
142	7-26-73	8-1	lukens	Nontypable
		8-5	R.thigh	Nontypable
		8-7	penrose drain	Nontypable
182	9-5-73	9-18	R.arm	15
			L.arm	15
			R.leg	15
28	2-9-74	2-15	lukens	5,7,9,11,15,18
10	1-19-74	2-15	sputum	5,7,9,11,15,18
		2-16	R.leg	5,7,9,11,15,18
		2-19	L.leg	5,7,9,11,15,18
		2-21	lukens	5,7,9,11,15,18
		2-24	lukens	5,7,9,11,15,18
		2-26	lukens	Nontypable
		2-28	lukens	Nontypable
		2-28	blood	5,7,9,11,15,18
		3-1	Foley	5,7,9,11,15,18
6	1-10-74	1-11	Wound	Nontypable
		1-14	I.V.	5,7,9,15,18
			Chest	Nontypable
			lukens	Nontypable
		1-15	lukens	5,7,9,15,18
		1-30	lukens	5,7,9,11,15,18

NO SERRATIA FOUND IN ANTEMORTEM SPECIMENS - SERRATIA FOUND IN POSTMORTEM SPECIMENS				
Patient Number	Admission Date	Culture Date	Source	Phage Type
35	3-1-73	3-1	Spleen	
			RUL	
			RLL	5,7,9,11,15,18
			LUL	
			LLL	
152	8-3-73	8-7	LLL	
			LUL	Nontypable
			RLL	
			RUL	
238	11-13-73	11-19	Tiss.#5	5
202	10-7-73	10-9	PM blood	5,11,15
139	7-24-73	10-20	Tiss. # 1 & 3	5,7,9,11,15,18
27	2-9 -74	2-13	Liver	
			LUL	5,7,9,11,15,18

Table 6. Patients from Whom Serratia was Isolated from Both Antemortem and Autopsy Specimens

Patient Number	Admission Date	ANTEMORTEM STUDY Date	Source	Phage Type	POSTMORTEM STUDY Date	Source	Phage Type
19	1-25-74	1-28	Blood		2-4	Brain	
		1-29	I.V.tip			Spleen	
			Chest			RUL	
		1-30	Lukens	5,7,9,11,15,18		RLL	
			Blood			LUL	5,7,9,11,15,18
		1-31	Blood			LLL	
						Tiss.# 1,3,4	
24	2-1-74	2-25	# 1 vein;		2-11	Liver	
			#2 vein, L. Arm	Nontypable		Spleen	
						Kidney	Nontypable
						LLL	
30	2-11-74	2-15	L.arm		2-20	Liver	
			Lukens	5,7,9,11,15,18		Spleen	5,7,9,11,15,18
		2-16	Blood			RLL	
						RUL	
						LLL	
						LUL	
33	2-22-74	2-22	R.leg		3-4	Liver	
		2-28	Lukens			Spleen	
			Blood			RLL	
			R.thigh	5,7,9,11,15,18		LLL	5,7,9,11,15,18
		3-1	Lukens			RUL	
		3-2	R.thigh			LUL	
35	2-25-74	3-2	R.thigh		3-12	Blood	
		3-4	R.thigh			Spleen	
			Chest			LLL	
		3-5	I.V.tip	5,7,9,11,15,18		LUL	5,7,9,11,15,18
		3-6	Chest			RLL	
		3-7	R.brachial vein			RUL	
			Saphenous vein			Tiss.# 2 & 3	
		3-9	Lukens				
			Foley				
		3-10	Lukens				
		3-11	Lukens				

12-8

of each episode was too small to occasion serious concern as to the scope of this nosocomial problem, but the potential for serious infection makes continued monitoring highly advisable.

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2. English VE, Lindberg RB, Mason AD, Jr, Pruitt BA, Jr: Bacteriophage types of Serratia marcessens from burn wounds of military personnel. USA Inst Surg Res, BAMC, Ft Sam Houston, TX, Ann Res Rpt FY73,Sect.13.

PRESENTATIONS

Lindberg RB: Important gram-negative organisms in hospital acquired infections. Seminar-Environmental Microbiology & Infection Control. Univ Georgia, Athens, GA. 7 December 1973.

PUBLICATION

Lindberg RB: Culture and Identification of Commonly Encountered Gram-negative Bacilli: Serratia. In Advances in Clin. Bact & Immunol. Series: Opportunistic Pathogens. Univ. Press, Baltimore, MD.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OE 6382	74 07 01	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
73 07 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	61102A	3A161102B71R	01	317
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) Five Per Cent Aqueous Sulfamylon Soaks Used In Topical Treatment of Burned Soldiers (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
71 10	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 74	.6	15
	CURRENT 75	.4	10

19. RESPONSIBLE DOD ORGANIZATION
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ASSOCIATE INVESTIGATORS
NAME: James Taylor, MAJ, MC
NAME: DA

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Burn; (U) Eschar separation; (U) 5% Sulfamylon Acetate Solution; (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) Ten per cent Sulfamylon acetate burn cream is an effective topical agent when applied to burn wounds to control bacterial population. During the latter stages of eschar separation where residual eschar is interspersed between areas of open wound tissue, the application of the cream is made difficult and the cream adheres poorly to areas of open granulation tissue. Five per cent Sulfamylon acetate solution is used to facilitate removal of the residual non-viable tissues in wounded soldiers.

24. (U) Five per cent Sulfamylon acetate is used as a debriding agent by applying gauze sponges soaked in the solution to the burn wound and wrapping the area. The gauze sponges are applied soaked with the solution or with normal saline, allowed to dry and removed dry every 6 or 8 hours.

25. (U) 73 07 - 74 06 By using the wet to dry dressings, residual non-viable tissue can be removed by mechanical action as the gauze dressing is changed. Less than 20% of the total body surface should be treated in such dressings at any given time. Quantitative wound cultures were done and it was determined that under the guidelines of this therapeutic regimen, there was no escape of the wounds from bacterial control.

*Available to contractors upon originator's approval

ABSTRACT

PROJECT NO. 3A161102B71R-01, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: FIVE PER CENT 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 July 1973 - 30 June 1974

Investigators: Daryl R. Erickson, MD, Major, MC
James W. Taylor, MD, Major, MC

Reports Control Symbol MEDDH-288(R1)

Ten per cent Sulfamylon acetate burn cream as a topical agent is applied to the wounds of burn patients to control the bacterial population. During the later stages of eschar separation, where residual eschar is interspersed between areas of open granulation, the application of the cream is difficult and the cream adheres poorly to areas of open granulation tissue. Its application at this time may dislodge adjacent homograft. To fill this therapeutic void, 5% Sulfamylon acetate solution soaked wet to dry dressings have been applied to such wounds in order to facilitate debridement of the residual nonviable tissue by mechanical action of dressing changes. Quantitative surface cultures done on 14 paired areas showed that if specific guidelines were followed, there was no escape of the wounds from bacterial control.

Burn
Eschar separation
5% Sulfamylon acetate solution
Humans

FIVE PER CENT AQUEOUS SULFAMYLON SOAKS USED IN TOPICAL TREATMENT OF BURNED SOLDIERS

Wet to dry, occlusive, coarse-mesh gauze dressings changed every six to eight hours have been used for many years to clean up untidy wounds. This study was done to answer the following question: Is 5% Sulfamylon acetate solution more effective than normal saline in controlling surface growth of bacteria under wet to dry dressings?

METHOD

Burn wounds were studied which had progressed to the point of nearly complete eschar separation, but were not clean enough for autografting. In most cases paired extremity wounds were selected. In three cases other separated areas were used. Wounds were either the result of full thickness burns or mixed full thickness and partial thickness burns. In all cases, the total area under dressings was 20% or less of the total body surface area.

Coarse-mesh gauze dressings soaked in either 5% Sulfamylon solution or normal saline were applied to symmetrical areas. The dressings were left in place six to eight hours and removed dry. After two to four days of this regimen, gauze pledgets saturated with sterile Ringer's lactate solution were placed on the study areas. These pledgets were removed after 10 seconds and submitted to the bacteriology laboratory for quantitative analysis. The bacteriology laboratory did not know which solution was being used for the area being cultured.

RESULTS

Ten patients had fourteen paired bacteriologic studies (Table I). The initial bacterial analysis performed two to four days after institution of the wet to dry therapy, revealed that there was no significant difference in the type of bacteria or the number of bacteria between the paired surfaces. The colony count varied from no growth to 10^5 organisms/cm^3 among the patients studied. No patient has more than a single log difference between the paired areas. When Pseudomonas organisms were present, they were present on the Sulfamylon side as well as on the saline side.

In three cases where the therapy was continued long enough to get a repeated study, the colony count and type of bacteria had not changed appreciably.

8/14 paired studies had colony counts on both areas of 10^4.

4/14 paired studies had from no growth to 10^3 colonies with a maximal difference between paired areas on one log.

TABLE I

Pt	Length of Therapy	Area	Bacteria	Colony Count	Type of Therapy
AW	4d	R arm	Providencia	3×10^4	N.S.*
		R leg	Providencia) Staph +)	5×10^4	S/S**
CM	2d	L thigh	Providencia) Staph +)	2×10^5	N.S.
		R thigh	Staph +) Corynebact)	5×10^4	S/S
	7d	L thigh	Staph +) Corynebact)	2×10^4	N.S.
		R calf	Corynebact	2×10^4	S/S
OJ	3d	L leg	No growth		N.S.
		R leg	No growth		S/S
AH	4d	Forehead	Staph -) Prot Mirab)	2×10^2	N.S.
		Neck	Staph -) Prot Mirab)	4×10^3	S/S
LC	3d	R arm	Staph -) Pseudo)	1×10^4	N.S.
		Chest	Staph -) Pseudo)	3×10^4	S/S
HR	2d	R leg	Pseudomonas	4×10^5	N.S.
		L leg	Pseudomonas) Candida)	2×10^4	S/S
	5d	R leg	Pseudomonas	1×10^4	N.S.
		L leg	Pseudomonas	4×10^4	S/S
	5d	R forearm	Pseudomonas	1×10^4	N.S.
		L forearm	Pseudomonas	2×10^4	S/S
GH	1.5d	R forearm	Staph +) Corynebact)	3×10^3	S/S
		L forearm	Staph +) Corynebact)	3×10^2	S/S
	3d	R forearm	Corynebact	5×10^4	N.S.
		L forearm	Corynebact	9×10^4	S/S

TABLE I

Pt	Length of Therapy	Area	Bacteria	Colony Count	Type of Therapy
MM	?	R leg	Staph + Corynebact	$1x10^4$	N.S.
		L leg	Staph +	$2x10^4$	S/S
BL	?	Abd	Staph +	$1x10^4$	N.S.
		L forearm	Staph - Providencia	$2x10^4$	S/S
JK	?	R buttock	No growth		N.S.
		L buttock	No growth		S/S

*N.S. = normal saline
**S/S = 5% Sulfamylon solution
?Length of therapy data not available

2/14 paired studies had a colony count of 10^5 organisms and in both cases this number of bacteria were present on only one side. In both cases the 10^5 level was on the saline treated side.

One patient (HR) developed invasive burn wound sepsis during the course of the study. He will be discussed later.

DISCUSSION

From this study, it is clear that when the wet to dry technique is properly used there is no bacteriologic difference between saline and 5% Sulfamylon solution. It should be emphasized that this technique must be applied only to untidy wounds which are nearly ready for autografting in patients who are metabolically stable.

In retrospect, the patient who developed invasive burn wound sepsis (HR) was not a candidate for this therapy. He differed from the other patients in three significant respects. First, his wounds were mainly partial thickness. Second, he was relatively early in his postburn course (this therapy began on postburn day 14) and was metabolically unstable in that he had markedly impaired renal function resulting from an episode of gram negative sepsis on postburn day 7. Finally, he was the only patient who had Pseudomonas as the sole organism on his burn wounds. While no conclusion can be derived from a single instance, it is interesting that the ecthyma gangrenosum lesions were located on the saline treated side.

This study corroborated the reproducibility of the quantitative culture technique used. However, it also demonstrated that the level of bacterial growth on the wound surface does not reflect what is happening beneath the surface of the burn wound.

CONCLUSION

Saline may safely be used in the wet to dry dressing technique if the following limitations are employed:

1. The patient is metabolically stable.

2. The wound is nearly free of eschar, but needs to be cleaned up before autografting.

3. The dressings should cover no more than 20% of the body surface area.

4. The wound has been shown to have multiple organisms if Pseudomonas sp are present at all.

SUMMARY

1. There is no difference in bacterial flora as to type or quantitation when saline is compared with 5% Sulfamylon solution in the wet to dry, coarse-mesh gauze debridement-dressing technique.

2. Limitations of the wet to dry coarse-mesh gauze debridement-dressing technique are discussed.

3. The moistened gauze-sponge technique of wound surface bacterial quantitation is supported.

PRESENTATIONS AND/OR PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 08 6982	74 07 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
73 07 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	61102A	3A161102B71R	01	223
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with security classification code): (U) Development of Prophylactic Topical Therapy for Use on Burn Wounds of Military Patients: Search for Improved Formulations (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
65 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 74	.4	14
	CURRENT 75	.5	12

19. RESPONSIBLE DOD ORGANIZATION
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NAME: DA

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with security classification code)
(U) Burn wound; (U) Sulfamylon-sulfadiazene; (U) Pseudomonas; (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) Assessment of topical antimicrobial agents in prevention of burn wound sepsis using an animal model of Pseudomonas infection to improve the care of thermally injured troops.

24. (U) Surveillance of phage type, antibiotic and Sulfamylon-tolerance and biotype categories has been focussed on observing the pathologic behavior of strains capable of establishing hospital microepidemics despite topical therapy with Sulfamylon. Response to experimental therapy of such strains is determined.

25. (U) 73 07 - 74 06 A major epidemic of a well-defined phage type (with 10 phage susceptible sites) presented a unique study opportunity when 2 loss-variants were detected: one with 6 phage-reacting sites, and one with 4 sites. The original type was extraordinarily virulent; deaths occurred in as little as two days post-seeding and topical therapy was ineffective. Sulfamylon sensitivity in vitro was of an order that in other strains had responded to treatment. The six-phage reacting variant was virulent but responded to therapy; the four-phage pattern was avirulent. Phage-mediated virulence manifested as an essential invasive phenomenon independent of extracellular toxins may aid in explaining the progressive emergence of Pseudomonas aeruginosa as an increasing factor in nosocomial infection.

*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 69 (FOR ARMY USE) ARE OBSOLETE

ABSTRACT

PROJECT NO. 3A161102B71R-01, RESEARCH IN BIOMEDICAL SCIENCE

REPORT TITLE: DEVELOPMENT OF PROPHYLACTIC TOPICAL THERAPY FOR USE ON BURN WOUNDS OF MILITARY PATIENTS: SEARCH FOR IMPROVED FORMULATIONS

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

Period covered in this report: 1 July 1973 - 30 June 1974

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

Reports Control Symbol MEDDH-288(R1)

Pseudomonas aeruginosa is today a widespread and increasingly serious nosocomial infecting agent in patients compromised by extensive burns, immunosuppression or neoplastic disease. Control of burn wound sepsis by topical Sulfamylon has been achieved in this Institute, but Pseudomonas sepsis remains a frequent complication of severe burns. Bacteriophage typing of isolates revealed a major monotype epidemic in the burn ward, with a strain moderately resistant to Sulfamylon (MIC0.312%) and with extreme virulence. Sulfamylon therapy was ineffective in the experimental infected burn model. Two variants of the epidemic strain were detected after their spontaneous appearance in patients. One was virulent but responded to Sulfamylon treatment in the experimental model. The other was not virulent for the burned and infected rat. This phage-mediated virulence and related Sulfamylon treatment tolerance is a new phenomenon, and makes the testing of virulence and therapy response potentially important in managing severe burns.

Burns
Sulfamylon-sulfadiazine
Pseudomonas
Rats

DEVELOPMENT OF PROPHYLACTIC TOPICAL THERAPY FOR USE ON BURN WOUNDS OF MILITARY PATIENTS: SEARCH FOR IMPROVED FORMULATIONS

The increasing importance of Pseudomonas aeruginosa as a cause of nosocomial infections, as a major cause of sepsis in burns, in immunosuppressed patients and in neoplastic disease has been extensively documented. The presence of a large population of susceptible burn patients in the Institute of Surgical Research presents an optimal opportunity for spread of this dangerous invasive organism, and specific strains have been observed to spread in epidemic manner. Recognition of such strains is done by phage typing (Lindberg RB, Latta RL, Brame RE, Moncrief JA. Bact Proc 1964: 81[1]), and characterization of strains as to virulence in burn wounds has been done using the seeded burned rat model. This same model has been indispensible for assessing agents proposed for control of burn wound infection by topical application. Experience with this model of Pseudomonas burn wound sepsis has, in most instances, been confined to the use of "typical" challenge strains, but the presumption that the syndrome of bacterial invasion of the burn wound is a standardized or well-characterized effect is illusory. Individual attributes of the infecting strain play a role in the nature of the tissue lesions that occur, and in the ultimate lethality of the infection.

The differences in response to topical Sulfamylon therapy as a function of virulence are illustrated in Figure 1. The three strains compared varied in virulence in this animal model. Strain 12-4-4 in this series of tests killed approximately 83% of all seeded untreated rats. Strains 8-28-3 and 3-23-5 killed 98% to 99% of untreated controls; strain 3-24-5 was regarded as the more lethal since it killed more quickly. With topical treatment, over 90% of animals seeded with 12-4-4 survived; 80% of those seeded with 8-28-3 and treated survived, while only 45% of animals seeded with 3-24-5 survived with topical treatment. These strains did not vary to a significant degree in their sensitivity to Sulfamylon in vitro.

Reproducibility of virulence in strains maintained frozen in sterile milk at -70° C is summarized in Table 1. The behavior of three topical challenge strains is shown in ascending order of virulence. The attribute bestowing virulence is consistent and appears to be a fundamental part of the biology of Ps. eruginosa.

A marked reduction in the incidence of burn wound sepsis (BWS) occurred when patients were treated with topical Sulfamylon in this Institute. Although strains of Ps aeruginosa vary markedly in virulence in the animal model, such virulence is not necessarily correlated with the behavior of the strain in the human. Precise strain identification is made routinely by phage typing so that at any given time the Pseudomonas population of the burn ward is definable. Thus, if treatment-resistant strains emerged, the probability is that they would be recognized. In practice, as soon as a predominant phage type reaches numbers suggestive of an epidemic proportion, its virulence and response to therapy are

14-2

Typical Behavour of Virulent Pseudomonas aeruginosa Strains on the Burned Rat

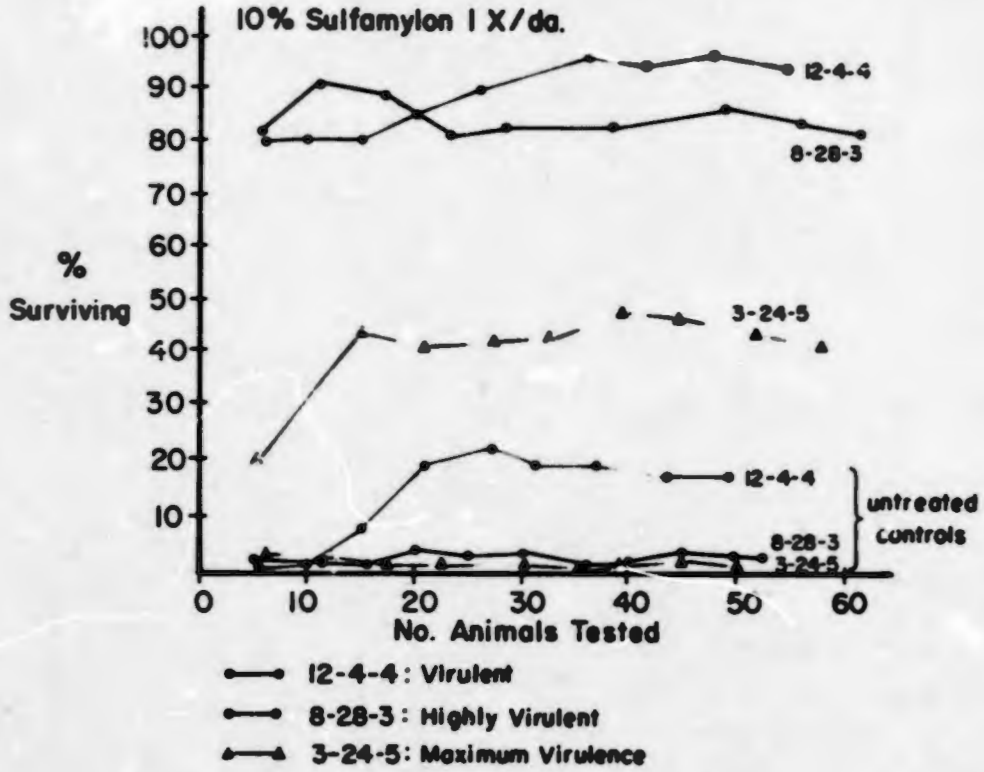

Figure 1

Table 1. Consistency of Virulence of Pseudomonas aeruginosa as Infecting Agent in Burned Rats

Strain	No. Rats Tested	No. of Experiments	Ave. No. Seeded Controls	Deaths/ Total	% Mortality
12-4-4	381	47	8.1	332/387	87.1
8-28-3	116	14	8.2	115/116	99.1
VA-134	105	17	6.1	105/105	100.0

tested in the rat model. Such a situation arose in 1973, when a unique epidemic of treatment-resistant Ps. aeruginosa appeared. The behavior of this strain was studied in detail since it presented a new aspect of virulence in relation to topical treatment, and revealed a possible hazard in control of pseudomonal burn wound sepsis that has not previously been seen.

The new phage type was designated by the code number A-71. The initial outbreak of this strain was homogeneous as to phage type, but 4 months after the onset, two phage types which were interpreted as loss variants appeared in a smaller number of patients. These were designated A-71 a and A-71b. The outbreak was classified as an epidemic; at its peak it was by far the predominant type on the burn ward, and risk of acquiring it was high for incoming patients. Patient-to-patient transmission rate rose to a peak just before its abrupt disappearance from the burn ward.

Table 2 summarizes the incidence of Type A-71, A-71a and A-71b. There were 23 patients involved. During this period, 10 additional phage types were seen on the ward, but none approached the incidence of A-71.

The two sub-type, designated as loss-variants, appeared during the latter half of this micro-epidemic. Fourteen out of 23 patients who harbored type A-71 died. The deaths could not, in all instances, be described as associated with Pseudomonas sepsis, but pulmonary infection and septicemia were observed in 9 of these fatalities. The organism can scarcely be described as benign.

The phage type patterns of A-71 and its variants are shown in Table 3. It was an elaborate pattern. The strain reacted with 13 out of 18 phages in the typing set. This pattern had been observed once before at the Institute of Surgical Research in 4 patients injured in a single episode in Iowa in 1965. Those burn patients arrived with the strain on

Table 2. Incidence of Ps aeruginosa Type A-71 1972-1973

Type	Month and Number of Patients Positive for First Time							
	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June
A-71	1	4	2	2	5	4	4	0
A-71a				2	1	2	2	0
A-71b				1		1	1	0

on their burns, but no extension to other patients in the ward occurred in that episode. In fact, the strain was displaced by other types present in the resident burn flora. The 1965 isolates had been held as unique additions to the Pseudomonas strain collection, and a strain from that time was compared with the current strains. No differences could be detected between the 1965 and the 1972-1973 isolates.

Table 3. Phage Type of Ps. aeruginosa A-71 and of Variants A-71a and A-71b

A-71	2, 7, 21, 24, 44, 1214, 68, 109, 352, 119X, F-7, F-8
A-71a	2, 7, 21, 24, 68, 119X, F-7, M-4
A-71b	7, 21, 68, F-7

A-71a and A-71b were not recovered until February 1973. They were recovered from patients whose wounds had already been colonized by A-71, and were at once regarded as probable loss-variants. They were recovered only on direct culture; serial transfers of A-71 retained their complete phage pattern.

The A-71 epidemic, once recognized, was persistent and the strains remained on the host despite presence of other phage types on adjoining patients. In view of this behavior, the virulence of several strains of A-71 were determined and its response to Sulfamylon therapy was tested on the burn rat model. It was at once obvious that the virulence was higher than had been observed with any of over 100 strains tested during the years of study of Pseudomonas burn wound sepsis. A-71 was completely lethal. Tests with A-71a and A-71b revealed differences that could be explained by a loss-variant hypothesis.

Table 4 summarizes the behavior of this remarkable epidemic strain. When burned rats seeded with A-71 were treated, only a slight degree of survival was effected. When twice-daily treatment was tested, there was a slight increase in survival, to 10% of seeded animals, but this still left the A-71 strains uniquely refractory to topical Sulfamylon. Death occurred, on an average, in less than a week in untreated animals, and some strains killed in 3 to 4 days. An unusually high degree of involvement of lung and nasopharynx occurred in seeded rats.

Table 4. Virulence and Response to Sulfamylon Topical Therapy of Ps aeruginosa A-71, A-71a and A-71b on Burned Seeded Rats

Treatment	Strains Tested; Total Dead/Total Tested					
	A-71		A-71a		A-71b	
	No.	%	No.	%	No.	%
Sulfamylon 10% 1x/day for 10 days	278/295	94.2	2/57	3.5	3/54	5.5
Controls (no treatment)	270/270	100	53/56	95.3	3/53	5.6
No. strains tested	10		3		2	

Five of the 10 strains tested left no survivors in the treated groups. The total survival for all 10 strains was only 6.8%. No previous experience with topical therapy had indicated the possible existence of such a virulent refractory epidemic strain.

When variant A-71a was tested, an immediate difference appeared. While survival was negligible in the untreated controls, the Sulfamylon-treated animals survived. The strain behaved in a manner entirely analogous to those virulent strains of Ps aeruginosa tested over a period of several years which can be effectively controlled with topical therapy.

It was initially assumed that strain A-71 was resistant to Sulfamylon in vitro. Ps aeruginosa strains have been monitored since the drug was introduced, and on a cumulative basis, the organisms have fluctuated around a median range of sensitivity that is essentially stable. Figure 2 shows the annual cumulative sensitivity of a total of over 2000 strains. It is evident that there has been no consistent trend toward emergence of more resistant strains during these years of observation.

Fifty-six isolates of strain A-71, 9 of A-71a and 8 of A-71b, were tested for sensitivity to Sulfamylon. The sensitivity of this strain and

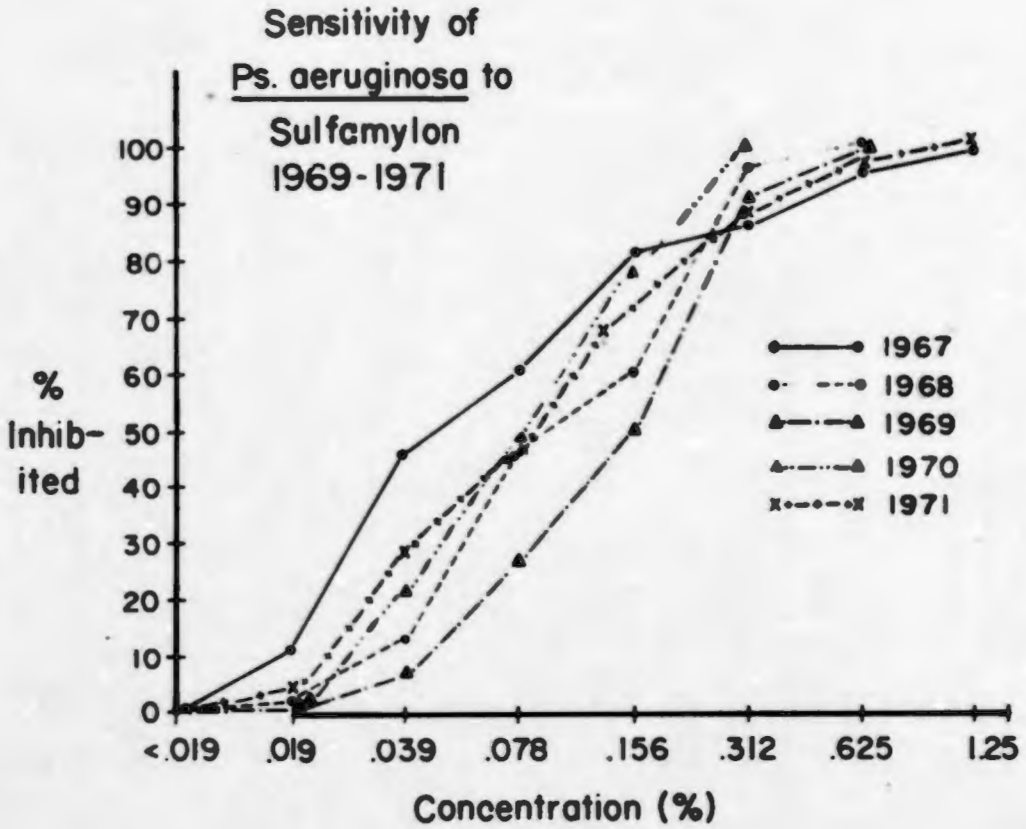
Sensitivity of
Ps. aeruginosa to
Sulfamylon
1969-1971
% Inhibited
100
90
80
70
60
50
40
30
20
10
0
<.019
.019
.039
.078
.156
.312
.625
1.25
Concentration (%)
1967
1968
1969
1970
1971

Figure 2

its variants was slightly shifted toward the higher range, since half of each group required 312% for inhibition. The median level for inhibition of A-71 was 0.201%. For A-71a it was 0.136% and for A-71b, 0.107%. The two loss-variants diminished in virulence and in parallel were increasingly sensitive to Sulfamylon. The level of Sulfamylon tolerance of A-71 strains was not, however, an explanation for the treatment-refractory state; the strains tested for virulence included three of the most sensitive, which were as virulent and as refractory to treatment as were the more resistant strains. This sensitivity is illustrated in Table 5.

Table 5. Sensitivity of Ps aeruginosa A-71 to Sulfamylon

Type	Concentration (%)					
	0.625	0.312	0.156	0.078	0.039	0.019
A-71	0	28	10	9	5	4
A-71a	0	4	1	3	1	0
A-71b	0	3	0	2	1	2
Strains Tested:			A-71 : 56			
			A-71a: 9			
			A-71b: 8			

The designation of strain A-71 as the parent strain of a sequence of loss-variants is, of course, arbitrary. In theory, the more complex phage pattern of A-71 might arise from the simpler A-71a or A-71b. However, the chronologic sequence of appearance and the decreasing degree of animal virulence of types A-71a and A-71b make the hypothesis of naturally occurring loss-variants plausible. Since the biologic attributes of virulence and drug tolerance were associated with phage susceptibility, the hypothesis that these attributes are phage-mediated is an attractive one. Experiments are under way to validate this presumption by transduction or by transferring DNA from virulent to non-virulent variants. Since the phenomenon occurred in nature it is all the more probable that the virulence attribute (as opposed to the various "toxins" that have been demonstrated in Ps aeruginosa) is a separate and transferrable entity. Such transformation makes the future role of Ps aeruginosa, even in the presence of effective chemotherapeutic agents, a continuing danger to severely traumatized patients.

In the system described, topical therapy was ineffective for a major epidemic type, in the experimental model. Systemic therapy with carbenicillin was, however, unexpectedly effective in permitting survival of seeded burned rats. A dose of 100 ug, intraperitoneally, once daily,

resulted in survival of 90% of two sets of 10 rats each. This is a higher survival rate than was possible with standard challenge strains similarly treated. The effect was not due to extreme sensitivity of the strains to carbenicillin: one strain had an MIC of 39, the other of 78 ug per ml.

Although this treatment-resistant epidemic strain disappeared from the burn ward population in the absence of any specific effort to eradicate it, the possibility of such treatment-refractoriness being manifest in burn patients is a real possibility. At the least, such experience emphasizes the need to assess the invasive capability and chemotherapeutic response of any strains which, on the basis of specific type designation, have become epidemic in a burn ward. Fluctuations in the extent and severity of nosocomial Pseudomonas infections may be uncovered by this approach and serve to explain otherwise unexplainable fluctuations of topical therapy effectiveness.

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PUBLICATIONS:

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 08 6978	74 07 01	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. DOWN GRADING	7. REGRADING	8a. GROUP INDIC	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
73 07 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	61102A	3A161102B71R	01	219
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Provide with Security Classification Code): (U) The Role of Fungi in Burn Wound Infection: Observations on Biopsy and Autopsy Tissues From Seriously Burned Soldiers (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
66 02	Cont	DA	C. In-House

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

18. RESOURCES ESTIMATE	PRECEDING/CURRENT YEAR	a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
FISCAL YEAR	74	.6	15
	75	.7	17

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21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Provide each with Security Classification Code)
(U) Fungi; (U) Mucor; (U) Candida; (U) Rhizopus; (U) Burns; (U) Phycomycosis; (U) Humans

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

23. (U) To determine the species of fungi in burn patients and determine the importance of such opportunistic invaders of burned soldier's wounds.

24. (U) Culture for fungi in tissues is routinely done. Continued modifications of technic of sampling and use of substrates is aimed at increasing recovery rates.

25. (U) 73 07 - 74 06 Predominant species of fungi in burn tissues appear to have reached a relatively consistent pattern in 1972 and 1973. Numerically, Fusarium sp. were the most frequently recovered, with the next most common genus being Aspergillus. Candida sp. (primarily C. albicans) were more frequently recovered than in previous years. Colonization with 13 genera of fungi occurred; this has been a characteristic of the burn wounds for at least the past 3 years. There were 80 patients with positive fungi and yeast cultures out of 91 cultured. This represented a positive rate of 88%. In previous years this rate had been in the 75% range. The fungi may well play a significant pathogenic role in the natural history of burn wound infection. Culture data such as is shown here offers a basis for study of this factor.

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*Available to contractors upon originator's approval

ABSTRACT

PROJECT NO. 3A161102B71R-01, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: 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

Period covered in this report: 1 July 1973 - 30 June 1974

Investigators: Robert B. Lindberg, PhD
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Harvey O.D. Smith, Jr, SP6
Virginia C. English, MA

Reports Control Symbol MEDDH-288(R1)

Cultivation of biopsy and autopsy tissues for fungi and yeasts is an essential part of definitive documentation of the microbiology of the burn wound. Thirteen genera of fungi were recovered, plus several species of Candida yeasts were far more common than fungi in burn wounds and autopsy tissues. Fusarium (and Cephalosporium) and Aspergillus were the most frequently encountered genera; Sepedonium and Alternaria also were recovered in approximately 8% of autopsies, although their occurrence was minimal in biopsied samples. Mucor and Rhizopus, the genera of phycomycetes most frequently associated with burn wound phycomycosis, were found in 2 patients, but the invasive disease was not seen. Fungi are frequently present in burn wounds, and surveillance is desirable, but the clinical significance of this group of organisms has lessened. The role of Candida sp in burn wounds is quite a different question; study of the systemic effect of this group of opportunistic colonizers of the burn wound is indicated.

Fungi
Mucor
Rhizopus
Candida
Burns
Phycomycosis
Humans

THE ROLE OF FUNGI IN BURN WOUND INFECTION: OBSERVATIONS ON BIOPSY AND AUTOPSY TISSUES FROM SERIOUSLY BURNED SOLDIERS

The occurrence of fungi and yeasts in burn wound infections appears to be increasing, and interest in fungi as opportunistic invaders in the compromised host has unquestionably increased. Previous reports on observation of fungi in burns have emphasized that the significance of fungi in the burn wound remains undefined. Cultural results offer some information as to the biologic identity of colonizing species of fungi. The relationship between species and ability to invade tissues can only be resolved by recovery and identification of the offending strains. This study undertakes to do this. There is a poor correlation between the histologic appearance of fungi invading burn wounds and the mycologic results of wound culture. However, the proportion of patients' wounds from which yeasts or fungi could be recovered rose from the range of 75% in 1971 and 1972 to 88% in 1973. This may reflect an increased incidence, but more probably is due to increased expertise in recovering fungi.

METHODS

Emphasis has been placed on collection of fresh biopsy samples, always positioned to include the live-dead interface of the burn area being tested. Autopsy samples were collected with a simiar emphasis. The planting of thin slivers of tissue has given best results in recovery rates (Lindberg RB, Townsend CH, Contreras AA, Pruitt BA, Jr. USA Inst Surg Res, BAMC, Ft Sam Houston, TX, Ann Res Rpt FY 1970, Sect. 26)[1]. Prompt planting has given more recoveries than were obtained with tissues refrigerated overnight in maintenance medium. Isolation was made on Sabouraud's agar, and an observation period of 10 days was maintained.

RESULTS

Biopsy. There was a drop from 201 to 106 patients biopsied and cultured for fungi from 1972 to 1973. Those patients who were biopsied, however, were sampled more often so that the number of strains of fungi recovered was 400 in 1973, in comparison with 362 in 1972. Results of biopsy culture are summarized in Table 1. The 1973 results are compared with the results obtained in 1972. As has been the case since we began culturing wounds for fungi, the most common group recovered was the several species of Candida. While, Candida albicans was prominent, strains of Candida stutzeri , Candida stellatoides, Candida guillermondi and other species, not albicans, were also recovered. The percentage of patients from whom Candida were recovered rose from 13.9 in 1972 to 53.7% in 1973. Whether this increase reflects active disease due to Candida burn wound invasion is not clear; the majority of the wounds from which Candida was recovered were not invaded. Fusarium sp was by far the most common genus encountered. Cephalosporium bears a close generic and physical relationship to Fusarium, and it is probable

Table 1. Biopsy Speciemns Positive For Fungi and Yeasts - 1972-1973

Genus	No. Patients Positive 1972	1973	No. Strains Recovered 1972	1973
Aspergillus	8	8	11	17
Cephalosporium	5	4	15	5
Fusarium	19	12	33	23
Sepedonium	0	1	0	1
Curvularia	3	2	3	2
Scopulariopsis	11	0	11	0
Alternaria	3	2	7	2
Diplosporium	1	0	1	0
Penicillium	1	1	1	1
Helminthosporium	0	4	0	9
Rhizopus	2	2	3	2
Mucor	2	2	2	2
Stemphyllium	1	0	1	0
Syncephalastrum	1	0	1	0
Geotrichum	1	0	1	0
Candida sp	28	57	46	141
No. Patients Cultured	201	106		
No. Tissue Samples	217<		362	400

that identification of these two genera are frequently crossed. Together these closely related genera accounted for 15.6% of all patients sampled who harbored fungi in their burn.

Other species appearing in numerically significant incidence on patients wounds included Aspergillus, and possibly Helminthosporium. The Phycomycetes were represented by two patients positive for Mucor sp and two with Rhizopus sp. These genera are noted because of the experience with phycomycosis in burned patients in earlier years. Such an infection may be fulminating and require radical surgical extirpation, with loss of large amounts of tissue if the disease is to be arrested. The rarity of these genera in 1972 and 1973 suggests that they are indeed casual contaminants of the burn wound in most circumstances; in these patients, clinical phycomycosis did not appear.

Autopsy. The fungi recovered from tissues at autopsy reflect the role of fungi in the burn more completely than do biopsy isolates, since dissection permits recognition of potentially invaded areas and collection of more adequate samples. The number of genera recovered, as shown in Table 2, lessened the past two years, from 17 in 1971 to 14 in 1973 Mucor sp was recovered from the wounds of three patients, one of whom also harbored the fungus in the lung. Rhizopus was found in two patients and Absidia not at all in 1973. It was evident that this dangerous group was consistently rare when sought in routine sampling.

Numerically predominant genera included Aspergillus and Fusarium. Helminthosporium, Sepedonium and Alternaria were the only other genera to appear in more than 2 or 3 patients. Penicillium was found in the lungs of five patients, but never in the burn wound. Candida, of course, was the most commonly encountered organism, and vastly overshadowed fungi in its occurrence.

Comparison of the incidence of fungi in burn patients who were sampled in 1973 is shown in Table 3. The incidence of patients positive is essentially parallel, and, of course, most of the patients positive at autopsy had been positive in biopsy. Mucor and Rhizopus strains were recovered with the same low frequency in both groups. Aspergillus sp was the second most commonly occurring genus, although true invasive mycotic infection with these strains was not described. Fusarium sp were by far the most common genus encountered; for at least three years this genus has been conspicuous as the most frequently encountered group in burn tissues and in viscera at autopsy as well. Generalized invasive mycotic infection due to Fusarium, or the closely related Cepahlosporum sp, is rare, but the hyphal elements are frequently described as invading adjacent viable tissue, and such tissue invasion is a potential source of toxicity or other systemic changes. There were 13 genera of fungi recovered from autopsy tissues; only 10 of these also appeared in biopsy. Scopulariopsis, Geotrichum and Clodosporium were not found in biopsy samples. The close parallel between findings in biopsy and in autopsy samples offers assurance of the validity of the sampling

Table 2. Autopsy Tissues Cultured for Fungi:
Fungi Recovered from Viscera (Lung, Liver, Spleen)
and Burn Wound at Autopsy - 1971-1973

Genus	Number of Patients Positive at Autopsy					
	Burn Wound			Viscera		
	1971	1972	1973	1971	1972	1973
Mucor	3	0	3	0	0	1
Rhizopus	1	1	2	1	1	0
Absidia	1	1	0	0	0	0
Aspergillus	6	11	19	3	2	5
Penicillium	8	1	0	5	6	5
Paecilomyces	0	0	0	1	2	0
Alternaria	2	3	6	0	0	0
Cephalosporium	2	4	3	0	4	4
Fusarium	9	30	25	7	20	19
Helminthosporium	4	1	4	0	0	2
Nigrospora	9	0	0	3	0	0
Scopulariopsis	5	3	1	1	2	0
Sepedonium	1	5	5	1	4	3
Diplosporum	1	2	0	0	0	0
Geotrichum	0	0	3	1	0	0
Fonsecaea	2	0	0	2	0	0
Curvularia	0	5	1	0	0	0
Microsporum	0	1	0	0	0	0
Cladosporium	0	0	1	0	0	3
Candida	13	32	67	11	25	50
Number patients positive	46	65	80			
Total patients cultured	61	89	91			
Number genera present	17	16	14			

Table 3. Autopsy and Biopsy Cultures for Fungi from 193 Patients, ISR - 1973

Genus	Number of Patients Positive	
	Autopsy	Biopsy
Mucor	3	2
Rhizopus	2	2
Aspergillus	11	8
Penicillium	1	1
Alternaria	6	2
Cephalosporium	3	4
Fusarium	25	12
Helminthosporium	4	4
Scopulariopsus	1	0
Sepedonium	5	1
Geotrichum	3	0
Curvularia	1	2
Clodosporium	1	0
Candida	67	57
Number of species recovered	14	11

technic. If the genera recovered represented simply surface contaminants falling on the burn wound, a more heterogeneous population would be expected, and correspondence between biopsy and autopsy would be less precise.

DISCUSSION

Prior to 1969, fungus cultures were not carried out routinely on all tissue samples, nor were previous culture technics reassuring in respect to the number of positive cultures obtained. However, the development of improved technics, the use of carefully selected slivers of tissue from the tissue samples, and the use of flat-sided one ounce tissue-culture flasks for initial isolation, greatly improved the recovery rate, and the consistency with which fungus species have been recovered. A marked change since 1969 was the virtual disappearance of Geotrichum sp as a major part of the fungal flora, and its replacement by Fusaium sp (and probably Cephalosporium sp) as the principal fungal entities populating burn wound tissues. There appeared to be less clinical disease produced by opportunistic fungi than was the case four years ago, no proven cases of phycomycosis were observed in 1973. Although its occurrence can be a lethal event, fungal wound sepsis is apparently a sporadic phenomenon, and in the vast majority of cases, the colonization and superficial invasion into viable tissue is not followed by clinically significant invasive mycosis. Monitoring of the fungal population is emphatically desirable, since only with such background material can the role of fungi in burn wound infection be elucidated.

The incidence of yeasts in burn wounds is, as the tables show, extremely high. Invasive candidiasis is rare, and the importance of yeast colonization of the burn is not well understood. Future research should include scrutiny of sera of burn patients for yeast antigen. This technic has been perfected to a point where it is feasible to apply it to a study of Candida colonization of the burn wound.

REFERENCES

1. Lindberg RB, Townsend CH, Contreras AA, Pruitt BA, Jr: Role of fungi in burn wound infections. USA Inst Surg Res, BAMC, Ft Sam Houston, TX, Ann Res Rpt FY 1970, Sect. 26.

PRESENTATIONS

Lindberg RB. The cultivation of yeasts and fungi from tissue biopsies. Seminar - Univ. Georgia, Athens, Georgia, October 7, 1973.

PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0E 6384	74 07 01	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISP'N INSTR'S	8A. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
73 07 01	H.TERMINATION	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	3A161102B71R	01	118
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with security Classification Code): (U) Development of Streptozotocin Model of Fungal Burn Wound Infection as it Occurs in Burned Military Personnel (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
72 01	74 05	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	PRECEDING	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	74	.1	4
CURRENCY			

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NAME: DA

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Burns; (U) Fungi; (U) Rats; (U) Hyperglycemia

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

23. (U) The significance of fungi in the burn wound as well as successful modalities of treatment are to be elucidated. Experimental model of fungal burn wound infection is required to perfect therapy of fungal infection in burned soldiers.

24. (U) Rats made hyperglycemic with Streptozotocin, were burned and seeded with various spore suspensions of the following fungi: Rhizopus, Aspergillus, Cephalosporium and Fusarium. All rats were verified as being hyperglycemic by blood glucose determinations. Both control and seeded rats were examined pathologically when they died or were sacrificed and examined at the end of one month.

25. (U) 73 07 - 74 05 The streptozotocin model of fungal burn wound infection is not a satisfactory animal model for the study of fungal burn wound infection other than that with Rhizopus species. Because all other fungi tested only sporadically produce wound invasion with deep tissue involvement it is felt no further studies for this animal model should be carried out and the study terminated as of 1 May 1974.

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*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

ABSTRACT

PROJECT NO. 3A161102B71R-01, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: DEVELOPMENT OF STREPTOZOTOCIN MODEL OF FUNGAL BURN WOUND INFECTION AS IT OCCURS IN BURNED MILITARY PERSONNEL

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

Period covered in this report: 1 July 1973 - 1 May 1974

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Robert B. Lindberg, PhD

Reports Control Symbol MEDDH-288(R1)

The streptozotocin animal model was used to determine the invasive capability of the following fungi Aspergillus, Fusarium, Cephalosporium and Rhizopus when seeded on the animal following a standard full thickness 20% scald burn. Animals were followed until death or sacrificed at 30 days. Only Rhizopus species consistently demonstrated burn wound infection and visceral spread, in 96% and 59% of the animals respectively. No evidence of fungal hematogenous infection was noted. Visceral invasion of the liver, spleen and kidneys was by direct spread from the infected burn wound. A high incidence of bacterial wound infection was present in all animals seeded with Rhizopus.

The streptozotocin rat model of fungal burn wound infection has not proven to be an acceptable model to study burn wound infection by any other fungi except Rhizopus species. Because burn wound invasion is erratic and cannot be consistently reproduced in large enough numbers to make it statistically significant further studies with this animal model will be terminated as of 1 May 1974.

Burns
Fungi
Rats
Hyperglycemia

DEVELOPMENT OF STREPTOZOTOCIN MODEL OF FUNGAL BURN WOUND INFECTION AS IT OCCURS IN BURNED MILITARY PERSONNEL

The presence of fungi in burn wounds has increased markedly in the past several years. (Nash, G, et al. JAMA 215 (10) 1664, 1971.)[1] The significance of fungi in burn wounds as well as their relationship to modalities of treatment remain to be elucidated. Bruck et al (Bruck, HM, et al. US Army Inst of Surg Res Anl Res Prog Rpt, 30 Jun 71, BAMC, FSHT, Sect 13.)[2] used the alloxan treated rat as a model to establish and evaluate fungal burn wound infection.

Streptozotocin, derived from Streptomyces achromogens, possesses antibiotic, antitumor and a hyperglycemic action. The development of frank hyperglycemia in rats and dogs treated with this drug, was first reported by Rakieten et al. (Rakieten R, et al. Cancer Chemother Rep 29:91-98, 1963.)[3] Streptozotocin has a highly effective beta-cytotoxicity similar to alloxan but more specific and with a wider margin of safety than alloxan.

The only success so far in establishing invasive fungal infection in an animal has been when a hyperglycemic state exists. Sprague-Dawley rats were made hyperglycemic by administering 65 mg/kg of streptozotocin intravenously. Hyperglycemia was verified four days later by blood glucose determination. Blood glucose levels ranged between 300 and 650 mg%. The animals were given a standard 20% total full thickness scald burn and immediately seeded with specific fungal suspensions in concentrations of between 10^6 and 10^7 spores per ml. Fungi tested included Aspergillus, Fusarium, Cephalosporium and two species of Rhizopus. All animals in the study were followed to death or sacrificed at 30 days post seeding.

Animals seeded with Rhizopus species demonstrated burn wound infection and visceral spread in 96% and 59% of cases respectively. Viscera (kidney, spleen, and liver) were infected by direct extension from the burn wound. No evidence of hematogenous fungal infection was demonstrated. A high incidence of heavy bacterial contamination of the burn wound was associated with all Rhizopus fungal infections.

Other fungi tested included Aspergillus, Cephalosporium and Fusarium. Rats were made hyperglycemic with an average dose of 65 mg/kg of streptozotocin. Four days later blood sugar levels were determined and ranged between 300-650 mg%. At this time a standard 20% total full thickness scald burn was created on the rats and spore suspensions of these fungi in concentrations of between 10^6 and 10^7 were seeded immediately on the burn surface with a cotton tip applicator. Control rats not seeded were included as were rats seeded with Rhizopus. Ten animals were seeded with each spore suspension and ten controls were used. At the end of 30 days all living animals were sacrificed. All control animals lived without any deaths and these were autopsied and found to have no significant bacterial or fungal infection by culture and microscopic examination.

Autopsy evaluation of the animals was directed at ascertaining wound colonization, invasion and systemic spread by the fungi. Ninety per cent of the rats seeded with Aspergillus had wound colonization, but only 25% of the wounds had demonstrable invasion by the fungus. No animal had evidence of systemic dissemination. Organ involvement was noted in the liver or spleen in less than 10% of the animals and was by direct invasion rather than systemic spread as ascertained by gross dissection of the animal. Animals seeded with Cephalosporium and Fusarium demonstrated erratic colonization of the wound and no animals exhibited evidence of either invasion or systemic spread.

The streptozotocin laboratory animal model of fungal burn wound infection has demonstrated that application of 10^6 or 10^7 spores of Rhizopus species will not only colonize but invade a burn wound and by means of direct extension spread to involve internal organs. Systemic spread other than by direct extension was not noted. Experimentation with other species of fungi has proven them to be erratic invaders with only occasional wound invasion and no systemic or direct spread to viscera. Because the animal model has only been useful with Rhizopus species, further studies on this animal model will be suspended.

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1. Nash, G, et al: Fungal burn wound infection. JAMA 215 (10) 1664, 1971.

2. Bruck, HM, et al: Studies on occurrence of significance of fungi in burn wounds. Development of laboratory model. US Army Inst of Surg Res Anl Res Prog Rpt, 30 Jun 71, BAMC FSHT, Sec 13.

3. Rakieten R, et al: Studies on the diabetogenic action of Streptozotocin. Cancer Chemother Rep 29:91-98, 1963.

PUBLICATIONS AND/OR PRESENTATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OF 6382	74 07 01	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. HANDLING INSTRN	8a. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
74 07 01	K. COMPLETION	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
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(U) Leukocyte Chemotaxis In Thermally Injured Military Personnel (44)

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FISCAL YEAR	74	.6	18
	CURRENT		

19. RESPONSIBLE DOD ORGANIZATION

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22. KEYWORDS (Provide EACH with Security Classification Code)

(U) Burns; (U) Leukocytes; (U) Chemotaxis; (U) Humans

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

23. (U) Evaluation of leukocyte chemotaxis in thermally injured military personnel.

24. (U) Leukocyte chemotaxis was evaluated by modification of the Boyden technique namely the ability of leukocytes to migrate through nucleopore filter against a complement dependent chemotactic agent, CASEN-SERUM.

25. (U) 73 07 - 74 06 Forty-four thermally injured patients with mean burn size 51.1% (range 145-92%) were evaluated. During first 72 hours postburn there was an inverse correlation between burn size and chemotactic index ($y=94.4 - 0.759x$; $r^2 = 0.51$) Following 72 hours the chemotactic index correlated with clinical status and ultimate survival (survivors - 97.9% ± 5.1; non-survivors 39.4% ± 5.5). Serum from burned patients treated with Sulfamylon and silver sulfadiazine depressed normal leukocyte chemotaxis (Sulfamylon - 58.3%, silver-sulfadiazine 43.6%), whereas serum from silver nitrate treated patients demonstrated no depression. Moreover, Sulfamylon and sulfadiazine when included directly with normal leukocytes, produced marked depression ofn normal leukocyte chemotaxis (Sulfamylon 31.5% decrease, sulfadiazine 75.4% decrease). Normal AB serum returned burn leukocyte chemotaxis to normal. These studies demonstrate a marked depression of leukocyte chemotaxis in thermally injured patients which appears due to both an intrinsic defect in metabolism caused by the thermal injury and the topical agents used for treatment of the wound.

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*Available to contractors upon originator's approval

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ABSTRACT

PROJECT NO. 3A161102B71R-01, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: LEUKOCYTE CHEMOTAXIS IN THERMALLY INJURED MILITARY PERSONNEL

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

Period covered in this report: 1 July 1973 - 30 June 1974

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Arthur D. Mason, Jr, MD
Basil A. Pruitt, Jr, MD, Colonel, MC

Reports Control Symbol MEDDH-288(R1)

Polymorphonuclear leukocytes from burned patients exhibit suppressed chemotaxis possibly related to the susceptibility of such patients to opportunistic infection. This study assesses the effect of normal serum upon burn-suppressed leukocytes and the effects of three commonly used topical chemotherapeutic agents upon the chemotaxis exhibited by granulocytes from normal controls.

In vitro incubation with normal serum restored chemotaxis to normal in the suppressed granulocytes from burned patients. The serum factor responsible for this restoration was heat labile. Serum albumin alone did not exhibit this effect.

Both mafenide and silver sulfadiazine suppressed the chemotactic function of granulocytes obtained from normal controls, while silver nitrate exhibited no such activity. Studies of the chemotactic function of control granulocytes after incubation with sera from burned patients yielded similar results only the sera from patients treated with silver nitrate failed to suppress normal leukotaxis. The chemotactic impairment found in leukocytes from burned patients, however, while related to burn size and predictive of prognosis, did not vary with the agent used for topical therapy.

These data suggest the presence of a reversible intrinsic defect in leukotaxis consequent to burn injury related to some factor deficient in burn serum. In addition, extrinsic impairment of normal granulocyte leukotaxis by two commonly used chemotherapeutic agents is demonstrated.

Burns
Leukocytes
Chemotaxis
Humans

LEUKOCYTE CHEMOTAXIS IN THERMALLY INJURED MILITARY PERSONNEL

The use of topical chemotherapeutic agents to control bacterial invasion in extensive burn wounds has become an accepted method of treatment. Mafenide acetate (Sulfamylon®), silver nitrate and silver sulfadiazine (Silvadene®) are widely used agents for such topical treatment. The advantages and disadvantages of each agent have been described and their effectiveness in decreasing the incidence of pseudomonas burn wound sepsis is well established. Despite this decreased incidence of pseudomonas burn wound sepsis, the mortality from extensive thermal injury remains high. The use of topical chemotherapy has been associated with increased frequency of infection by other opportunistic organisms. Infection remains the most frequent cause of death in patients who have sustained large burns.

The susceptibility of burn patients to opportunistic infection is not understood. Several defense mechanisms are known to be impaired, including the epithelial barrier, immune mechanisms and leukocyte function. We have introduced an assay of leukocyte chemotaxis to assess one aspect of polymorphonuclear leukocyte function in thermally injured patients and have demonstrated an inverse correlation between leukocyte chemotaxis and burn size (Warden GD, Mason AD, Jr, Pruitt BA, Jr. J Clin Invest. in press)[6]. In addition, suppression of leukocyte chemotaxis has been predictive of mortality in burn patients. This study of the mechanism of chemotactic suppression following burn injury assesses the effects of normal serum and of topical chemotherapeutic agents upon granulocyte chemotaxis.

MATERIALS AND METHODS

Preparation of Leukocytes. On the day of testing 10 cc of heparinized blood (200 units heparin per 10 cc blood) was collected in a glass syringe. The aliquant was placed in an equal volume of clinical dextran (6%) in physiologic saline solution, containing glucose (400 mg/500 ml) and heparin (20 mg/100 ml). Erythrocyte-mononuclear sedimentation was accomplished in a 50 ml conical tube at 37° C for 45 minutes. The leukocyte rich supernatant was removed with a Pasteur pipette and used within one hour of preparation. Average yields of 10^6 cells were obtained from 10 ml of blood. Ninety-nine per cent viability was confirmed by the trypan blue exclusion method after sedimentation.

Chemotactic Agent. Previous studies by Baum and Mowat (Baum J, Mowat AG, Kirk JA. J Lab Clin Med 77:501, 1971)[3] have demonstrated a mixture containing two parts of casein solution and one part of human serum to furnish reliable chemotactic attraction. Casein was prepared in a concentration of 5 mg per ml in Hank's solution. Human serum, type AB, from healthy donors was used throughout the study. The serum was obtained in the following manner: (1) 500 ml of peripheral blood was removed via the antecubital vein and placed in 50 ml conical tubes

and allowed to clot at room temperature; (2) After clot retraction, the sample was centrifuged at 2000 g and the serum removed; (3) The serum was stored at -73° C in 3 ml aliquots.

Evaluation of Leukocyte Chemotaxis. Perspex chambers similar to Boyden's design were constructed (Fig. 1). Two ml of leukocyte-rich supernatant were diluted with 8 ml of Hank's solution and 2 ml of this mixture, containing approximately 4×10^6 cells, were placed in the upper chamber and 1.5 ml of chemotactic agent (casein-serum mixture) were placed in the lower chamber. A nuclepore* filter, 0.5 micron pore size, 25 mm diameter was interposed between the upper and lower chambers. The chamber was incubated at 37° C for 120 minutes. The nuclepore filter was removed, stained with Wright-Giemsa stain for 4 minutes. Wright's buffer for 4 minutes, cleared with xylene and mounted with xylene-permount (3:1 ratio) on a glass slide. The nuclepore filter was examined microscopically under high power and the cells migrating through the nuclepore filter (bottom side) were counted as were the cells remaining on the starting side (top side) in each microscopic field. A total of 400 cells was counted. Chemotaxis was evaluated in the following manner, negating the necessity to count the cells before placement in the upper chamber.

$$\text{Chemotactic Index} = \frac{\text{number cells (attracting side)} \times 1000}{\text{number cells (starting side)}}$$

Leukocytes from thermally injured patients were compared with cells from healthy volunteers and the functional chemotactic index expressed as the per cent of the chemotactic index of the control leukocytes:

$$\text{Functional chemotactic index} = \frac{\text{chemotactic index burn patient}}{\text{chemotactic index normal volunteer}} \times 100$$

Duplicate chambers were used for each patient sample and simultaneous control samples were also performed in duplicate.

Preparation of Patient Serum. On the morning of each test, 10 cc of peripheral blood was obtained and allowed to clot. After clot retraction the sample was centrifuged at 2000 g and the serum removed. The serum was stored at -73° C or used within one hour of preparation.

Experimental Group. Studies were performed on 46 thermally injured patients with a mean burn size of 50.9% total body surface (range 14.5% to 92.0%). The overall mortality among these patients was 65.2%. The mean burn size in those patients who expired was 59.0% (range 31.5% to 92.0%) while the surviving patients had an average burn size of 35.9% (range 14.5% to 70.5%). Autopsy examination was performed on 25 of the 30 non-surviving patients. The mean day of death was 14.9 days (range 3 to 38 days). Among

* Nuclepore Corporation, 7035 Commerce Circle, Pleasanton, CA 94566.

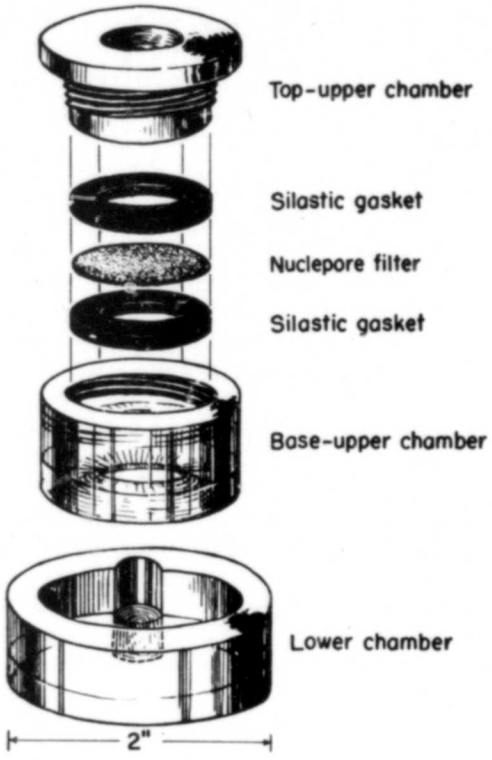

Figure 1. Chemotactic chamber.

the autopsied cases, infection as the major cause of death occurred in 23 of the 25 cases (92.0%). Septic complications included pneumonia, burn wound sepsis, and septicemia. The mean day of clinical diagnosis of the septic complications was 9.8 days (range 3 to 19 days). None of the 16 surviving patients had systemic sepsis.

The patients' cells were tested at admission and then weekly until discharge or expiration. The patients were treated with topical mafenide acetate 10% ointment (Sulfamylon®), silver nitrate 5% solution in dressings, or silver sulfadiazine 1% cream (Silvadene®) using standard procedures of the Institute of Surgical Research.

Evaluation of Effect of Burn Serum on Leukocyte Chemotaxis. The leukocyte-rich supernatant obtained from normal volunteers was incubated in 1:1 ratio for 20 minutes at 37° C with serum obtained from thermally injured patients, normal serum obtained from healthy type AB donors or Hank's solution. This suspension was then diluted with Hank's solution and 2 ml of this mixture, containing approximately 4×10^6 cells were placed in the upper compartment of the chamber for evaluation of leukocyte chemotaxis.

Evaluation of Effect of Topical Chemotherapeutic Agents on Leukocyte Chemotaxis. The topical agents were dissolved in Hank's solution and incubated with 2 ml of leukocyte rich supernatant obtained from normal volunteers with final concentrations of: mafenide acetate 10 mg%, and 100 mg %, p. carboxybenzenesulfonamide 10 mg%, sulfadiazine 5 mg%, sodium sulfadiazine 5 mg%. In addition acetazolamide (Diamox®), 50 ug/ml and 100 ug/ml and gentamycin (Garamycin®) 4 ug/ml were also included in duplicate chemotactic chambers. Hank's solution without added drug was used in the same manner for control studies, which were performed in duplicate.

Evaluation of Effect of Dialyzed Burn Serum on Leukocyte Chemotaxis. Serum from thermally injured patients was dialyzed in cellulose casing (Visking, medical grade 23/32)* with diphosphate buffer 0.15 M, pH 7.4 for 48 hours at 4° C. The dialyzed serum was then added to 2 ml of the leukocyte-rich supernatant from normal volunteers in 1:1 ratio and the mixture incubated at 37° C for 20 minutes. After dilution with Hank's solution, 2 ml of the mixture containing 4×10^6 cells, was placed in the upper compartment of the chemotactic chamber for evaluation of leukocyte chemotaxis. Normal AB serum was maintained at 4° C for 48 hours and then incubated in the same manner as a simultaneous control.

Evaluation of Effect of Normal Serum on Chemotaxis. Serum from normal AB donors was used to assess the effect of normal serum upon leukocytes from burned patients. AB serum was added to 2 ml of the leukocyte-rich supernatant in 1:1 ratio and the mixture incubated at 37° C for 20 minutes. After dilution with Hank's solution, 2 ml of the mixture, containing approximately 4×10^6 cells, was placed in the upper compartment of the chemotactic

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* Visking Company, Chicago, Illinois.

chamber for evaluation of leukocyte chemotaxis. Simultaneous controls were run using Hank's solution instead of serum.

Evaluation of Effect of Heated Normal Serum on Leukocyte Chemotaxiss. Serum from normal AB donors was heated at 56° C in a water bath for 20 minutes. The leukocyte-rich supernatant from thermally injured patients was incubated with the heated AB serum at 37° C for 20 minutes. This suspension was diluted with Hank's solution and 2 ml of this mixture containing approximately 4×10^6 cells were placed in the upper compartment of the chemotactic chamber for evaluation of leukocyte chemotaxis. As controls, Hank's solution and normal serum type AB were incubated in dupiicate.

Evaluation of the Effect of Albumin on Leukocyte Chemotaxis of Thermally Injured Patients. Two ml of the leukocyte-rich suspension obtained from thermally injured patients was incubated at 1: 1 ratio with salt poor, human albumin* (25 g/100 cc) for 20 minutes. The suspension was diluted with 5 ml Hank's solution and 2 ml of this mixture (approximately 4×10^6 cells) was placed in the upper chamber of the chemotactic chamber for evaluation of leukocyte chemotaxis. As controls, incubation with Hank's solution and normal serum type AB was performed in duplicate.

RESULTS

Chemotaxis in Thermally Injured Patients. As simultaneous daily control values, the chemotactic index was measured in a total of 44 normal, healthy volunteers (ages 23 to 54 years). A mean value of 764.4 with 95% confidence limits of 750 to 780 was observed.

During the first 72 hours following injury 24 patients were studied. Burn size and functional chemotactic index were inversely related, with a regression of $y = 85.3 - 0.59x$ (y = functional chemotactic index, x = burn size), and $r^2 = 0.34$ (Fig. 2).

After 72 hours, the patients separated into two groups, survivors and nonsurvivors, with the surviving patients demonstrating improvement in leukocyte chemotactic function during their hospital courses (Fig. 3). The nonsurviving patients demonstrated neither significant increase or decrease in leukocyte chemotaxis from admission until death. The average functional chemotactic index in the surviving groups was 97.7, SE + 2.3; whereas the average in the nonsurviving was 39.9, SE $\pm$ 2.3 (Table 1). No patient with a functional chemotactic index below 60 beyond 72 hours after injury survived; only one patient with an index greater than 60 expired. Statistical comparison revealed a significant difference between the mean indices of these groups ($P \ll 0.01$).

Diminished chemotactic function was observed prior to clinical infection in all patients who ultimately succumbed to infection. The onset of clinical

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* Cutter Laboratories, Berkeley, California.

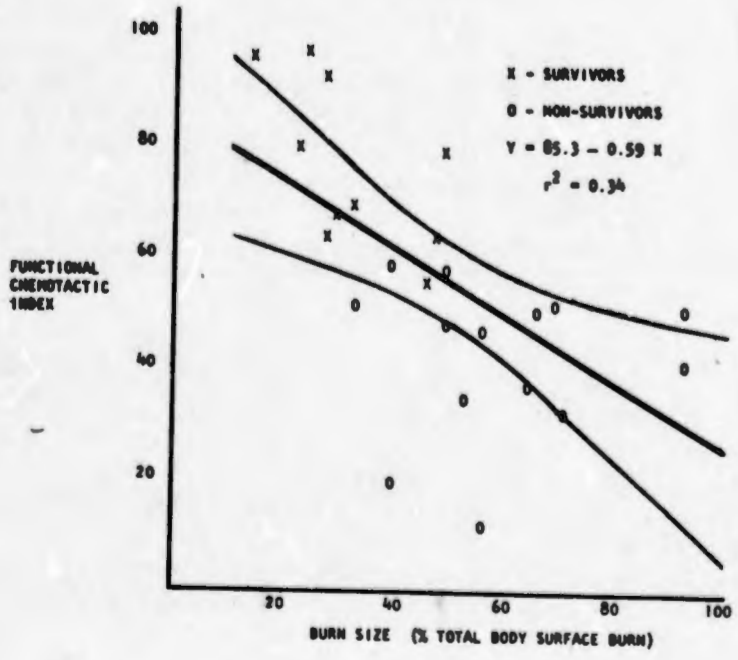

Figure 2. Functional chemotactic index versus burn size during first 72 hours postburn.

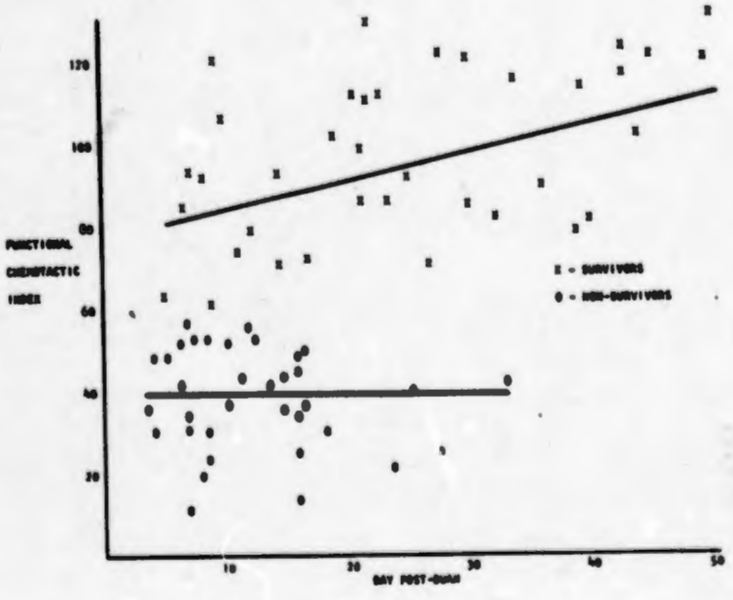

Figure 3. Functional chemotactic index versus day postburn after 72 hours postburn.

Table 1. Functional Chemotatic Index after 72 Hours

	Survivors	Non-Survivors
Number - Patients	12	23
Number - Determinations	36	34
Funtional Chemotactic Index	97.7%	39.9%
Significance	P = < 0.01	
Range	61.2 - 130	14.6 - 75
Burn Size - Mean	44.6%	57.9%
Range	25.5 - 70.5	31.5 - 92

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infection was not associated with any change in the functional index. Functional chemotactic index did not vary with either the agent used for topical chemotherapy or with other identifiable differences in treatment. Age and sex were also without effect.

Effect of Burn Serum on Normal Leukocyte Chemotaxis. Serum obtained from 27 burn patients treated with mafenide acetate with a mean burn size of 46.3% total body surface (range 18.0 to 75%) produced significant suppression ($P \ll 0.01$) of chemotaxis of normal control leukocytes, decreasing the chemotactic index to 54.0% of control values (Table 2). The serum of 14 burn patients treated with silver sulfadiazine (mean total body surface burn 65.0%, range 30.5 to 96.0%) also suppressed normal control leukocyte chemotaxis to 42.4% of control ($P < 0.01$). However, the silver nitrate patients' serum produced no suppression of normal leukocyte chemotaxis although burn size was similar to the mafenide acetate treated group. Simultaneous control AB serum had no effect on normal leukocyte chemotaxis.

Effect of Topical Chemotherapeutic Agents on Normal Leukocyte Chemotaxis. Mafenide acetate and its metabolite, p. carboxybenzenesulfonamide at a physiologic concentration (10 mg%) usually obtained during treatment of major thermal injuries produced significant suppression ($P < 0.01$) of normal control leukocyte chemotaxis to 68.5% (SE $\pm$ 1.9) and 58.1% (SE $\pm$ 2.0) respectively, of baseline values (Table 3). Increasing the concentration of mafenide acetate to 100 mg% resulted in a more pronounced depression (54.7% SE $\pm$ 2.7) of normal leukocyte chemotaxis ($P < 0.01$). Sulfadiazine and sodium sulfadiazine at concentration of 5 mg% produced marked suppression of normal leukocyte chemotaxis to 24.6% and 32.8% respectively. Gentamycin at a concentration of 4 ug/ml produced no significant change of normal leukocyte chemotaxis. The suppressions exhibited by adding the topical agent directly to the cell suspension are similar to the suppressions obtained by incubating with burn serum from thermally injured patients treated with these agents.

Effect of Acetazolamide on Normal Leukocyte Chemotaxis. Since mafenide acetate inhibits carbonic anhydrase, the effect of acetazolamide on normal leukocyte chemotaxis was also evaluated at concentrations of 50 ug/ml and 100 ug/ml. Animal carbonic anhydrase inhibition occurs at serum levels of 50 ug/ml. Acetazolamide at 50 ug/ml produced no suppression of normal leukocyte chemotaxis; whereas at 100 ug/ml there was slight depression, to 85.2% of control ($P < 0.01$). We conclude that carbonic anhydrase inhibition by mafenide acetate was not responsible for the depression seen with burn serum or mafenide acetate.

Effect of Dialyzed Burn Serum on Normal Leukocyte Chemotaxis. Nondialyzed burn serum obtained from 10 patients treated with mafenide acetate with a mean burn size of 50.2% total body surface (range 18.0 to 70.5%) produced suppression of normal leukocyte chemotaxis to 43.9% of control values. Dialyzing the burn serum for 48 hours with a phosphate buffer significantly reduced ($P \ll 0.01$) the degree of suppression by burn serum to a mean value of 84.0% (Table 4). Dialyzed burn serum obtained from nine patients treated with silver sulfadiazine (mean burn size 69.7%, range 30.5 to 5.96%) also

Table 2. Effect of Burn Serum on Normal Leukocyte Chemotaxis

	Sulfamylon	Silver Nitrate	Silver Sulfadiazine
Number	27	3	14
Mean Functional Chemotactic Index	54.0%*	111.8%	42.4%
Standard Error	± 3.8	± 5.8	± 2.3
Burn Size - Mean	46.3%	40.8%	65.0%
Range	18 - 70.5	35.5 - 48	30.5 - 96

* Significantly different from control, $P < 0.01$.

Table 3. Effect of Chemotherapeutic Agents *in vitro* on Normal Leukocyte Chemotaxis

Chemotherapeutic Agent	Concentration	No.	Mean Functional Chemotactic Index
Mafenide acetate	10 mg%	6	68.5% ± 1.9
Mafenide acetate	100 mg%	6	54.7% ± 2.7
p-carboxybenzenesulfonamide	10 mg%	6	58.1% ± 2.0
Sodium sulfadiazine	5 mg%	4	36.7% ± 2.0
Sulfadiazine	5 mg%	4	24.6% ± 3.6
Gentamycin	4 ug/ml	4	97.8% ± 7.4

Table 4. Effect of Dialyzed Burn Serum on Normal Leukocyte Chemotaxis

	Sulfamylon		Silver Sulfadiazine	
	Burn Serum	Dialyzed Burn Serum	Burn Serum	Dialyzed Burn Serum
Number	10	10	9	9
Mean Functional Chemotactic Index	43.7%	84.0%	42.0%	82.8%
Significance	P = < 0.001		P = < 0.01	
Burn Size - Mean	50.2%		69.7%	
Range	18.0 - 70.5		30.5 - 96.0	

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produced significantly ($P<0.01$) less suppression than did the non-dialyzed burn serum with mean values increasing from 42.0% to 82.8%. These studies suggest that dialysis removed a chemotaxis-suppressing substance or substances, presumably the topical therapeutic agents mafenide acetate and silver sulfadiazine.

Effect of Normal Serum on Burn Leukocyte Chemotaxis. In 33 patients with a mean burn size of 46.8% total body surface area (range 14.5 to 70.5%) and having a mean baseline chemotactic index of 61.1% (SE ± 5.7) there was a marked increase of leukocyte chemotaxis as incubation with normal AB serum (Table 5). Levels increased to 107.7% (SE ± 48) of simultaneous normal controls. Normal AB serum incubated with normal cells had no effect on leukocyte chemotaxis. The improvement of chemotaxis by normal serum was not related to the agent being used for topical chemotherapy and was not a dilution effect, since similar dilution with Hank's solution in the control runs had no effect.

Effect of Heated AB Serum on Leukocyte Chemotaxis. Six patients with a mean burn size of 50.0% total body surface burn (range 32 to 63%) and a mean functional chemotactic index of 70.6% (range 35.6 to 119%) demonstrated a significant ($P<0.05$) increase of functional chemotactic index (mean 102.6%; SE ± 3.25; range 93.115%) when their leukocyte-rich suspension was incubated with normal type AB serum. However, when the normal type AB serum was heated for 20 minutes at 56° C, cooled at 37° C and then incubated with leukocyte rich suspension, there was no increase or decrease in the functional chemotactic index (mean 72.5%; range 41.3 to 106.7%) (Table 6). This experiment suggests that the factor or factors in normal serum responsible for improving the chemotaxis of leukocytes from thermally injured patients are heat labile.

Effect of Albumin on Leukocyte Chemotaxis. Four patients with a mean burn size of 26.5% total body surface (range 27 to 30%) and a mean functional chemotactic index of 86.4% (range 44.9 to 119.4%) demonstrated no significant increase in leukocyte chemotaxis when their leukocytes were incubated with salt poor human albumin. The mean functional chemotactic index after incubation with salt poor albumin was 74.8% (range 35.3 to 111.2%). Although the albumin used was commercial salt poor albumin, this experiment suggests that the albumin fraction is not the active factor in normal serum responsible for increasing chemotaxis of leukocytes from thermally injured patients.

DISCUSSION

Chemotaxis is an important biologic phenomenon determining the direction of motion of bacteria, plant and animal cells in reproduction, nutrition, cellular organization and inflammation. Various methods have been used to study chemotaxis but the method devised by Boyden in 1962 (Boyden S. J Exp Med 115: 453, 1962)[4], based on the ability of leukocytes to migrate through a filter toward a chemotactic agent, has been the most useful because of its simplicity and reproducibility. A number of modifications of this technic have been devised (Ward PA, Lepow IH, Newman LJ. Amer J Path 52: 725, 1968;[7]

Table 5. Effect of Normal Type AB Serum of Leukocyte Chemotaxis of Thermally Injured Patients

	Baseline	Normal Serum Incubation
Number	33	33
Mean Functional Chemotactic Index	61.1%	107.7%
Standard Error	± 5.7	± 4.8
Significance	P = < 0.01	
Burn Size	46.8% (Range - 14.5 - 70.5)	

Table 6. Effect of Heated AB Serum on Leukocyte Chemotaxis

	Baseline	Normal Serum Incubation	Heated Serum Incubation
Number	6	6	6
Mean Functional Chemotactic Index	70.6%	102.6%	72.5%
Standard Error	± 8.8	± 3.3	±10.7
Significance *	-	P = <0.05	N.S.
Burn Size		50% (Range 32 - 63%)	

* Multiple comparisons with Baseline (Scheffe Test).

Ward PA. J Exp Med 128: 1201, 1968; [6] Zigmond SH, Hirsch JC. J Exp Med 137: 387, 1973[9]). The importance of chemotaxis in the function of polymorphonucleocytes is demonstrated in figure 4, since even leukocytes fully capable of ingesting and killing microorganism are incapcitated if unable to sense and be directed towards the invading pathogen.

This study demonstrates (1) an intrinsic defect in the chemotaxis of leukocytes from burned patients and (2) suppression of the chemotaxis of normal leukocytes by serum from burned patients treated with either mafenide acetate or silver sulfadiazine.

Chemotaxis is suppressed soon after burn injury and this suppression is proportional to the extent of injury. That this suppression is not a drug effect is indicated (1) by its occurrence in patients treated with silver nitrate; (2) by its reversal upon incubation with normal serum, an effect removed by heating the serum, and (3) by the failure of dilution alone to duplicate the effect of normal serum. In addition, the serum from one patient did not suppress the chemotaxis of normal cells during treatment with silver nitrate but inhibited such chemotaxis during treatment with Sulfamylon soaks (Fig. 5). The chemotaxis of the patients' own cells was suppressed during both forms of treatment and subsequently returned to normal.

The effects of mafenide acetate and silver sulfadiazine upon normal leukocyte chemotaxis are readily demonstrated at concentrations which occur in serum during treatment. Silver nitrate does not exhibit such an effect <u>in vitro</u>.

Susceptibility to opportunistic infection is a hallmark of burn injury. The etiology of this susceptibility is not understood, but one proposed explanation is failure of granulocyte defense. Others have demonstrated functional impairment of granulocytes from burned patients (Alexander JW, Moncrief JA. Arch Surg 93: 75, 1966; [1] Alexander JW, Wixon D. Surg Gynec Obstet 130: 431, 1970; [2] Curreri PW, Heck EL, Browne L, Baxter CR. Surgery 74: 6, 1973[5]), and this study suggests that delivery of these impaired granulocytes may also be faulty. The study further suggests a dilemma: the use of either mafenide or silver sulfadiazine to protect the burn wound from infection may cause further suppression of the delivery of granulocytes and thereby enhance the susceptibility of the patient to infection by other routes. Such a hazard of therapy has not been examined previously and its importance is not defined at this time.

Incubation in normal serum restores chemotactic function to the granulocytes obtained from burned patients. This restoration of function appears due to a heat labile component of normal serum. If similar restoration of function can be achieved <u>in vivo</u>, this observation may have clinical importance, since such restoration would permit a direct test of the relationship between chemotactic suppression and susceptibility to opportunistic infection in burned patients.

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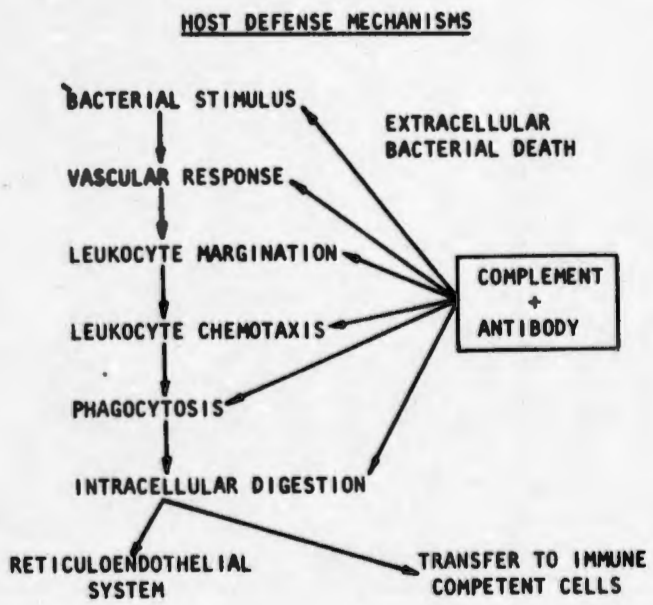

Figure 4. Host defense mechanism against bacterial invasion.

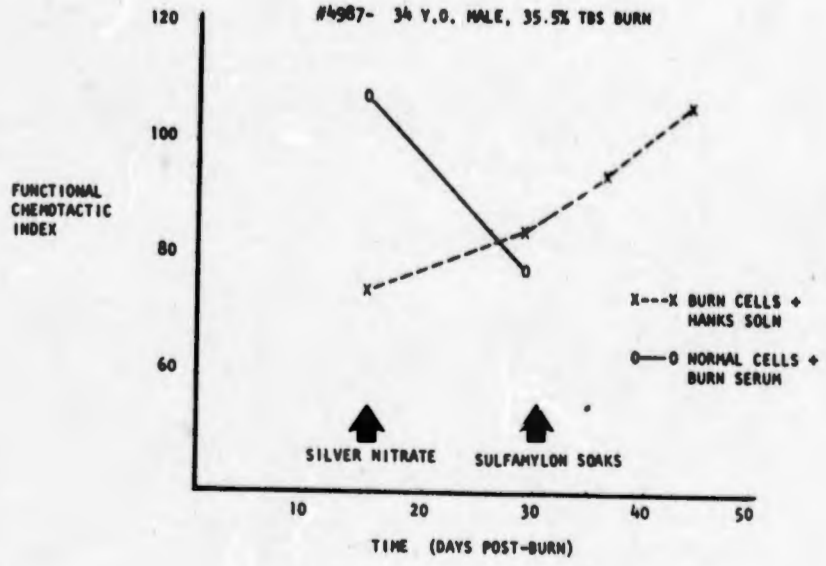

Figure 5. Functional chemotactic index versus day postburn demonstrating suppression of normal leukocyte chemotaxis by burn serum after beginning mafenide acetate topical therapy.

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PUBLICATIONS

None

PRESENTATIONS

Warden GD: Evaluation of leukocyte chemotaxis 'n thermally injured patients. Presented at American Burn Assoc. meeting, Cincinnati, Ohio, 5 April 1974.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0E 6985	74 07 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DOD'N INSTR'N	8b. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
74 03 27	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	3A161102B71R	01	083
b. CONTRIBUTING	62110A	3A162110A821	00	
c. CONTRIBUTING				

11. TITLE (Procede with Security Classification Code): (U) Complement Components in The Thermally Injured Soldier (Total, C_1, C_3, C_5, and C_8 Levels) and Their Relationship to Bacteremia (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS:
003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
74 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 74	.6	5
	CURRENT 75	.5	12

19. RESPONSIBLE DOD ORGANIZATION
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TELEPHONE: 512-221-2720

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Metabolic Branch
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SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS
NAME: Arthur D Mason, Jr, MD
NAME: James Murray, SP4 DA

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Procede EACH with Security Classification Code): (U) Burned soldier; (U) Complement; (U) Thermal injury; (U) Infection; (U) Host resistance

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

23. (U) Study complement components and their relationship to infection in the thermally injured soldier.

24. (U) Obtains prospective serum samples on thermally injured patients. Measure total complement and C_1, C_3, C_5, C_8 components by hemolytic assay before, during and if possible, subsequent to proven septic episodes in those patients.

25. (U) 74 02 - 74 06 To date no sequential studies of complement factors in the thermally injured patient have been made. We are setting up the assay procedure and attempting to collect serum specimens from appropriate patients.

*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

ABSTRACT

PROJECT NO. 3A161102B71R-01, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: COMPLEMENT COMPONENTS IN THE THERMALLY INJURED SOLDIER (TOTAL, C_1, C_3, C_5, and C_8 LEVELS) AND THEIR RELATIONSHIP TO BACTERMIA

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

Period covered in this report: 1 July 1973 - 30 June 1974

Investigators: W. Abe Andes, MD, Major, MC
J. Murray, SP4
Arthur D. Mason, Jr., MD

Reports Control Symbol MEDDH-288 (R1)

The role of complement in the thermally injured patient is undefined. Occasional studies have been done at various stages postburn. No study has attempted to measure functional levels of C_1, C_3, C_5, and C_8 in the seriously burned soldier. This protocol is intended to evaluate changes in such complement components with the onset of bacteremia and the patients subsequent course. With such information, the existence of deficiencies, complement activation, and correlation with other studies current in this institution may be made.

To date no sequental studies of complement factors in the thermally injured patient have been made. We are setting up the assay procedure and attempting to collect serum specimens from appropriate patients.

Complement
Thermal injury
Infection
Burned soldier

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OE 6972	74 07 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
73 07 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	61102A	3A161102B71R	01	126
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) The Correlation of Thymic Humoral Factor Levels and Cellular Immunity in Burned Military Personnel (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
73 05	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 74	.4	7
	CURRENT 75	.3	4

19. RESPONSIBLE DOD ORGANIZATION
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Burn Study Branch
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SOCIAL SECURITY ACCOUNT NUMBER
ASSOCIATE INVESTIGATORS
NAME: Allan Goldstein, MD
NAME: DA

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Thymus; (U) Burns; (U) Immunity; (U) Thymic humeral factor; (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 determine what alterations in circulating thymic humeral factor occur following thermal injury of military personnel.

24. (U) Lymphocyte mediated cellular immunity has been shown to be decreased in patients with severe burns and in experimentally burned animals. The development of a radioimmuno assay for thymic humeral factor will allow us to determine if a deficiency in the hormonal output of the thymus gland plays a role in this immunosuppression.

25. (U) 73 07 - 74 06 Blood samples from 21 burned patients have been drawn within the first week following injury and at two week intervals thereafter. Ten of these patients survived their burns and have been followed with blood samples to the time of discharge. Such samples have been frozen and sent to Dr. Allan Goldstein's laboratory in Galveston where they are awaiting analysis.

*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

ABSTRACT

PROJECT NO. 3A161102B71R-01, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: THE CORRELATION OF THYMIC HUMORAL FACTOR LEVELS AND CELLULAR IMMUNITY IN BURNED MILITARY PERSONNEL

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

Period covered in this report: 1 July 1973 - 30 June 1974

Investigators: Norman S. Levine, MD, Major, MC
Roger E. Salisbury, MD, Major, MC
Arthur D. Mason, Jr. MD, Major, MC
Allan L. Goldstein, MD*
Basil A. Pruitt, Jr. MD, Colonel, MC

Reports Control Symbol MEDDH-288(R1)

The purpose of this investigation is to determine if alterations in circulating thymic humoral factor occur following thermal injury of military personnel. Blood samples from 21 burn patients have been drawn within the first week following thermal injury and at two week intervals thereafter. Ten of these patients survived their burns and have been followed with blood samples to the time of discharge. The remaining 11 patients were studied at two week intervals until the time of death. These samples have been frozen and sent to Dr. Allan Goldstein's laboratory in Galveston, Texas, where they are awaiting analysis.

Thymus
Burns
Immunity
Thymic humoral factor
Burn patients

*Biochemistry Department, University of Texas Medical School at Galveston, Galveston, Texas 77550

THE CORRELATION OF THYMIC HUMORAL FACTOR LEVELS AND CELLULAR IMMUNITY IN BURNED MILITARY PERSONNEL

It has been demonstrated that both severely burned patients and severely burned animals show a depression of lymphocyte mediated cellular immunity. In burned patients, Rapaport[1] has shown that the ability to exhibit a delayed hypersensitivity reaction is impaired both with regard to primary sensitization (DNCB) and with regard to the anamnestic response to a variety of skin tests. In animals with experimental burns, the anamnestic response to tuberculin skin testing is altered,[2] allograft rejection is prolonged,[3] and this prolongation has been shown to be proportional to the size of burn.[4] Moreover, the ability of lymphocytes from a burned animal to elicit a graft versus host reaction in an F_1 hybrid animal is depressed.[5] These changes may correlate with the lymphocytopenia observed in patients following thermal injury. Recent electron microscopic studies and animal studies suggest a selective loss of thymic-derived lymphocytes following thermal injury.[6,7]

There is considerable evidence to show that the cellular immune response is in part controlled by a thymic humoral factor. This factor plays a critical role in the prenatal and neonatal development of cellular immunocompetence and has been shown to be needed for the maintenance of cellular immunocompetence throughout adult life. Neonatally thymectomized animals become immunoincompetent: the absolute number of blood lymphocytes to these animals is depressed, as is the capacity of these animals to reject allograft, induce a graft versus host reaction, and maintain body weight and lymphoid tissue size.[8,9] It has been demonstrated that such "immunoincompetence" could be reversed in these animals by implanting isogeneic thymus tissues inclosed in a millipore filter chamber. This suggested that the thymic tissue elaborates a humoral factor capable of restoring "immunocompetence" to such animals. Recently, a purified fraction of thymus gland, thymosin, has been isolated which, when administered to neonatally thymectomized mice, can restore their cellular "immunocompetence" in terms of the tests mentioned above. Moreover preparations of human thymosin have been made available and have been used in treating neonatal disorders involving thymic insufficiency.

The purpose of this investigation was to determine if a thymic insufficiency is present in severely burned military personnel.

MATERIALS AND METHODS

Serum samples from 15 normal individuals and from 21 thermally injured patients were drawn. In the patients with thermal injury, the initial sample was drawn within the first week following burning, and samples were drawn at two week intervals until recovery or death occurred. Of the 21

thermally injured patients studied, 11 died, and 10 survived the burn. These samples were immediately frozen and transported to Dr. Allan Goldstein's laboratory at the University of Texas in Galveston, Texas, where they are awaiting analysis of thymic humoral factor levels. Because of difficulties encountered in that laboratory in the performance of a radioimmunoassay, the determinations on the samples have not yet been performed. It is anticipated that these will be done within the next year.

SUMMARY

Serum samples on 21 burn patients, collected within the first week after thermal injury and every two weeks thereafter have been frozen and conveyed to Dr. Allan Goldstein's laboratory in Galveston, Texas, where they are awaiting analysis of serum thymic humoral factor levels. Frozen samples on 15 normal individuals have also been sent. It is anticipated that these assays will be performed within the next year.

REFERENCES

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2. Rapaport FT, Milgrom F, Kano K, Gesner B, Solowey AC, Casson P, Silverman HI, Converse JM: Immunologic sequelae of thermal injury. Ann N Y Acad Sci 150:1004-1008, 1968.

3. Rapaport FT, Converse JM, Horn L, Ballantyne DL Jr, Mulholland JH: Altered reactivity to skin homografts in severe thermal injury. Ann Surg 159:390-395, 1964.

4. Chambler K, Batchelor JR: Influence of Defined Incompatibilities and area of burn on skin-homograft survival in burned subjects. Lancet 1:16-18, 1969.

5. Munster AM, Eurenius K, Mortensen RF, Mason AD Jr: Ability of splenic lymphocytes from injured rats to induce a graft-versus-host reaction. Transplantation 14:106-108, 1972.

6. Ritzmann S., et al: Personal communication.

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8. Asanuma Y, Goldstein AL and White A: Reduction in the incidence of wasting disease in neonatally thymectomized CBA/W mice by the injection of thymosin. Endocrinology 86:600, 1970.

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responses in normal and immunologically deficient mice. J of Immunology 104:359-366, 1970.

PUBLICATIONS AND PRESENTATIONS

None.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0A 6980	74 07 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
73 07 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	61102A	3A161102B71R	01	165
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Procede with Security Classification Code): (U) Studies of Disturbance of Protein Turnover in Burned Troops - Use of an Animal Model (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
65 07	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 74	.9	26
	CURRENT 75	.9	22

19. RESPONSIBLE DOD ORGANIZATION
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20. PERFORMING ORGANIZATION
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21. GENERAL USE: FOREIGN INTELLIGENCE NOT CONSIDERED

ASSOCIATE INVESTIGATORS
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NAME: Arthur D Mason, Jr, MD DA

22. KEYWORDS (Procede EACH with Security Classification Code)
(U) Protein; (U) Burn; (U) Trauma; (U) Turnover; (U) Rats; (U) Albumin

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

23. (U) To determine the cause of the dysproteinemia observed following burn injury and to determine if the more marked dysproteinemia seen in the presence of infection of the burn wound is an effect caused by some action of the bacteria. It is hoped that this will aid in understanding similar changes which are observed in burned soldiers.

24. (U) The amount of C-14 incorporated into the serum proteins of burned, burned-infected, treated burned-infected, and control rats has been measured. Study of ^{14}C incorporation at the subcellular level will be done to corroborate the in vivo results. The intravascular/extravascular distribution of albumin is being measured by radio-immunoassay using plasmas and extracts of tissues obtained on the sixth day postburn.

25. (U) 73 07 - 74 06 Albumin content has been measured in blood and eviscerated carcasses of groups of control, burned, and burned-infected rats. These results have confirmed the earlier ones which indicated that the tissue albumin pool size in the injured rats was elevated at a time when the plasma albumin pool size was lower than normal. The increased tissue pool size was a result of the high content of albumin in the burn wound. Other tissues were equal to control values. The study of protein turnover is continuing.

*Available to contractors upon originator's approval

ABSTRACT

PROJECT NO. 3A161102B71R-01, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: 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 report: 1 July 1973 - 30 June 1974

Investigators: Wanda L. Brown, MS
Eleanor G. Bowler, PhM
Arthur D. Mason, Jr, MD

Reports Control Symbol MEDDH-288(R1)

Measurements of 2-^{14}C glycine incorporation into serum proteins have been made on the following groups of rats: control, burned, burned-infected, and burned-infected treated topically with Sulfamylon acetate. Incorporation of ^{14}C into all serum electrophoretic fractions was greater in the injured rats than in the controls. Concentrations of alpha-1, alpha-2, and beta globulins were also elevated in the serum of the injured rats. Serum albumin concentrations were depressed. However, measurements of albumin content of the tissues of burned and burned-infected rats showed higher levels than control tissues. The increased body albumin could be accounted for by the elevated content of the burn wound. A manuscript is in preparation to report the complete results of this phase of the study of protein turnover.

Protein
Burn
Trauma
Turnover
Rats
Albumin

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0E 6974	74 07 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
73 07 01	K. COMPLETION	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	3A161102B71R	01	124
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE: (U) The Effect of Adrenergic Blockade on The Hypermetabolic Response Following Thermal Injury of Military Personnel (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
73 01	74 01	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	PRECEDING	a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
FISCAL YEAR	74	.6	19
	CURRENCY		

19. RESPONSIBLE DOD ORGANIZATION
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20. PERFORMING ORGANIZATION
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Surgical Study Branch
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TELEPHONE: 512-221-5712
SOCIAL SECURITY ACCOUNT NUMBER:
ASSOCIATE INVESTIGATORS
NAME: James M Long, MAJ, MC
NAME: Arthur D Mason, Jr, MD DA

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS: (U) Adrenergic blockade; (U) Hypermetabolism; (U) Oxygen consumption; (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) The purpose of this study will be to determine the effect of adrenergic blockade on the metabolic response following thermal injury of military personnel.

24. (U) Hypermetabolic patients will be studied between the 4th and 21st postburn days. Basal oxygen consumptions will be performed in the early morning and regitine and propanalol administered to achieve alpha and beta adrenergic blockade. Blood glucose, insulin, and free fatty acids will be obtained before and after blockade and core temperature and oxygen consumption will be obtained following blockade. Adequacy of beta block will be tested by giving 2 mcg of isuprel IV at the end of the infusion and monitoring heart rate and serum free fatty acids.

25. (U) 73 07 - 74 01 Nine studies were performed in six patients. No consistent change in metabolic rate was seen with alpha blockade alone, mean basal metabolic rate was 70.7 ±7.9 Kial/m^2/hr before blockade and 68.1 ± 4.6 Kial/m^2/hr following blockade (N=3). A significant decrease in metabolic rate was associated with combined alpha and beta (N=6, 69.6 ± 5.3 vs 57.4 ± 5.2) or beta blockade alone, associated with a decrease in pulse rate, blood pressure, minute volume and free fatty acids.

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* 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.

ABSTRACT

PROJECT NO. 3A161102B71R-01, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: THE EFFECT OF ADRENERGIC BLOCKADE ON THE HYPERMETABOLIC RESPONSE FOLLOWING THERMAL INJURY OF MILITARY PERSONNEL

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

Period covered in this report: 1 July 1973 - 30 June 1974

Investigators: Douglas W. Wilmore, MD, Lieutenant Colonel, MC
James M. Long, MD, Major, MC
Arthur D. Mason, Jr., MD

Reports Control Symbol MEDDH-288(R1)

Nine studies were performed in six patients. No consistent change in metabolic rate was seen with alpha blockade alone, mean basal metabolic rate was 70.7 ± 7.9 Kcal/m^2/hr before blockade and 68.1 ± 4.6 Kcal/m^2/hr following blockade (N=3). A significant decrease in metabolic rate was associated with combined alpha and beta (N=6, 69.6 ± 5.3 vs. 57.4 ± 5.2) or beta blockade alone, associated with a decrease in pulse rate, blood pressure, minute volume, and free fatty acids.

Adrenergic blockade
Hypermetabolism
Oxygen consumption
Burn patients

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THE EFFECT OF ADRENERGIC BLOCKADE ON THE HYPERMETABOLIC RESPONSE FOLLOWING THERMAL INJURY OF MILITARY PERSONNEL

Nine studies were performed in six patients (Table 1). The patients were placed in a warm environment in the early morning and allowed to rest and equilibrate with the ambient conditions for several hours. Basal studies of pulse rate, blood pressure, respiratory rate, minute ventilation, metabolic rate, serum free fatty acids, blood sugar, and insulin were performed. Blockade was effected by the intravenous administration of phentolamine (Regitine^{R}), 75 mg (alpha blockade), or phentolamine, 75 mg, and propanolol (Inderal^{R}), 75 mg infused over 15 to 30 minutes (combined alpha and beta blockade). The adequacy of beta blockade was determined by the absence of response in heart rate or increase in serum free fatty acids following intravenous administration of 2 ug isoproterenol (Isuprel^{R}). Orthostatic hypotension and nasal stuffiness were present in patients following alpha blockade. After adequate blockade, the initial studies were repeated.

Effect of Adrenergic Blockade

No consistent change in metabolic rate was seen with alpha blockade alone, and the mean basal metabolic rate was 70.5 ± 7.9 Kcal/m^2/hr before blockade and 68.1 ± 4.6 Kcal/m^2/hr following blockade (n=3). However, there was a significant decrease in metabolic rate associated with combined alpha and beta blockade (Table 2) or beta blockade alone, and this response was associated with a decrease in pulse rate, blood pressure, minute ventilation, and free fatty acids. The dose of propanolol required for competitive blockade of the beta receptor system in these hypermetabolic burn patients was greater than the dose required for normal man, and a persistent sympathetic break-through occurred with the administration of smaller doses of the drug per unit time. Metabolic activity returned to its preblockade level within two to three hours following drug administration.

PUBLICATIONS AND/OR PRESENTATIONS

None

Table 1.

DESCRIPTION OF PATIENTS STUDIED FOLLOWING ALPHA AND BETA ADRENERGIC BLOCKADE

Patient	Age (Years)	Weight (Kg)	Body Surface Area (m^2)	Total Body Surface Burn (Per Cent)	Per Cent 3°	Postburn Day Studied
1	38	113.0	2.15	51	22	22,24
2	34	77.0	1.91	66.5	39.5	13
3	18	70.0	1.92	55	18	24,28,31
4	22	48.0	1.59	76	33.5	37
5	21	77.7	2.05	28.5	4	9
6	14	52.9	1.54	57	27	27
Mean	24	73.1	1.86	55.5	24.0	24
Range	14-38	48.0-113.0	1.54-2.15	28.5-76	4-39.5	9-37

Table 2.

EFFECT OF ALPHA AND BETA BLOCKADE ON HYPERMETABOLISM FOLLOWING THERMAL INJURY

	Pulse (Beats/Min)		Blood Pressure (mmHg)		Respiratory Rate (Breaths/Min)		Minute Ventilation (L/Min)		Metabolic Rate (Kcal/m²/Hr)		Free Fatty Acids (mEq/L)	
	PRE	POST	PRE	POST	PRE	POST	PRE	POST	PRE	POST	PRE	POST
Alpha Blockade												
1a	120	110			29.0	32.9	38.2	29.1	80.2	76.8	2.0	8.0
1b	126	118			25.6	24.9	24.3	19.7	76.8	66.7	1.3	3.5
3	100	84	142/70	;20/50	12.0	12.5	12.1	16.1	55.0	60.9		
Mean*	115	104			22.2	23.4	24.9	21.6	70.7	68.1	1.6	5.8
S.E.	8	10			5.2	5.9	7.5	3.9	7.9	4.6	0.4	2.2
Alpha and Beta Blockade												
2	118	84	116/72	88/52	40.0	38.0	35.4	32.2	68.0	59.9	4.0	1.7
3a	96	88	160/82	124/68	16.0	19.5	18.0	9.4	66.8	44.9	4.6	2.3
3b	98	84	128/80	100/70	22.7	24.0	12.0	10.1	46.9	42.4	5.0	3.5
4	120	96	120/100	90/_	17.7	14.0	22.6	19.0	78.7	64.7		
5	120	84	118/80	80/60	19.4	17.5	19.4	17.5	72.0	55.8	4.9	3.6
6	144	96	115/80	86/_	17.7	14.6	20.5	17.2	85.1	76.6	2.2	1.3
Mean	116	89	126/82	95/63	22.2	21.3	21.4	17.6	69.6	57.4	4.1	2.5
S.E.	7	2	7/4	6/4	3.7	3.7	3.2	3.4	5.3	5.2	0.5	0.5
p	<0.01		<0.001/<0.01		>0.10		<0.02		<0.01		<0.01	

*Effect not different by paired t test.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0C 6978	74 07 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8A. DISC'N INSTR'N	8B. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
73 07 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	61102A	3A161102B71R	01	300
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) Evaluation of Gastrointestinal Absorption and Nutritional Efficacy of Standard High Protein Diet 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
69 07	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 74	.3	7
CURRENT FISCAL YEAR 75	.3	5

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

21. GENERAL USE: FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code): (U) Gastrointestinal absorption; (U) High protein diet; (U) Trace elements; (U) Humans

23. TECHNICAL OBJECTIVE. 24. APPROACH. 25. PROGRESS (Furnish individual paragraphs identified by number. Precede text of each with appropriate security classification code.)

23. (U) To evaluate the nutritional efficacy of standard high protein diets, nutritional supplements and elemental diets in burned patients. To determine the hormonal and dietary factors which influence nitrogen balance.

24. (U) The effect of nutritional support and nitrogen loss was evaluated in 43 studies in normal and injured man. Measurements included caloric and nitrogen intake, metabolic rate, insulin, cortisol, catecholamines, growth hormone, glucose, fatty acids, urea nitrogen, and nitrogen excretion.

25. (U) 73 07 - 74 06 Four major factors interacted to determine nitrogen balance. A positive correlation existed between nitrogen balance and nitrogen intake, non-protein caloric intake and basal insulin. As metabolic rate increased, positive nitrogen balance decreased. Other factors appeared to influence these major variables.

*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

ABSTRACT

PROJECT NO. 3A161102B71R-01, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: EVALUATION OF GASTROINTESTINAL ABSORPTION AND NUTRITIONAL EFFICACY OF STANDARD HIGH PROTEIN DIET IN BURNED SOLDIERS

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

Period covered in this report: 1 July 1973 - 30 June 1974

Investigators: Douglas W. Wilmore, MD, Lieutenant Colonel, MC
Amanda Din, First Lieutenant, AMSC

Reports Control Symbol MEDDH-288(R1)

The effect of nutritional support and nitrogen loss was evaluated in 43 studies in normal and injured man. Measurements included caloric and nitrogen intake, metabolic rate, insulin, cortisol, catecholamines, growth hormone, glucose, fatty acids, urea nitrogen, and nitrogen excretion.

Four major factors interacted to determine nitrogen balance. A positive correlation existed between nitrogen balance and nitrogen intake, non-protein caloric intake and basal insulin. As metabolic rate increased, nitrogen balance decreased. Other factors appeared to influence these major variables.

Gastrointestinal absorption
High protein diet
Trace elements
Humans

EVALUATION OF GASTROINTESTINAL ABSORPTION AND NUTRITIONAL EFFICACY OF STANDARD HIGH PROTEIN DIET IN BURNED SOLDIERS

The effect of nutritional support on metabolic rate and nitrogen loss was evaluated in 43 studies in normal and injured men. The patients, with a mean burn size of 53 per cent of the body surface (Table 1), received a wide range of nutritional support administered by the enteral, parenteral, or enteral-parenteral routes. The normal individuals were studied the day before epinephrine infusion as previously described. Resting metabolic rate was determined in all subjects between 6:00 A.M. and 8:00 A.M. at least three hours after cessation of ad libitum oral caloric intake. However, calories and nitrogen administered by the intravenous route were continued at a constant infusion rate during the measurement period. Blood was obtained for determination of glucose, urea, free fatty acids, insulin, human growth hormone, glucagon, cortisol, and catecholamines and a timed urine specimen was obtained for catecholamine determination. The caloric and nitrogen intake for the accompanying 24-hour period was determined from the composition as listed by the manufacturer, and food intake was determined by the dietitian from known lots of food prepared in the research metabolic kitchen. Urine was collected in 24 hour pools and concentrations of sodium, potassium, creatinine, urea, and total nitrogen determined. Generous estimates of stool losses (2-3 gm nitrogen/day) were made for the few patients receiving enteral residue-containing diets. Wound loss was not measured. Insulin was administered as required to those patients with elevated blood or urine glucose and human growth hormone, 10 international units, was given daily by intramuscular injection to nine subjects to evaluate the effect of endogenous reset of insulin output.

The mean values of all measurements obtained over three to seven days in the patients were computer-processed with the control data to determine the interaction and effect of caloric and nitrogen support and metabolic rate on nitrogen excretion.

Increased nitrogen excretion was associated with hypermetabolism and/or with increased dietary protein intake. Nitrogen excretion decreased with administration of nonprotein calories and/or an increase in plasma insulin (Tables 2, 3). Metabolic rate was related to urinary catecholamines as previously described, and a positive correlation also existed between glucagon levels and catecholamine excretion. Each of these three variables relates in similar fashion to protein metabolism, with a positive correlation existing between urinary catecholamines, metabolic rate, or glucagon and nitrogen excretion. Metabolic rate and catecholamine excretion were not reduced by calorie and nitrogen

Table 1.

CHARACTERISTICS OF SUBJECTS STUDIED

Subject	Age (Years)	Weight (Kg)	Body Surface Area (m^2)	Total Body Surface Burn (%)	% 3°	Mean Postburn Day Studied
Control						
1	23	55.8	1.66	0	0	–
Patient						
1	18	52	1.61	58	28.5	a. 25 b. 63
2	17	60.6	1.76	58	14.5	6
3	33	72.6	1.98	50.5	2.5	a. 12 b. 26
4	29	70.3	1.83	47.5	14	15
5	63	73.6	1.87	31.5	25	17
6	64	95.2	2.20	59	54	10
7	51	95.0	2.19	58	27.5	6
8	64	57.2	1.66	39	23	5
9	39	90.0	2.08	23*	10	9
10	29	50.0	1.59	47	0	18
11	60	67.3	1.84	62	53	42
12	25	54.3	1.64	78.5	11	31
13	41	53.0	1.65	60	8	a. 77 b. 85
14	19	77.0	1.98	33.5**	33.5	a. 255 b. 248

(Continued)

Table 1. (Continued)

CHARACTERISTICS OF SUBJECTS STUDIED

Subject	Age (Years)	Weight (Kg)	Body Surface Area (m^2)	Total Body Surface Burn (%)	% 3°	Mean Postburn Day Studied
Patient						
15	28	58.0	1.90	75.5	57	a. 49 b. 42
16	16	62.0	1.79	38	14	a. 24 b. 17
17	18	66.0	1.88	64	22	a. 17 b. 24
18	18	66.0	1.88	64	22	a. 32 b. 26
19	21	76.5	1.95	45	26	a. 20 b. 24
20	34	85.0	2.00	60	16.5	a. 24 b. 17
21	22	48.0	1.59	56.5	33.5	a. 44 b. 37
22	14	42.0	1.54	57	27	a. 36 b. 43
Mean	33	66.9	1.84	53	24	29+
Range	14-64	42.0-95.2	1.54-2.20	23-78.5	0-57	6-255

*Multiple fracture.

**Initial injury 75 per cent; wound partially grafted before transfer.

+Excluding patient No. 14.

Table 2.

METABOLIC RATE, CALORIC AND NITROGEN INTAKE, NITROGEN EXCRETION AND BASAL INSULIN IN NORMAL AND INJURED MAN

Subject	Nitrogen Excretion ($gm/m^2/Day$)	Metabolic Rate ($Kcal/m^2/Day$)	Nitrogen Intake ($gm/m^2/Day$)	Non-Protein Caloric Intake ($Kcal/m^2/Day$)	Insulin ($\mu U/ml$)
Controls (Fed)					
1	7.90	888	9.00	1196	20.7
1c	8.06	769	9.12	810	17.5
4	7.52	808	9.00	1014	13.8
5	8.23	871	10.10	1104	14.0
6	7.70	889	8.71	1133	9.4
7	8.52	835	10.50	990	17.0
Controls (Fasting)					
1b	5.33	750	0	0	9.5
2	4.33	823	0	0	11.0
3b	8.93	919	0	0	12.5
Patients					
1a	8.79	2030	6.52	2258	6.0
1b	11.42	1545	20.20	2468	24
2	11.52	1718	5.68	1392	9.5
3a	14.0	2244	12.38	1436	17.5
3b	10.1	1404	13.00	1893	13.0

Table 2. (Continued)

METABOLIC RATE, CALORIC AND NITROGEN INTAKE, NITROGEN EXCRETION AND BASAL INSULIN IN NORMAL AND INJURED MAN

Subject	Nitrogen Excretion ($gm/m^2/Day$)	Metabolic Rate ($Kcal/m^2/Day$)	Nitrogen Intake ($gm/m^2/Day$)	Non-Protein Caloric Intake ($Kcal/m^2/Day$)	Insulin ($\mu U/ml$)
Patients					
4	13.10	1524	15.84	1654	14.5
5	10.40	1312	9.51	1187	24
6	7.92	1495	0	913	37
7	12.44	1680	0	548	19.5
8	7.99	1022	4.34	850	13.5
9	15.74	1608	0	515	36
10	10.48	2200	8.01	2460	40
11	9.9	1526	8.50	1280	61
12	7.5	2026	7.75	2006	25
13a	8.6	1375	12.2	2009	17
13b	4.4	1375	12.2	2009	31
14a	6.0	1207	15.8	2055	23
14b	4.7	1207	15.8	2055	80
15a	9.7	1716	13.6	1934	24
15b	6.5	1716	13.6	1934	49
16a	12.8	1658	17.1	1907	17.5
16b	9.9	1658	17.1	1907	23.7

Table 2. (Continued)

METABOLIC RATE, CALORIC AND NITROGEN INTAKE, NITROGEN EXCRETION AND BASAL INSULIN IN NORMAL AND INJURED MAN

Subject	Nitrogen Excretion ($gm/m^2/Day$)	Metabolic Rate ($Kcal/m^2/Day$)	Nitrogen Intake ($gm/m^2/Day$)	Non-Protein Caloric Intake ($Kcal/m^2/Day$)	Insulin ($\mu U/ml$)
17a	15.1	1616	16.1	1466	10.7
17b	10.7	1666	16.1	1466	20.1
18a	9.1	906	13.5	1458	26
18b	7.3	906	13.5	1458	42
19a	15.6	1440	17.5	1733	9.2
19b	12.5	1440	17.5	1733	28
20a	15.0	1894	13.8	1560	12.0
20b	16.0	1894	13.8	1560	15.5
21a	12.7	1878	22.0	2450	30
21b	9.5	1878	22.0	2450	46
22a	12.6	1794	14.0	1841	16.8
22b	8.0	1794	14.0	1841	45.6

Table 3.

MATHEMATICAL RELATIONSHIPS BETWEEN ENERGY PRODUCTION, FOOD INTAKE, NITROGEN EXCRETION, AND BASAL INSULIN

N_{IN} = Nitrogen Intake (Kcal/m^2/Day)

N_{EXC} = Nitrogen Excretion (Kcal/m^2/Day)

N_{BAL} = Nitrogen Balance [N_{IN} - N_{EXC}] (Kcal/m^2/Day)

MR = Metabolic Rate (Kcal/m^2/Day)

NPC = Non-Protein Caloric Intake (Kcal/m^2/Day)

BI = Basal Insulin (μU/ml)

$p < 0.05$, Factors listed in order of F-Ratio

$$N_{EXC} = -1.398 + 0.4153\ N_{IN} - 0.004530\ NPC - 0.07746\ BI + 0.01696\ MR - 0.000004\ MR^2$$

N = 35 (Eight values with error > 25% were eliminated)

$R^2 = 0.8246$

$$N_{BAL} = -3.830 + 0.6072\ N_{IN} - 0.006599\ MR + 0.004376\ NPC + 0.05570\ BI$$

N = 43

$R^2 = 0.9001$

administration, confirming previous studies, but the protein-wasting resulting from the hypermetabolism was diminished or reversed with nonprotein calorie intake, nitrogen administration, and/or maneuvers which increased basal insulin levels (Table 3).

DISCUSSION

The factors which affect protein metabolism in injured man can be identified by inspection or analysis of our data. First, the influence of dietary factors: protein intake increases excretion of urinary nitrogen, and nonprotein calorie intake decreases nitrogen excretion. The nitrogen conserving effect of calorie intake is in part mediated by insulin stimulation, and a variety of metabolic studies confirm the positive correlation between protein sparing and insulin during calorie administration following injury. On the other hand, our data indicate that metabolic rate describes an effect on protein metabolism which is antagonistic to the anabolic activity of insulin. Nitrogen metabolism appears to be determined by the interaction between insulin (the dominant anabolic hormone) and sympathetic activity, as measured or expressed by catecholamine excretion rate, metabolic rate, or glucagon. Thus, a specific relationship between nitrogen balance and nitrogen and nonprotein calorie intake exists in injured man, if an adjustment is made for the various factors which alter insulin levels (such as the proportion of fat and carbohydrate in the nonprotein component of the diet, the route of food administration, and the exogenous administration of insulin) and the level of sympathetic activity in the patient. Finally, protein and calorie intake does not diminish the hypermetabolic response to injury, but feeding the patient minimizes or prevents severe protein wasting which may alter optimal body function.

PUBLICATIONS AND/OR PRESENTATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
	DA OE 6971	74 07 01	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8A. LVL OF INSTR'N	8B. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
73 07 01	K. COMPLETION	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	3A161102B71R	01	131
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)

(U) Vitamin K Deficiency in The Thermally Injured Soldier (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
72 10	74 04	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 74	.5	13
FISCAL YEAR: CURRENT		

19. RESPONSIBLE DOD ORGANIZATION

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NAME: Joseph P Baron, SP4 DA

21. GENERAL USE

FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Vitamin K; (U) Thermal injury. (U) Prothrombin time; (U) Burned soldier

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

23. (U) To study the incidence of abnormal prothrombin times and the response to Vitamin K1 in the thermally injured soldier. In some instances this will also serve as an indicat'on of Vitamin K deficiency in such patients.

24. (U) One-stage prothrombin times will be performed each week for four weeks on patients admitted to the US Army Institute of Surgical Research ward. After a patient is found to have less than 70% prothrombin complex activity on two consecutive days he will be treated with liberal doses of Vitamin K and his prothrombin time remeasured.

25. (U) 73 07 - 74 04 Fifty patients have been studied serially for periods of time greater than two weeks postburn. Fourteen have been available for treatment and adequate followup as outlined in the protocol. Five have shown marked improvement in prothrombin activity and two have had a partial response. Antibiotics, inadequate diet, and prolonged hospitalization may all be etiologic agents in the production of Vitamin K-responsive prolongation of the prothrombin time.

Available to contractors upon originator's approval

ABSTRACT

PROJECT NO. 3A161102B71R-01, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: VITAMIN K DEFICIENCY IN THE THERMALLY INJURED SOLDIER

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

Period covered in this report: 1 July 1973 - 30 June 1974

Investigators: W. Abe Andes, MD, Major, MC
Daniel E. McEuen, SP4
Joseph P. Baron, SP4

Reports Control Symbol MEDDH-288 (R1)

A vitamin K deficient diet or treatment in an intensive care unit may predispose patients to the onset of hypoprothrombinemia due to vitamin K deficiency within seven days to four months. The sometimes inadequate diet of the thermally injured soldier and other conditions which seem to be favorable to the production of vitamin K deficiency prompted the investigation of the frequency with which vitamin-K responsive prolongation of the prothrombin time might occur in such patients. Weekly blood studies were drawn following thermal injury until the patient was fully ambulatory and eating a normal diet without symptoms. Vitamin K_1 (0.25 mg/kg) was administered intravenously to those patients who manifested an isolated defect in prothrombin time. Treatment was instituted when less than 70% of normal activity was manifested on two or more occasions. Fifty patients have been studied for two or more weeks following thermal injury since the inception of the protocol. Fourteen patients were treated and available for adequate followup. Five patients had a complete response evidenced by shortening of their prothrombin time to normal. Two had a partial response. Seven had no response to this regimen. Four of the five responsive patients were on total parenteral nutrition or minimal oral carbohydrate intake. The fifth had been on a reduced diet for several months postburn without vitamin supplementation. The responsive patients were treated between 8 and 188 days postburn and had rapid correction of their prothrombin time. No patient had other evidence of a vitamin deficiency syndrome. The occurrence of vitamin K deficiency has been documented following thermal injury and seems to respond adequately to therapy when severe liver impairment is absent.

Thermal injury Vitamin K
Prothrombin time Burned soldier

VITAMIN K DEFICIENCY IN THE THERMALLY INJURED PATIENT

Hemorrhage, abnormal coagulation studies, and marked weight loss are well known in the critically injured patient (Pruitt BA, Jr, Foley FD, Mason AD: USAISR, Ann Prog Rpt FY 1971, Sec 32;[3] Eurenius K, McManus WF, McEuen DD: USAISR Ann Prog Rpt FY 1971, Sec 39;[4] Ham JM: Med J Aust 2: 716-718, 1971)[2]. Some of these patients develop vitamin K deficiency as evidenced by an abnormal prothrombin time correctable by administration of the vitamin. Clinically disabled, elderly patients may manifest abnormal prothrombin times responsive to vitamin K. (Hazell K, Baloch KH: Gerontol Clin (Basel) 12: 10, 1970)[5]. The activity of vitamin K is confined to a role in the production of coagulation factors II, VII, IX, and X. (Woolf IL, Babior BM: Amer J Med 53: 261, 1972)[6]. The usual exogenous sources of the vitamin are dietary vegetables although synthetic vitamins and endogenous elements are available for treatment purposes.

In this study we have attempted to define the prevalance, significance, possible clinical causes, and response to treatment with vitamin K of isolated abnormal prothrombin times in the thermally injured patient. Whole blood studies were drawn as seemed clinically indicated but at least weekly for weeks whenever possible following thermal injury. Specimens were drawn, mixed with citrate and immediately chilled on ice. Coagulation studies were then performed by methods used previously in this lab. Such studies have included prothrombin time, activated partial thromboplastin time, fibrinogen concentration, fibrin-fibrinogen degradation product titers, platelets, plasma hemoglobin, studies for circulating anticoagulants, peripheral blood smears and other studies as seemed necessary in the individual patient.

Fifty patients have been studied for at least two weeks postburn. There have been sixteen survivors of this group of fifty patients. Fourteen patients fulfilled the criteria for treatment and were evaluable for followup study. Of these fourteen, seven were judged nonresponders, five responded fully and two manifested partial responses. The mean burn size of the group was 45% of the total body surface area. The mean burn size of the survivors was 41% and the mean burn size of the nonsurvivors was 48%. Of those treated, the mean burn size was 43% and the responsive patients had a mean burn size of 51%. All of the patients treated in whom studies were available had at least one abnormal liver enzyme test and several were jaundiced. The five patients who responded to vitamin K administration were treated between 8 and 188 days postburn. Four of the five had been on almost continuous intravenous hyperalimentation and antibiotics. The fifth was poorly nourished and had been on multiple antibiotics for five months because of skin infections. Two patients with active bleeding had clinical improvement with correction of their prothrombin time.

The nonresponders and partial responders all had liver function test or pathological hepatic abnormalities sufficient to explain their unresponsiveness. Antibiotic therapy and an inadequate diet do seem to be important etiologically in the group of patients who respond to vitamin K.

An abnormal prothrombin time should be investigated in the thermally injured patient with careful clinical examination and appropriate laboratory tests, although vitamin K deficiency has been documented in the postburn period. No allergic reactions or other clinical ill effects were noted following treatment with vitamin K. However, a sense of effectiveness induced by such treatment should not cause one to overlook other causes for an abnormal prothrombin time in the postburn period.

REFERENCES:

1. Frick PG, Riedler G, Brogli H: Dose response and minimal daily requirement for vitamin K in man. J Appl Physiol 23: 387-389, 1967.

2. Ham JM: Hypoprothrombinaemia in patients undergoing prolonged intensive care. Med J Aust 2: 716-718, 1971.

3. Pruitt BA, Jr., Foley FD, Mason AD: Diagnosis and treatment of acute gastrointestinal ulceration in burn patients. US Army Institute of Surgical Research. Ann Prog Rpt FY 1971, BAMC Fort Sam Houston, Texas, Section 32.

4. Eurenius K, McManus WF, McEuen DD: Coagulation abnormalities in thermally injured soldiers. US Army Institute of Surgical Research Ann Prog Rpt FY 1972, BAMC, Fort Sam Houston, Section 39.

5. Hazell K, Baloch KH: Vitamin K deficiency in the elderly Gerontol Clin. (Basel) 12: 10-17, 1970.

6. Woolf IL, Babior BM: Vitamin K and warfarin: metabolism function and interaction. Amer J Med 53: 261-267, 1972.

PRESENTATIONS AND/OR PUBLICATIONS:

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OE 6982	74 07 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
74 03 27	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	3A161102B71R	01	119
b. CONTRIBUTING	61101A	3A161101A91C	00	
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) A Therapeutic Trial of Antacid in Prevention of the Clinical Complications Associated with Gastric Mucosal Disease 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
74 01	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 YR 74	.7	17
CURRENT FISCAL YR 75	.8	19

19. RESPONSIBLE DOD ORGANIZATION
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ASSOCIATE INVESTIGATORS
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21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Curling's ulcer; (U) Burned soldiers; (U) Gastritis; (U) Antacid

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 if neutalization of hydrogen ions by antacid administration in burned soldiers who manifest disruption of the gastric mucosal barrier will prevent progressive gastric mucosal damage, gastric hemorrhage and perforation.

24. (U) All patients admitted to the Institute of Surgical Research within 72 hours after sustaining greater than 35% total body surface injury will be considered for this study. A lithium flux test will be performed as has been previously described within the 72 hour postburn period. Half of the patients who manifest disruption of the gastric mucosal barrier on lithium flux testing will be randomly assigned to receive a standard antacid preparation. The remainder of the patients with disruption of the gastric mucosal barrier will receive no intragastric neutralization of acid. The patients with disruption of the gastric mucosal barrier who receive antacid therapy will be statistically compared as regards hemorrhage and perforation to those with disruption of the gastric mucosal barrier who receive no antacid therapy.

25. (U) 74 01 - 74 06 This project has just been initiated and there is insufficient data to analyze at this time.

275<

*Available to contractors upon originator's approval

ABSTRACT

PROJECT NO. 3A161102B71R-01, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: A THERAPEUTIC TRIAL OF ANTACID IN PREVENTION OF THE CLINICAL COMPLICATIONS ASSOCIATED WITH GASTRIC MUCOSAL DISEASE IN BURNED SOLDIERS

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

Period covered in this report: 1 July 1973 - 30 June 1974

Investigators: Joseph C. McAlhany, Jr., M.D., Maj, MC
Albert J. Czaja, M.D., Maj, MC
Basil A. Pruitt, Jr., M.D., Colonel, MC

Reports Control Symbol MEDDH-288(R1)

A lithium flux technique has been utilized to assess the integrity of the gastric mucosal barrier (GMB) following thermal injury. Within 72 hours post burn, disruption of the GMB was correlated with endoscopic progression of gastric mucosal disease, gastric hemorrhage or perforation in 7 of 8 patients. No instance of gastric hemorrhage or perforation was encountered in 10 patients with a normal GMB. This data suggests that the lithium flux technique may be a useful index of clinical gastric complications occurring after thermal injury.

Patients admitted to the U.S. Army Institute of Surgical Research within 72 hours after sustaining greater than 35% total body surface injury will be considered for a therapeutic trial of antacid in prevention of the clinical complications associated with gastric mucosal disease after burns. A lithium flux test will be performed, as previously described, within 72 hour post burn period. The patient population will then be randomly assigned to receive a standard liquid antacid preparation or no intragastric neutralization of acid.

The data generated by the study will allow statistical comparison as regards the incidence of hemorrhage and perforation. The patients with disruption of the gastric mucosal barrier who receive antacid therapy will be contrasted to those with disruption of the gastric mucosal barrier who receive no antacid therapy. These comparisons will clarify the effectiveness of antacid in prevention of clinical complications associated with progressive gastric mucosal disease after burns.

Curling's ulcer
Burned soldiers
Antacid
Gastritis

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0F 6380	74 07 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. OVER'N INSTR'N	8A. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
74 03 27	K. COMPLETION	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	3A161102B71R	01	080
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Procede with Security Classification Code)

(U) Diarrhea in Thermally Injured Military Personnel (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
74 01	74 06	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 74	1.3	20
FISCAL YEAR CURRENT		

19. RESPONSIBLE DOD ORGANIZATION

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21. GENERAL USE

FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Procede EACH with Security Classification Code) (U) Nutritional intake; (U) Diarrhea; (U) Postburn; (U) Nature; (U) Course; (U) Age; (U) Physical findings; (U) Humans

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

23. (U) To describe the characteristics of diarrhea occurring in thermally injured military personnel during the first month postburn. To identify the nature, time of occurrence postburn, and cause of the disorder, its icidence by age; associated physical findings; and its relationship to nutritional intake.

24. (U) Patients, admitted within 72 hours postburn, are observed periodically for four categories of data for four weeks: (1) physical findings, (2) characteristics of stools, (3) medical therapy pertinent to the gastrointestinal tract, and (4) nutritional intake.

25. (U) 74 01 - 74 06 Bowel habits of 29 patients aged 11 months to 76 years, with 18-96% TBS burns, were studied for up to 28 days. Characteristics of stools were correlated with alterations in diet, drugs, and physical examination. Eighteen patients developed loose stools during the first 2 weeks postburn, up to 14 times daily, for up to 16 days duration. Seven of 9 children (78%) had diarrhea, compared to 11 of 20 adults (55%). Seven adult patients, surviving for 3 to 14 days postburn, had no stools. Milk intolerance was clearly related to diarrhea in 3 patients, and may have existed in 4 others. Diarrhea was noninfectious and did not appear related to burn size. In 10 patients, the nature of the diarrhea suggested a small bowel, nonsecretory etiology. In 5 patients, diarrhea was clearly related to gastrointestional bleeding. A secretory etiology of undetermined nature was probable in the other 3 cases. Except for bleeding patients, the diarrhea seemed self-limiting and easily controlled with symptomatic treatment.

277<

*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

ABSTRACT

PROJECT NO. 3A161102B71R-01, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: DIARRHEA IN THERMALLY INJURED MILITARY PERSONNEL

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

Period covered in this report: 1 July 1973 - 30 June 1974

Investigators: Madeline L. Bluemle, LTC, ANC
Albert J. Czaja, M.D., Major, MC
Amanda Din, 1LT, AMSC

Reports Control Symbol MEDDH-288(R1)

Frequency and character of diarrhea after thermal injury was determined by evaluation, for up to 28 days postburn, of bowel habits of 29 consecutive patients aged 11 months to 76 years, with 18-96% total body surface burn. Character and frequency of stools were correlated with alterations in diet, drugs, and physical examination. Eighteen patients developed loose stools, during the first two weeks postburn, up to 14 times daily, for up to 16 days duration. Seven of nine children (78%) had diarrhea, compared to 11 of 20 adults (55%). Seven adult patients, surviving for three to 14 days postburn, had no stools. Diarrhea in three patients appeared clearly related to an intolerance of milk products, and in one patient also to an intolerance for fruits. Milk intolerance may have existed in four other patients. Diarrhea was noninfectious, and did not appear related to burn size, sepsis, or antibiotic therapy. Diarrhea, which occurred frequently in patients who were fed in the early postburn period, probably reflected a mechanical dysfunction of the bowel immediately after burn injury. In some patients, an unexplained secretory component was suggested by loose stools unrelated to feeding. Gastrointestinal bleeding, which occurred in five diarrheic patients, probably added a cathartic effect to these other effects. Except for the patients with bleeding problems, the diarrhea seemed self-limited and easily controlled with symptomatic treatment. When milk intolerance was suspected, restriction of milk products was effective.

Diarrhea	Postburn	Nutritional intake		Course
Physical findings	Age	Nature	Humans	

DIARRHEA IN THERMALLY INJURED MILITARY PERSONNEL

Diarrhea is a frequently occurring management problem in the thermally injured patient. Such diarrhea may cause the loss of considerable amounts of fluid, electrolytes, and other nutrients at a time when the balance of these elements is critical. Nursing management can be complicated by repeated fecal contamination of burned areas or wound dressings.

The problem of diarrhea in the burned patient has not been evaluated. This prospective study was conducted to describe the incidence, characteristics, and course of diarrhea in the thermally injured patient during the first month postburn.

METHODS

Thermally injured patients admitted to the burn unit within 72 hours postburn were studied for up to four weeks postburn. Patients with electrical injury, known abdominal trauma, a history of gastrointestinal surgery, chronic diarrhea, or drug abuse were excluded.

Physical findings, frequency and character of stools, clinical course, and dietary intake were monitored in each patient. Observations of clinical status were made as soon as possible after admission, at least weekly thereafter, or more frequently if loose stools were noted. Signs and symptoms related to the abdomen and gastrointestinal tract, daily body temperature, daily weight, and presence of sepsis were noted. A loose, semiliquid, or watery stool was considered abnormal.

In patients with diarrhea, rectal contents of fresh stool specimens were tested for pH, using pH indicator dipsticks with ranges of 4.0 to 7.0 and 6.5 to 10.0. Accuracy of these indicators was verified by comparison with similar ranges of pH buffer solutions. After emulsification in one ml of distilled water, stool specimens were tested for reducing substances with urine sugar reagent tablets. Testing with known concentrations of glucose showed this method to be sensitive to solutions as weak as .05% glucose. Stools were also tested for occult blood, using blood detection reagent tablets or guaiac-impregnated filter paper. Smears, stained with crystal violet solution, were examined immediately for white blood cells. If loose stools developed, a stool culture was obtained by rectal swab and one stool specimen was examined for ova and parasites.

Observations concerning medical therapy were limited to notations of antibiotic therapy, treatment of diarrhea and the patient's response to it, and any medications or procedures which might have had an effect on gastrointestinal function.

The type of diet, estimated amounts of supplemental and total milk products, and total fluid intake and output were monitored daily.

RESULTS

Patients

Twenty nine patients, ranging in age from 11 months to 76 years, with 18% to 96% total body surface thermal injuries, were studied for one to 28 days after admission (see Table 1). Eighteen patients developed loose stools during the first two weeks postburn. Eleven of these patients expired; only two of the surviving patients had uncomplicated recoveries. Seven adult patients, with 53.5% to 96% total body surface burns, surviving for three to 14 days postburn, had no stools. Only four patients had normal bowel movements throughout the study period.

There was a higher incidence of diarrhea among male patients (68.8%) than among females (42.9%). Also, all patients who developed gastrointestinal bleeding as well as diarrhea were males.

Loose bowel movements varied from one to 14 per day, with an average of 6.8 per day. Diarrhea in each patient ranged from one to 16 days, with an average duration of 7.4 days. Sixteen of the 18 patients with diarrhea had at least three watery or loose stools per day. Diarrhea was intermittent in nine cases.

Although diarrhea could develop anytime within the first fourteen days postburn, for 15 patients, its onset was during the first week, most commonly on the third or fourth hospital day. In patients studied four weeks, diarrhea generally abated during the third or fourth week even when symptomatic treatment was not ordered.

The incidence of diarrhea by race or ethnic background is presented in Tables 1 and 2. Seven of nine patients with pigmented skin had loose stools. Two blacks and one patient of Spanish ancestry showed a strong relationship between milk ingestion and diarrhea. Diarrhea improved or abated for all three within one

Table 1. Characteristics of 29 Burned Patients Evaluated for Diarrhea

Characteristics		Diarrhea Group; N=18	Non-Diarrhea Group; N=11	Total; N=29
Age:	11 months-16 years	7	2	9
	17 years-76 years	11	9	20
Sex:	Male	15	7	22
	Female	3	4	7
Race or Ethnic Background:				
	Non-Spanish Caucasian	11	9	20
	Spanish Caucasian	4	0	4
	Negro	2	2	4
	Eskimo	1	0	1
% TBS Burn:	Range	19.5-90	18-96	18-96
	Mean	46.8	62.9	52.9
Survival:		7	2	9

Table 2. Characteristics of Diarrhea in 18 Thermally Injured Patients

	Number of Patients	Maximum Number of Stools/Day (Mean)	Average Duration (Mean Number of Day.)	Positive Stool Cultures	Significant Number of WBC's in Stools	Blood / Stools		Stools with pH of 6.5 or Less	Presence of Reducing Substances	Probable Milk Intolerance
						Frank	Occult			
Children	7	7.3	9.3	0	0	1	6	5	5	4
Non-Spanish Caucasian	4	6.8	16.0			1	3	2	2	1
*Others	3	8.0	11.0			0	3	3	3	3
Adults	11	6.5	6.2	0	0	6	11	8	1	3
Non-Spanish Caucasian	7	4.7	4.9			4	7	5	0	0
*Others	4	9.8	8.5			2	4	3	1	3
Total	18	6.8	7.4	0	0	7	17	13	6	7
Percentage				0	0	38.8	94.4	72.2	33.3	38.8

*"Others" include Spanish Caucasian, Negro, and Eskimo patients.

day after exclusion of milk products from the diet. In one black patient, diarrhea appeared to be aggravated by the ingestion of fruit. Although diarrhea in this patient continued with decreased frequency after milk product restriction, it ceased only after exclusion of all fruits from the diet. The return of fruits to her diet a week after continued clinical improvement did not precipitate a recurrence of symptoms.

Four other patients showed a possible relationship of loose stools to milk ingestion. Three patients were of Spanish ancestry, one was a non-Spanish Caucasian infant. Variations in frequency of loose stools were strongly related to quantities of milk ingested in two patients and showed a weaker relationship in the other two patients. Stools of all four patients had an acid pH and those of three patients contained reducing substances. Oral feedings were discontinued for two patients. Milk products were not withheld from the other two patients, one of whom had diarrheal stools until he expired, whereas the other had a steady decline in frequency of stools with decreased milk ingestion until he was discharged three weeks postburn.

In only three of the 18 patients with diarrhea was there a strong relationship between the onset of diarrhea and institution of a diet after a period of food restriction. Standard hospital diets, with supplements of fruit juices, milk, or high protein, high calorie, milk-based supplements were provided in all patients in a form appropriate to their conditions. Dietary intake varied, in that diets were changed frequently or withheld as necessitated by fluctuations in patients' clinical conditions. Most patients with very large burns were usually not fed initially; many patients in this category expired before diets were initiated.

Only three patients received specific antidiarrheic medications; treatment was successful in two cases. One of these patients was also treated by milk restriction. The third patient had some initial relief of symptoms, but later developed colonic hemorrhage which required total colectomy. Two additional patients received numerous sedatives, but these had no definite effect on controlling their diarrhea. The remaining patients either expired or had spontaneous cessation of diarrhea. No specific relationship of diarrhea to antibiotic therapy or any other medication or treatment was apparent.

Physical Findings

Physical findings were not helpful in distinguishing diarrheic from non-diarrheic patients. Decreased bowel sounds, mild

to moderate abdominal distention, and hyperthermia occurred in both groups of patients with sufficient frequency to preclude differentiation by these criteria.

Only four of the 10 patients with diarrhea who could respond to questioning complained of abdominal pain, usually cramping in nature. In two of these patients who had possible milk intolerance, pain and diarrhea abated with restriction of milk products. Another patient developed gastrointestinal bleeding along with the diarrhea and abdominal cramping.

There were very low incidences of increased bowel sounds, nausea, vomiting, abdominal rigidity, hypothermia, and sepsis. Again, frequencies were about equally divided between the two groups. Weight changes and burn size were also examined and found to have no apparent relationship to incidence of diarrhea.

Stool Characteristics

Stool consistency, color, and amount were variable, being liquid to loose-soft and generally small in volume. In only seven patients were stools expelled forcefully.

Seventeen of the 18 patients with diarrhea had stools positive for occult blood; eight had stools containing frank blood. All rectal cultures were negative for abnormal organisms, as were all stool specimens for ova and parasites. White blood cell counts of rectal smears were normal in all cases.

In 17 patients with diarrhea whose stools were tested for reducing substances, those from six patients (35.3%) were positive; five of these patients also had clinical evidence of milk intolerance.

Stools from the same 17 patients were found to have a pH of 6.5 or less in 13 patients (72.2%), as shown in Table 2. Stools with a pH of 7.0 were found in seven patients (38.8%), as were stools with a pH of greater than 7.0. In 11 of the 13 patients with acid stools, the pH was less than 6.0. Fifty five per cent of patients with diarrhea had one or two acid stools.

Two patients who had reducing substances in their stools also had acid stools with a pH less than 6.0. Of the other four patients with stools positive for reducing substances, the pH was 6.0 or greater in three patients, two of whom had diarrhea. One patient, who was probably milk intolerant, had an alkaline pH and

some reducing substances in one specimen, but no diarrhea at the time of testing.

DISCUSSION

Diarrhea developed in 63.1% of patients following thermal injury. Loose-watery bowel movements, up to seven per day, commonly complicated the clinical course of these patients during the first week postburn and continued, in some cases, intermittently for up to three weeks postinjury. Although over 50% of adults (11 of 21 patients) developed diarrhea, children (seven of nine patients) were more prone to suffer this complication, and the course of their diarrhea was usually more prolonged. In all patients, the diarrhea was noninfectious and self-limited, usually resolving within nine days even when left untreated. Although volume losses were never difficult to manage, and significant weight changes did not occur as a result of the diarrhea, patient comfort did suffer and nursing management was complicated by frequent wound cleansing or dressing changes in an effort to minimize contamination. These increased nursing care activities reduced time available to patients for needed rest or sleep.

Stool volumes and consistency were variable and were indicative of both small and large bowel dysfunction. Diarrhea in these patients, probably functional, usually occurred when feeding was begun in the presence of abdominal distention and decreased bowel sounds. Under these circumstances, abnormal bowel function might be anticipated, since secretory functions and intestinal motility are probably altered in this early period postburn by maximal humoral (catecholamine, glucagon) and neurological (sympathetic) stimulation as well as by alterations in visceral blood flow (Harrison TS, Seaton JF, Feller I, Ann Surg 165:169-172, 1967; Wilmore DW, et al, Lancet 1:73, 1974; Hamilton JR, Radde IC, Johnson G, Amer J Med 44:453-462, 1968).[1-3] As bowel motility recovered, the diarrhea tended to improve spontaneously even with continued feeding of the patients. In these patients, the diarrhea was not severe and did not mitigate against the importance of oral feedings. Symptoms might be better managed by limiting quantities of feedings administered during the first week postburn.

Over 38% of patients with diarrhea demonstrated an intolerance to milk. These were primarily patients with pigmented skin who are recognized to have a high incidence of hereditary disaccharidase deficiency (Newcomer AD, Mayo Clinic Proceedings 48:648-652, 1973; Bayless TM, J Amer Diet Ass 60:478-482, 1972; Dill JE, et al, Amer J Clin Nutr 25:869-870, 1972).[4-6] In these patients, this

enzyme activity might have been further diminished by their increased caloric requirements during the early postburn period. A "relative" malnutrition could limit enzyme activity (Bowie MD, Brinckman GL, Hansen JDL, Lancet 2:550, 1963; Bowie MD, Brinckman GL, Hansen JDL, J Pediat 66:1083, 1965; Kumar V, Chase HP, Amer J Clin Nutr 25:485-489, 1972).[7-9] This lower tolerance for milk was usually challenged in the first week postburn by frequent administration of milk-based nutriments. Loose stools with low pH and with reducing substances following milk administration justified the diagnosis of lactase deficiency (Newcomer AD, Mayo Clinic Proceedings 48:648-652, 1973; Lifshitz F, Coello-Ramirez P, Contreras-Gutierrez M, J Pediat 79:612-617, 1971).[4,10] These patients often complained of crampy abdominal pain and their symptoms improved rapidly when milk was restricted. In infants and young children this type of diarrhea could be further aggravated by fructose ingestion (Lifshitz F, Coello-Ramirez P, Contreras-Gutierrez M, J Pediat 79:612-617, 1971; Lifshitz F, Coello-Ramirez P, Gutierrez-Topete G, J Pediat 77:595, 1970).[10-11] The milk substrate was better tolerated late in the convalescent period, indicating only a transient compromise in disaccharidase activity. Symptoms could be controlled in these patients by the restriction of milk-based nutriments during the early postburn period. These nutriments, if needed, could be best tolerated during the second or third week postburn and thereafter.

Several patients continued to have loose stools even when fasting. In these cases, a secretory component to the diarrhea was likely (Fordtran J, Fed Proc 26:1405-1414, 1967).[12] Elevations of plasma glucagon in the immediate postburn period may have contributed to this secretion (Fasth S, Hulten L, Acta Physiol Scand 83:169-173, 1971);[13] stimulation of intestinal cyclic 3', 5' AMP by circulating toxins, possibly endotoxin, might also have occurred (Fordtran J, Fed Proc 26:1405-1414, 1967; Field M, New Eng J Med 284:1137-1144, 1971).[12,14] This diarrhea also improved spontaneously, presumably as the humoral stimulation diminished. The incidence and types of diarrhea were not related to burn size, occurrence of sepsis, or use of antibiotics.

Although only about one third of the patients with loose stools had obvious gastrointestinal bleeding, stools of almost all patients with diarrhea contained occult blood. This high incidence of occult blood was anticipated, in view of findings concerning the incidence of gastrointestinal bleeding recently described at this institute.

In summary, diarrhea following thermal injury may occur frequently in patients who are fed in the early postburn period. This

diarrhea is noninfectious and self-limiting, and probably reflects a mechanical dysfunction of the bowel immediately after burn. In some patients, an inherited propensity to milk intolerance may be transiently accentuated; in others, an unexplained secretory component may predominate. Gastrointestinal bleeding can produce a cathartic effect, which could be additive to these other effects. Recognition of this tendency for bowel dysfunction soon after thermal injury, and reassurance as to the benign natural history should permit early, effective, symptomatic control, often with dramatic results, especially in patients with a transient milk intolerance. Recognition of the high incidence of diarrhea in children can alert medical and nursing personnel to the necessity for careful monitoring of bowel function in these susceptible patients.

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PUBLICATIONS AND/OR PRESENTATIONS:

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0D 6985	74 07 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
73 07 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	61102A	3A161102B71R	01	311
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) Inhalation Injuries - Pathogenesis and Treatment 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
72 05	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 74	.4	10
FISCAL YEAR — CURRENT 75	.5	12

19. RESPONSIBLE DOD ORGANIZATION
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21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code): (U) Inhalation injury; (U) Xenon lung scans; (U) N_2O_4; (U) Surfactant; (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 create a pulmonary injury by inhalation of nitrogen oxides and to evaluate the effect of various treatment modalities on such an injury.

24. (U) To date approximately 10 goats have received N_2O_4. Nearly all those so treated have developed either abnormal xenon lung scans or chest x-rays. Three goats showed a marked fall in pulmonary dynamic compliance following N_2O_4 inhalation. All the treated animals have shown pathological changes in the lungs at autopsy. These changes varied from inflammatory changes to pneumonia. Many of the goats also had obliterative emphysema. At the present time the effect of steroids on the N_2O_4 inhalation injury is being studied.

25. (U) 73 07 - 74 06 In summary, instillation of N_2O_4 into the goat trachea produces an injury which results in changes in pulmonary mechanics, chest x-ray, and xenon lung scan. Present plans call for a similar series of goats to undergo the inhalation of N_2O_4 while being treated with steroids in an attempt to determine the efficacy of steroid treatment on inhalation injuries.

*Available to controlling upon originator's approval

DD FORM 1 MAR 65 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

ABSTRACT

PROJECT NO. 3A161102B71R-01, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: INHALATION INJURIES--PATHOGENESIS AND TREATMENT IN BURNED TROUPS

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

Period covered in report: 1 July 1973 - 30 June 1974

Investigators: Gary W. Welch, MD, Major, MC
Peter A. Petroff, MD, Major, MC
Edwin W. Hander, ILT, MSC

Reports Control Symbol MEDDH-288(R1)

The purpose of this study is to create a pulmonary injury by inhalation of nitrogen oxides and to evaluate the effect of various treatment modalities on such an injury. Adult goats were anesthetized and intubated, following which 30 to 80 cc of N_2O_4 (nitrogen tetroxide) were instilled via the endotracheal tube.

To date, approximately 10 goats have received N_2O_4. Nearly all those so treated have developed either abnormal xenon lung scans or chest x-rays. Three goats showed a marked fall in pulmonary dynamic compliance following N_2O_4 inhalation. All the treated animals have shown pathological changes in the lungs at autopsy. These changes varied from inflammatory changes to pneumonia. Many of the goats also had obliterative emphysema. At the present time, the effect of steroids on the N_2O_4 inhalation injury is being studied.

In summary, instillation of N_2O_4 into the goat trachea produces an injury which results in changes in pulmonary mechanics, chest x-ray, and xenon lung scan. Present plans call for a similar series of goats to undergo the inhalation of N_2O_4 and thereafter receive systemically administered steroids in an attempt to determine the efficacy of steroid treatment on inhalation injuries.

N_2O_4
Inhalation injury
Xenon lung scans
Surfactant
Goats

290<

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OD 6980	74 07 01	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISP'N INSTR'S	8a. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
73 07 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	61102A	3A161102B71R	01	309
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) Use of 133 Xenon in Early Diagnosis of Inhalation Injury in Burned Military Personnel (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS:
003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
71 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	PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
FISCAL YEAR PRECEDING 74	.6	14
FISCAL YEAR CURRENT 75	.4	8

19. RESPONSIBLE DOD ORGANIZATION
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21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede each with Security Classification Code)
(U) Inhalation injury; (U) 133 Xenon lung scan; (U) Blast injury; (U) Humans

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

23. (U) To determine the reliability of the 133 Xenon lung scan in detecting inhalation injury in combat wounded personnel.

24. (U) All patients with flame, steam or blast injuries and suspected inhalation injury admitted to the USAISR receive a 133 Xenon lung scan on admission. The results of the scan are compared with clinical and pathologic diagnoses of inhalation injury to determine the reliability of the 133 Xenon scintiphotography.

24. (U) 73 07 - 74 06 Use of the 133 Xenon lung scan over the past year has demonstrated a high correlation of radiation densities with subsequent clinical pathological findings and changes on standard roentgenograms.

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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

ABSTRACT

PROJECT NO. 3A161102B71R-01, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: USE OF ^{133}XENON IN EARLY DIAGNOSIS OF INHALATION INJURY IN BURNED MILITARY PERSONNEL

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

Period covered in this report: 1 July 1973 - 30 June 1974

Investigators: Robert N. Agee, M.D., Major, MC
Glenn D. Warden, M.D., Major MC
John L. Hunt, M.D., Lieutenant Colonel, MC

Reports Control Symbol MEDDH-288(R1)

Xenon scans were performed on 104 patients admitted to the United States Army Institute of Surgical Research during 1973. This represents 40% of the total number of burn patients admitted (261). Average burn size of those undergoing xenon scans was 47.3%. Seventeen of the patients were children less than 16 years old.

Twenty nine patients had positive scans, 74 had negative scans and one had a technically inadequate scan. Three of the positive scans were "false positive" (3%) and there were 16 (15.4%) false negative scans.

Two patients underwent initial scanning more than 72 hours post burn and both scans were interpreted as being negative. Subsequent clinical changes in these patients indicated that the scans were falsely negative.

Three patients had early positive scans with follow up negative scans with post burn day 2, 4, 5, and 6 respectively.

292<

Inhalation injury
^{133}Xenon lung scan
Blast injury
Humans

USE OF ^{133}XENON IN EARLY DIAGNOSIS OF INHALATION INJURY IN BURNED MILITARY PERSONNEL

Injury to the respiratory tract from incomplete products of combustion or from steam is a common accompaniment of cutaneous thermal injury. Pulmonary damage does not usually become manifest either clinically or by roentgenography until the 4th or 5th post burn day. Clinical symptoms and signs during this period are unreliable as are conventional laboratory tests. Recently ^{133}Xenon scanning and fiberoptic bronchoscopy have found to be valuable in establishing the diagnosis early. The xenon washout ventilation scan technique experience at the USA-ISR during 1973 will be presented in this report.

Moylan et al[1] reported the early diagnosis of inhalation injury using 133Xenon lung scan in 1972. Fifty consecutive burn patients admitted to the USA-ISR were studied using this method. Fifteen were found to have abnormal scans. No false positive or false negative scans were obtained as shown by clinical-pathological findings.

During 1973 261 thermally injured patients were admitted to the USA-ISR. One hundred and four patients were studied by ^{133}Xenon scan (40%) and are included in this report. Those patients excluded from the report include scald, electrical and chemical burns, those minor burns not thought likely to have an associated inhalation injury, and those patients who could not be stabilized hemodynamically for movement to the Nuclear Medicine Service.

The average cutaneous burn size was 47.3%. Seventeen of the patients were children less than 16 years of age.

Of the 104 patients examined initially, 29 had positive scans and 74 had negative scans. One patient had a "technically inadequate scan". Three of the positive scans were "falsely positive" and there were 16 (15.4%) "falsely negative" scans based on clinical pathological findings.

Two of the falsely negative scans were in patients who underwent initial scanning more than 72 hours post burn. It is possible that scans performed earlier, prior to the onset of significant hyperventilation, would have been positive. Three patients whose initial scans were positive had follow up negative scans on post burn date 5, 6, and 24 respectively. No scan performed after day 5 was positive unless an accompanying chest x-ray infiltrate was present.

SUMMARY

^{133}Xenon scanning performed within the first 72 hours post burn is a reliable means of detecting an inhalation injury early. Efficacy of treatment may depend on early diagnosis. Scientific evaluation of treatment demands early documentation of inhalation chemical tracheo-

bronchitis.

REFERENCES

1. Moylan, J.A. Jr., et. al.: Use of ^{133}Xenon in Early Diagnosis of Inhalation Injury in Burned Military Personnel. Ann. Surg. 176:477, 1972.

PUBLICATIONS AND/OR PRESENTATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 00 6385	74 07 01	DD DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. SMRY'S HISTORY	9A. SPECIFIC DATA-CONTRACTOR ACCESS	B. LEVEL OF SUM
73 07 01	K. COMPLETION	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	3A161102871R	01	313
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Provide with Security Classification Code):
(U) Coagulation Abnormalities 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
69 01	74 04	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. FUNDING (In Thousands)
FISCAL YEAR: PROCEEDING	74	.5	13
FISCAL YEAR: CURRENT			

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NAME: DA

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Provide Key Words, Phrases or Codes Followed by Security Classification Code): (U) Coagulation; (U) Platelets; (U) Burned soldiers; (U) Disseminated intravascular clotting

23. TECHNICAL OBJECTIVE. 24. APPROACH. 25. PROGRESS (Provide Individual paragraphs identified by number. Precede text with Code and Security Classification Code.)

23. (U) To measure coagulation changes as a function of time following thermal injury and to evaluate their effects in soldiers with hemorrhagic complications.

24. (U) Twenty patients admitted within 24 hours post-injury to the US Army Institute of Surgical Research with a greater than 40% total body surface burn have been studied since the last report. Serial assays of coagulation indices in this group of patients were obtained as early as possible postburn but within 24 hours in all cases.

25. (U) 73 07 - 74 04 Twenty patients were studied and found to exhibit marked coagulation and erythrocyte changes. Each patient had erythrocyte fragmentation in his peripheral blood smear on admission. Nine patients had nucleated erythrocytes within twenty-four hours postburn. Hyperfibrinolysis was evidenced by prolonged thrombin times, and plasminogen depletion in many of these patients. This hyperfibrinolysis seemed to decrease during the hospitalization of each patient to at least some degree. Platelet counts fell in 17 of the 20 patients during their hospitalization and within 24 hours of injury in each of the six patients studied at least twice during that period. An abnormality in the prothrombin time or activated partial thromboplastin time was also noted frequently. These studies indicate that there was at least a transient, and in some cases more prolonged, period of intravascular and probably burn wound coagulation with the development of hyperfibrinolysis in response to the coagulation. Erythrocyte morphologic changes disappeared within 72 hours postburn.

ABSTRACT

PROJECT NO. 3A161102B71R-01, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: COAGULATION ABNORMALITIES IN THERMALLY INJURED SOLDIERS

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

Period covered in this report: 1 July 1973 - 30 June 1974

Investigators: W. Abe Andes, MD, Major, MC
Joseph P. Baron, SP4

The variety and severity of metabolic, hemodynamic, and biochemical changes in acute severe thermal injury are often striking. Coagulation, fibrinolytic, and erythrocyte morphologic changes are often transient or unmeasured in the complexity of caring for such patients and inasmuch as the importance of such changes is unknown. This study was initiated because of the opportunity for studying such patients with marked hemolysis, pathological bleeding, and the therapeutic challenges they offer. Twenty patients were studied with a mean burn size of 76%. All were available for study within 2 to 21 hours after burn. Five patients initially had fibrinogen values less than 100 mg %, but 15 of the 18 patients had subsequent elevations with the mean rising from 218 mg % to 400 mg % by postburn day one. Platelet counts fell significantly in each of the six patients studied at least twice in the day of burning and later in the hospitalization of all patients (with one exception) who were available for testing more than once. Accelerated fibrinolysis was evidenced in every patient as early as two hours postburn by elevated firbin-fibrinogen degradation product levels. The serial thrombin time as a measure of activated plasmin was positive in five of 15 patients initially. The thrombin time as a reflection of degradation products was more than 5 seconds longer than the control in six of 15 patients. A mean prothrombin time of 17.4 seconds with a control of 13 seconds and a mean activated partial thromboplastin time of 61 seconds were noted.

These findings indicate that severe thermal injury causes a rapid onset of coagulation and secondary fibrinolysis. Such changes may become exaggerated because of infection, shock, or other insults with intravascular coagulation in normal tissues, pathological bleeding, and organ dysfunction or damage.

Disseminated Intravascular Clotting
Coagulation
Burned Soldiers
Platelets

COAGULATION ABNORMALITIES IN THERMALLY INJURED SOLDIERS

The variety and severity of metabolic, hemodynamic, and biochemical changes in acute, severe thermal injury are often striking. Coagulation, fibrinolytic and erythrocyte morphologic changes are often transient or unmeasured in the complexity of caring for such patients and inasmuch as the importance of such changes is unknown. This study was initiated because of the opportunity for studying such patients with marked hemolysis, pathological bleeding and the therapeutic challenges they offer. The patient population we evaluated included twenty patients of which 13 were men, 6 women, and one child. Ages ranged from 38 months to 85 years. Burn size had a mean of 76% and range from 40 to 98% of the total body surface area. The patients were all available for study within 2 to 21 hours after burn injury. A wide spectrum of burn etiologies was included.

METHODS

Erythrocyte morphology was studied after venipuncture in wet preparations, routine Wright's stained smears and by scanning electron microscopy after slow glutaraldehyde fixation by the method of Morel. (Morel FMM, Baker RF, Wayland H: J Cell Biol 48: 91-100, 1971)[1]. Microcytic erythrocytes less than 3μ in diameter were counted in peripheral blood smears by the method of Topley. (Topley E:J Clin Path 14:295-297, 1961)[2]. Normally none are found. Platelet counts were done by phase microscopy. Coagulation studies were done by the usual methods in this laboratory. Fibrinogen was measured by turbidimetric or thrombin-clottable methods. Fibrinolysis was evaluated by a number of methods.

Fibrin-fibrinogen degradation products were measured by the staphylococcal clumping method. (Leavelle DE, Mertens BF, Bowie EJW, et al: Am J Clin Path 55: 452-457, 1971)[3]. Serial thrombin times were performed by measuring the thrombin time of plasma incubated at 37° for up to one hour. (Brodsley I, Lesis HD: Am J Clin Path 45: 61-69, 1966)[4]. Positive tests are considered a greater than two fold increase in time to clot after incubation. Plasminogen was measured by the caseinolytic method of Sherry. (Sherry S, Lindgmeyer RI, Fletcher AP, Alkjaersig N: J Clin Invest 38: 810-822, 1959)[5].

RESULTS

Every patient exhibited erythrocyte fragmentation to some degree. Wet preparations, scanning electron microscopy in routine Wright's stained smears confirmed the presence of the characteristic fragments. These disappeared quickly and were very rarely found more than 72 hours postburn in any case. Nine patients were found to have circulating normoblasts with normal or elevated hematocrits during the first 24 hours.

No patient with less than 2% microcytes had an abnormal red cell mass or fall in hematocrit greater than 5 during the first 24 hours. Each patient with a reduced red cell mass or hematocrit less than 40 had 3% or more microcytes when first studied. Six patients had more than 5% microcytes and five of these required early transfusion or died in unreversed shock. These comments do include one patient with 2% microcytes whose other values were as seen in the following:

GW - 20 years old

87% burn - gasoline

19 hours postburn - Prothrombin Time: $\frac{56 \text{ sec (Patient)}}{13 \text{ sec (Control)}}$

Activated partial thromboplastin time: > $\frac{180 \text{ sec (Patient}}{39 \text{ sec (Control)}}$

Fibrinogen: < 100 mg %

Platelets: 460,000 78,000

Fibrin-Fibrinogen Degradation Products: 1:64 (N < 1:4)

Plasminogen: 0.27 U (N = 2-3)

Serial Thrombin Time: 0 - $\frac{38 \text{ sec}}{11 \text{ sec}}$

1/2 hour - 140 seconds

1 hour - Unclottable

A-PTT $\frac{\text{Patient + Control (1:1)}}{\text{Control}} : \frac{47}{33}$

Summary: Hypofibrinogenemia

Thrombocytopenia

Hyperfibrinolysis

Circulating Anticoagulant Present (FDP?)

A 20 year old patient was transferred to our unit within fourteen hours of thermal injury of 87% of the total body surface with early signs of inhalation injury necessitating intubation. Marked pigmenturia,

acidosis, persistent hypotension, and oliguria were also present. Hemolysis as well as burn wound and escharotomy bleeding developed. This resulted in a 60% red cell mass deficit within 24 hours. Replacement therapy controlled bleeding somewhat but shock, sepsis and a fatal outcome supervened. These findings were not unique. Five patients initially had fibrinogen values less than 100 mg %, but 15 of 18 patients had subsequent elevations with the mean rising from 218 mg % to 400 mg % by postburn day one. Each of the three patients with a fall in fibrinogen at 24 to 36 hours postburn died in less than 48 hours. Platelet count significantly fell in each of the six patients studied at least twice during the day of burning and later in the hospitalization of the rest who were available for testing more than once.

Accelerated fibrinolysis was evidenced in every patient as early as 2 hours postburn by elevated fibrin-fibrinogen degradation product levels. These levels fell from an initial mean of 60 μg/ml to 30 μg/ml on the first postburn day. The serial thrombin time as a measure of activated plasmin was positive in 5 of 15 patients initially but was normal in all patients by day 1 postburn. The thrombin time as a reflection of degradation products, antithrombin activity was more than 5 seconds longer than the control in 6 of 15 patients.

A mean prothrombin time of 17.4 seconds with a control of 13 seconds and an activated partial thromboplastin time of 61 seconds (control 37 to 41 seconds) were noted. Clinically unusual bleeding from the upper gastrointestinal tract, venipuncture sites, burn wound, or escharotomy incisions was noted in seven patients during the first 24 hours postburn. Six of 8 patients with acute renal failure but not in shock had both early pigmenturia and acidosis. Postmortem examinations were obtained in 13 cases but only 4 patients expired during the first 50 hours postburn and were available for more detailed comment.

Three of these four patients had clinical inhalation injury with pathological confirmation. Diffuse to occasional pulmonary microthromboses were found in two patients and severe congestion and/or small hemorrhagic lesions in the others. Marked renal tubular casts were found in all patients and two of the four patients had glomerular microthrombosis. One patient had unilateral adrenal hemorrhage.

COMMENT

Acute studies of severely burned patients are fraught with difficulties. The rapid change in clinical condition, variety of treatment regimens, and complexity of postburn dynamics make serial investigations during the resuscitation period rare. Direct microcinemaphotographic studies of burn wounds have documented capillary or venule erythrocyte sludging followed by platelet aggregation and eventual thrombosis. In our extensively burned patients such changes could account for an early fall in platelets and elevation of FDP. However, if caused solely by a circumscribed burn wound it would seem that such changes should return

rapidly to normal since the burn wound represents only a small portion of the total vascular bed. Other factors undoubtedly play a role and hemolysis, with release of phospholipids and thromboplastic materials, reduction of oxygen delivery, and possibly microvascular sludging due to the presence of less deformable erythrocytes, might contribute.

Other forms of trauma rarely exhibit significant hemolysis. A progressive fall in platelets noted here and in previous studies together with hyperfibrinolysis, and evidence of widespread organ dysfunction could also be taken to indicate systemic activation and effects of coagulation. Postmortem findings, although available in only a few of our patients during the resuscitation period, would seem to corroborate such an interpretation. Causes for accelerated activation of coagulation may be easily recognized if not measured. Local or systemic hypoxia or acidosis, direct skin or other organ damage, elevation of catecholamines and vasoconstriction all may contribute. Fibrinogen or coagulation factors V and VIII measurements may only represent net levels inasmuch as others have shown that burn injury stimulates their elevation. Thus, although several patients had no documentation of hypofibrinogenemia it seems inescapable that fibrinogen turnover was accelerated.

Therapeutic implications of some of these studies would seem to indicate administration of fresh frozen plasma, whole blood, or, probably rarely, platelet concentrates in cases of specific deficiencies. Each of our bleeding patients had at least one abnormal coagulation index. Appropriate therapy ameliorated much of the bleeding. In addition, heparin should be considered for cases associated with bleeding and coagulation factor consumption. It might also be considered for clinically unexplained renal failure with severely deranged coagulation tests, especially when characteristic patterns of change can be measured. The slowing of fibrinolytic activity documented here and elsewhere probably indicates that antifibrinolytic agents are usually unnecessary or that a severe deficiency of plasmin has already been induced.

In summary it would seem that some information regarding the extent of hemolysis to expect may be gained by observing an early peripheral blood smear in the severely burned patient. Coagulation tests and fibrinolytic activity, while often abnormal initially, may be interpreted and used to best advantage by evaluating serial studies. Coagulation indices, clinical events, and postmortem findings indicate that a period of widespread intravascular coagulation may occur in the thermally injured patient very early after the initial injury.

REFERENCES:

1. Morel FMM, Baker RF, Wayland H: Quantitation of Human Red Blood Cell Fixation by Glutaraldehyde. J Cell Biol 48: 91-100, 1971.

2. Topley E: The Usefulness of Counting 'Heat-Effected' Red Cells as a guide to the Risk of the Later Disappearance of Red Cells after Burns. J Clin Path 14: 295-297, 1961.

3. Leavelle DE, Mertens BF, Bowie EJW, et al: Staphylococcal Clumping on Microtiter Plates: A Rapid, Simple Method for Measuring Fibrinogen Split Products: Am J Clin Path 55: 452-457, 1971.

4. Brodskey I, Lesis HD: Evaluation of Fibrinolysis in Hepatic Cirrhosis, Relation of Serial Thrombin Time and Euglobulin Lysis Time. Am J Clin Path 45: 61-69, 1966.

5. Sherry S, Lindemeyer RI, Fletcher AP, Alkjaersig N: Studies on enhanced fibrinolytic activity in man. J Clin Invest 38: 810-822, 1959.

PRESENTATIONS:

Andes W. Abe. Hematologic Changes in Acute, Severe Thermal Injury American Burn Association Meeting, Cincinatti, Ohio, April 4, 5, and 6 1974.

PUBLICATIONS:

Andes WA: Hyperacute Hematologic Findings in the Severely Burned Patient. (Abs) Am Soc of Hematology, 1973.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6954	74 07 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
73 07 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	61102A	3A161102B71R	01	251
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) Effect of Extracellular Volume on Renal Bicarbonate Reabsorption -A Laboratory Model of Renal Changes Observed in Injured Soldiers (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
68 07	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 74	.4	16
	CURRENT 75	.3	6

19. RESPONSIBLE DOD ORGANIZATION
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NAME: David B Olin, MAJ, MC

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

DA

22. KEYWORDS (Precede each with Security Classification Code): (U) Bicarbonate reabsorption; (U) Sodium; (U) Extracellular volume; (U) Burned soldiers; (U) Prostaglandins

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

23. (U) Disorders of acid-base hemostasis are extremely common in injured or ill soldiers. These disorders are perpetuated, compensated, or corrected by changes in renal bicarbonate reabsorption. This study was undertaken to examine the effect of prostaglandin E1 (PGE_1) on renal bicarbonate reabsorption in the dog.

24. (U) These studies were performed on lightly anesthetized mongrel dogs, in whom both ureters were cannulated and a 26 gauge needle placed in a retrograde fashion into the left renal artery. PGE_1 was infused into the left renal artery at the rate of 2µg/min. A systemic infusion of 0.9 molar sodium bicarbonate was infused to maintain a plasma bicarbonate concentration o approximately 30 mEg/L. Similar studies were also performed on adrenalectomized dog: approximately two weeks after bilateral adrenalectomy. Similar studies were also performed on animals receiving a systemic infusion of 20% glucose and a buffered solution of NaH_2PO_4 and Na_2HPO_4.

25. (U) 73 07 - 74 06 The infusion of PGE_1 into the left renal artery at 2µg/min was found to cause a significant increase in flow rate and natriuresis on the infused side with no change on the control side. Bicarbonate reabsorption was unchanged during the infusion of PGE_1. Studies performed in adrenalectomized animals also showed no significant difference in bicarbonate reabsorption. Studies of glucose and phosphate reabsorption were then performed to further evaluate the effect of PGE_1 on proximal tubular function. The infusion of PGE_1 resulted in a significant decrease in glucose reabsorption. During the infusion of the buffered phosphate solution fractional phosphate excretion increased significantly on the infused side. These studies suggest that PGE_1 inhibits sodium reabsorption in the proximal tubule and must also affect an energy transport mechanism for glucose and phosphate independent from that of bicarbonate reabsorption.

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(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

ABSTRACT

PROJECT NO: 3A161102B71R-01, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: EFFECT OF EXTRACELLULAR VOLUME ON RENAL BICARBONATE REABSORPTION - A LABORATORY MODEL OF RENAL CHANGES OBSERVED IN INJURED SOLDIERS: THE EFFECT OF PGE_1 ON BICARBONATE, GLUCOSE, AND PHOSPHATE REABSORPTION IN THE DOG

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

Period covered in this report: 1 July 1973 - 30 June 1974

Investigators: Philip W. Rogers, MD, Major, MC
Daniel A. Nash, Jr., MD Major, MC
David B. Olin, MD, Major, MC
Donald J. Johnson, Major, VC
John W. Beason, SP4

Reports Control Symbol MEEDH-288(R1)

Subdepressor doses of prostaglandin E_1 infused into the renal artery of dogs results in diuresis, natriuresis, and increase in free water excretion independent of significant change in glomerular filtration rate or redistribution of intrarenal blood flow. The above effects cannot be entirely attributed to the renal vasodilating properties of PGE_1. Previous studies have suggested that PGE_1 might have a specific action on a particular portion of the nephron by inhibiting sodium reabsorption. These studies were designed to evaluate the effect of subdepressor doses (2μg/min) of PGE_1 on sodium reabsorption in the proximal tubule by utilizing bicarbonate, glucose, and phosphate reabsorption as markers of proximal tubular function. These studies show no change in bicarbonate reabsorption in the intact animal; however, there is a significant ($p<0.01$) decrease in bicarbonate reabsorption with selective aldosterone deficiency. Glucose and phosphate reabsorption in intact animals also decreased significantly ($p<0.001$) with the infusion of PGE_1. A marked natriuresis, increase in urine flow rate, and slight increase in renal plasma flow was noted in the infused kidney with no significant change in these parameters in the control kidney. These studies suggest that PGE_1 does have a specific tubular effect on sodium reabsorption and that the portion of the nephron involved is the proximal tubule.

Bicarbonate reabsorption Sodium Extracellular volume
Prostaglandins Burned soldiers

EFFECT OF EXTRACELLULAR VOLUME ON RENAL BICARBONATE REABSORPTION - A LABORATORY MODEL OF RENAL CHANGES OBSERVED IN INJURED SOLDIERS: THE EFFECT OF PGE_1 ON BICARBONATE, GLUCOSE, AND PHOSPHATE REABSORPTION IN THE DOG

The prostaglandins are a group of 20-carbon unsaturated fatty acids containing a five-membered ring(Bergstrom S, Ryhage R, Samuelson B, and Sjovall J. Acta Chem Scand 16: 501-502, 1962)[1]. Three biological classes of prostaglandins, the PGEs, the PGFs, and the PGAs have all been isolated from renal medullary extracts of several animals species as well as man(Lee JB, Crowshaw K, Takman BH, Attrep KA, and Gougoutas JZ. Biochem J 105: 1251-1260, 1967;[2] Daniels EG, Hinman JW, Leach BE, and Muirhead EE. Nature (London) 215:1298-1299, 1967)[3] In high doses, the prostaglandins have been found to reduce blood pressure(Lee JB, Covino BG, Takman BH, and Smith ER. Circulation Res 17: 57-77, 1965)[4]. Low doses of prostaglandin E_1 (PGE_1) initiate, by poorly understood mechanisms, diuresis and natriuresis independent of significant change in glomerular filtration rate, or decrease in renal vascular resistance as evidenced by enhanced renal plasma flow, or reduction in para-aminohippurate extraction. This response is characteristic of that produced by other renal vasodilators (Harvey RB Am J Physiol 211: 487-492, 1966,[5] Vander AJ. Am J. Physiol 206:492-498, 1964)[6]. or expansion of intravascular volume, (Earley LE and Friedler RM. J Clin Invest 44: 929-941, 1965;[7] Earley LE and Friedler RM. J Clin Invest 45: 542-561, 1966)[8]. Previous studies infusing subdepressor doses of PGE_1 directly into the renal artery of dogs have shown an increase in urine volume, urinary sodium excretion, free water clearance, and renal plasma flow (Gross JB and Bartter FC. Am J Physiol 225: 218-224, 1973;[9] Martinez-Maldonado M, Tsapparas N, Eknoyan G and Suki WN. Am J Physiol 222: 1147-1152, 1972;[10] Vander AJ. Am J Physiol 214:218-221, 1968;[11] Carriere S, Friborg J and Guay JP. Am J Physiol 221: 92-98, 1971;[12] Johnston MH, Herzog JP and Lavier DP. Am J Physiol 213: 939-946, 1967;[13] Berl T and Schrier RW. J Clin Invest 52: 463-471, 1973)[14]. Since PGE_1 is a known renal vasodilator, these changes are thought to be manifestations of production of increased renal blood flow or intrarenal redistribution of blood flow. Recent studies by Arendshorst, et al (Arendshorst WJ, Johnston PA and Selkurt EE. Am J Physiol 226: 218-225, 1974)[15] in hydropenic and volume expanded animals have demonstrated no significant redistribution of plasma flow to the superfical cortex or to the inner medulla with the infusion of 0.1 - 2.0 μg/min of PGE_1 into the renal artery of dogs. The above studies suggest that the natriuresis, increase in urine volume, and increased free water clearance may result from a direct tubular action of PGE_1. The most likely portion of the nephron in which PGE_1 might exert a tubular effect would appear to be the proximal tubule. This study was designed to examine the effect of subdepressor doses of PGE_1 on sodium reabsorption in the proximal tubule utilizing bicarbonate, glucose, and phosphate reabsorption as markers of proximal tubular function.

METHODS AND MATERIALS

Experiments were performed on mongrel dogs of either sex weighing from 9 to 25 kg. Surgical anesthesia was induced with 30 mg/kg pentobarbital intravenously, and light anesthesia maintained by subsequent small doses as necessary, judged from corneal reflexes. An intratracheal tube fitted with an inflatable cuff was placed in the trachea and connected to a Bird respirator. The PCO_2 was maintained between 35 and 43 mm Hg. Indwelling polyethelene catheters were placed in the left jugular vein and the left femoral vein and artery. Blood pressure was monitored in the femoral vein and artery. Blood pressure was monitored in the femoral artery with a Statham strain guage transducer and recorded on a Sanborn 9 ; Multichannel recorder.

A midline suprapubic abdominal incision was made and both ureters isolated and cannulated for timed urine collections in separate containers. The left renal artery was isolated through a left flank incision and careful dissection utilizing the retroperitoneal approach. Care was taken to prevent interruption of renal innervation. A 26- gauge needle attached to a polyethylene catheter was inserted directly into the left renal artery allowing infusion into the renal artery in a retrograde manner. This needle was left in place throughout the experiment and isotonic saline, with or without prostaglandin was infused continuously into the renal artery at the rate of 0.375 ml/min. Animals with multiple left renal arteries were not utilized.

Crystalline PGE_1 (7.6 mg) kindly supplied by Dr J.E. Pike, UpJohn Company, Kalamazoo, Michigan, was mixed in 47 ml normal saline and 3 ml absolute ethanol were added to bring the crystals into solution. The stock solution was stored at 4° Centigrade and dilutions made in normal saline to permit an infusion rate of 2µg/min when infused directly into the left renal artery at .375 ml/min.

Renal plasma flow and glomerular filtration rate were calculated from the clearance of para-aminohippurate (PAH) and [^{125}I] iothalmate respectively, infused into the left femoral vein at the rate 1 ml/min. The infusion of PAH produced plasma levels of approximately 3 mg/100 ml.

The animals were allowed to equilibrate for approximately one hour before commencing the studies. Four groups of animals were studied in the following manner:

Group I (bicarbonate infusion in the intact dog). A solution of 0.9 molar sodium bicarbonate was systemically infused into 8 animals at 1 ml/min. After an equilibration period of approximately

60 minutes, 2 to 4 control clearance periods were obtained with infusion of normal saline into the left renal artery at 0.375 ml/min. PGE_1 was then infused into the left renal artery at 2μg/min and three to four experimental periods obtained. The PGE_1 infusion was then discontinued and a normal saline infusion resumed and 2 to 3 post experimental periods obtained. All clearance periods were ten minutes in duration.

Group II (bicarbonate infusion in adrenalectomized dogs). This group of 6 dogs was studied as was the first group except that the animals had previously been subjected to bilateral adrenalectomy. Complete adrenalectomy was assured by the development of hyperkalemia and metabolic acidosis. Studies were performed two weeks after surgery with maintenance therapy consisteing of 0.5 mg of desoxycorticosterone acetate (DOCA) and 0.75 mg of dexamethasone injected intramuscularly daily. DOCA was withheld for 72 hours prior to the study. We have previously shown that this procedure results in selective aldosterone deficiency.

Group III (glucose infusion in the intact dog). This group of 6 dogs was studied exactly as outlined for group one except that the animals received a systemic infusion of 20% glucose at 3 ml/min.

Group IV (phosphate excretion after thyroparathyroidectomy). The effect of PGE_1 on phosphate excretion was studied in 7 dogs. Thyroparathyroidectomy was performed 72 hours prior to each study. This was done to obviate any effect of parathyroid hormone on phosphate excretion. Animals were studied in the hydropenic state receiving the combined infusion of PAH and [125^I]iothalamate at 1 ml/min. During the infusion of PGE_1, urinary losses were replaced with equal volumes of 1/4 normal saline. Collection periods were 60 minutes in length. Input and output records were maintained during the study and weights recorded before and after each study. These parameters demonstrated no volume expansion in the animals.

The methods used to measure GFR, RPF, sodium, potassium, chloride, PCO_2 Ph and glucose in the blood and urine were identical to those previously described as were the calculations and statistical analyses. Plasma and urine PO_4 were measured according to the method of Fiske and Subbarow, adapted to the Autoanalyzer.

RESULTS

Group I - The effect of PGE_1 on renal bicarbonate reabsorption during control and experimental periods is shown in Table I and Table II respectively. Mean bicarbonate reabsorption during the control period in the non-infused kidney was 26.5 ± 0.31 mEq/L GFR and was 27.5 ± 0.38 during the experimental periods. The values are not statistically significantly different. The mean bicarbonate

TABLE I

SYSTEMIC INFUSION OF 0.5M $NaHCO_3$ AT 1.0 ml/min; INFUSION OF PAH_1 (Naphuline) INTO LEFT RENAL ARTERY

	LEFT KIDNEY (INFUSED SIDE)								RIGHT KIDNEY (NON-INFUSED SIDE)							
CONTROL	FLOW ml/min	GFR ml/min	RPF ml/min	FF %	$P_{HCO_3^-}$ mEq/L	$\frac{C_{Na}}{GFR} \times 100$ %	$\frac{C_{Cl}}{GFR} \times 100$ %	$T_{HCO_3^-}$ mEq/L GFR	FLOW ml/min	GFR ml/min	RPF ml/min	FF %	$P_{HCO_3^-}$ mEq/L	$\frac{C_{Na}}{GFR} \times 100$ %	$\frac{C_{Cl}}{GFR} \times 100$ %	$T_{HCO_3^-}$ mEq/L GFR
1	1.22	32.7	76.4	0.43	38.1	5.4	0.5	31.4	1.74	53.3	79.4	0.67	38.1	4.7	0.4	31.1
2	1.05	37.0	74.1	0.50	36.3	4.4	0.2	30.9	1.80	48.3	72.1	0.55	36.5	6.8	1.0	28.8
3	1.25	33.9	71.9	0.47	31.7	5.1	0.7	26.8	1.80	52.6	75.0	0.70	31.7	3.4	0.2	27.2
4	1.40	28.2	86.7	0.33	31.0	6.0	0.9	24.9	1.20	31.0	84.3	0.36	31.0	5.2	0.4	25.8
5	1.09	21.8	76.8	0.29	25.6	6.8	2.2	23 1	0.60	23.5	77.8	0.30	29.6	4.7	0.1	23.5
6	0.95	25.7	78.8	0.36	31.0	3.6	0.5	27.4	0.37	30.7	85.6	0.38	31.0	3.7	0.1	27.1
7	0.88	17.4	54.9	0.32	30.8	6.6	2.2	22.7	0.84	19.7	55.4	0.36	30.8	6.6	1.3	20.9
8	0.45	23.3	83.8	0.29	33.3	3.3	0.2	28.9	0.45	23.2	88.1	0.29	33.3	3.8	0.1	28.3
MEAN	0.97	27.5	76.4	±0.37	32.8	5.2	0.9	26.8	0.98	33.5	79.0	0.45	32.8	4.9	0.5	26.5
STD DEV	±0.34	±6.7	99.6	±0.07	± 3.0	±1.3	±0.8	± 0.34	±0.51	±13.5	± 9.7	±0.16	± 3.0	±1.3	±0.5	± 0.31

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TABLE II

SYSTEMIC INFUSION 0.9% $NaHCO_3$ AT 1.0 ml/min; INFUSION PGE_1 (10_{-6}g/min) INTO LEFT RENAL ARTERY

	LEFT KIDNEY (INFUSED SIDE)								RIGHT KIDNEY (NON-INFUSED SIDE)							
EXPERIMENTAL	FLOW ml/min	GFR ml/min	RPF ml/min	FF %	P_{HCO_3} mEq/L	$C_{\frac{U_{HCO_3}V}{P_{HCO_3}}}$ GFR %	$C_{\frac{U_{CL}V}{P_{CL}}}$ GFR %	T_{HCO_3} mEq/L GFR	FLOW ml/min	GFR ml/min	RPF ml/min	FF %	P_{HCO_3} mEq/L	$C_{\frac{U_{HCO_3}V}{P_{HCO_3}}}$ GFR %	$C_{\frac{U_{CL}V}{P_{CL}}}$ GFR %	T_{HCO_3} mEq/L GFR
1	2.95	33.6	82.6	0.41	44.9	8.3	3.0	35.0	1.70	38.6	88.3	0.44	44.9	6.3	0.4	36.0
2	2.70	43.2	85.1	0.51	35.6	6.8	2.9	28.2	1.68	29.2	69.3	0.42	35.6	9.7	1.2	23.5
3	3.88	48.1	104.8	0.38	30.9	9.2	5.0	21.5	1.11	44.4	79.6	0.56	30.9	4.0	0.1	25.2
4	4.25	30.1	128.4	0.25	31.8	12.3	7.4	23.4	0.95	31.9	87.5	0.36	31.8	4.0	0.3	27.5
5	2.88	26.3	109.7	0.24	31.8	12.3	8.4	23.9	0.55	25.3	58.7	0.43	31.8	4.1	0.1	26.7
6	1.46	23.5	88.1	0.27	32.5	7.3	4.5	28.2	0.34	34.4	69.1	0.35	32.5	3.8	0.1	29.8
7	1.35	17.6	46.4	0.38	30.8	9.2	6.7	23.8	0.35	19.5	73.5	0.27	30.8	3.7	0.1	25.4
8	0.51	25.9	121.7	0.92	31.0	6.4	2.7	25.5	0.51	23.4	104.7	0.22	32.5	4.1	0.1	26.3
MEAN	2.63	29.8	94.9	0.33	33.7	9.0	5.1	26.2	0.89	29.6	77.8	0.39	33.9	5.0	0.31	27.5
STD DEV	±1.26	± 8.75	±24.9	±0.10	± 4.68	±2.3	±2.2	±4.3	±0.54	± 8.4	±13.9	±0.10	± 4.7	±2.1	±0.38	±3.8
L vs R	P<0.01	n.s.	P<0.10	n.s.	n.s.	P<0.02	P<0.001	n.s.								

reabsorption in the infused kidney during the control period was 26.2 ± 0.43 mEq/L GFR and was 26.8 ± 0.34 mEq/L GFR during the infusion of PGE_1. These values are also not significantly different. Urine flow rate, glomerular filtration rate, renal plasma flow, fractional sodium excretion, and fractional chloride excretion did not change significantly in the non-infused kidney. In the infused kidney, mean urinary flow rate during the control period was 0.97 ± 0.34 ml/min and increased to 2.43 ± 1.26 ml/min with the infusion of PGE_1. No significant difference in glomerular filtration rate or renal plasma flow was noted with the infusion of PGE_1. Fractional sodium and chloride excretion increased on the infused side with a mean fractional sodium excretion of 5.2 ± 1.3% and fractional chloride excretion 0.9 ± 0.8% during the control period which increased to 9.0 ± 2.3% and 5.1 ± 2.2% respectively during the experimental period. These studies suggest that the infusion of subdepressor doses of PGE_1 results in an increased urine flow rate and increased excretion of sodium chloride with no significant change in bicarbonate reabsorption, and therefore suggest that PGE_1 has no effect on proximal tubular reabsorption of bicarbonate.

Since bicarbonate reabsorption also takes place in the distal convuluted tubule further studies were indicated to determine the contribution of the distal tubule, if any, to the bicarbonate reabsorptive rates as measured by the clearance technique in Group I.

Group II - The effect of the infusion of 2 μg/min of PGE_1 into the left renal artery on bicarbonate reabsorption in aldosterone deficient dogs was also measured. The data from control and experimental periods is presented in Table III and Table IV respectively. Mean bicarbonate reabsorption, fractional sodium and chloride excretion and flow rates did not change in the non-infused kidney with the infusion of PGE_1. Mean bicarbonate reabsorption in the infused kidney during the control period was 24.1 ± 4.2 mEq/L GFR and decreased to 22.48 ± 5.5 mEq/L GFR with the infusion of PGE_1. Compared to the right control kidney this decrease is significant ($p<0.01$). Urine flow rate, fractional sodium and chloride excretion increased significantly on the infused side with the infusion of PGE_1.

Previous studies from this laboratory have shown that aldosterone deficiency markedly impairs distal hydrogen ion secretion; thus this group of dogs was studied to ascertain if increased distal hydrogen ion secretion might be obscuring the effect of PGE_1 infusion on proximal hydrogen ion secretion. The infusion of PGE_1 resulted in a statistically significant decrease in bicarbonate reabsorption in the adrenalectomized group of animals and yet resulted in no change in bicarbonate reabsorption in the intact group of animals studied. These studies suggest that PGE_1 does depress bicarbonate reabsorption in the proximal tubule; however, the bicarbonate leaving the proximal tubule is then reabsorbed distally thus accounting for no significant net change in bicarbonate excretion in the intact animals. Only

TABLE III

SYSTEMIC INFUSION OF 0.9% $NaHCO_3$ AT 1.0 ml/MIN IN ADRENALECTOMIZED DOGS. INFUSION OF PGE_1 (2μg/ml) INTO LEFT RENAL ARTERY

	LEFT KIDNEY (INFUSED SIDE)								RIGHT KIDNEY (NON-INFUSED SIDE)							
CONTROL	FLOW ml/min	GFR ml/min	RPF ml/min	FF %	P_{HCO_3} mEq/L	$C_{HCO_3}/100$ GFR %	$C_{CH_2O}/100$ GFR %	T_{HCO_3} mEq/L GFR	FLOW ml/min	GFR ml/min	RPF ml/min	FF %	P_{HCO_3} mEq/L	$C_{HCO_3}/100$ GFR %	$C_{CH_2O}/100$ GFR %	T_{HCO_3} mEq/L GFR
1	0.96	10.6	25.2	0.42	32.2	10.0	4.4	22.2	0.82	12.5	26.2	0.48	32.2	8.8	2.8	23.0
2	0.36	15.2	31.6	0.48	35.5	4.9	0.5	30.1	0.31	16.1	32.7	0.49	35.5	5.3	0.4	30.0
3	2.60	26.5	67.6	0.39	30.2	13.1	10.7	20.7	0.75	20.6	56.2	0.31	30.2	5.6	2.7	22.2
4	0.39	21.2	66.3	0.32	35.5	6.6	0.8	28.7	0.61	21.1	65.9	0.32	35.5	6.1	0.4	29.2
5	0.67	16.7	65.5	0.32	26.2	5.8	2.0	20.6	0.67	16.7	61.5	0.35	26.2	5.8	2.0	20.6
6	0.58	15.1	64.3	0.34	30.3	6.9	0.2	22.5	0.54	15.9	51.7	0.31	30.3	6.2	0.2	22.5
MEAN	0.98	17.2	66.8	0.38	31.7	8.0	3.1	24.1	0.62	16.5	65.6	0.38	31.7	6.30	1.6	24.58
STD DEV	±0.82	± 5.7	±1.6	±0.06	±3.6	± 3.2	±4.0	± 4.2	±0.18	± 3.5	±16.7	±0.07	± 3.6	± 1.27	±1.2	±39.8

TABLE IV

SYSTEMIC INFUSION OF 0.5M $NaHCO_3$ AT 1.0 ml/min IN ADRENALECTOMIZED DOGS; INFUSION OF PGE_1 (2µg/min) INTO LEFT RENAL ARTERY

	LEFT KIDNEY (INFUSED SIDE)								RIGHT KIDNEY (NON-INFUSED SIDE)							
EXPERIMENTAL	FLOW ml/min	GFR ml/min	RPF ml/min	FF %	P_{HCO_3} mEq/L	$\frac{C_{HCO_3}}{GFR}$ %	$\frac{C_{CH_2O}}{GFR}$ %	T_{HCO_3} mEq/L GFR	FLOW ml/min	GFR ml/min	RPF ml/min	FF %	P_{HCO_3} mEq/L	$\frac{C_{HCO_3}}{GFR}$ %	$\frac{C_{CH_2O}}{GFR}$ %	T_{HCO_3} mEq/L GFR
1	1.45	8.3	31.7	0.26	32.7	17.4	9.3	18.8	0.52	12.1	22.3	0.54	32.7	6.5	1.1	24.8
2	1.48	17.7	66.4	0.27	36.5	7.5	6.7	31.2	0.31	14.1	22.7	0.63	36.5	4.2	0.1	32.6
3	5.45	28.5	78.9	0.36	30.9	20.9	20.3	19.6	0.50	18.0	47.6	0.38	30.9	4.7	1.3	25.8
4	1.97	23.1	75.8	0.35	35.7	10.7	6.5	26.6	0.61	21.9	58.4	0.38	35.7	5.7	0.2	29.2
5	1.33	18.8	58.9	0.32	28.6	7.2	3.7	22.3	0.67	20.1	53.3	0.38	28.6	4.7	1.2	22.9
6	1.52	16.4	53.5	0.31	27.4	10.8	6.6	16.4	0.48	14.6	46.3	0.32	27.4	5.2	0.1	20.7
MEAN	2.20	18.8	60.7	0.31	31.9	12.4	8.5	22.48	0.50	16.8	41.6	0.40	31.9	5.2	0.7	26.0
STD DEV	±1.60	± 6.8	±17.2	±0.04	± 3.8	± 5.6	±6.1	± 5.51	±0.13	± 3.8	±15.5	±0.08	± 3.8	±0.8	±0.6	± 4.3
L vs R	p<0.05	N.S.	p<0.05	N.S	N.S	p<0.02	p<0.05	p<0.01								

with selective aldosterone deficiency and impaired distal hydrogen ion secretion was this proximal effect in bicarbonate reabsorption noted.

Group III - The effect of PGE_1 on glucose reabsorption was also studied in a group of six dogs. The data obtained from the studies during control and experimental periods is presented in Tables V and VI respectively. No change in glucose reabsorption was noted in the non-infused kidney with a mean control value of 2.38 and 0.42 mg/ml GFR and 2.33 ± 0.56 mg/ml GFR during the infusion of PGE_1. The urine flow rate, glomerular filtration rate, renal plasma flow, fractional sodium excretion did not differ significantly between the control and experimental periods in the non-infused kidney. Glucose reabsorption, however, decreased in the infused kidney during the infusion of PGE_1 with a mean control glucose reabsorptive rate of 2.36 ± 0.45 mg/ml GFR which decreased to 1.92 ± 0.58 mg/ml GFR during the experimental period. Again urine flow rate, fractional sodium excretion and fractional chloride excretion increased significantly in the infused kidney with the infusion of PGE_1.

Group IV - The effect of PGE_1 on phosphate reabsorption was also studied in dogs 72 hours after thyroparathyroidectomy. The results of these studies during control and experimental periods are presented in Tables VII and VIII respectively.

No significant change in fractional phosphate excretion was noted in the non-infused kidney with the infusion of PGE_1 with mean values of 2.85 ± 2.20 during the control period and 2.54 ± 2.91 PGE_1 infusion. Urine flow rate, glomerular filtration rate, renal plasma flow, fractional sodium and chloride excretion also did not change significantly. Phosphate excretion in the infused kidney increased from a mean control value of 2.35 ± 1.57 to 13.41 ± 11.72 ($p<0.02$) during the infusion of PGE_1. No significant changes in fractional calcium excretion were noted with the infusion of PGE_1. As previously noted urine volume and fractional sodium excretion did increase. The changes in phosphate excretion are not more highly significant and this may be attributed to the small number of animals studied to date and a somewhat blunted response due to volume contraction. More animals will be studied with the infusion of 0.5% normal saline to prevent severe volume contraction or volume expansion.

DISCUSSION:

The results of this study are in general agreement with data presented by other investigators. The infusion of comparable doses of PGE_1 into the renal artery have been shown to result in the

TABLE V

SYSTEMIC INFUSION OF 20% GLUCOSE AT 3 ml/MIN; INFUSION OF PGE_1 (2μg/MIN) INTO LEFT RENAL ARTERY

	LEFT SIDE (INFUSED SIDE)							RIGHT SIDE (NON-INFUSED SIDE)						
CONTROL	FLOW ml/min	GFR ml/min	RPF ml/min	FF %	$\frac{C_{Na}}{GFR} \times 100$ %	$\frac{C_{Cl}}{GFR} \times 100$ %	$T_{GLUCOSE}$ mg/ml GFR	FLOW ml/min	GFR ml/min	RPF ml/min	FF %	$\frac{C_{Na}}{GFR} \times 100$ %	$\frac{C_{Cl}}{GFR} \times 100$ %	$T_{GLUCOSE}$ mg/ml GFR
1	0.97	24.7	52.6	0.76	3.0	3.8	2.55	0.37	21.7	30.4	0.71	0.1	0.1	2.45
2	0.27	32.2	50.8	0.63	0.1	0.1	2.53	0.27	31.2	58.2	0.54	0.1	0.1	2.50
3	0.95	30.3	48.6	0.62	0.6	0.8	2.58	0.80	29.5	43.2	0.68	0.2	0.3	2.63
4	1.30	20.7	84.4	0.34	2.2	4.9	2.11	1.05	29.2	84.8	0.34	1.4	1.5	2.04
5	1.00	27.9	70.2	0.40	0.7	2.4	1.66	1.00	27.8	55.5	0.50	0.4	0.6	1.73
6	1.30	20.2	52.0	0.39	1.2	1.2	3.11	1.10	26.4	77.0	0.34	0.3	0.3	3.01
7	0.73	25.5	35.3	0.72	0.8	0.8	2.30	0.53	24.10	24.7	0.97	0.1	0.0	2.50
MEAN	0.93	27.2	56.1	0.47	0.84	1.4	2.36	0.82	27.8	53.6	0.52	0.4	0.5	2.38
STD DEV	±0.35	±3.9	±16.1	±0.15	±0.69	±1.4	±0.45	±0.31	±2.8	±21.0	±0.25	±0.5	±0.5	±0.42

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TABLE VI

SYSTEMIC INFUSION OF 20% GLUCOSE AT 3 ml/MIN; INFUSION OF PGE_1 (2µg/MIN) INTO LEFT RENAL ARTERY

	LEFT KIDNEY (INFUSED SIDE)							RIGHT KIDNEY (NON-INFUSED SIDE)						
EXPERIMENTAL	FLOW ml/min	GFR ml/min	RPF ml/min	FF %	$\frac{C_{Na}}{GFR} \times 100$ %	$\frac{C_{Cl}}{GFR} \times 100$ %	$T_{Glucose}$ mg/ml GFR	FLOW ml/min	GFR ml/min	RPF ml/min	FF %	$\frac{C_{Na}}{GFR} \times 100$ %	$\frac{C_{Cl}}{GFR} \times 100$ %	$T_{Glucose}$ mg/ml GFR
1	2.47	23.8	45.4	0.52	7.5	9.8	1.76	0.46	10.5	44.5	0.45	0.1	0.1	2.36
2	1.20	29.7	74.1	0.63	2.4	3.4	1.65	0.38	25.5	42.3	0.60	0.1	0.1	1.98
3	3.90	33.3	78.0	0.43	6.7	8.8	2.34	0.95	29.3	37.2	0.78	0.1	0.2	2.70
4	2.30	27.6	86.6	0.32	4.8	5.4	2.16	1.00	27.7	66.7	0.42	0.9	0.9	2.39
5	3.60	27.2	94.2	0.29	4.8	2.2	1.38	1.00	24.8	50.9	0.47	0.1	0.2	1.98
6	7.50	21.9	87.5	0.25	4.5	5.0	2.73	0.9	22.2	49.5	0.45	0.3	0.3	3.19
7	1.87	26.1	74.8	0.35	3.1	4.1	2.09	0.67	24.8	38.8	0.63	0.0	0.0	2.48
MEAN	2.55	27.0	90.0	0.32	4.5	5.5	1.92	0.87	24.9	47.0	0.51	0.23	0.4	2.33
STD DEV	±0.94	± 3.8	±19.9	±0.08	±1.1	±2.6	±0.58	±0.32	±2.8	±10.2	±0.15	±0.2	±0.5	±0.56
L vs R	p<0.001	p<0.05	p<0.001	p>.01	p<0.001	p<0.001	p<0.001							

TABLE VII

HYDROPENIC TETRAHYDROAMINOBENZOCYCLOHEPTENE 200 MG. RENAL SALINE INFUSION IN LEFT RENAL ARTERY 0.375cc/MIN

	LEFT KIDNEY (INFUSED SIDE)						RIGHT KIDNEY (NON-INFUSED SIDE)					
CONTROL	FLOW ml/min	GFR ml/min	RPF ml/min	$\frac{C_{Na}}{GFR} \times 100$ %	$\frac{C_{PD_4}}{GFR} \times 100$ %	$\frac{C_{Cr^{51}}}{GFR} \times 100$ %	FLOW ml/min	GFR ml/min	RPF ml/min	$\frac{C_{Na}}{GFR} \times 100$ %	$\frac{C_{PD_4}}{GFR} \times 100$ %	$\frac{C_{Cr^{51}}}{GFR} \times 100$ %
1	0.07	11.68	25.9	0.59	3.04	0.50	0.04	11.60	17.4	0.27	3.79	0.22
2	0.07	26.68	83.5	0.07	0.07	0.83	0.07	26.89	86.1	0.07	0.06	0.73
3	0.11	26.74	35.8	0.33	4.66	2.72	0.08	40.72	60.5	0.12	6.54	1.22
4	0.08	34.90	61.6	0.26	2.94	1.60	0.07	37.98	64.3	0.26	2.82	1.31
5.	0.10	42.75	50.8	0.21	1.91	0.84	0.10	43.34	45.3	0.20	1.51	0.69
6	0.05	15.77	32.8	0.22	1.50	1.14	0.05	19.75	42.0	0.10	2.40	0.71
MEAN	0.08	26.42	48.4	0.28	2.35	1.27	0.07	30.03	52.6	0.17	2.85	0.81
STD DEV	±0.02	±11.52	±21.5	±0.17	±1.57	±0.80	±0.02	±2.71	23.4	±0.09	±2.20	±0.4

TABLE VIII

HYDROPENIC THYROPARATHYROIDECTOMIZED DOG. PGE_1 (2 µg/MIN) INFUSION IN LEFT RENAL ARTERY

	LEFT KIDNEY (INFUSED SIDE)						RIGHT KIDNEY (NON-INFUSED SIDE)					
EXPERIMENTAL	FLOW ml/min	GFR ml/min	RPF ml/min	$\frac{C_{Na}}{GFR} \times 100$ %	$\frac{C_{PAH}}{GFR} \times 100$ %	$\frac{C_{Cr^{++}}}{GFR} \times 100$ %	FLOW ml/min	GFR ml/min	RPF ml/min	$\frac{C_{Na}}{GFR} \times 100$ %	$\frac{C_{PAH}}{GFR} \times 100$ %	$\frac{C_{Cr^{++}}}{GFR} \times 100$ %
1	0.64	10.35	30.6	3.78	21.17	1.98	0.04	10.34	14.2	0.17	2.27	0.19
2	0.42	34.53	72.8	0.50	0.18	3.67	0.06	27.63	67.9	0.02	0.08	0.39
3	0.38	35.88	38.2	1.05	12.38	7.72	0.08	39.87	32.6	0.08	0.21	1.02
4	0.97	38.78	34.1	2.09	32.49	17.09	0.04	41.2	46.2	0.17	1.97	0.09
5	0.24	48.66	56.7	0.36	9.03	1.79	0.08	46.88	46.1	0.02	0.72	0.46
6	0.24	21.44	34.8	0.40	5.21	3.49	0.06	21.53	22.9	0.03	1.97	0.65
MEAN	0.48	31.61	44.5	1.43	13.41	5.96	0.06	31.24	36.3	0.08	2.54	0.60
STD DEV	±0.20	±13.60	±16.6	±1.31	±11.72	±5.85	±0.01	±13.87	±18.9	±0.07	±2.91	±0.31
	< 0.07	NS	< 0.07	< 0.05	<0.05	NS						

following: (1) an increase in urine flow, (2) a increase in sodium excretion, (3) a variable rise in renal plasma flow, (4) no significant change in glomerular filtration rate or systemic arterial blood pressure (Martinez-Maldonado M, Tsaparas N, Eknoyan G and Suki WN. Am J Physiol 222: 1147-1152, 1972;[10] Vander AJ. Am J Physiol 214: 218-221, 1968;[11] Carriere S, Friborg J, and Guay JP. Am J Physiol 221: 92-98, 1971;[12] Johnston HH, Herzog JP, and Lavler DP. Am J Physiol 213: 939-946. 1967;[13] Berl T and Schrier RW. J. Clin Invest 52: 463-471, 1973)[14]. In addition previous investigators have also noted an increase in free water excretion (C_{H_2O}) and a decrease in free water reabsorption (T_{CH_2O}) (Martinez-Maldonado M, Tsaparas N, Eknoyan G and Suki WN. Am J Physiol 222: 1147-1152, 1972;[10] Berl T and Schrier RW. J Clin Invest 52: 463-471, 1973)[14]

The intrarenal mechanism of action of PGE_1 is still poorly understood. Since PGE_1 is a vasodilator previous studies suggested that the changes induced by the infusion of PGE_1 might all be related to renal vasodilitation. Bresler(Bresler EH. Am Heart J 62: 1-6, 1961)[16] along with other investigators (Vander AJ. Am J Physiol 206: 492-498, 1964;[6] Earley LE, and Friedler RM J Clin Invest 44: 929-941;1965;[7] Earley LE and Friedler RM. J Clin Invest 45: 542-561, 1966)[8] has suggested that peritubular hydrostatic and oncotic forces influence sodium reabsorption in the proximal convuluted tubule and that the changes induced by vasodilitation may depress sodium reabsorption. Our studies as well as those of previous investigators have shown a slight increase in renal plasma flow without a change in glomerular filtration rate suggesting a decrease in oncotic pressure and increased hydrostatic pressure in the peritubular capillaries. Earley and Friedler(Earley LE and Friedler RM. J Clin Invest 45: 542-561, 1966)[8] earlier proposed that the natriuresis resulting from vasodilitation might be related to increased medullary blood flow, thus reducing medullary hypertonicity and decreasing reabsorption of water from the decending limb of Henle's loop. This would overload the reabsorptive capacity in the ascending limb resulting in a natriuresis. Another mode by which vasodilitation might result in a natriuresis is based on the work of Rector, Seldin, and Brunner (Rector FC, Brunner FP, and Seldin DW. J Clin Invest 45: 590-602, 1966;[17] Rector FC, Brunner FP and Seldin DW. J Clin Invest 45: 603-611, 1966)[18] who have demonstrated that sodium and water reabsorption may be directly related to tubular diameter.

Martinez-Maldonado, et al(Martinez-Maldonado M, Tsaparas N, Eknoyan G and Suki WN. Am J Physiol 222: 1147-1152, 1972)[10] and Berl, et al (Berl T and Schrier RW: J. Clin Invest 52: 463-471, 1973)[14] have shown an increase in free water excretion and decreased free water reabsorption with the infusion of PGE_1. With the current knowledge of renal physiology of the function of the loop of Henle in the concentrating and diluting mechanism, our studies would suggest that the increased free water excretion is related to depressed sodium reabsorption in the proximal tubule due to a direct effect of

PGE_1 has an effect on sodium transport other than through hemodynamic alterations. The studies by Beri, et al(Beri T and Schrier RW. J. Clin Invest 52: 463-471, 1973)[14] showed no antagonistic action of PGE_1 to the effect of vasopressin thus suggesting no decreased water reabsorption in the distal tubule or collecting ducts.

Since recent studies by Arendshorst, et al(Arendshorst WJ, Johnston PA, and Selkurt EE. Am J Physiol 226: 218-225, 1974)[15] were unable to document marked redistribution of plasma flow to the superfical cortex or to the inner medullar with the infusion of PGE_1 or volume expansion, this makes a specific tubular effect of PGE_1 on sodium reabsorption more likely.

Our studies suggest that the infusion of 2 μg/min of PGE_1 into the renal artery of a dog results in a natriuresis and increased urinary flow rate related to depressed sodium reabsorption in the proximal tubule. Our data, however, are inadequate to explain the specific mechanism involved. It appears as if the proximal tubule is involved since there is a significant ($p<0.001$) decrease in glucose and phosphate reabsorption without a change in the reabsorptive capacities of these substances in the control kidney. Since bicarbonate reabsorption takes place in the distal convuluted tubule as well as the proximal convuluted tubule, studies were performed in both the intact and adrenalectomized animals. No change in bicarbonate reabsorption in the intact animals was noted; however, the possibility that a depressive effect on proximal bicarbonate reabsorption was being masked by coinciding stimulatory effect on distal bicarbonate reabsorption had to be explored. Aldosterone deficiency results in a selective inhibition of distal hydrogen ion secretion, (Beri T and Schrier RW. J. Clin Invest 52: 463-471. 1973)[14] thus if increased distal bicarbonate reabsorption were masking a proximal effect, aldosterone deficiency should uncover such an effect. Indeed when the aldosterone deficient animals were studied, a significant ($p<0.01$) decrease in bicarbonate reabsorption was demonstrated. Although previous studies have suggested a specific tubular effect of PGE_1 on sodium reabsorption, such an effect had not been clearly demonstrated prior to these studies. This effect, however, is small and its role in the overall physiologic function of the prostaglandins has yet to be explained.

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PRESENTATIONS AND/OR PUBLICATIONS:

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
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3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. SMRY'S INSTR'S	9. SPECIFIC DATA-CONTRACTOR ACCESS	10. LEVEL OF SUM
73 07 01	D. CHANGE	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

11. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
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c. CONTRIBUTING				

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23. KEYWORDS (Provide RAER and Security Classification Code): (U) Hypertension; (U) Renal artery stenosis; (U) Sodium; (U) Renin-angiotensin; (U) Dogs

24. TECHNICAL OBJECTIVE. 25. APPROACH. 26. PROGRESS (Furnish individual paragraphs identified by number preceded text of each DoD Security Classification Code.)

23. (U) to define the relationship of sodium balance to the renin angiotensin system in unilateral and bilateral renal artery stenosis in the dog with respect to similar problems in the hypertensive soldier.

24. (U) Moderate renal artery stenoses (50%) were created in two dogs and severe stenoses (90%) created in the other two animals. After a control period, unilateral stenoses were created followed by a 14-day equilibration period, then comparable stenoses created on the other side followed by a 14-day equilibration period, and finally the right kidney was removed and the animals followed for 14 days. Sodium balance, measurement of peripheral plasma renin activity, weights, glomerular filtration rates and direct measurement of blood pressure were obtained serially throughout the study.

25. (U) 73 07 - 74 06 The studies are incomplete at the time of this report and more animals will have to be studied. Unilateral moderate stenoses resulted in moderate hypertension associated with marked elevation of plasma renin activity and a progressive negative sodium balance which persists for approximately six days with gradual return to normal. Unilateral, severe stenosis is associated by an even more marked hypertension and increase in plasma renin activity and a more negative sodium balance. The hypertension persists for a longer period of time. During the period of bilateral stenoses the blood pressure and plasma renin activity rise acutely followed by a gradual return to normal of the plasma renin acitivity with persistent hypertension. The same trend is noted in the third study period after right nephrectomy. These studies suggest that hypertension is initiated on a pressor basis via renin-angiotensin-aldosterone and may be maintained by a pressor or volume mechanism.

*Available to contractors upon originator's approval

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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

ABSTRACT

PROJECT NO. 3A161102B71R-01, RESEARCH IN BIOMEDICAL SCIENCES

PROJECT TITLE: RELATIONSHIP OF SODIUM BALANCE TO PLASMA RENIN CONCENTRATION IN TROOPS WITH RENOVASCULAR HYPERTENSION: A CANINE MODEL

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Period covered in this report: 1 July 1973 - 30 June 1974

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Hypertension of renal etiology may be related to increased levels of angiotensin II or secondary to extracellular fluid volume expansion with renal parenchymal disease. The current studies were undertaken to determine the relationship of plasma renin activity, sodium balance, and glomerular filtration rate to hypertension resulting from acute and chronic unilateral and bilateral renal artery stenosis in the conscious dog. Animals with approximately 50% renal artery occlusion (moderate stenosis) and approximately 90% occlusion (severe stenosis) were studied after unilateral renal artery stenosis, bilateral renal artery stenosis, and after removal of the right kidney leaving the left kidney supplied by 2 stenosed vessels in place.

Unilateral renal artery stenosis results in transient hypertension with markedly elevated plasma renin activity and a negative sodium balance. The changes are more severe in the group of animals with bilateral renal artery stenosis. Moderate stenosis produces an initial rise in plasma renin activity, and a transient period of negative sodium balance followed by persistent hypertension with normal or low plasma renin activity and a persistently positive sodium balance. This again is more marked in the group with severe renal artery stenosis. Nephrology in the animals with moderate vessel stenosis resulted in transient increase in plasma renin activity, mean arterial blood pressure, and no real change in glomerular filtration rate. Plasma renin activity and mean arterial blood pressure gradually returned to normal. The animals with the severe renal artery stenosis and right nephrectomy had acute reduction in glomerular filtration

rate with a markedly positive sodium balance and persistent elevation of the mean arterial blood pressure. Plasma renin activity was modestly elevated immediately after nephrectomy and returned to normal levels.

Although the data from this study are incomplete and the numbers of animals are insignificant at the present time, important observations have been made. These studies would suggest that renal ischemia initially causes hypertension by generation of renin and angiotensin II, sodium retention occurs and extracellular fluid volume is expanded. In the absence of the contralateral kidney, extracellular fluid volume remains expanded suppresses renin release from the ischemic kidney and in all probability maintains hypertension. In the presence of the contralateral kidney, the sodium load reabsorbed by the ischemic kidney is readily excreted by the contralateral kidney with a slightly decreased plasma volume and hypertension persists on a pressor basis.

Hypertension
Sodium
Dogs
Renal artery stenosis
Renin-angiotensin

RELATIONSHIP OF SODIUM BALANCE TO PLASMA RENIN CONCENTRATION IN TROOPS WITH RENOVASCULAR HYPERTENSION: A CANINE MODEL

Interference with the blood supply to one kidney in humans and experimental animals results in hypertension. This form of hypertension is characterized by renin release by the involved kidney. The exact mechanisms responsible for this form of hypertension, however, are not entirely clear but are most likely related to increased formation of the potent pressor agent angiotensin II. This unilateral stenosis leads to impaired excretion of sodium by the proximal tubule in the contralateral kidney due to the increased blood pressure thus resulting in an overall negative sodium balance and persistence of high angiotensin II levels. If, however, the contralateral kidney has been removed, there is no mechanism present to excrete the retained sodium. This results in a positive sodium balance and increased blood volume possibly supressing plasma renin activity and angiotensin II. This has been shown in recent studies by Swales and co-workers (Swales JD, Thurston H, Queiroz FP, and Medina A: J Lab Clin Med 80: 539-547, 1972)[1]. Studies by Koletsky and co-workers (Koletsky S, Pavlicko KM, Rivera-Velez JM: Lab Invest 24: 41-44, 1971)[2] have shown decreased juxtaglomerular granulation and renin content with normal plasma renin and aldosterone secretion in the rat with clip hypertension and contralateral nephrectomy. Studies by Regoli and co-worker (Regoli D, Brunner H, Peters G, and Gross F: Proc Soc Exp Biol 109: 142-145, 1962)[3] have measured renal renin concentration in normal rats and with rats with unilateral and bilateral renal artery stenoses, have shown increased renin concentration in the kidney with unilateral renal artery stenoses depending upon the severity of the stenoses. The data from all of these studies suggest that hypertension from unilateral renal artery stenosis is directly related to the elevated renin concentrations (pressor hypertension) and the hypertension from bilateral renal artery stenoses may be related to retention of sodium and water (volume hypertension).

Since there appears to be some relationship between hypertension on a pressor basis and that related to volume, the current studies were undertaken to determine the relationship of plasma renin activity, sodium balance, and glomerular filtration rate to hypertension resulting from acute and chronic unilateral and bilateral renal artery stenosis in the conscious dog.

METHODS AND MATERIALS

Studies were conducted on conscious. trained, adult female purebred Beagle dogs weighing 9 to 15 kg. A carotid loop was created in each animal prior to study to allow access to the arterial circulation without exciting the animal. The dogs were maintained in stainless steel, metabolic cages and were fed a measured amount of Wayne Dog Chow and given an additional 5 mEq/kg of sodium in the form of a gelatin capsule. Two groups of animals were studied.

Group I consisted of dogs with approximately 50% occlusion (moderate stenosis) of the main renal artery while group II consisted of animals with approximately 90% occlusion (severe stenosis) of the main renal artery. The study periods consisted of a 7 day control period, stenosis of the right renal artery created on day 8 with a 14 day post operative study period, stenosis of the left renal artery performed on day 22 with another 14 day post operative study period, and right nephrectomy performed on day 35 with a 14 day post operative study period. The animals were then sacrificed.

Daily measurement of intake and output was recorded as well as daily weights. Daily serum and urinary electrolytes were obtained. Direct systemic arterial blood pressure measurements were made by the percutaneous insertion of a 21 gauge needle into the carotid loop attached to a Statham strain guage manometer. Blood pressures were measured daily or as frequenty as possible. Peripheral venous blood for plasma renin activity was collected before and after every surgical procedure, hourly to 8 hours after the surgical procedure, daily for the first three post operative days, then every other day. Plasma renin activity was determined by the radioimmunoassay technique as described by Haber(Haber E, Koerner T, Page LB, et al; J Clin Endo and Metab 24: 1349-1355, 1969)[4] and is well established in our laboratory (normal 1-3 ng/ml/hr).

The main renal arteries were approached through separate flank incisions and the stenosis of each vessel created with a silver clip.

After nephrectomy, the kidneys were perfused with saline, the vessels filled with hypaque, and silk ligatures placed around the most proximal portion of the vascular stump. Radiographs were taken to determine the degree of stenosis of each renal artery as well as to determine possible areas of infarction.

RESULTS

Group I (moderate renal artery stenosis). Shown in Figure 1 are the data from a representative dog with moderate renal artery

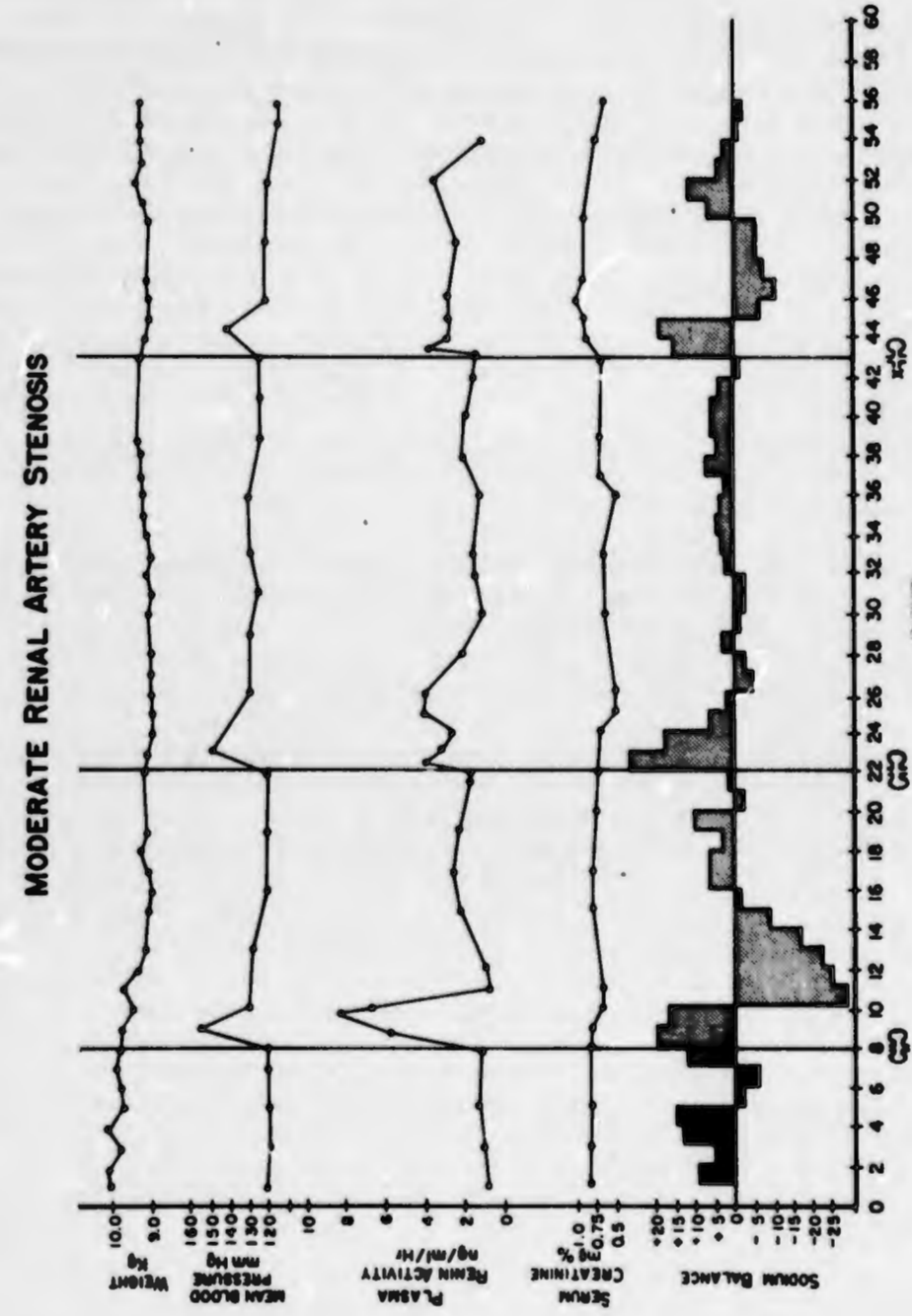
MODERATE RENAL ARTERY STENOSIS
WEIGHT kg
MEAN BLOOD PRESSURE mm Hg
PLASMA RENIN ACTIVITY ng/ml/Hr
SERUM CREATININE mg %
SODIUM BALANCE
DAYS

Figure I

stenoses. Right renal artery stenosis as performed on day 8 results in a sharp increase in plasma renin activity and mean systemic arterial blood pressure with avid sodium retention which persisted for approximately two days. There followed a rapid decline in plasma renin activity, increased sodium excretion and a return toward normal of the mean systemic arterial blood pressure. Stenosis of the left renal artery to approximately the same degree as that previously performed on the right side again results in an increase in plasma renin activity, but not as great as that noted with unilateral stenosis. There is also more avid sodium retention and an increase in blood pressure to the same level as with unilateral stenosis. The blood pressure again tends to return to near control blood pressure levels with slightly positive sodium balances. Acute reduction of total renal mass by right nephrectomy leaving the moderately stenosed kidney on the left side insitu results in a modest rise in plasma renin activity, transient increase in blood pressure persisting for two days, and a markedly positive sodium balance for the same length of time. Plasma renin activity gradually returns to normal as do blood pressure and sodium balances.

Group II (severe renal artery stenosis). Shown in figure 2 are the data from a representative animal with approximately 90% stenoses of the renal arteries. A creation of 90% stenosis of the right renal artery on day 9 results in a marked increase in plasma renin activity, increased mean arterial blood pressure, and a positive sodium balance that persists for approximately 3 days. As the plasma renin activity returns toward normal the mean arterial blood pressure also decreases but does not reach the control value of 120 mm Hg. The creation of a similar stenosis on the left side again results in an increase in plasma renin activity but not of the magnitude reached in the unilateral stenosis; a further increase in blood pressure, and a positive sodium balance. Five days after bilateral stenosis, the mean arterial blood pressure has stabilized at 155 mm Hg with a control value of 120 mm Hg and plasma renin activity has returned to a normal value of approximately 1.0 ng/ml/hr. Of interest is the daily positive sodium balance of approximately 20 mEq of sodium. Acute reduction of the renal mass by right nephrectomy on day 44 again resulted in a somewhat blunted plasma renin response but a definite increase in mean arterial blood pressure and a persistently positive sodium balance. This is of course associated with a marked increase in serum creatinine representing a marked reduction in renal function. The change in weight during this period has to be attributed to mild starvation.

The data shown in Figures 1 and 2 are representative of only 2 animals in each group and certainly don't indicate definitive conclusions at this time. More animals in each group will have to be studied before definitive conclusions can be made.

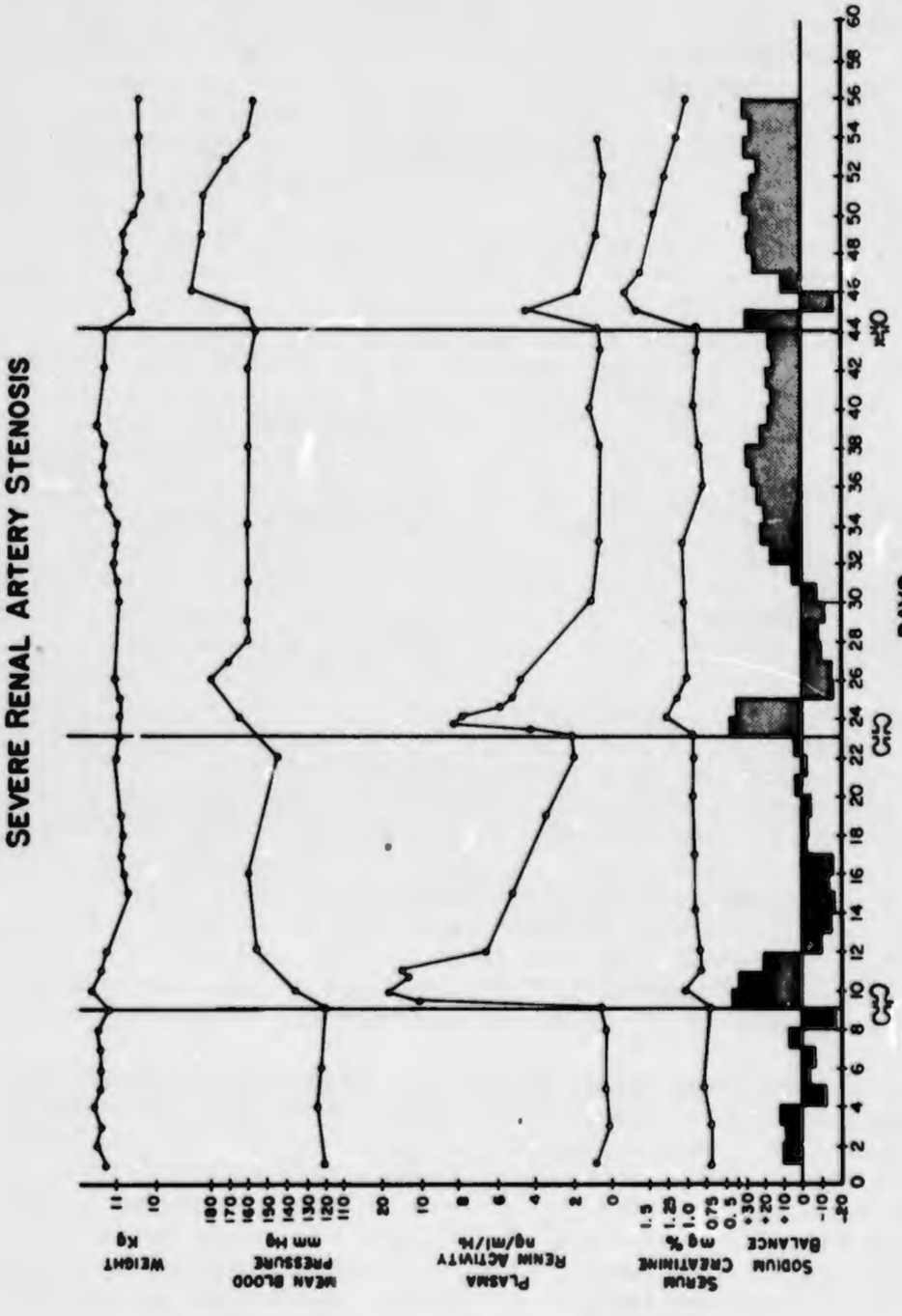
SEVERE RENAL ARTERY STENOSIS
WEIGHT kg
MEAN BLOOD PRESSURE mm Hg
PLASMA RENIN ACTIVITY ng/ml/hr
SERUM CREATININE mg %
SODIUM BALANCE
DAYS
CLIP
CLIP
OFF

Figure 2

DISCUSSION

Although the data from this study are incomplete and the numbers of animals are insignificant at the present time, important observations have been made. At the present stage of renal physiology, hypertension may be secondary to an increase in plasma volume due to excessive salt and water retention, or an increase in renin and angiotensin II, or possibly other substances such as that advocated by Grollman and Krishnamurty(Grollman A, and Krishnamurty VSR: Am J Physiol 221: 1499-1506, 1971)[5]. The pathogenesis of hypertension associated with unilateral renal vascular stenosis is probably related to increased secretion of renin by the ischemic kidney. This enzyme's action upon a circulating globulin substrate produces angiotensin I which is then converted in the lungs by a converting enzyme to the potent pressor angiotensin II. Although the stimulus to renin release remains debatable at the present time, it is strongly suggested that decreased renal arterial pressure is a stimulus controlling renin release from the juxtaglomerular apparatus. Injections of renin or angiotensin into animals results in hypertension, and clipping of the renal artery to produce unilateral renal artery stenosis in the rat is associated with elevated circulating plasma renin activity as well as persistent hypertension. The hypertension of end stage renal disease occasionally is due to increased renin secretion and responds to bilateral nephrectomy; however, in most instances the hypertension associated with end stage renal disease is related to expanded extracellular fluid volume and the hypertension responds dramatically to the removal of salt and water by hemodialysis. Plasma renin activity is normal or low in such patients. The presence of increased mineralocorticoid substances such as aldosterone and 18-hydroxy desoxycorticosterone also produce hypertension by salt retention and expansion of extracellular fluid volume. In such instances, plasma renin activity is suppressed by the expanded extracellular fluid volume. The studies by Grollman and Krishnamurty have not been reproducible therefore the presence of another pressor substance remains to be defined. Thus the most clearly defined mediators of hypertension of renal orgin are increased renin release and volume expansion secondary to impaired salt excretion.

The data from the animals with a moderate unilateral renal artery stenosis is in agreement with Goldblatt's original observations (Goldblatt H, Lynch J, Hanzel RF: J Exp Med 59: 347-379, 1934)[6]. Goldblatt attempted to simulate human arterionephrosclerosis feeling that many tiny vascular lesions in both kidneys may give rise to essential hypertension. He attempted to reduce circulation to both kidneys with clamps. After unilateral clamping, he observed a small, transient elevation in blood pressure and only after clamping of both renal arteries or removal of the contralateral kidney

did blood pressure rise significantly and remain significantly elevated. Our unilateral moderate renal artery stenosis data are in agreement with Goldblatt's observations; however, severe unilateral renal artery stenosis in our animals resulted in a significant and sustained hypertension. The response in plasma renin activity to stenosis was almost twice as great with the severe stenosis as with the moderate stenosis.

Of interest is the association of glomerular filtration rate, plasma renin activity, and hypertension during moderate and severe bilateral renal artery stenosis. With moderate bilateral renal artery stenosis the mean arterial blood pressure is only slightly increased whereas the plasma renin activity which was initially elevated returns to levels at the upper limits of normal with persistent mildly positive sodium balances and no significant change in serum creatinine. This would suggest that the mild blood pressure elevation is related primarily to retention of sodium and water as shown by slight increase in the weight of the animal.

Bilateral severe renal artery stenosis resulted in a sustained significantly elevated mean arterial blood pressure with plasma renin activities that were initially elevated but were depressed to normal or low levels with persistent and markedly positive sodium balance and an increase in weight. These changes are very similar to those seen in the bilateral moderate renal artery stenosis but are of greater magnitude. These data suggest that renal ischemia results in activation of the renin-angiotensin aldosterone system and initial pressor hypertension. When total renal perfusion is markedly decreased beyond the limits of autoregulation, glomerular filtration rate decreases allowing sodium retention, suppression of plasma renin activity and hypertension on a volume basis.

Renal ischemia gives rise to increased distal renal tubular sodium reabsorption under the influence of the renin-angiotensin aldosterone system, but also results in increased sodium reabsorption by the proximal convuluted tubule. Sustained hypertension, however, has been shown to result in depressed proximal tubular sodium reabsorption takes place in the proximal tubule, the amount of sodium r reabsorbed distally under the influence of aldosterone is not great enough to restore plasma volumes to normal. This results in a continued release of the renin and a pressor hypertension. If, however, renal ischemia is such that there is under perfusion of the total renal mass, then glomerular filtration rate decreases and there is no mechanism by which retained sodium maybe excreted. This results in a positive sodium balance and increased blood volume. This has been shown in a recent study by Swales and co-workers (Swales JD, Thurston H, Queiroz FP, and Medina A. J Lab Clin Med 80: 539-547, 1972).

Unilateral nephrectomy resulted in no change in sodium balance or blood pressure; however unilateral renal artery constriction resulted in a progressive negative sodium balance and significantly elevated blood pressure. Renal artery constriction with contralateral nephrectomy however resulted in a positive sodium balance and significant hypertension. Similar studies performed by Regoli and co-workers(Regoli D, Brunner H, Peters G, and Gross F. Proc Soc Exp Biol 109: 142-145, 1962)[3] measured renal renin concentration in normal rats and in rats with unilateral and bilateral renal artery stenoses have shown increased renal renin concentration in the kidney with the unilateral renal artery stenosis and normal renal renin concentration in rats with bilateral renal artery stenosis. Very similar studies by other investigators (Gavras H, Brunner HR, Vaughan ED Jr., and Laragh JH: Science 178: 1369-1371, 1973;[7] Miller ED, Jr., Samuels AI, Haber E, and Barger AC. Inhibition of angiotensin conversion in experimental renovascular hypertension. Science 177:1108-1109, 1972)[8] have shown that the hypertension resulting from unilateral renal artery stenosis presumably on a pressor basis, can be reversed by passive immunization of the rats to angiotensin II or by the administration of an angiotensin antiogonist; however, the administration of these same agents to rats made hypertensive by bilateral renal artery stenosis results in no change in the mean arterial blood pressure. This hypertension is persumed to be secondary to sodium and water retention or on a volume basis. Our studies are in agreement with those studies just cited and show the concurrent changes of plasma renin activity, glomerular filtration rate and sodium balance to changes in mean arterial blood pressure.

The changes observed during the last period of our studies in which the renal mass was acutely reduced by removal of the right kidney leaving the ischemic kidney in place are very similar to the changes that took place during bilateral renal artery stenosis. The moderate stenosis resulted in initial changes with return to normal by autoregulation of the kidney; however, severe stenosis with reduction in glomerular filtration rate and severe total under perfusion resulted in a significant increase in blood pressure with normal plasma renin activity and a persistently markedly positive sodium balance.

The sum of these observations coupled with those observations cited from the literature suggest that renal ischemia initially causes hypertension by generation of renin and angiotensin II, sodium retention occurs and extracellular fluid volume is expanded. In the absence of the contralateral kidney, extracellular fluid remains expanded supresses renin release from the ischemic kidney and in all probability itself maintains hypertension. In the presence of the contralateral kidney, the sodium load reabsorbed by

the ischemic kidney is readily excreted by the contralateral kidney with a slightly decreased plasma volume and hypertension persists on a pressor basis.

These studies further suggest that decreasing extracellular fluid volume by salt depletion during total renal underperfusion does not change the blood pressure but uncovers the turned on renin angiotensin system and probably converts a volume mediated hypertension to a pressor mediated hypertension. Further studies are anticipated to show changes in plasma renin activity and blood pressure during bilateral renal stenosis with the administration of furosemide and volume concentration.

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1. Swales JD, Thurston H, Queiroz FP, and Medina A: Sodium balance during the development of experimental hypertension. J Lab Clin Med 80: 539-547, 1972.

2. Koletsky S, Pavlicko KM, Rivera-Velez JM. Renin-angiotensin activity in hypertensive rats with a single ischemic kidney. Lab Invest 24: 41-44, 1971.

3. Regoli D, Brunner H, Peters G, and Gross F. Changes in renin context in kidneys of renal hypertensive rats. Proc Soc Exp Biol 109: 142-145, 1962.

4. Haber E, Koerner T, Page LB, et al: Application of a radioimmunoassay for angiotensin I to the physiologic measurements of plasma renin activity in normal human subjects. J Clin Endo and Metab 24: 1349-1355, 1969.

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6. Goldblatt H, Lynch J, Hanzel RF: Studies on experimental hypertension. The production of persistent elevation of systolic blood pressure by means of renal ischemia. J Exp Med 59: 347-379, 1934.

7. Gavras H, Brunner HR, Vaughan ED, Jr., and Laragh JH. Angiotensin-sodium interaction in blood pressure maintenance of renal hypertensive and normotensive rats.

8. Miller ED, Jr., Samuels AI, Haber E, and Barger AC. Inhibition of angiotensin conversion in experimental renovascular hypertension. Science 177: 1108-1109, 1972.

PRESENTATIONS AND/OR PUBLICATIONS:

None

333<

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0D 6380	74 07 01	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DOD'N INSTR'N	8A. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
73 07 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	61102A	3A161102B71R	01	308
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) An Evaluation of the Use of Enzymatic Debridement of Burn Wound Eschar to Decrease Morbidity 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
70 01	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 74	.3	9
CURRENT FISCAL YEAR 75	.3	8

19. RESPONSIBLE DOD ORGANIZATION
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ASSOCIATE INVESTIGATORS
NAME: William R Smith, CPT, MSC
NAME: DA

21. GENERAL USE: FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code): (U) Enzymatic debridement; (U) Eschar; (U) Thermal injury; (U) Rats

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

23. (U) Rapid removal of burn eschar by enzymatic means in rats as a possible future therapy in burned soldiers.

24. (U) Commercially available sutilains will be tested in humans for efficacy of escharal debridement and problems presented in bacterial control.

25. (U) 73 07 - 74 06 Studies have commenced (under a separate protocol) to test the efficacy of the use of Sutilains enzyme in debriding the burned hand of burned soldiers. A concurrent study (as part of this protocol) is planned to study the efficacy of such an enzyme preparation in debriding small area burns. Appropriate controls of Plastibase R ointment (in which the enzyme is prepared), no topical chemotherapeutic agent, and topical chemotherapy will be included. Time course for eschar separation, grafting, and bacterial colonization will be performed to study the liabilities as well as advantages of such an enzymatic product.

*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 69 (FOR ARMY USE) ARE OBSOLETE

ABSTRACT

PROJECT NO 3A161102B71R-01, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: AN EVALUATION OF THE USE OF ENZYMATIC DEBRIDEMENT OF BURN WOUND ESCHAR TO DECREASE MORBIDITY IN BURNED TROOPS

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

Period covered in this report: 1 July 1973 - 30 June 1974

Investigators: Norman S. Levine, MD, Major, MC
Hugh D. Peterson, DDS, MD, Colonel, MC
Arthur D. Mason, Jr., MD
William R. Smith, Captain, MSC

Reports Control Symbol MEDDH-288(R1)

An investigation of the use of enzymatic debridement for burned hands was commenced. Three patients with bilateral hand burns were studied. Sutilains enzyme was used on one hand; saline soaks were used on the other hand. One of the three patients in the study died. In the two remaining patients, the following observations were made: (1) sutilains enzyme did effectively debride the burned wound; (2) this debridement was incomplete, and a deep layer of necrotic tissue was left undebrided; (3) debridement with sutilains enzyme did not result in any shortening of the time from burn to grafting; (4) there was no grossly observable difference in function in the hands treated with sutilain enzyme when compared to the controls. These observations are merely clinical impressions gained in the treatment of these three patients. It is apparent that satisfactory evaluation of this enzyme preparation will require a revised protocol with proper experimental design.

Enzymatic debridement
Eschar
Thermal injury
Rats

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0F 6385	74 07 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'S	8b. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
74 07 01	K.COMPLETION	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	3A161102B71R	01	301
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) A Laboratory Model of Electric Injury as Occurs in a Military Population (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
73 07	74 03	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	PRECEDING/CURRENT FY	a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
FISCAL YEAR	74	.1	1

19a. RESPONSIBLE DOD ORGANIZATION
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NAME: Travis Masterson, BS DA

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Provide EACH with Security Classification Code)
(U) Electric injury; (U) Rats; (U) Experimental model; (U) Pathophysiology

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

23. (U) The creation of an reproducible electric injury model in animals to investigate the pathophysiology of the injury.

24. (U) Sprague-Dawley rats had brass electrodes placed in a subcutaneous pocket in the medial portion of each hand limb. The animals were anesthesized and then shocked with various voltages. Electrode size and depth, distance between electrodes and integrity of electrode contact were evaluated. Tissue temperature voltage, arpage and time of electric contact were recorded.

25. (U) 73 07 - 74 03 Both cutaneous and deep tissue injury was produced in the animal model. An inverse relationship was observed between the voltage and time of application necessary for current arcing. Tissue injury was directly related to voltage. Tissue temperature was the critical factor in determining the magnitude of tissue injury prior to current arcing.

*Available to contractors upon originator's approval

336<

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

ABSTRACT

PROJECT NO. 3A161102B71R-01, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: A LABORATORY MODEL OF ELECTRIC INJURY AS OCCURS IN A MILITARY POPULATION

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

Period covered in this report: 1 July 1973 - 30 June 1974

Investigators: John L. Hunt, MD, Lieutenant Colonel, MC
Arthur D. Mason, Jr, MD
Travis S. Masterson, MS

Reports Control Symbol MEDDH-299(R1)

A reproducible electric injury model was developed in Sprague-Dawley rats to investigate the pathophysiology of acute electric injury. Rats were shocked with various voltages and an inverse relationship was observed between the voltage and time of application necessary for current arcing. Tissue injury was directly related to voltage. Electrode size and depth, distance between electrodes and integrity of electrode contract were important variables in influencing tissue damage. Injury in this model was manifest by both cutaneous and deep tissue damage. In each animal a cutaneous injury characterized by coagulation necrosis occurred, and local but not proximal deep leg muscle damage was evident. Simultaneous measurement of amperage and tissue temperature, both local and proximal to contact points, revealed a slow parallel rise in each which rapidly peaked at the onset of current arcing. Amperage then fell precipituously to zero, but temperature slowly fell to preshock levels. This three phase response of amperage and temperature to electric shock was due to alterations in local tissue resistance. Tissue temperature adjacent to the contact site always exceeded 60° C and was associated with deep muscle necrosis, whereas tissue temperature in leg muscles more distant from the contact site never exceeded 60° C and those muscles survived. The result of the present investigation revealed that an electric burn is a self limiting injury; once current arced, further skin and muscle damage was impossible because amperage fell to zero. Tissue damage associated with electric injury occurred when electrical energy was converted to thermal energy. Tissue temperature was the critical factor in determining the magnitude of tissue injury prior to current arcing. Muscle injury occurred at the time of initial thermal insult and subsequent progressive or "de novo" muscle necrosis did not occur.

Electrical injury
Rats
Experimental model
Pathophysiology

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OD 6978	74 07 01	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. GROUP INDIC'N	8b. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM / 10. WORK UNIT
73 07 01	D. CHANGE	U	U	NA	NL	☒ YES ☐ NO	

10. NO CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A161102B71R	01	194
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Provide 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: 003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
70 07	Cont	DA	C. In-House

17. CONTRACT GRANT: Not Applicable
a. DATE/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 PROG'D: 74	.3	5
FISCAL YEAR CURRENT: 75	.2	5

19a. RESPONSIBLE DOD ORGANIZATION

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NAME: Robert B Lindberg, PhD
NAME: John L Hunt, LTC, MC — DA

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Provide 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. Provide total 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.

24. (U) Laboratory assessment of bacterial barrier 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 postoperative cultures at margin of operative field. Temperature monitoring to determine heat transmission characteristics.

25. (U) 73 07 74 06 The draping material has undergone several fabrication modification and the laminate as now produced does not possess reliable bacterial barrier properties. Although drops of bacterial broth cultures did not appear to penetrate the material within four hours, incubation of the blood agar test plates for 48 hours after removal of the drape material revealed growth of the test organisms, Pseudomonas species, Escherichia coli, Klebsiella, Serratia and Staphylococcus in one third, one fourth, one sixth, one third and one fourth of test areas respectively. Unless the previously present bacterial barrier can be restored to this material by altering fabrication technics this material has no advantage over cotton drapes and the study will be terminated.

*Applicable 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.

ABSTRACT

PROJECT NO. 3A161102B7IR-01, RESEARCH IN BIOMEDICAL SCIENCES

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 July 1973 - 30 June 1974

Investigators: Basil A. Pruitt, Jr., MD, Colonel, MC
Robert B. Lindberg, Ph D
John L. Hunt, MD, Lieutenant Colonel, MC

Reports Control Symbol MEDDH-288(R1)

Available surgical drapes are either uncertain bacterial barriers or possess undesirable physical properties which limit their usefulness and clinical acceptance. A synthetic sheeting of Spunbonded Olefin was initially evaluated in both laboratory and clinic and found to be a reliable bacterial barrier but to drape poorly and to permit quantitative fluid run-off.

Subsequent modification of the sheeting has resulted in the present lamination of one layer of Spunbonded Olefin between two layers of cellulosic material to diminish fluid run-off and improve the draping characteristics of the sheeting. However, the lamination and softening processes appear to have destroyed the bacterial barrier as assessed in the laboratory. Eighteen-hour broth cultures of Pseudomonas aeruginosa, Klebsiella pneumoniae, Serratia marcescens, Escherichia coli and Staphylococcus aureus were prepared with a concentration of organisms of approximately 10^9 per cubic milliliter, and used as the inoculum. Discs of the synthetic sheeting were cut to cover a Petri dish area, sterilized in ethylene oxide, and then placed on fresh blood agar plates, using sterile technic. Four drops of each test culture were placed on the disc in a square pattern with each drop approximately 1.2 cm from the edge of the disc. The drops were left in place for four hours at room temperature, following which the discs were then carefully removed with the drops in place. The blood agar plates were subsequently incubated for 48 hours and then read for the presence of bacterial growth in the area underlying the prior position of each drop of bacterial broth culture.

The synthetic sheeting laminate appeared to be nonwettable since the drops of broth culture were still in place four hours after placement on the test material. The material, however, was permeable to each of the bacterial species tested with Pseudomonas penetration noted at 4 of 12 test sites, E. coli at 3 of 12 test sites, Klebsiella at 2 of 12 test sites, Serratia at 4 of 12 test sites, and Staphylococcus aureus at 3 of 12 test sites. It should be noted that there were five discs of the material tested through which none of the bacteria penetrated. This variable penetrability is puzzling and suggests a mechanical defect in the integrity of the sheeting. The manufacturer has suggested that in the lamination process, which entails focal application of adhesive, the laminating device may penetrate the sheeting to create a defect through which the bacteria easily pass.

Further testing is underway with microscopic examination of the test material to identify defects in the area of broth culture drop placement. If passage of bacteria can be correlated with such defects, further modification of the lamination process will be necessary. If such gross defects are not identifiable, it is felt that even more extensive manufacturing modifications will be necessary since in its present form this material offers no significant advantage over standard cotton drapes, which are freely permeable to bacteria.

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Military burn unit
Operating room based infections
Surgical drapes
Surgical gowns

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0D 6976	74 07 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
73 07 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	083
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code) (U) Studies of the Effect of Variations of Temperature and Humidity on Energy Demands of the Burned Soldier in a Controlled Metabolic Room (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS
003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
71 07	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 74	1	24
	CURRENT 75	1	10

19. RESPONSIBLE DOD ORGANIZATION
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21. GENERAL USE
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22. KEYWORDS (Precede EACH with Security Classification Code) (U) Metabolism; (U) Heat loss; (U) Evaporative water loss; (U) Controlled environment; (U) Humans; (U) Critical temperature

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 relationship between surface cooling and hypermetabolism in a controlled ambient environment, to determine the mediator of the profound hypercatabolic response following thermal injury.

24. (U) The use of a controlled environmental study room to measure metabolic rate at various temperatures; concomitantly, measurements of water loss, heat production, core and mean skin temperature, urine and plasma catecholamines with calculation of heat transfer coefficients and routes of heat loss.

25. (U) 73 07 - 74 06 Twenty noninfected burned adults (mean burn size 45%, range 7-84%) and four normals studied in an environmental chamber at two or more temperatures between 19 and 33° C (vapor pressure constant) demonstrated that the patients were hypermetabolic at all temperatures with core and mean skin temperature significantly elevated above control values. Between 25 and 33° C ambient, metabolism was unchanged in controls and burns < 40% total body surface (48.9 ± 4.6 Kcal/m^2/hr vs. 48.9 ± 4.5), but BMR decreased in larger burns in the warmer environment (72.0 ± 1.9 vs 65.8 ± $p<0.01$). At 21° C, BMR and catecholamines increased, except in four nonsurvivors who became hypothermic with decreased catechol elaboration. BMR in six patients with bacteremia was below predicted levels (49.1 ±2.8 measured, 75.3 ± 1.2 predicted, $p<0.001$) while catecholamines were markedly elevated suggesting that infection acts physiologically like competitive adrenergic blockade.

*Available to contractors upon originator's approval

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ABSTRACT

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: STUDIES OF THE EFFECT OF VARIATIONS OF TEMPERATURE AND HUMIDITY ON ENERGY DEMANDS OF THE BURNED SOLDIER IN A CONTROLLED METABOLIC ROOM

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Period covered in this report: 1 July 1973 - 30 June 1974

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Twenty noninfected burned adults (mean burn size 45%, range 7-84%) and four normals studied in an environmental chamber at two or more temperatures between 19 and 33° C (vapor pressure constant) demonstrated that the patients were hypermetabolic at all temperatures with core and mean skin temperature significantly elevated above control values. Between 25 and 33° C ambient, metabolism was unchanged in controls and burns <40% total body surface (48.9 ± 4.6 Kcal/m^2/hr vs. 48.9 ± 4.5), but BMR decreased in larger burns in the warmer environment (72.0 ± 1.9 vs. 65.8 ± 1.7, $p < 0.01$). At 21° C, BMR and catecholamines increased, except in four nonsurvivors who became hypothermic with decreased catechol elaboration. BMR in six patients with bacteremia was below predicted levels (49.1 ± 2.8 measured, 75.3 ± 1.2 predicted, $p < 0.001$) while catecholamines were markedly elevated suggesting that infection acts physiologically like competitive adrenergic blockade.

Metabolism
Heat loss
Evaporative water loss
Controlled environment
Humans
Critical temperature

STUDIES OF THE EFFECT OF VARIATIONS OF TEMPERATURE AND HUMIDITY ON ENERGY DEMANDS OF THE BURNED SOLDIER IN A CONTROLLED METABOLIC ROOM

Hypermetabolism characterizes the metabolic response to thermal injury, and the magnitude of this post-traumatic physiologic alteration is closely related to the extent of injury. Negative nitrogen balance, loss of other intracellular constituents, and a rapid decrease in body weight are consequences of the increase in metabolic activity, and extensive loss of protoplasmic mass may result in severe erosion of energy and protein stores essential to optimal body function. Similarities between the thermally injured and individuals with thyrotoxicosis prompted early metabolic and endocrine studies, but the increased oxygen consumption has not been related to abnormal thyroid function (Cope O, Nardi GL, Quijano M, Rovit RL, Stanbury JB, Wright A, Ann Surg 137:165, 1953).[10] Increased evaporative water loss from the burn wound results in surface cooling, which could stimulate metabolic activity to maintain normal heat balance and core temperature. The purpose of this study was to define the relationship between surface cooling and hypermetabolism following thermal injury in a controlled ambient environment.

MATERIALS AND METHODS

Patients

Twenty noninfected burn patients, between the ages of 14 and 49 years, were initially studied along with four normal individuals of approximately the same age. Burn patients were selected to represent a range in size of total body surface injury, all except one were male, and none had significant pre-existing disease before thermal injury (Table 1). The patients were studied between the sixth and 33rd post-burn days, with the mean day of study being the 11th day following injury. All patients in this initial group were stable following initial resuscitation and during the study period, and were alert and cooperative during the studies. These patients did not have systemic infection, as determined by stable body temperature, absence of clinical signs of infection, and negative blood cultures and endotoxin levels before, during, and after the study period.

The control individuals were healthy male subjects working on the Burn Unit, who were accustomed to the techniques and methodology of respiratory and metabolic testing. There was no history of chronic disease in this group, and all were vigorous, active individuals.

Place of Study

The studies took place in an environmental chamber on the Burn Ward. This rectangular room, previously described in detail (Wilmore

Table I

CHARACTERISTICS OF PATIENTS STUDIED AT TWO OR MORE AMBIENT TEMPERATURES

SUBJECTS	AGE (Years)	WEIGHT (kg)	BODY SURFACE AREA (m^2)	TOTAL BODY BURN (Per Cent)	PER CENT THIRD DEGREE	POSTBURN DAY STUDIED	COMMENT
Control							
1*	25	75.0	1.90	0	0		
2*	20	72.2	1.98	0	0		
3*	23	88.0	2.17	0	0		
4*	34	78.0	2.04	0	0		
Mean	26	78.3	2.02				
Range	20-34	72-88	1.90-2.17				
Patients							
1*	43	69.5	1.82	7	7	8-9	
2	14	59.0	1.72	10	0	7	
3*	28	63.0	1.79	15	2	7-8	
4	17	55.7	1.93	23	4	7	
5*	15	48.0	1.56	26	18	17-18	Associated head injury; tracheostomy
6*	49	87.5	2.01	34.5	0	8-9	
7	18	70.0	1.79	39	12	8	
8*	24	87.0	2.09	40	14	8-9	
9	43	118.0	2.36	41	3	10	
10	32	50.0	1.48	46	17.5	6	Tracheostomy, female
11	40	80.0	1.99	50	0	12	
12	38	113.0	2.15	51	22	7	
13*	26	65 9	1.78	52	8	10-11	
14	18	60.5	1.77	53	9.5	14	
15*	34	85.0	2.00	60	16.5	14-15	
16*	18	66.0	1.88	63.5	21.5	10-11	
17	19	96.0	2.23	66	25.5	14	
18	34	77.0	1.91	66.5	39.5	14	Tracheostomy
19	22	48.0	1.59	76	33.5	32-33	Tracheostomy
20*	25	68.0	1.85	84	76	8-9	Tracheostomy
Mean	28	73.4	1.88	45.0	16.5	11	
Range	14-49	48-118	1.48-2.36	7-84	0-76	6-33	

*Studied over two consecutive 24-hour periods.

DW, Mason AD Jr, Johnson DW, Pruitt BA Jr, In Press, J Appl Physiol)[51] can maintain a selected temperature and humidity between +15° C and +40° C (range ± 2 per cent), limited by a -1.1° C and 35° C dewpoint. The maximum air velocity in the chamber is less than 50 feet per minute at the level of the bed, three feet above the floor. The room maintains good thermal stability with electronic equipment and three individuals present, and clinical monitoring, patient care, and burn wound treatment were continued while each patient was housed in the chamber.

Study Design

The four normals and the first nine patients were studied in the chamber for two consecutive 24-hour periods after entering the chamber at approximately 9:00 A.M. During one 24-hour interval, the environmental temperature was set at 25° C, vapor pressure at 11.88 mmHg, and, during the other 24-hour study period, the environmental temperature was set at 33° C with the same vapor pressure. The order of the study periods was sequentially altered, and tests were made between the 18th and 24th hours of each study day. As more patients were evaluated, shorter equilibration times were allowed, so that the final 11 patients were studied at two or more temperatures on the same day. These patients entered the chamber in the morning between 6 and 8 A.M., were allowed to rest 4-6 hours in the 33° C environment, and then studied. A new ambient temperature was then selected, a period of equilibration allowed, and additional measurements made. The ambient temperatures selected for this study were 33, 29, 25, 21, and 19 degrees centigrade, and all individuals were studied while in two or more ambient conditions. Vapor pressure was maintained at 11.88 mmHg throughout the range of ambient temperatures. Patients who were studied in the cooler environments (less than 25° C) were evaluated in these ambient conditions as the final test in the series, and were exposed for only one to two hours to the cooler ambient conditions before the measurements were made. In all other instances, the equilibration period was four hours or more.

Study Methods

The methods of study have been previously described in detail (Wilmore DW, Mason AD Jr, Johnson DW, Pruitt BA Jr, In press, J Appl Physiol).[51] In summary, all patients were treated by the open method with topical mafenide (Sulfamylon^{R}) cream applied to the burn wounds. The individuals were maintained in the supine position, on a water-resistant mattress, several hours before each test. All patients were fasted three or more hours before and while measurements were made, but received water by the oral or parenteral routes if required to maintain a normal state of hydration. Expiratory gas was collected in two 200 liter Douglas bags to determine oxygen consumption and carbon

dioxide production. A mouth piece and nose clip or nose plugs were used as an interface with the patient except in those individuals with tracheostomies in whom a direct connection with the tracheostomy tube could be made. All patients were previously trained with the equipment, and application of topical anesthesia to the lips and nose was utilized in patients with facial burns to allow patient acceptance of the equipment by minimizing pain and discomfort. After a steady state was achieved, the expiratory gas was collected and analyzed and the metabolic rate calculated from the oxygen consumption corrected for the respiratory quotient and expressed in kilocalories per square meter body surface area per hour. Core temperature was continuously monitored from rectal and tympanic probes, and a small hand-held thermocouple was used to measure skin temperature. Between 20 and 50 skin temperatures were taken from areas not in contact with the mattress and the mean skin temperature calculated. These three basic measurements (metabolic rate, core temperature, and mean skin temperature) were carried out at each ambient condition after the period of equilibration.

The core to skin heat transfer coefficient was calculated by the following equation:

$$\text{Core-skin } (\text{Kcal}/\text{m}^2/\text{hr}/^\circ\text{C}) = \frac{\text{metabolic rate } (\text{Kcal}/\text{m}^2/\text{hr})}{\text{core temp } (^\circ\text{C}) - \text{skin temp } (^\circ\text{C})}$$

In selected patients, urine and/or plasma was collected for the determination of catecholamines, measured by an automated fluorimetric method (Viktora JK, Baukal A, Wolff FW, Clin Pharmacol Ther 5:398, 1964).[46] The urine was collected during the final hour of each ambient study condition, usually by an indwelling catheter which was present in most of these patients, the volume excreted per unit time recorded, the specimen acidified and frozen for analysis. Blood samples were immediately chilled and plasma immediately analyzed for catecholamine levels.

Septic Patients

Eighteen studies were performed in 10 septic individuals (Table 2), all with proven bacteremia demonstrated by a positive blood culture obtained at the time of study. All patients maintained an adequate urine output at the time of study and did not demonstrate hypotension or signs of cardiovascular instability. Metabolic rate, core and skin temperature, urine, and/or plasma catecholamines were measured in selected patients as previously described.

Table 2

CHARACTERISTICS OF PATIENTS WITH BACTEREMIA

SUBJECT	AGE (Years)	WEIGHT (kg)	BODY SURFACE AREA (m^2)	TOTAL BODY SURFACE BURN (Per Cent)	PER CENT 3°	POSTBURN DAY STUDIED	ORGANISM CULTURED FROM BLOOD	ENDOTOXIN
1	18	70.0	1.92	55	12	8	Staphylococcus aureus	*
2	38	70.5	1.88	56.5	56.5	10	Klebsiella	Positive
3	48	105.0	2.15	61	13	12	Staphylococcus aureus	*
4	39	78.0	2.00	73	49	4	Aeromonas liquefaciens	*
5	34	65.0	1.85	76	28	7	Providencia stuarti	*
6	22	84.0	2.02	82	49	5	E. coli	Positive
7	43	57.4	1.73	45	15	17	Klebsiella	*
8	63	73.6	1.87	31.5	25	17	Providencia stuarti	Negative
9	37	71.6	1.81	67	46	3	Bacillus species	*
10	64	95.2	2.20	49	44	10	Providencia stuarti	*
Mean	4	77.0	1.94	59.5	34.0	9		
Range	18-64	57.4-105	1.73-2.20	31.5-82.0	12-56.5	3-17		

*Not measured.

RESULTS

Body Temperature and Heat Transfer

Core temperature did not change significantly between the ambient conditions at 25 and 33° C, but skin temperature was altered and moved in the direction of the environmental temperature. However, a marked difference was noted in the core and skin temperatures when comparing normal individuals with burn patients; the average core and skin temperatures were consistently 1-2° C higher in the injured patients when compared with controls (Table 3). This difference was present at all ambient conditions studied between 33 and 25° C, and was also found at the cooler temperature in those patients who were able to increase their metabolic rates in the 21 and 19° C environments. Heat transfer coefficients demonstrated a two-fold increase in core to skin conductance of heat in the burn patients when compared with control individuals, and this two-fold increase was present at all temperatures studied.

Effect of Burn Size and Ambient Temperature on Metabolic Rate

Metabolic rate increased with burn size. The relationship appeared curvilinear, metabolism approaching 70-75 Kcal/m^2/hr when measured in the 33 and 25° C environment as burn size exceeded 50 per cent total body surface (Table 4). In the controls and patients with smaller burns (less than 40 per cent total body surface), there was no change in mean metabolic rate between 33° and 25° C (48.9 ± 4.6 Kcal/m^2/hr at 33° vs. 48.9 ± 4.5, mean ± S.E.M.). However, it appeared that a consistent decrease in caloric expenditure occurred in patients with larger burn injuries in the warmer environment when compared with 25° C. Mean metabolic rate at 25° C was 72.0 ± 1.9 Kcal/m^2/hr, which decreased to 65.8 ± 1.7 Kcal/m^2/hr at 33° C ($p < 0.001$ by paired t test). As the ambient temperature decreased below 25° C, metabolism increased in both the controls and those patients who eventually survived. However, oxygen consumption rarely exceeded two and one-half times basal levels (Fig. 1). In contrast, four patients with more extensive burns became hypothermic at 21° C, with decreased metabolic rate and a marked fall in core and skin temperature.

Relationship Between Metabolic Rate and Catecholamines

The urinary excretion of catechqiamines per unit time was related to metabolic rate measured during the same time period. The relationship appeared linear until metabolic rate approached two times basal levels, then increased excretion in urinary catecholamine was associated with only a slight increase in metabolic rate, with the predicted metabolic response never exceeding two and one-half times basal levels for normal man (Fig. 2). The ability to generate additional heat in a cool environment was variable in patients with burns greater than 40 per cent

Table 3

MEASUREMENTS FROM FOUR NORMALS AND 20 PATIENTS

AT TWO AMBIENT TEMPERATURES

(Mean ± S.E.)

	NORMAL	BURNS	p
25° C			
Metabolic rate (Kcal/m^2/hr)	35.6 ± 2.0	66.8 ± 2.6	< 0.001
Core temperature (°C)	36.7 ± 0.2	38.4 ± 0.2	< 0.001
Skin temperature (°C)	31.4 ± 0.2	33.1 ± 0.2	< 0.001
Core-skin heat transfer coefficient (Kcal/m^2/hr/°C)	6.6 ± 0.4	13.3 ± 1.0	< 0.001
33° C			
Metabolic rate (Kcal/m^2/hr)	36.3 ± 1.6	62.6 ± 2.3	< 0.001
Core temperature (°C)	36.9 ± 0.1	38.1 ± 0.2	< 0.001
Skin temperature (°C)	34.2 ± 0.3	35.9 ± 0.2	< 0.01
Core-skin heat transfer coefficient (Kcal/m^2/hr/°C)	13.9 ± 1.4	30.6 ± 2.5	< 0.001

Table 4

RELATIONSHIP BETWEEN METABOLISM AND PER CENT TOTAL BODY SURFACE BURN AT VARYING TEMPERATURES

y = Metabolic rate in Kcal/m^2/hr

x = Per cent body surface burn

T = Ambient temperature °C (T 19-33)

$p < 0.05$ for all equations

Ambient Temperature (°C)	Relationship	R^2
21	$y = 42.70 + 1.539\ x - 0.01290x^2$	0.877
25	$y = 35.50 + 1.050\ x - 0.006698x^2$	0.889
33	$y = 36.15 + 1.001\ x - 0.007694x^2$	0.795
T*	$y = 188.8 + 1.211\ x - 10.38T - 0.009274x^2 + 0.1701\ T^2$	0.849

*Based on 72 measurements of metabolic rate at five different temperatures; hypothermic patients not included.

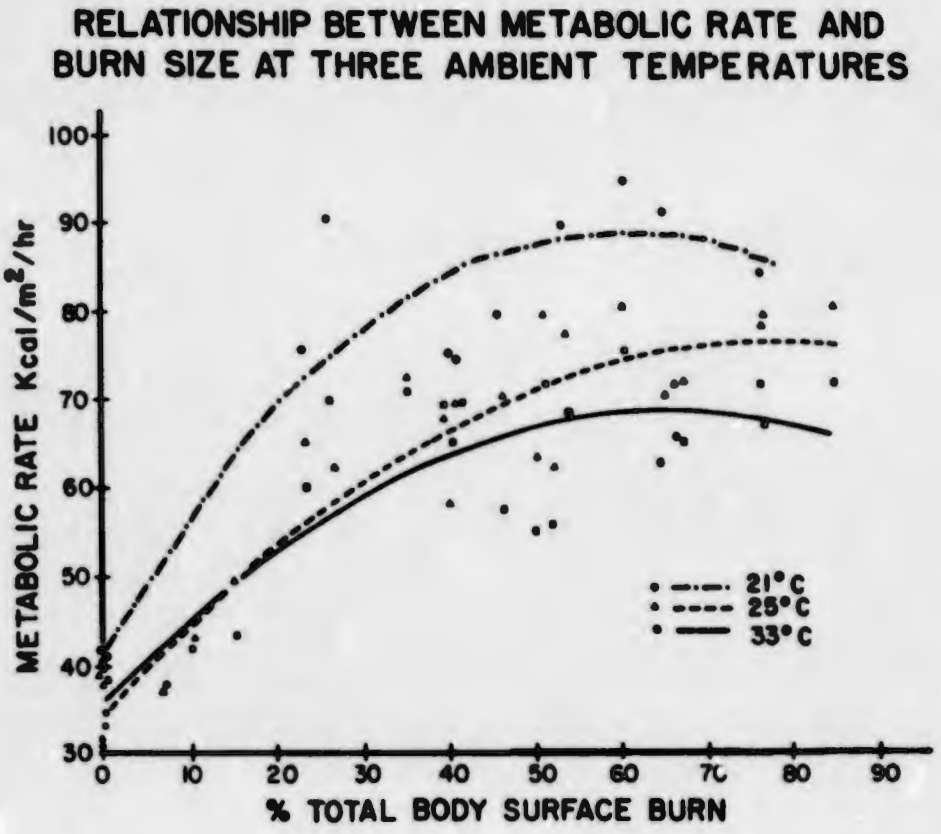

Figure 1. The relationship between metabolic rate and environmental temperature is demonstrated by the individual regression curves derived for 21, 25, and 33° ambient, as calculated for individual equations in Table 4. Both normal men and burn patients increased metabolic rate at 21° C when compared with 25 and 33° C. Only a small decrease in metabolism occurred in patients with large thermal injury studied at 25 and 33° C, and the reduction was in the order of 5-8 Kcal/m^2/hr. Metabolic rate did not return to normal when the patients were studied in a warm environment.

RELATIONSHIP BETWEEN METABOLIC RATE AND URINARY CATECHOLAMINES

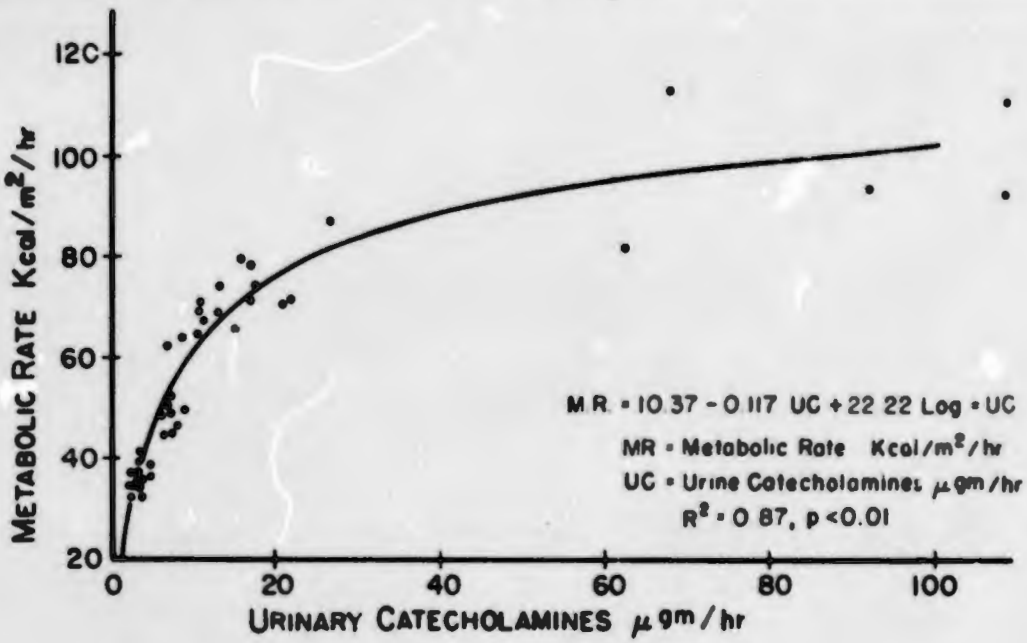

Figure 2. The response curve to a dose of catecholamine or to nerve stimulation has the shape of a rectangular hyperbole, with a large quantitative difference in response elicited by a small change in dose. As the catecholamines increase, a plateau is reached and increasing doses exert little effect on metabolic rate. Our data expressed as a hyperbolic function is:

$$MR = 100.93 - \frac{580.56}{UC} + \frac{1{,}584.58}{UC^2} - \frac{1351.64}{UC^3}$$

$N = 55$, $R^2 = 0.84$, $p < 0.05$.

total body surface (Table 5). In four patients with an average burn size of 57 per cent (range 41-76), there was a marked accentuation of hypermetabolism, and this compensatory increase in heat production maintained core temperature and was accompanied by an increase in excretion of urinary catecholamines. However, four patients with a mean burn size of 65 per cent (range 51-84) studied in a cool ambient temperature failed to maintain heat balance and became hypothermic. These "non-responders" had a sudden decrease in their catecholamine excretions during exposure to the cooler ambient temperature (Fig. 3).

Septic Patients

Metabolic rate was significantly decreased in 10 septic patients studied. The mean metabolic rate was 50.3 ± 2.2 Kcal/m^2/hr compared with predicted or measured rates during nonseptic intervals which averaged 73.0 ± 1.2 Kcal/m^2/hr (Table 2). Urinary catecholamines were markedly elevated in these patients, averaging 910.8 ± 406 µgm/hr, and this level of catecholamine excretion was inappropriately high for the metabolic response measured (See Fig. 2) (Table 6).

DISCUSSION

Hypermetabolism characterizes the metabolic response following thermal injury, and this alteration in metabolic activity correlates well with an increased evaporative water loss from the burn wound (Harrison HN, Moncrief JA, Duckett JW Jr, Mason AD Jr, Surgery 56:203, 1964).[28] Decreasing wet or dry heat loss in small animals has been reported to return metabolic rate towards normal (Caldwell FT Jr, Ann Surg 155:119, 19622;[6] Lieberman ZH, Lansche JM, Surg Forum 7:83, 1966).[37] Burn patients treated in a warm environment (32° C) demonstrate lower metabolic rates than when treated in a cool environment (22° C) (Barr P-O, Birke G, Liljedahl S-O, Plantin L-O, Lancet 1:164, 1968),[1] and this evidence has been interpreted to support the thesis that hypermetabolism in the burn patient is a response to increased surface cooling due to increased evaporative water loss. In contrast, Zawacki and associates covered burn wounds with a water-impermeable membrane and thus blocked evaporative water loss, but found no consistent alterations in metabolic rate in burn patients at approximately 25° C ambient temperature (Zawacki BE, Spitzer KW, Mason AD Jr, Johns LA, Ann Surg 171:236, 1970).[56]

In this study, the average core temperature of the burn patients was elevated above normal, and the mean skin temperature was increased in all nonhypothermic patients at all ambient conditions studied when compared with normal man, demonstrating that the burn patients are internally warm and not externally cold. The usual response of normal man to a cold environment involves both vasoconstriction to achieve

Table 5

VARIABLE RESPONSE OF PATIENTS WITH LARGE THERMAL INJURY TO DECREASING ENVIRONMENTAL TEMPERATURE

	METABOLIC RATE (Kcal/m²/hr)					URINARY CATECHOLS (µgm/hr)					SKIN TEMPERATURE (°C)					TYMPANIC TEMPERATURE (°C)				
PATIENT	33	29	25	21	19	33	29	25	21	19	33	29	25	21	19	33	29	25	21	19
1	69.4		69.2	74.0		13.5		12.0	13.5		36.0		33.7	32.3		37.8		39.0	39.3	
2	57.6		70.0	79.2	82.7	8.4		28.0	91.0		36.3		33.5	32.2	32.1	37.5		38.5	38.8	38.7
3	63.6		69.9	91.9		15.3		18.5	34.2		36.1		33.0			38.7		37.5		
4	67.0	66.3	79.3	84.0		6.6	8.2	16.2	96.0							38.6	38.7	38.8	38.1	
5	63.5	52.6	50.8			9.2	11.8	10.2			34.8	33.6	32.3			37:2	36.8	36.2		
6	71.7		78.7	69.3		22		17.5	10.8		34.4		31.4	29.5		38.2		37.5	37.9	
7	84.0		83.2	57.6							34.6		32.1	30.4		36.3		35.4	35.9	
8	80.2	87.0	81.5			22.5	27.6	12.5			34.5	33.3	31.5			37.3	37.0	36.5		
9	65.2	72.3	71.4	68.4		16.8		17.6	7.6		35.1	34.6	32.5	30.9		37.2	36.9	37.1	36.7	
10	71.4		79.7	67.0							35.0		32.0	29.3		37.0		38.2	38.0	

Table 5 (A)

DESCRIPTION OF PATIENTS STUDIED - MULTIPLE TEMPERATURES

PATIENT	AGE	WEIGHT (kg)	BODY SURFACE AREA (m^2)	TOTAL BODY SURFACE BURN (Per Cent)	PER CENT 3°	POSTBURN DAY STUDIED
1	43	118.0	2.36	41	3	10
2	32	50.0	1.48	46	17.5	6
3	18	66.0	1.88	63.5	21.5	10-11
4	22	48.0	1.59	76	33.5	33
5	74	66.4	1.71	46	27	6
6	38	113.0	2.15	51	22	7
7	22	64.0	1.86	57.5	19.5	7
8	60	67.3	1.84	62	53	11
9	34	77.0	1.91	66.5	39.5	14
10	25	68.0	1.85	84	76	8-9

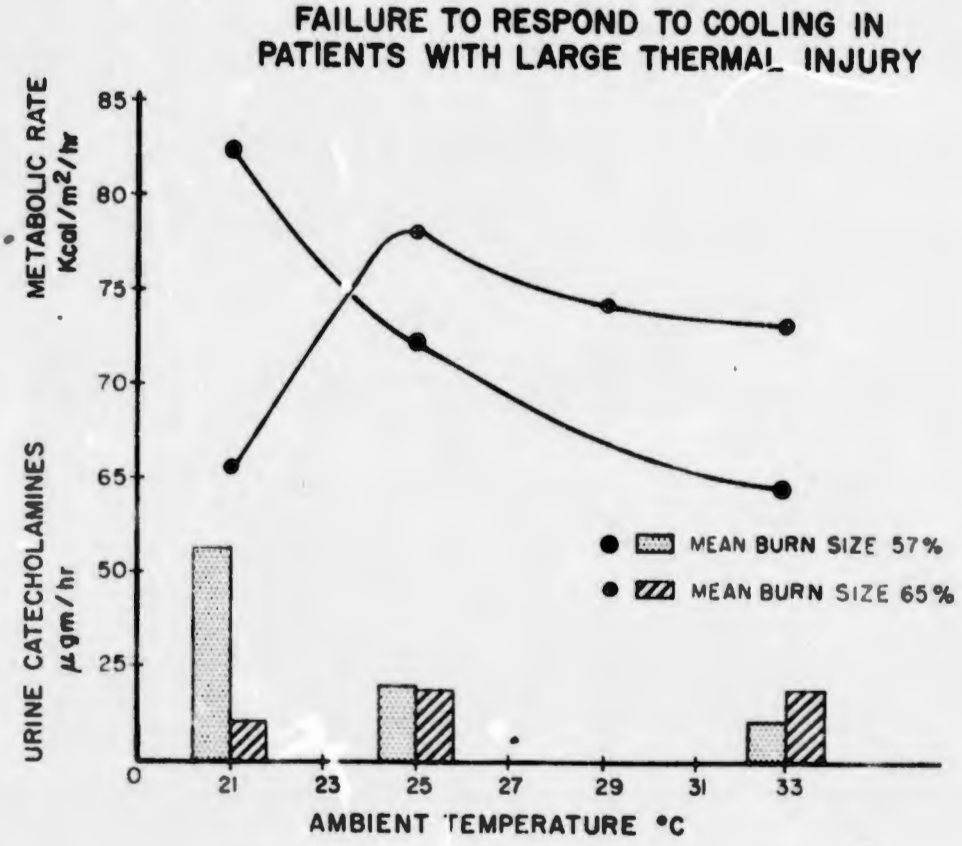

Figure 3. The ability to generate additional heat to cold stress is variable in patients with large thermal injury, but appears to depend primarily on burn size. Individuals with smaller injuries and available metabolic reserve respond to cooling by increasing metabolic rate and this response is associated with increased urinary catecholamines (solid dots, stippled bars), while others lack catechol or tissue reserves, fail to maintain heat balance, and become hypothermic (crossed dots). These "nonresponders" are apparently at maximal rates of energy production and cannot respond appropriately to catechol mediated stress, such as cooling, infection, and hemorrhage. Two elderly patients studied reacted physiologically like poikilotherms in the varying ambient temperatures.

Table 6

METABOLIC REQUIREMENTS IN PATIENTS WITH BACTEREMIA

PATIENT	AMBIENT TEMPERATURE	METABOLIC RATE (Kcal/m^2/hr) PREDICTED*	MEASURED	URINARY CATECHOLAMINES (ugm/hr)
1	21	84.5	66.2	668
	25	74.3	51.7	195
	33	70.2	58.8	22.8
2	25	74.5	40.6	
	29	69.8	35.6	
3	33	71.0	60.4	
4	25	74.7	54.5	
5	21	84.4	51.3	
	25	74.2	53.8	
	29	69.4	48.2	
	33	70.1	45.5	
6	25	72.7	35.9	
	29	67.9	35.9	
7	25	71.4	44.4	930
	25	71.4	54.0	1,391
8	25	64.7	54.7	264
9	25	75.2	52.0	3,498
10	25	72.8	62.3	318

*Based on regression equation, measurements in five patients before septic episode are comparable to the predicted values.

core insulation and decreased sweat secretion. Further cooling results in hypermetabolism, which occurs in an individual with a normal or slightly decreased core temperature and a cool dry skin. Our patients, however, were hypermetabolic in a warm environment, with elevated core and skin temperatures, and, when equilibrated with a cooler environment, continued or slightly increased their hypermetabolism while their core and skin temperature remained higher than those of the normal controls. Evaporative water loss and surface cooling in the burn patient is not the primary stimulus for the hypermetabolic state but rather the hypermetabolic response is related to an endogenous reset in metabolic activity. Vaporizational heat loss serves as a convenient route for transfer of this large heat load from the body.

The inability of the burn patient to return metabolic rate to normal levels in a warm environment is comparable to reports by Swedish investigators, who found a decrease of metabolic rate between 22° and 32° C, but failed to demonstrate a return of metabolism to basal levels in patients in warm dry air (Barr P-O, Birke G, Liljedahl S-O, Plantin L-O, Lancet 1:164, 1968).[1] As noted in the present study, hypermetabolism occurs in normal man at 21° C, and other reports suggest that the critical temperature for man is approximately 24-25° C (range 22-27) (Erikson H, Krog J, Anderson KL, Scholander PF, Acta Physiol 37: 35, 1956; Wilkerson JE, Raven PB, Horvath SM, J Appl Physiol 33:451, 1972).[12,50] Exposure to ambient conditions below this temperature results in hypermetabolism. Increased heat production, accentuated nitrogen excretion, and weight loss occur in fasted normal man (Iampetro PF, Bass DE, J Appl Physiol 17:947, 1962),[33] burn patients (Birke G, Carlson LA, Von Euler US, Liljedahl S-O, Plantin L-O, Acta Chir Scand 138:321, 1972),[4] and individuals with fractures (Cuthbertson DP, Fell GS, Smith CM, Tilstone WJ, Nutr Metab 14:92 (supp), 1972)[11] when studied below critical temperature, and this is a normal physiologic response to cold exposure. We interpret our data and the other clinical reports as indicating that warm environments do not abate the metabolic response to injury but rather that cold environments accentuate or augment the post-traumatic metabolic response to injury.

What is the mediator for the increased energy production following thermal injury? Hormonal stimulation of heat production occurs with the elaboration of thyroid hormones or catecholamines (Hsieh ACL, Carson LD, Amer J Physiol 190:243, 1957).[31] Thyroid function has been carefully studied and hyperthyroidism does not appear to cause the increased oxygen consumption following burn trauma (Cope O, Nardi GL, Quijano M, Rovit RL, Stanbury JB, Wright A, Ann Surg 137:165, 1953).[10] Catecholamines are elevated following thermal injury (Goodall McC, Stone C, Haynes BW Jr, Ann Surg 145:479, 1957),[18] and adrenergic activity, as measured by urinary excretion of catecholamines (Von Euler US, Clin Pharmacol Ther 5:398, 1968),[47] is related to the extent of stress or severity of injury and to the oxygen consumption of the

patient (Harrison TS, Seaton JF, Feller I, Ann Surg 165:169, 1967).[29] With wound healing, catecholamines and metabolic rate return to normal.

Cannon demonstrated the importance of the sympathetic nervous system to the maintenance of body homeostasis following a wide variety of stresses, and suggested that increased heat production, which characteristically occurs with sympathoadrenal discharge, may be one of the most important physiologic effects of these hormones (Cannon WB, The Wisdom of the Body, New York, Norton, 1967, pp 177-201).[7] Catecholamines infused into animals increase metabolic rate, and epinephrine or norepinephrine (Stone DJ, Keltz H, Sarkar TK, Singzon J, J Appl Physiol 34:619, 1973)[42] infusions in normal man produce hypermetabolism. Rats in which adrenergic function is completely blocked die in three hours when exposed to 4° C ambient environment, while normal animals adjust to the temperature by increasing their metabolic rate (Brodie BB, Davies JI, Hynie S, Krishna G, Weiss B, Pharm Review 18:273, 1966).[5] Totally sympathectomized cats can be maintained in a carefully controlled laboratory environment, yet are unable to defend against hypoxia, fluid restriction, stresses of environmental temperature, hemorrhage, and exercise (Cannon WB, Newton HF, Bright EM, Menkin V, Moore RM, Amer J Physiol 89:84, 1929).[8] The importance of the adrenergic beta receptors in mediating calorigenesis was demonstrated by Estler and Ammon (Estler C-J, Ammon HPT, Canad J Physiol Pharm 47:427, 1969),[13] and was confirmed in our patient studies. Alpha blockade did not affect heat production in the burn patients, while combined alpha and beta blockade and beta blockade alone significantly reduced metabolic rate. Thus, the increased heat production appears to be mediated by catecholamines, which have a direct effect on cellular calorigenic activity. However, the increase in sympathetic activity and catecholamine elaboration in the burn patients is not the immediate effect of cold stimulation of peripheral or central nervous system receptors.

Other factors interact with the basic reset in internal metabolic activity to alter the final sympathetic-mediated physiologic response. First, ambient temperature affects the basic reset to increase metabolic rate as environmental temperature falls below 25° C. Moreover, patients with burns greater than 40 per cent of the total body surface demonstrate a slight reduction in heat production between 25 and 33° C. This response is due to the inability of patients with large thermal injury to achieve adequate core to skin insulation and reduce surface temperature in response to cooling (Wilmore DW, Mason AD Jr, Johnson DW, Pruitt BA Jr, In Press, J Appl Physiol),[51] as demonstrated in this study by the increased core-skin heat conduction in the burn patients as compared to controls. One explanation for the impairment in insulative function and inability to regulate skin temperature and limit heat loss is that tissue injury results in increased blood flow to the skin, apparently to aid oxygenation and improve nutrient supply to insure wound healing. This thesis is supported by studies in burns demonstrating a hyperdynamic circulatory state, increased cutaneous blood

flow, and peripheral shunting, associated with normal visceral flow and oxygen extraction (Gump FE, Price JB Jr, Kinney JM, Surg Gynec Obstet 130:23, 1970).[26]

Secondly, the ability to respond to a stimulus requiring catecholamine-mediated calorigenesis depends upon the availability of catecholamine reserves and the ability of tissue to respond to increased catechol stimuli. Goodall and Haynes reported that patients with large thermal injury may show depletion of adrenal medullary catecholamines (Goodall McC, Haynes BW Jr, J Clin Invest 39:1927, 1960),[19] and Goodall and Moncrief demonstrated that severe thermal injury could deplete monoamine stores in the sympathetic nerve endings and sympathetic ganglia (Goodall McC, Moncrief JA, Ann Surg 162:893, 1965).[20] Labelled dopamine administered to patients with large thermal injury demonstrated an increased turnover compared with normals, and there was a marked shift of this precursor toward noradrenaline synthesis and utilization (Goodall McC, Alton H, J Clin Invest 48:1761, 1969).[21] The turnover and excretion of dopamine was so rapid that Goodall suggested precursors be administered to burn patients to insure adequate adrenergic stores. These study patients with burns greater than 40 per cent of the body surface appear to maintain maximal or near maximal rates of catechol synthesis and utilization. Exposing these patients to a cool environment (21° C) results in a mild cold stress, ordinarily a stimulus for the elaboration of additional catecholamines. Patients who eventually survived responded by increasing heat production as a result of increased elaboration of catecholamines. In contrast, the patients who lacked catecholamine reserve or tissue responsiveness to these mediators failed to generate additional heat to maintain heat balance in the 21° environment and became hypothermic, with the decrease in catecholamine excretion reflecting a predictable hormonal response during hypothermia. All of these nonresponding patients subsequently died from complications of their injury. Like Cannon's sympathectomized cats, the nonresponders lacked homeostatic reserve, for injury had reset their rate of energy production at a maximum level. Additional sympathetic nervous system reserve was unavailable for catecholamine-mediated responses to cooling, infection, or hemorrhage.

The third factor which interacts with the internal post-traumatic reset in metabolic activity is associated injuries and infection. Burn patients with associated injuries, such as fractures, head injuries, or visceral trauma, have metabolic rates which exceed values predicted for their burn wound size. This, however, only occurs if the burn injury is smaller than 40 per cent of the total body surface and their metabolic activity is not at maximal levels. Associated injuries in patients with large burns exert little or no additional metabolic effect, for the thermal injury has already caused a maximal stress response.

Infection is commonly associated with a febrile response and an increase in metabolic rate (Coleman W, Dubois EF, Arch Int Med 15:887, 1915).[9] This is true in patients with small burns who have "calorigenic reserve," for infection will cause an additional expenditure of energy which abates as the septic process is controlled. Sepsis evokes a sympathoadrenal response (Feldman J, Gellhorn E, Endocrinology 29:141, 1941; Griffiths J, Groves AC, Leung FY, Surg Gynec Obstet 6:897, 1973; Groves AC, Griffiths J, Leung F, Meek RN, Ann Surg 178:102, 1973),[14,24,25] but the physiologic effects of the catecholamines appear to be blunted as a consequence of the infection in patients with large thermal injury, a response similar to pharmacologic competitive adrenergic blockade. The marked increase in catecholamines in the blood and urine of our septic patients was accompanied by an inappropriately low physiologic response to these neurohumoral mediators, resulting in inadequate heat production, increased skin blood flow, and progressive hypothermia. While these high levels of catecholamines may represent a massive response of the sympathetic nervous system to infection associated with tissue refractoriness, these infected patients are similar to others with large thermal injury with limited catecholamine stores and minimal homeostatic reserve. This large increase in excretion of urinary catecholamines is observed when the tissue inactivation process is inhibited, as seen with receptor blockade, which results in transmitter overflow (Iverson LL, The Uptake and Storage of Noradrenaline in Sympathetic Nerves, London, Cambridge Univ Press, 1967, p 87)[34] and a diminution of the end-organ physiologic response. We have not observed in nonseptic burn patients the very high plasma and urine concentrations of catecholamines measured in the infected patients.

Increased sympathetic activity occurs following burn injury but is also characteristic of major trauma (Frankson C, Gemzell CA, Von Euler US, J Clin Endocr 14:608, 1954),[15] cold exposure (Hsieh ACL, Carson LD, Amer J Physiol 190:243, 1957),[31] severe exercise (Von Euler US, Hellner S, Acta Physiol Scand 26:183, 1952),[48] and other stresses (Vetter NJ, Strange RC, Adams W, Oliver MF, Lancet 1:284, 1974).[45] The sympathetic nervous system directs the mobilization of substrate from tissue stores to provide specific body fuels by having direct effects on body tissues, by altering circulation, and by interacting with other hormones to regulate substrate flow (Himms-Hagen J, Pharm Reviews 19: 367, 1957; Robinson GA, Butcher RW, Sutherland EW, Cyclic AMP, New York Academic press, p 145)[30,41] In addition, catecholamines stimulate calorigenesis by a direct effect on cellular metabolic activity to increase heat production (Griffith FR Jr, Physiol Reviews 31:151, 1951).[23] As the stress decreases, sympathetic activity falls to normal, and, with parasympathetic activity, substrate is directed into tissue synthesis and energy storage. Early physiogists recognized this interaction and characterized the sympathetic nervous activity as catabolic, directing

the expenditure of energy, and the parasympathetic activity as anabolic, conserving and restoring body fuel (Best CH, Taylor NB, The Physiological Basis of Medical Practice, Baltimore, Williams and Wilkins, 1955, p 737).[2]

What then directs and regulates the sympathetic response following thermal injury? The reflex arc is composed of nervous and/or humoral afferent stimuli to the hypothalamus, which in turn initiate responses along sympathetic and motor efferent pathways (Gordon ML, Endocrinology 47:347, 1950; Griffith FR Jr, Physiol Reviews 31:151, 1951).[22,23] Stimulation of the ventral medial nucleus of the hypothalamus in animals produces increased sympathetic activity associated with hyperthermia, hyperglycemia, hyperglucagonemia, and insulinopenia (Frohman LA, In Parabiology Annual, edited by H. L. Ioachim, New York, Appleton-Century-Croft, 1971, p 353).[16] This and other related hypothalamic nuclei integrate the function of the autonomic nervous system and regulate body temperature, the flow of energy substrates, metabolic rate, and other endocrine functions in a coordinated manner. The metabolic control center of the hypothalamus is closely integrated with the activity of the thermal regulatory centers, specifically with the posterior area described by Isenschmidt and Krehl, which regulates the cold response, and some authors have suggested that this thermal regulatory area and the sympathetic center are the same (Best CH, Taylor NB, The Physiological Basis of Medical Practice, Baltimore, Williams and Wilkins, 1955, p 737).[3]

Burn patients appear to have reset their thermal regulatory set point upward, thus increasing the discharge of sympathetic impulses to stimulate heat production and substrate mobilization in order to maintain a new and elevated core temperature. Burn patients increase metabolic rate in a cool environment at core and skin temperatures above the set points recorded for normal man (Wilmore DW, Mason AD Jr, Johnson DW, Pruitt BA Jr, In Press, J Appl Physiol).[51] If burn patients are allowed to control the ambient temperature, they select "comfort" environments between 28 and 35° C and maintain elevated core and skin temperatures while subjectively comfortable (Wilmore DW, Mason AD Jr, Johnson DW, Skreen RW, Pruitt BA Jr, USA Institute of Surgical Research Annual Progress Report, MEDD-288(R1), 1973, 39-23).[55] Fasting blood glucose is above normal levels following injury, and the hyperglycemia is related to the extent of the injury and the increased catecholamine excretion rate. Fasting levels of growth hormone are also elevated above control values in spite of hyperglycemia (Orcutt TW, Wilmore DW, Pruitt BA Jr, Unpublished Data).[38] Afferent neural or hormonal stimuli from the injury appear to influence hypothalamic centers to elevate central temperature (and/or metabolic) set point, increasing sympathetic nervous system activity, and resulting in the hypermetabolism characteristic of thermal injury.

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PRESENTATIONS

Wilmore DW. Catecholamines: Mediator of Hypermetabolic Response to Thermal Injury. American Surgical Society, Colorado Springs, Colorado, May 1974.

Wilmore DW. Metabolic Rate, Ambient Temperature, Catecholamines: Interreaction Following Thermal Injury. Army Surgical Society, West Point, New York, June 1974.

PUBLICATIONS

Wilmore DW, Long JM, Skreen RW, Mason AD Jr, Pruitt BA Jr. Catecholamines: Mediator of the Hypermetabolic Response to Thermal Injury. In Press, Ann Surg.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0E 6953	74 07 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. OSD'N INSTR'N	8b. SPECIFIC DATA CONTRACTOR ACCESS	9. LEVEL OF SUM
73 07 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	084
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) Evaluation of Gastric Physiologic Disturbances Associated With Thermal Injury in a Military Population (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
72 01	Cont	DA	C. In-Hosue

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 74	.5	1
FISCAL YEAR CURRENT 75	.4	5

19. RESPONSIBLE DOD ORGANIZATION
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21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code): (U) Evaluation; (U) Gastric physiologic distrubances; (U) Thermal injury; (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 study the gastric pathophysiology of the thermally injured soldier, so as to better define etiologic factors responsible for Curling's ulcer.

24. (U) Evaluation to be carried out on thermally injured patients with greater than 30% TBS area injury admitted to the USAISR. Study will be stratified so that a group of patients in the 30 to 50% TBS area injury and the second group in the 50 to 70% TBS area injury will be included. Investigative procedures: Will be performed within 24 hours if possible and at 72 hours postburn. Burns of greater than 50% body surface area will also be studied at 5 to 7 days post injury and all patients will then be studied between the 9th and 12th day post burn and at 30 days at discharge. Studies will encompass: (1) gastric endoscopy with photography and biopsy for semiquantitative mucous determination, (2) ion flux across the gastric mucosa, (3) coagulation studies, (4) measurements of gastric clearance of radioactive isotopes, and (5) evaluation of the role of bacteremia.

25. (U) 73 07 - 74 06 Studies have been performed on 15 patients admitted to the USAISR. Preliminary endoscopic findings document the existence of definite gastric mucosal abnormalities occurring early post burn which are persistent. The true incidence of acute gastroduodenal lesions or Curlin's ulcers would appear to be much higher than previously determined by clinical course operative and autopsy findings. Measurements of mucous, ion flux, coaculation and gastric clearance of radioactive isotope will be evaluated at a later date.

*Avail. this to contractors upon originator's approval

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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

ABSTRACT

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: EVALUATION OF GASTRIC PHYSIOLOGIC DISTURBANCES ASSOCIATED WITH THERMAL INJURY IN A MILITARY POPULATION

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

Period covered in this report: 1 July 1973 - 30 June 1974

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*Samuel S. Spicer, M.D.

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Gastric pathophysiology in the thermally injured soldier was studied in order to describe factors which may be important in the etiology of Curling's ulcer. The methods for clinical study encompassed 1) gastric endoscopy with photography and biopsy for semiquantitative mucous determinations, 2) ion flux across the gastric mucosa, 3) coagulation studies 4) measurements of gastric clearance of a radioactive isotope.

Thirty-two adult patients were evaluated with burns of greater than 25% total body surface (TBS) sustained within one week of admission to the US Army Institute of Surgical Research. Initial studies were performed within 72 hours post burn and were repeated during the second and third week post injury.

Gastroduodenoscopy demonstrated that superficial gastric and duodenal mucosal lesions occurred soon after thermal injury in most patients (86%) with burns involving more than 35% of their total body surface. Deeper, ulcerative lesions developed later in areas of intense early mucosal injury. Gastric and duodenal abnormalities frequently coexisted without clinical signs or symptoms. Twenty-one gastric biopsies in 9 patients demonstrated normal quantities of superficial and deep cellular mucosubstances as determined by mucous histochemistry. Diffuse gastric mucosal disease was present in 78% of the patients with normal cellular mucosubstance. Permeability of the gastric mucosal barrier to hydrogen back diffusion was studied by a lithium flux technique. Diffuse gastric mucosal lesions were present in 7 of 10 patients with a normal mucosal barrier which suggests that an increased back diffusion of hydrogen ions is not an etiologic factor in the development of these early gastric lesions. The disruption of the gastric mucosal barrier in eight patients correlated with endoscopic

and clinical progression of mucosal disease. Gastric clearance of radioactive technectium was too variable for analysis. There was no correlation between changes in coagulation studies and the endoscopic or clinical manifestations of gastroduodenal mucosal disease.

Evaluation
Gastric physiologic disturbances
Thermal injury
Burn patients

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EVALUATION OF GASTRIC PHYSIOLOGIC DISTURBANCES ASSOCIATED WITH THERMAL INJURY IN A MILITARY POPULATION

Gastric pathophysiology of the thermally injured patient has been studied to better define the etiologic factors responsible for Curling's ulcer. This clinical study encompassed 1) gastric endoscopy with photography and biopsy for semiquantitative mucous determinations, 2) measurement of ion flux across the gastric mucosa, 3) measurement of coagulation indices, and 4) measurements of the gastric clearance of a radioactive isotope.

Thermally injured patients with greater than 25% total body surface area injury were evaluated within one week of admission to the U.S. Army Institute of Surgical Research. Written informed consent was obtained from all patients prior to study. A minimum age limit of 15 years was established for the study. Clinical investigative procedures were performed within 24 hours, if possible, and at 72 hours post burn. Further evaluation was carried out during the second and third week post injury.

Gastroduodenal Endoscopy

To determine the true incidence, morphology and behavior of acute gastroduodenal disease following thermal injury, early and serial fiberoptic gastroduodenoscopies were performed in 32 burn patients. A history compatible with chronic peptic ulcer disease or gastritis, evidence of previous gastrointestinal surgery, or a history suggestive of excessive alcohol, aspirin, or steroid consumption eliminated the patient from consideration. Resuscitation fluids, systemic and topical antibiotics, vitamins, antacids, analgesics, anesthetics, and nutritional support were managed independently by each attending physician. Gastroduodenoscopy was performed with the Olympus GIF-D fiberoptic pan-endoscope. Premedication consisted of sufficient intravenous diazepam to induce drowsiness (up to 20 mgs). This was occasionally supplemented by intravenous Meperidine (up to 25 mgs). Each endoscopic procedure thoroughly evaluated the distal two-thirds of the esophagus, the entire stomach, and the first portion of the duodenum. Photographs were taken of each area examined and representative mucosal biopsies were procured. In patients with nasogastric tubes in place, superficial gastric lesions that were in a linear distribution or that were localized to a discrete area of the stomach were attributed to the effects of mechanical irritation and were discounted from the study.

During the eight month period between January and August 1973, 77 thermally injured patients who fulfilled the study criteria regarding age, burn size, and post burn interval were admitted to the U.S. Army Institute of Surgical Research. Thirty-nine of these eligible patients (51%) entered into the investigation and 32 were successfuly examined. All of the patients studied with one exception were male. Ages ranged from 16 to 74 years (average age 34.8 years) and the burn size varied from 23% to 96% total body surface (Average burn size 56.6%). Larger

burns predominated in the study group with 19 patients having injuries greater than 50% of their total body surface (Table 1). Mortality within the study population was 75% (24 of 32 patients). Mortality of patients eligible for study but not evaluated by endoscopy was 73% (33 of 45 patients).

Fifty-three gastroduodenoscopies were performed without complication in the 32 patients. Six patients were studied on three occasions at intervals designated by the protocol. Nine patients were examined twice, while 17 patients were evaluated only once.

Twenty-three of the 32 patients (72%) were studied within 72 hours after injury. Four of these patients were examined within 24 hours of injury (one as early as 12 hours post burn). The remaining nine patients were evaluated 4 to 20 days after injury.

Thirty-five biopsy specimens (32 gastric and 3 duodenal) were obtained from 19 of the patients evaluated within the initial 72 hour period. Postmortem examinations in 19 of 24 patients confirmed the findings described at gastroduodenoscopy.

Superficial Gastric Mucosal Injury

Superficial mucosal abnormalities were recognized at gastroscopy in 25 of the 32 patients (78%) (Table 1). Gastric mucosal injury was identified as early as 12 hours post burn and was present in 17 of the 23 patients evaluated within 72 hours after thermal injury. Three patients with burns involving less than 30% of their total body surface had repeatedly normal examinations. Twenty-nine patients had burns involving more than 35% of their total body surface. In this group of larger burns, gastric mucosal abnormalities were recognized soon after thermal injury in 25 of the 29 patients (86%). Three types of superficial gastric mucosal lesions were recognized. Most commonly, small punctate erythematous lesions, a few millimeters in diameter, were diffusely scattered over the rugal crests of the fundus and body. Areas of central pallor were frequently present within these small circumscribed areas of erythema. A second variety of lesion suggested a conglommeration of the smaller lesions, appearing as a large (often greater than 2 cm in diameter), irregularly shaped, confluent area of erythema and mucosal hemorrhage. Discrete erosions with circumferential erythema represented the third type of lesion usually encountered. All three varieties of lesions could be present within the same stomach as early as 24 hours after injury. The histology of these lesions revealed microvascular congestion, mucosal hemorrhage with mild inflammation, and cellular disruption above the muscularis mucosae.

The gastric lesions were always distributed over the fundus and body of the stomach. While disease was never isolated to the antrum, in 12 of 25 patients (48%), the antrum was also abnormal. Serial evaluations indicated that the antrum was usually less extensively involved than the

Table I

Incidence of Acute Gastric and Duodenal Disease in Relation to Burn Size

TBS Injury (%)	No. Patients	No. Gastric Abnormality	No. Duodenal Abnormality
0-20	0	0	0
20-30	3	0	0
30-40	2	2	1
40-50	8	6	6
50-60	7	7	7
70-80	4	4	2
80-90	3	3	3
90-100	3	2	2
Total	32	25	25
% Abnormality		78%	72%

more proximal areas.

Gastric Ulceration

Seven patients in the study group (22%) had gastric ulcers by endoscopy (Table II). Three patients had multiple gastric ulcers and two of these had concomitant duodenal ulcerations. All seven patients had superficial gastric mucosal disease and the ulcerations were located in areas of diffuse mucosal abnormalities. The earliest detection of a discrete gastric ulcer was at 96 hours post burn: two other patients developed ulcerative lesions in areas of superficial mucosal injury during the first week post burn.

Superficial Duodenal Inflammation

Twenty-three of the 32 patients (72%) had diffuse mucosal abnormalities of the proximal duodenum at the time of their initial endoscopy (Table I). Acute duodenal changes were evident in 14 of the 23 patients (61%) who were evaluated within 72 hours post burn. All patients with duodenal involvement had burns of greater than 35% total body surface, and in all but two cases, acute gastric mucosal disease was also present. Erythema, edema, increased mucosal friability, and erosions of the bulb and proximal duodenum characterized the endoscopic picture of "duodenitis" in these patients. In some cases, there appeared to be actual mucosal sloughs within the duodenal cap. The histology of representative areas demonstrated microvascular congestion with mucosal hemorrhage, increased round cell infiltration, and occasionally, cystic dilatation of Brunner's glands.

Seven of the 23 patients with "duodenitis" also had duodenal ulcers at the time of their first examination. In these patients mucosal erythema and congestion were most pronounced in the vicinity of the ulcer, although the mucosa of the entire duodenal bulb appeared abnormal. Two other patients with erosive duodenal disease recognized within 72 hours post burn later evolved a frank duodenal ulceration in the area of early superficial injury. Both ulcers were clinically inapparent and healed uneventfully.

Only two patients had duodenal changes without accompanying gastric disease. One of these patients with isolated "duodenitis" demonstrated a reactivated chronic duodenal ulcer and the other had biochemical evidence of pancreatitis.

Duodenal Ulceration

Nine of the 32 patients had duodenal ulcerations (28%); two of these patients also had multiple gastric ulcers (Table II). Duodenal ulcerations were not observed within 72 hours after injury, the earliest lesion being detected on the 4th post burn day. Seven of the 9 ulcers were in the anterior - inferior portion of the duodenal bulb; the other

Table II

Types of Gastroduodenal Disease
Encountered at Endoscopy

	Number	Total Patients
Erosive gastritis*		25 (78%)
With gastric ulcer	7	
With duodenitis	13	
With duodenal ulcer	6	
Gastric ulcer		7 (22%)
With duodenal ulcer**	2	
Duodenitis		23 (72%)
With duodenal ulcer	9	
With pancreatitis	1	
Duodenal ulcer		9 (28%)
Chronic, reactivated	1	

*Fundus and body were always involved; antral involvement in 48%.

**Associated with multiple gastric ulcers.

two ulcers were located on the posterior wall of the duodenum. All duodenal ulcer patients had an accompanying "duodenitis"; in two of these patients an ulceration was actually observed to evolve in the area of early erosive disease. With one exception, all patients with duodenal ulcers also had acute gastric mucosal disease.

Clinical Behavior of the Acute Gastroduodenal Disease

Although over 70% of the patients evaluated within 72 hours post burn had endoscopic abnormalities of the stomach and duodenum, and 75% of this population succumbed to complications of their burn disease, the incidence of brisk hemorrhage and perforation in this group of severely ill patients was only 28% (9 of 32 patients) (Table III). Surgical intervention for control of life threatening hemorrhage was required in two patients, both with duodenal ulcers. Ulcer perforation (one gastric and one duodenal) occurred in two other patients. In the entire study group, only four patients (12.6%) developed gastrointestinal complications which necessitated a laparotomy (Table III).

Successive endoscopic evaluations were performed in 15 of the 32 patients. Progression of the gastroduodenal abnormalities was documented in 9 of 15 patients by an increased number and distribution of lesions as well as by ulcer formation. Endoscopic evidence of disease progression closely paralleled a deterioration in the clinical course of the patient. Six of the 7 patients with progression of their mucosal disease were septic and four of these were also hypotensive and hypoxic. The two patients whose disease progressed in spite of an uncomplicated clinical course had erosive "duodenitis"; an asymptomatic duodenal ulceration developed later in both patients.

The gastric mucosal disease in four patients improved coincidental with the stabilization of vital signs, resumption of oral diet, and evidence of wound healing. Only one patient actually healed this superficial gastric mucosal disease completely during the two week period of observation. Although rapid clinical improvement was evident in the three other patients, their gastric mucsoal abnormalities appeared to improve slowly.

Gastric Mucosubstance Histochemistry

A change in the mucous protective barrier has been suggested as a basis for acute gastric ulcerations. An alteration in this barrier might result from decreased mucous production or a change in the character of the mucous produced, thereby rendering the gastric mucous membrane more susceptible to damage. Histochemical techniques provide a means of studying directly the cells of the gastric mucosa that produce the mucous protective barrier. The purpose of this study was to evaluate with histochemical methods the mucosubstance present in the various gastric epithelial cells and to correlate the content of mucosubstance with acute ulcerative disease of the gastric mucosa.

Table III

Incidence of Clinically Significant Complications
in 32 Thermally Injured Patients

Types of Lesions	Total No. of Cases	Hemorrhage*	Perforation	Operation
Erosive gastritis	25	1	0	0
Duodenitis	23	0	0	0
Duodenal ulcer	9	3	1	3
Gastric ulcer	7	3	1	1
Total complications		7	2	4
% Incidence		22%	6.3%	12.6%

*Three or more blood transfusions over 24 hours.

Table IV

GASTRODUODENAL LESIONS AFTER THERMAL INJURY
(9 PATIENTS)

		TOTAL NUMBER
ACUTE GASTRODUODENAL DISEASE		7 (78%)
EROSIVE "GASTRITIS"	7	
GASTRIC ULCER	1	
EROSIVE "DUODENITIS"	3	
DUODENAL ULCER	2	
NO LESIONS		2 (22%)
		9 (100%)

Table V

GASTRIC MUCOSUBSTANCE HISTOCHEMISTRY
AFTER THERMAL INJURY

(9 PATIENTS)

	NUMBER	SUPERFICIAL CELLS	CHIEF CELLS
ACUTE GASTRIC LESIONS	7 (78%)	+(7/7)	0 (7/7)
NO LESIONS	2 (22%)	+(7/7)	9 (7/7)

+ = 3 - 4^+ GRADING

0 = 0 - 2^+ GRADING

Nine patients with a mean age of 28 years and a mean burn size of 56% were studied by fiberoptic gastroduodenoscopy and gastric biopsy. Gastroduodenoscopy was performed on all patients within five days post burn. Under direct vision, biopsies were taken from areas of intact mucosa in the body of the stomach. Sections of each specimen were stained with hemotoxlyn and eosin for morphologic examination and with histochemical methods for visualizing and differentiating carbohydrate secretions of the stomach. The histochemical methods included the alcian blue (pH 2.5) periodic acid Schiff (PAS) method of Mowry and Winkler and a sequency of an aldehyde fuchsin followed by alcian blue at pH 2.5 and azure A at pH 4.5. The surface epithelium in mucous neck cells were evaluated particularly with the alcian blue - PAS sequence which colors neutral mucosubstance red and acid mucosubstance turquoise. The chief cells were appraised mainly with the aldehyde fuchsin - alcian blue sequence which stains sulfated mucosubstance purple. Specimens were coded without knowledge of the endoscopic findings and mucosubstance was graded semi-quantitatively (0.4+).

Gastroduodenal lesions were present on initial endoscopic examination in 7 of 9 patients (78%). The spectrum of gastroduodenal disease apparent endoscopically included superficial gastric mucosal lesions in all seven, gastric ulcer in one, superficial duodenal mucosal lesions in three and duodenal ulcer in two patients (Table IV).

The gastric biopsies revealed normal staining of neutral and acidic mucosubstance in superficial and deep foveola cells; however, chief cells were depleted of their sulfated mucosubstance in all samples (Table V).

ION FLUX ACROSS THE GASTRIC MUCOSA

A protective gastric mucosal barrier has been described and documented by Davenport.[1] The epithelial cells of the gastric mucosa have been described as the true barrier. Disruption of the gastric mucosal barrier (GMB) has been documented in critically ill patients and is believed to reflect poor vascular perfusion and subsequent mucosal injury.[2] This is said to result in increased permeability of the gastric mucosa to hydrogen ion which in turn may lead to progressive mucosal damage and the development of "stress" ulcerations. In order to define the possible pathogenic influences for the development of gastroduodenal lesions after thermal injury, a study to determine the status of the GMB after thermal injury was instituted.

Determinations of the permeability of the gastric mucosa in thermally injured patients were performed concurrently with the serial study of gastroduodenal mucosal changes as determined by endoscopy. The technique utilized for determination of GMB integrity and ion flux involved changes in the ratio of lithium/PEG (polyethylene glycol - a nonabsorbable marker) over an established time period. Lithium has been documented to diffuse across the gastric mucosa in a logarithmic relation to hydrogen ion and the quotient of the permeability coefficients of lithium and hydrogen are

constant in a variety of experimental conditions that increase mucosal permeability.[3] The LI/PEG changes reflect only the back diffusion of lithium from lumen to mucosa because lithium is neither secreted nor neutralized.

A dobule lumen nasogastric tube was positioned to allow free aspiration of gastric contents after an overnight fast. Gastric contents were aspirated and the stomach was washed by three instillations and withdrawals over one hour of 200 cc of an iso-osmotic baseline solution containing 140 mEq/L HCL, 10 mEq/L NaCl, 5 mEq/L LiCl and 1 gram/L of PEG. After the one hour wash and equilibration, 200 cc of the same solution was instilled and mixed. At one minute, a baseline 30 cc sample was withdrawn. After a 15 minute flux period the gastric contents were removed as a final sample. The test period was repeated and all samples were analyzed for concentrations of hydrogen, lithium, sodium, potassium and PEG.

Ion fluxes were inferred by changes in the ratio of Li/PEG over the 15 minute test period. The change in LI/PEG ratios for each patient study were averaged. An increase in the ratio change represents a positive ion flux (net gain of luminal ion) and a decrease in the ratio represents a negative flux (net loss of luminal ion). Luminal volume changes, pyloric losses, and duodenal reflux do not change the ratio of LI/PEG.

To establish the validity of this determination of gastric mucosal permeability, seven male adult volunteers with no previous history of gastrointestinal disease underwent 29 periods of testing as described perviously. After establishing control values, four of the same volunteers underwent 17 periods of testing utilizing the same test solution with the addition of acetylsalacylic acid (20 mm ASA). The concentration of ASA utilized has been previously documented to disrupt the GMB in humans and produce an increase in lithium and hydrogen back diffusion.[4] Analysis of the data revealed the presence of lithium back diffusion after testing with ASA test solution. The difference between the values for control (mean change LI/PEG = + .15± .04) and ASA test period (mean change Li/PEG = + .08 ± .03) was significant statistically (P less than .01). Back diffusion of hydrogen and lithium has been documented to be a normal characteristic of the gastric mucosa; however, the methodology for determining lithium flux in our hands consistently gave positive flux values in the control situations indicating no back diffusion and representing gastric PEG adsorption despite a prior one hour wash. This test however, does document the negative net flux of lithium when the GMB is disrupted as evidence by the ASA test group. Since the purpose of the study was to determine whether GMB disruption was a factor related to gastric mucosal changes after thermal injury, the data from patient studies were analyzed as to the presence or absence of GMB disruption.

Twenty-five thermally injured patients (23 male and 2 female) with

an age range of 16-71 years (mean age of 34 years) were studied. Burn size ranged from 34% to 90% total body surface with the mean burn size being 58%. It was possible to evaluate the physical status of the gastric mucosa at autopsy in 18 of the 25 patients.

Based on the lithium flux test, the 18 patients were divided into two groups of 1) GMB disruption or 2) normal GMB. Ten patients revealed a normal GMB; however, 7 or the 10 (70%) demonstrated gastric mucosal lesions by endoscopy. The spectrum of mucosal disease included superficial areas of erythema, mucosal hemorrhage, discrete erosions, and acute gastric ulcerations. Of the eight patients with disruption of the gastric mucosal barrier, six demonstrated gastric mucosal lesions. Seven of the eight patients with disruption of the gastric mucosal barrier (87.5%) developed gastric bleeding, perforation or progression of the mucosal disease on serial endoscopic examinations (Table VI). Two of the ten patients with normal gastric mucosal barr'ers manifested endoscopic progression only. Progression of gastric lesi ns was defined as 1) an increase in the number and distribution of lesions 2) the appearance of distinct new gastric lesions as manifested usually by progression to definite mucosal ulcerations, or 3) a clinical catastrophe such as gastrointestinal bleeding or perforation of an existing lesion. Significant bleeding was defined as a necessity for 3 or more units of whole blood over 24 hours to stabilize vital signs and maintain the hematocrit.

Conversely, if the patients are divided into those who manifested progression of disease and those who demonstrated no progression, we can determine if the relationship between disruption of the GMB and progression of mucosal disease still exists. There were 9 patients in each group. Studies of the GMB in patients who manifested progression revealed a mean Li/PEG ration change of -.05; however the GMB in patients with no progression of gastric disease revealed a mean Li/PEG ratio change of +.24 (Table VII).

SUMMARY AND CONCLUSIONS

Gastroduodenoscopy indicates that damage to the gastric and duodenal mucosa occurs soon after thermal injury in most patients (86%) with greater than 35% total body surface burn. The early occurrence, morphology and histology of the lesions suggest that mucosal ischemia is a primary etiologic factor. The proximal duodenum is involved almost as frequently as the gastric mucosa. Deeper, ulcerative lesions develop later in areas of intense, early mucosal injury. Severe gastric and duodenal abnormalities frequently coexist without clinical signs or symptoms. Subsequent changes of the injured mucosa depend mainly upon the patient's subsequent clinical course.

Gastric mucosal disease was encountered despite normal quantities of superficial and deep cellular mucosubstance. This finding would suggest that loss of the gastric mucous protective barrier is not an

Table VI

GASTRIC MUCOSAL BARRIER DISRUPTION
72 HOURS AFTER THERMAL INJURY

	NO.	GASTRIC LESIONS	PROGRESSION GASTRIC	PROGRESSION DUODENAL
NEGATIVE LI/PEG	8	6/8	7/8	2/7
POSITIVE LI/PEG	10	7/10	2/10	4/10

Table VII

PROGRESSION OF GASTRIC LESIONS

	NO.	LI/PEG
PROGRESSION	9	- .05
NO PROGRESSION	9	+ .24

important etiologic factor in the development of acute gastric lesions after thermal injury. Depletion of anti-peptic sulfated mucosubstance in the pepsin producing chief cells may reflect augmentation of pepsin neutralization; however, this depletion of sulfated mucosubstance may enhance the potential for peptic injury to an already damaged mucosa.

The presence of acute gastric mucosal lesions in 70% of patients with a normal mucosal barrier suggests that an increased back diffusion of hydrogen (H+) ion is not an etiologic factor in the development of these early gastric lesions. Gastric mucosal barrier disruption did correlate with endoscopic and clinical progression of mucosal disease which suggests that back diffusion of hydrogen (H+) ion plays a contributory role in the progression of this disease and may be a useful prognostic index.

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3. Chung, RSK, Field, M and Silen, W: Gastric Mucosal Permeability to Hydrogen and Lithium: A New Method for Quantitation of the Gastric Mucosal Barrier. Surg. Forum, 21:297, 1970.

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PRESENTATIONS:

1. Czaja AJ, McAlhany JC, Jr., Pruitt BA, Jr: Acute gastroduodenal disease following thermal injury: an endoscopic evaluation of incidence and natural history.

2. Histochemical study of gastric mucosubstances after thermal injury: correlation with gastroduodenal endoscopic findings. McAlhany JC, Jr., Czaja AJ, Cathcart RS III, Spicer SS, Foley FD. Presented at American Burn Assoc, Cincinnati, Ohio, Apr 1974.

3. Serial endoscopic evaluation of acute gastroduodenal disease following thermal injury. Czaja AJ, McAlhany JC, Jr., Pruitt BA, Jr. Presented at the American College of Physicians, New York City, New York, 1 Apr 1974.

4. Acute duodenal disease after thermal injury: assessment by

early and serial endoscopy. Czaja AJ, McAlhany JC, Jr., and Pruitt BA, Jr. Presented at American Gastroenterological Assn. San Francisco, Calif., 22 May 1974.

PUBLICATIONS

None

EXHIBITS

Serial endoscopic evaluation of acute gastroduodenal disease following thermal injury. American College of Physicians, N.Y. 1-4 Apr 74 and the American Gastroenterological Assn., San Francisco, Calif. 21-24 May 74.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0E 6979	74 07 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
74 03 27	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	085
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) Assessment of Thermal Conductivity for The Measurement of Gastric Mucosal Blood Flow (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS:
003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
73 11	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.

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FISCAL YEAR PRECEDING 74	.2	1
FISCAL YEAR CURRENT 75	.2	3

19. RESPONSIBLE DOD ORGANIZATION
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ASSOCIATE INVESTIGATORS
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NAME:

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

DA

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Thermal conductivity; (U) Stomach; (U) Mucosal blood flow; (U) Dogs

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

23. (U) To evaluate the possibility of utilizing thermal conductivity as a means of estimating regional gastric mucosal blood flow in the canine stomach as a laboratory simulation of the burned soldier.

24. (U) The study will be performed on mongrel dogs weighing between 10 and 15 kilograms prepared with deinnervated gastric pouch. Aminopyrine clearance will be utilized to obtain reference gastric blood flow measurements. A flexible catheter with a tip thermistor will be obtained and modified to enable self heating. The thermistor tip will be heated above that of "core gastric temperature" and then placed on the gastric mucosa to obtain a temperature record. The temperature records will then be correlated with documented aminopyrine clearance to determine if the degree of cooling of the tip thermistor correlates with mucosal blood flow.

25. (U) 73 11 - 74 06 A thermistor probe has been obtained and the equipment is being calibrated.

387<

*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

ABSTRACT

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: ASSESSMENT OF THERMAL CONDUCTIVITY FOR MEASUREMENT OF GASTRIC MUCOSAL BLOOD FLOW

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

Period covered in this report: 1 July 1973 - 30 June 1974

Investigators: Joseph C. McAlhany, Jr., M.D., Maj, MC
Arthur D. Mason, Jr., M.D.
Travis S. Masterson, Jr., M.S.

Reports Control Symbol MEDDH-288(R1)

The purpose of this study is to evaluate the use of thermal conductivity as a means of estimating regional gastric mucosal blood flow. A catheter with a tip thermistor is commercially available and can be modified to enable self heating. In principle the thermistor tip will be heated above that of "core gastric temperature" and then placed on the gastric mucosa to obtain a temperature record. We propose that cooling will occur to a greater degree with increased mucosal blood flow. Initial evaluation will be performed on mongrel dogs weighing between 10 and 15 kilograms prepared with a deinnervated gastric pouch drained by a large bore Thomas cannula. Aminopyrine clearance will be utilized to obtain gastric blood flow measurement with which to compare thermal conductivity.

Current work is centered on the development of a suitable thermistor catheter which can be effectively passed and maneuvered through the fibroptic gastroscope. After the development of a suitable catheter, the experimental study will be performed to determine the sensitivity and reliability of thermal conductivity in estimating regional mucosal blood flow.

Stomach
Thermal conductivity
Dogs
Mucosal blood flow

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
	DA OC 6971	74 07 01	DD-DR&E(AR)636

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
73 07 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				

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69 07	Cont	DA	C. In-House

17. CONTRACT GRANT Not Applicable
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e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 74	.5	8
	CURRENT 75	.5	6

19. RESPONSIBLE DOD ORGANIZATION
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NAME: DA

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22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Shunt; (U) PV work; (U) Burns; (U) Lung mechanics; (U) Pulmonary diffusion; (U) Blood gases; (U) Wounded soldiers; (U) Ventilation/perfusion abnormalities; (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 identify the types of pulmonary lesions in burned patients; to define their pathophysiology. Study is relevant to military situation since most burn deaths are due to pulmonary disease.

24. (U) Classify patients into inhalation and noninhalation injury. In those with inhalation injury, study serially lung volumes (static and dynamic), flow-volume loops, single breath nitrogen tests for closing volumes, static and dynamic compliance, and diffusion capacity. Compare with results of xenon scan and bronchoscopy. In noninhalation injuries, continue prior work with attention to CO_2 response curve, better measurement of static lung volumes and use of flow-volume loop, single breath tests, and cardiac output.

25. (U) 73 07 - 74 06 Patients treated with silver nitrate topically have been studied in their early postburn course, observing ventilation parameters. Three patients were switched to Sulfamylon to determine its effects. Normal subjects were studied to determine the effect of carbonic anhydrase inhibition.

*Available to contractors upon originator's approval

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ABSTRACT

PROJECT NO. 3A161101A91C, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: PULMONARY PATHOPHYSIOLOGIC CHANGES 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 July 1973 - 30 June 1974

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Reports Control Symbol MEDDH-288(R1)

Seven burned patients treated with silver nitrate dressings were studied during the first 10 days after injury. Minute ventilation, oxygen consumption, and ventilatory equivalent were measured. Minute ventilation was increased two- to three-fold, as was oxygen consumption. Ventilatory equivalent was only slightly increased. Three patients were initially treated with silver nitrate and, then, when clinically stable, switched to Sulfamylon. They showed a 50 per cent rise in ventilation, tidal volume, ventilatory equivalent, and a slight increase in respiratory rate and in VD/VT. In addition, their PO_2 increased and base excess fell.

Five normal subjects were then given Diamox, and their minute ventilation, O_2 consumption, and ventilatory equivalent were measured at rest, with standard exercise, and with an added dead space. Diamox produced only a 25 per cent increase in minute ventilation and ventilatory equivalent. The results suggest that, although some of the increased ventilation of Sulfamylon is due to carbonic anhydrase inhibition, another factor, such as pain, caused by the topical agent, also plays a role.

Shunt
PV work
Burns
Lung Mechanics
Pulmonary diffusion
Blood gases
Wounded soldiers
Ventilation/perfusion abnormalities
Humans

PULMONARY PATHOPHYSIOLOGIC CHANGES FOLLOWING THERMAL INJURY IN BURNED SOLDIERS

Many factors have been proposed to explain the hyperventilation of the burned patient. The metabolic response to injury, with its attendant increased oxygen requirement, pain associated with second degree component of the burns, and topical therapy are all factors in the complex of hyperventilation. We set out to separate the components of hyperventilation by first noting that the ventilatory equivalent (i.e., the liters of total ventilation per liter of oxygen consumed) was constant for varying steady state oxygen demands over a small range (Comroe JH, Physiology of Respiration, Yearbook Med Pub, Chicago, 1965, pp. 192-195),[1] so that, for a given O_2 consumption, we could estimate excess ventilation or wasted ventilation. We then reported earlier studies, using a carbonic anhydrase inhibitor in normals, to quantitate the ventilatory effect of this factor. Lastly, we switched patients from silver nitrate to Sulfamylon when they were felt to be clinically stable, again following ventilatory parameters. By doing this, we could separate the components of hyperventilation into:

1) Increased oxygen consumption

2) Excess ventilation in silver nitrate

3) Carbonic anhydrase inhibition

4) Excess ventilation with Sulfamylon.

METHOD

Part A

Seven burned subjects treated with silver nitrate were studied. None was felt to have an inhalation injury, clinically, and all had negative xenon scans. Table I (Group I) shows age, sex, per cent total body surface burn, and admission vital capacity, peak flow, and flow at 50 per cent vital capacity in individual patients. Pulmonary function tests were performed using an Ohio Model 840 dry spirometer. Minute ventilation, oxygen consumption, carbon dioxide production, respiratory quotient, and ventilatory equivalent were measured, using the Douglas bag technique, with a Lloyd valve (60 cc dead space). VD/VT, that is, the dead space to tidal volume ratio, was measured with a separate collection of expired gas and simultaneous arterial blood gas. The tests were done daily, where possible, for the first 10 days postburn. Arterial blood gases were measured, using an IL 131 blood gas analyzer; dynamic compliance

Table I

Patient	Age	Sex	Per Cent Burn	VC (L)	VC Per Cent Predicted	C Dyn	Peak Flow (L/Sec)	Per Cent Predicted	Flow at 50 Per Cent VC (L/Sec)	Per Cent Predicted	Outcome
Part I											
1	17	M	41.5	3.90	78	.173	6.52	78	4.31	85	Alive
2	20	F	56.0	2.50	89	.082	5.18	86	3.41	78	Alive
3	29	M	58.0	3.19	55	.149	6.58	79	4.23	83	Alive
4	31	M	53.0	5.98	115	.153	10.42	126	7.59	149	Died*
5	34	M	35.5	4.00	89	.177	13.0	157	5.18	94	Alive
6	29	M	47.5	4.29	95	.152	8.65	104	5.56	109	Alive
7	40	M	39.5	4.84	88	.259	7.52	90	6.06	110	Alive
Part II											
1**	29	M	[illegible] 5	4.20	95	.152	8.65	104	5.56	109	Alive
2	17	M	58.0	2.96+	59	.089	6.32	76	3.16	62	Alive
3	33	M	50.5	4.72	84	.174	10.57	127	4.55	83	Alive

*Staphylococcus sepsis

**Patient is same as Patient 6 in Group I

+Severe restrictive disease

and pulmonary resistance were measured with a Statham pressure transducer and a 12 cm esophageal balloon placed with its tip 42 cm from the nares. Pulmonary mechanics were assessed in a sitting position.

Part B

Five normal subjects, all male, age 19 to 31, were studied. Minute ventilation, O_2 consumption, carbon dioxide production, and ventilatory equivalent were measured at rest, during a standard bicycle exercise, and with a 250 cc dead space added. Following three days of Diamox, 1 gram per day, these studies were repeated. Douglas bag technique was again used, but a 100 cc dead space valve (Otis McKerrow with vane) was substituted.

Part C

Three burned subjects (age, sex, pulmonary function in Table 1, Group II) were studied. Ventilatory indices were measured daily during five days of treatment with 0.5% silver nitrate soaks and then for five days after the patient was switched from silver nitrate soaks to Sulfamylon burn cream. Each patient was felt to be clinically stable at the time of the switch. Patient 1 was switched on Day 12 postburn, Patient 2 on Day 5 postburn, and Patient 3 on Day 7 postburn.

RESULTS

Part A

Table 2 shows the mean minute ventilation, ventilatory equivalent, dead space/tidal volume ratio, and arterial PO_2 for the patients. Normal minute ventilation is 5-7 liters per minute, BTPS, the normal ventilatory equivalent for resting subjects is 25, the normal VD/VT is .3-.4, and the normal PaO_2 in our laboratory is greater than 80.

Table 3 shows the PaO_2 on admission to the unit on Day 3, after resuscitation is essentially complete, and also shows the day and value for the lowest PaO_2 recorded during the first 10 days postburn, after postburn Day 3. In addition, A-aO_2 gradients, while breathing 100 per cent oxygen, were measured in four subjects in the first three days.

Table 4 shows the results in Patient 3. He was a 29 year old male, with 58 per cent total body surface burn, in which 34 per cent was third degree. He was injured in a gas explosion following igniting the pilot light on a hot water heater. Past history was significant in that, at age two, he had bilateral closed chest tube

Table 2

Patient	Mean (MV (L/Min)	Mean VE (L BTR/L STPO)	VD/VT Mean	PO_2 Mean	PCO_2 Mean
1	13.9		.363	84.1	34.1
2	14.7		.388	76.6	31.0
3	13.7	30.1	.295	81.8	31.3
4	12.1	28.2	.447	77.5	33.5
5	16.5	33.0	.326	73.3	31.9
6	14.2	34.2	.413	75.6	35.1
7	14.7	33.2	.473	72.3	32.5
Mean	14.3	31.7	.386	77.5	32.8

Table 3

Patient	Earliest PO_2	PO_2 on Day 3	Lowest PO_2	PBD	A-a O_2 (n=<100) (Day 1-3)
1	78	98	79	6	
2	87	77	69	6	145
3	83	95	57	7	330
4	70	76	72	6	300
5	59	68	54	7	
6	77	83	64	9	
7	67	74.5	67	2	280

Table 4

Patient 3 (58 Per Cent Total Body Surface Burn

PBD	1	2	3	4	5	6	7	8	9	10
MV	–	11.8	9.0	12.9	13.8	14.1	15.1	19.2	–	–
PO_2	83	95	92.5	72	80	75	57	76	92	95
VD/VT	–	.133	.339	.349	.268	–	–	.376	–	.307
VE	–	–	27.9	27.5	29.4	27.0	36.4	32.3		
X-Ray	CLEAR									
Weight	–	84.2	84.2	85.2	91.7	–	–	82.0	78.5	76.0
VC		3.19		2.95		3.54		3.48		3.60
FRC(ZVC)		24.1		40.0		40.0		27.2		38.7
PV		6.58		7.40		7.05		6.19		7.40
V 50		4.23		3.41		2.47		2.90		3.05
V 25		1.53		2.70		1.17		1.52		2.11
C Dyn		.149		.157		.134		–		.152
R Pulm		4.54		1.58		2.81		–		2.14

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thoracostomies because of pneumonia. He also smoked one pack of cigarettes per day. His lungs were clear, and chest x-rays were negative throughout his hospital course. On Day 7 postburn, he developed violent vomiting and shivering, and by Day 9 postburn had become markedly confused with a distended abdomen and was felt to be septic, although blood cultures were negative. Minute ventilation was "normal" for the first three days, then increased to about 14 liters per minute. At the time he became confused, his minute ventilation increased dramatically. Similar increases were apparent in the VD/VT and ventilatory equivalent, suggesting that some of the increased ventilation is "wasted ventilation." Yet, at this time, the only evident change in pulmonary mechanics was a fall in his FRC.

Patient No. 5 is shown in Table 5. This was a 34 year old male with 35.5 per cent total body surface burn, in which 1 per cent was third degree. He was injured when he was trapped in his burning car. Lungs were clear on admission, as was his chest x-ray. On Day 7, he was noted to have decreased breath sounds at the bases, and increased vasculature on x-ray. His VD/VT increased, and PO_2 fell. He hyperventilated markedly during this time. Subsequent to Day 12, he developed a right lower lobe infiltrate and was felt to have had a pulmonary infarct on the basis of macroaggregate albumin lung scan, which showed defects in perfusion in areas other than the area of the infiltrate.

Part B

Table 6 and 7 show the results of Part B. Minute ventilation is in liters BTPS, O_2 consumption in liters STPD. As can be seen, despite the doubling of the oxygen consumption with exercise, ventilatory equivalent did not change, either in the Diamox or no Diamox studies. In addition, the oxygen consumption was very nearly the same in the Diamox and no Diamox study, so that minute ventilation can be compared between these two groups of studies. As can be seen, Diamox consistently produces an increase in minute ventilation despite the initial condition, i.e., a 36 per cent increase at rest, 14 per cent increase with added dead space, 27 per cent increase with exercise, and 17 per cent increase with exercise and dead space. A similar effect on ventilatory equivalent is noted with Diamox (a 29 per cent increase at rest, a 16 per cent increase with dead space, 23 per cent increase with exercise, and a 15 per cent increase with exercise plus dead space). Lastly, all effects were found to be additive. No interaction between experimental conditions could be found.

Table 5

Patient 5 (35.5 Per Cent Total Body Surface Burn

PBD	2	3	4	5	6	7	8	9	10	11	12
MV	14.9	14.2	12.0	17.1	15.8	17.8	14.2	23.2	19.7	22.0	18.9
PO_2	59	68	76	68	78	54	62	71	62	49	75
$\dot{V}D/VT$	.241	.269	.274	.291	.303	–	.475	.354	.400	.560	.273
VE	35.8	33.0	27.9	31.5	35.9	33.5	32.4	33.8	33.6	46.9	34.0
X-Ray						← INCREASED VASCULATURE →					
Weight	–	102.4	104.6	–	106.8	108.1	105.4	106.5	106	106	102.9
RV	1.47				1.41		1.42				1.34
TLC	6.54				5.83		5.72				5.64
FRC (% VC)	27.61				11.4		0				0
PV	13.0				13.4		12.8				10.74
$\dot{V}$ 50	5.18				4.93		4.80				3.79
$\dot{V}$ 25	2.53				1.26		1.14				1.01
C Dyn	.177				.155		.213				.161
R Pulm	2.43				2.04		2.64				2.90
CV	25.32				28.54		34.85				29.03

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Table 6

	Diamox				No Diamox			
	Rest		Exercise		Rest		Exercise	
	NDS	DS	NDS	DS	NDS	DS	NDS	DS
MV	7.55	9.9	16.19	18.50	5.53	8.62	12.72	15.77
VO_2	.238	.251	.517	.487	.227	.250	.506	.484
VE	31.71	40.67	30.96	38.41	24.51	34.93	25.11	33.27
RQ	.795	.740	.747	.784	.793	.758	.783	.835

Table 7

	MV	VE
Diamox	+2.39 L/min	+5.97 L/LO_2
Exercise	+7.88 L/min	No effect
Dead Space	+2.72 L/min	+8.73 L/LO_2

All effects additive; no interactions

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Part C

Table 8 summarizes the results of this part of the experiment. Each number is the mean of daily values for five days before and after changing topical therapy from 0.5 per cent $AgNO_3$ soak to Sulfamylon burn cream. As can be seen, minute ventilation increased 59 per cent, ventilatory equivalent 41.5 per cent, VD/VT 27 per cent. Most of the effect on ventilation was due to an increase in tidal volume. That these changes were not due to pulmonary disease is suggested by a concomitant rise in PaO_2.

Table 9 shows the results in Patient 2. It is of interest that on the afternoon of the second day of Sulfamylon therapy he became dyspneic and orthopneic. Although he had no rales and his chest x-ray was clear, he improved dramatically after a diuretic to the extent that, by the following morning, he was asymptomatic. It is also of interest that he had a severe restrictive disorder at the time of admission which persisted, although improving at the time of discharge.

Tables 10 and 11 show results in the other two patients in this group and illustrate how consistent the results were. There was essentially no change in pulmonary function associated with the change of topical agents.

DISCUSSION

This study documents that the uncomplicated burn patients have a minute ventilation twice that of healthy normal subjects. In addition, the ventilatory equivalent is increased, suggesting that a portion of that hyperventilation is "wasted ventilation." When the patient is placed in Sulfamylon, his ventilation increases another 50 per cent, due largely to an increased tidal volume associated with a further increase in ventilatory equivalent and dead space/tidal volume ratio.

The cause of the hyperventilation is in a large part due to the increased metabolic demands of the burn patient. The oxygen consumption in the burn patient has been shown to be as much as twice basal levels; but exercise, causing a rise in O_2 consumption to the same extent, did not produce a rise in ventilatory equivalent in our normal subjects. There then appears to be a component of "wasted ventilation" in the uncomplicated burn patient. This may be due to pulmonary disease, blood volume changes, or psychogenic factors.

Table 8

Effect of Sulfamylon

(Three Patients)

	BE	PO_2	MV^{V}	TV	VD/VT	DS	VE
Before	+3	79.4	14.65	.633	.395	.227	32.10
After	-2.6	93.5	23.36	.984	.503	.422	45.43
Per Cent Change	_	18	59	55	27	86	41.5

Table 9

Patient 2 (58 Per Cent TBS Burn) - X-Ray Clear

PBD	1	2	3	4	5	6	7	8	9	10	Discharge
MV		11.57	11.75	11.95	14.53	16.34	19.36	23.22	20 [illegible]	20.12	
PO_2	73	80	77	92	89	98	93	[illegible]	98	90	
VE		26.3	30.2	33.6	29.2	35.8	40.8		58.7	43.6	
Topical Agent	← SILVER NITRATE →					← SULFAMYLON →					
Weight	61.1	62.2	64.8	62.4	62.4	60.6	61.9	61.5	61.2	60.0	
Clinical Course						Pulm Edema					
VC (5.7)		2.96		2.52				2.36		2.28	3.92
FRC (2VC) (35.0)		21.3		25.0				21.0		19.4	29.0
PV		6.32		4.68				4.05		3.92	8.04
V 50		3.16		3.04				3.54		2.78	2.65
V 25		1.52		1.77				1.57		1.01	.91
C Dyn		.089		.065				.073		.056	.068
R Pulm		4.11		2.94				7.06		4.98	7.09
CV		25.0		0						0	0

Table 10

Patient 3 (50.5 Per Cent TBS Burn)

DPB	2	3	4	5	6	7	8	9	10	11	12
MV	16.87	12.29	13.53	14.58	17.17	15.52	25.56	23.18	22.28	29.68	30.88
PO_2	101	–	92	84	85	83	96	97	98	104	98
VD/VT	.313	–	.290	.259	.259	.382	.396	.453	.350	.428	.454
VE	29.54	31.18	30.14	30.66	31.33	31.29	50.12	44.15	44.47	46.23	46.36
Weight	75.7	75.4	–	74.8	75.8	74.3	–	72.6	72.3	69.6	68.4
Topical Agent	← SILVER NITRATE →						← SULFAMYLON →				
VC	4.72		5.56				5.25				5.44
PV	10.57		10.42				11.88				7.84
V 50 (%)	4.55		3.92				4.55				4.42
V 25 (%)	1.14		1.64				1.14				1.52
C Dyn	.174		.145				.214				.210
R Pulm	2.610		1.838				2.043				1.479
CV	0		0				13.3				22.1

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Table 11

Patient 1 (47.5 Per Cent TBS Burn)

DPB	7	8	9	10	11	12	13	14	15	16
MV	17.74	15.45	20.05	15.02	16.15	26.66	24.43	22.18	21.74	24.10
PO_2	74	70	64	69	73	84	70	100	96	86
VD/VT	.470	.417	.442	.438	.365	.460	.558	.423	.488	.459
VE	39.16	34.96	36.33	32 51	31.73	40.98	43.01	44.36	51.02	47.25
Weight	78.2	77.2	76.9	75.1	–	74.6	72.8	70.9	70.3	70.0
Topical Agent	← SILVER NITRATE →					← SULFAMYLON →				
VC		3.67					3.86			3.41
PV		7.43					10.24			9.15
V 50 (%)		4.15					5.18			6.06
V 25 (%)		2.48					3.41			1.39
C Dyn		.111					.157			.127
R Pulm		2.18					1.34			2.604
CV (% VC)		12.5					–			16.3

All patients with a history of active lung disease or clinical findings suggestive of inhalation injury were excluded from the study. In addition, xenon scans were negative, and all admission chest x-rays were negative. Nevertheless, two of the nine patients had "restrictive disease," as evidenced by decreased vital capacities with flow rates appropriate to their vital capacity. In addition, the mean PaO_2 was low in all but two patients and the mean $A\text{-}aO_2$ gradient on room air was 30, indicative of a fairly severe ventilation perfusion abnormality (Mays EM, Chest 63:793-799, 1974).[2] Four of the patients had $A\text{-}aO_2$ gradients measured between Day 1 and 3 and three of these were markedly increased, indicating "shunting" was present. Whether the ventilation/perfusion ratio abnormalities were due to pulmonary disease or changes in pulmonary perfusion, they could account for the wasted ventilation.

The patients all had some pain and two patients had evidence of psychiatric problems as well, and these may have been contributing factors.

Sulfamylon and its metabolic products are carbonic anhydrase inhibitors and, as such, cause renal bicarbonate wasting, which may lead to a metabolic acidosis (White MG, Asch MJ, New Eng J Med 284:1281-1286, 1971).[3] The three patients switched from silver nitrate to Sulfamylon showed the expected fall in base excess, but their arterial pH remained above 7.4. Nevertheless, they showed ventilatory changes similar to those seen in the normal subjects given carbonic anhydrase inhibitors (Tomashefshi JF, Chinn HI, Clark RT Jr, Am J Physiol 177:451, 1954; Chiesa A, Stratton TB, Massoud AAE, Howell JBL, Clin Sci 37:689-706, 1969; Carter ET, Clark RT Jr, J Appl Physiol 13:42-46, 1958; Doyle D, Harris EA, Slawson KB, Brit J Pharm 22:228-237, 1964),[4-7] although the changes were much more marked.

Carter and Clark (Carter ET, Clark RT Jr, J Appl Physiol 13: 42-46, 1958)[6] suggest that the hyperventilation is due in part to increased tissue stores of CO^2 resulting from the inhibition of the $H+$ and $HCO_3- \rightarrow CO^2 + H_2O$ reaction. The hyperventilation increases the CO_2 gradient from blood to alveoli by lowering the alveolar CO_2. However, Chiesa, et al (Chiesa A, Stratton TB, Massoud AAE, Howell JBL, Clin Sci 37:689-706, 1969)[5] have shown that the ventilatory changes could be mimicked by giving an ammonium chloride load and have suggested that the metabolic acidosis is the more important factor. If this is the case, it appears that the burned organism behaves as if a pH of 7.45-7.50 is the expected pH

and that a lower pH rasults in hyperventilation and wasted ventilation.

The complications of a prolonged hyperventilation at rates two to three times normal are unknown. Acute hypercapneic hyperventilation produces an increased flow resistance (Newhouse MT, Becklake MR, Macklem PT, McGregor M, J Appl Physiol 19:745-749, 1964)[9] in humans, and decreased compliance, increased airway resistance, and ventilation/perfusion abnormalities in anesthetized dogs (Monkcom W, Patterson RW, J Thor Cardiov Surg 63:577-584, 1972).[8] In addition, it may be expected that, since the pressure-volume curve of the lung is sigmoidal in shape, further increases in tidal volume (as may occur in sepsis) will result in marked increases in the work of breathing. This could be aggravated still more if the patient has pneumonia, shock lung, or pulmonary edema, in which the lungs are less compliant.

We would suggest that, in the patient being treated with Sulfamyion, ventilation in excess of 25 L/min is highly suggestive of a secondary underlying disease such as sepsis, an acute abdomen, etc., and that simply changing the topical agent may result in a significant decrease in ventilation.

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PUBLICATIONS AND/OR PRESENTATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OF 6384	74 07 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
74 07 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	090
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) Inhalation Injury: Development of an Animal Model of Pulmonary Injury as it Occurs 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
73 10	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 74	.2	3
	CURRENT 75	.3	6

19. RESPONSIBLE DOD ORGANIZATION
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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: Edwin W Hander, ILT, MSC
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21. GENERAL USE: FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code): (U) Smoke Inhalation; (U) Pulmonary; (U) Goats; (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 produce an animal model which represents the pathological and physiological findings of smoke inhalation injury in order to identify the major offending agent and the effect of various modes of therapy. Because of the use of tanks in modern warfare, closed space injuries have become exceedingly important and the proper care of these soldiers critical.

24. (U) Goats will be exposed to smoke of a burning mattress for 10-20 minutes. Blood gases, static compliance, dynamic compliance, and pulmonary resistance will be measured before and after the inhalation. CO, CO_2, O_2, No_x, SO_2, TDI, HCN, and hydrocarbons will be measured during the inhalation. The goats will be sacrificed three hours to 72 hours after injury and their lungs studied pathologically.

25. (U) 73 10 - 74 06 Five goats have been studied to date. Four goats were exposed for 10 minutes, one for 20 minutes. All the goats showed a fall in PO_2, and dynamic compliance postinjury. In all but the goat with the long exposure, the values returned to normal over a 24-hour period of time. In the latter goat, bronchiolitis and peribronchiolitis was observed at post.

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*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

ABSTRACT

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: INHALATION INJURY: DEVELOPMENT OF AN ANIMAL MODEL OF PULMONARY INJURY AS IT OCCURS IN BURNED SOLDIERS

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

Period covered in this report: 1 July 1973 - 30 June 1974

Investigators: Peter A. Petroff, MD, Major, MC
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In order to establish an animal model of inhalation injury, goats were exposed to smoke of a burning mattress for 10 to 15 minutes. Xenon washout was prolonged, PaO_2 fell, as did the sum of PaO_2 and $PaCO_2$, and C dyn decreased. In one experiment, a severe metabolic acidosis occurred. To date, however, there have not been any pathological changes. Further work is being proposed.

Smoke inhalation
Pulmonary
Goats
Animal model

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INHALATION INJURY: DEVELOPMENT OF AN ANIMAL MODEL OF PULMONARY INJURY AS IT OCCURS IN BURNED SOLDIERS

Acute pulmonary damage due to inhalation of smoke occurring during a fire continues to be a major problem in the burn patient. In order to gain an understanding of the pathophysiology of this disorder, we are developing an animal model of this disease. Goats are exposed for a short period of time to smoke from a burning mattress. Since they are in a separate chamber, 8-10 feet from the fire, they inhale the noxious gases but do not sustain a cutaneous burn. During this period, we have conducted three such exposures.

In the first exposure, five tracheostomized goats were used. Blood gases and dynamic compliances were measured preburn. Blood gases were measured again at 10 minutes postburn and 24 hours postburn. C dyn was measured at 3-8 hours, 20 hours and in two goats at 42 hours (see Table 1 for results). Three goats were sacrificed at 20 hours and the other two at 42 hours. All showed tracheitis related to the tube and only one goat showed more peripheral changes consistent with an inhalation injury.

In the second exposure, eight untracheostomized goats were used. Again blood gases were measured but, since a tracheostomy was not used, compliance was not measured. All the goats developed a metabolic acidosis and in all but one PaO_2 fell. In all the goats, the sum of PaO_2 and pCO_2 fell, indicating ventilation perfusion abnormalities. Four goats had xenon scans. In one, the scan was clearly positive and in two was suggestive of inhalation injury. There were no pathologic findings at postmortem in any goat (see Table 2).

Lastly, the preceding experiment was repeated. In addition, serum lactate and pyruvates were measured and xenon scans were done pre- and postinjury. In one goat, the pre-exposure scan was inadequate. Pathology is not completed as yet. In this experiment, the exposure was much less satisfactory from the standpoint of concentration of noxious agents. The goats did not develop a metabolic acidosis the serum lactate increased, and the lactate pyruvate ratio remained constant. PaO_2 fell as before, as did the sum of PaO_2 and $PaCO_2$ (Table 3). Pathology was grossly negative. Four of the goats received atropine pre-exposure but did not differ from the other four. Excretion of xenon was delayed in all seven studied at four hours or 28 hours.

From these three experiments, it appears that noxious gases induce an immediate disturbance in ventilation/perfusion rate and prolonged

Table 1

Dynamic Compliance

Pre	3 Hours	8 Hours	20 Hours	42 Hours
.154		.109		.128
.134		.122	.137	
.117	.034		.092	
.125	.047		.134	.169
.173		.137	.050	

	Pre	10 Min. Post	20 Hours Post
PO_2	63.2	44.4	73.8
pCO_2	32.6	33.9	34.8
pH	7.441	7.418	7.483

Table 2

Blood Gases (Six Goats)*

	Pre	10 Min.	2 Hours	4 Hours	8 Hours	24 Hours
PO_2	82.2	69.7	76.7	69.7	75.8	71.5
pCO_2	35.2	21.2	32.3	35.2	32.8	31.7
pH	7.378	7.319	7.448	7.443	7.471	7.468
B.E.	-3	-14.0	-1/2	0		
<u>All Eight Goats</u>						
Hemoglobin (gm)		13.0				
O_2 Saturation (%)		64.9				
CO (%)		29.1				

*Two goats sacrificed at eight hours not included.

Table 3

Blood Gases (All Goats)

	pH	pCO_2	B.E.	PO_2	Lactate	Pyruvate	Ratio
Pre	7.39	35.1	-3	80.2	31.7	2.19	14.47
0	7.43	28.9	-4.5	73.0	54.1	4.05	13.36
2	7.46	29.6	-2.0	77.9			
4	7.46	30.7	-1.5	69.8			
8	7.43	28.1	-4.5	82.2			
24	7.42	29.0	-4.5	82.1			
48	7.45	30.7	-2.0	83.2			
	Mean hemoglobin			10.8			
	Postinjury % saturation (0 hour)			76.9			
	CO			15.2			
				Atropine			
Pre	7.39	35.1		79.5	29.2	1.8	16.2
0	7.42	29.8		74.2	54.5	4.1	13.3
2	7.49	28.1		75.0			
4	7.46	30.8		62.8			
8	7.45	29.2		79.0			
24	7.41	29.1		82.5			
48	7.45	30.1		86.2			
	Saturation	78.3					
	Hemoglobin	10.9					
	CO	14.8					
				No Atropine			
Pre	7.39	35.0		81	34.2	2.4	14.2
0	7.43	28.1		71.8	54.1	4.0	13.5
2	7.43	31.0		80.8			
4	7.46	30.6		76.8			
8	7.42	26.9		85.5			
24	7.43	28.9		81.8			
48	7.45	31.2		80.2			
	Saturation	75.6					
	Hemoglobin	10.6					
	CO	15.6					

washout of xenon. However, the severe tracheitis seen in human patients with inhalation injury was not observed, and the disease was not lethal when the exposure was only 10-15 minutes. The goat, because of his long trachea, may have a greater dead space/tidal volume ratio than humans and, by hyperventilating at low volumes, may suffer hypoxemia without getting the full-blown disease of inhalation injury.

We plan to continue the experiments but will try using a tracheostomy during the exposure only (so that we can avoid the problems of prolonged intubation). If we are then successful in producing pathologic changes in addition to hypoxemia and prolonged washout, we will evaluate steroids in treating the disorder and will again measure indices of pulmonary function, i.e., tidal volume, ratio, compliance and resistance.

PRESENTATIONS AND/OR PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OE 6954	74 07 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. SRDS'R INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
73 07 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	076
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Provide with Security Classification Code)

(U) Excision of Eschar in Burned Soldiers (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 Clinical Medicine

13. START DATE	a. ESTIMATED COMPLETION DATE	14. FUNDING AGENCY	16. PERFORMANCE METHOD
71 07	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 74	.2	5
FISCAL YEAR — CURRENT 75	.1	2

19. RESPONSIBLE DOD ORGANIZATION

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21. GENERAL USE

FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Provide Each with Security Classification Code) (U) Cryosurgery; (U) Liquid nitrogen; (U) Laser; (U) Escharotomy; (U) Excision; (U) Eschar; (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 investigate the use of a carbon dioxide laser to excise burns with a view towards minimizing blood loss involved in such excisions in burned soldiers.

24. (U) Symmetrical excisions have been performed on patients who are candidates for excision. Laser excisions were compared to both scalpel and electrocautery excisions from the standpoint of blood loss, time for excision, graft take, and pre-and post excision quantitative microbiology.

25. (U) 73 07 - 74 06 The burn wounds of ten patients have been excised using the laser. Laser excisions involve less blood loss and less operative time than did scalpel excisions. Comparison of the laser to the cutting electrocautery in a few patients has not shown any dramatic advantage of using the laser rather than the electrocautery. Although the laser generally involves less blood loss than the electrocautery, this was not dramatic. The electrocauter[illegible] on the other hand, usually required less operative time than the laser; however, this likewise was not dramatic. Further evaluation is being continued.

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*Available in unclassified form upon originator's approval

DD FORM 1498 MAR 68 — PREVIOUS EDITIONS OF THIS FORM OBSO[illegible] AND 1498 1 MAR 68 (FOR ARMY [illegible] USE OPT[illegible]

ABSTRACT

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: EXCISION OF THE ESCHAR IN BURN SOLDIERS: THE USE OF A CARBON DIOXIDE LASER IN THE DEBRIDEMENT OF THIRD DEGREE BURN ESCHAR

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

Period covered in this report: 1 July 1973 - 30 June 1974

Investigators: Norman S. Levine, MD, Major, MC
Roger E. Salisbury, MD, Major, MC
Basil A. Pruitt, Jr., MD, Colonel, MC

Reports Control Symbol MEDDH-288(R1)

The purpose of this study has been to investigate the use of a carbon dioxide laser with a view towards minimizing blood loss in surgical excisions in thermally injured soldiers. Symmetrical excisions have been performed on patients who are candidates for excision, comparing laser excisions either scalpel or electrocautery excisions from the standpoint of blood loss, time for excision, and graft take. The burn wounds of 10 patients have been excised using the laser. Laser excisions involved less blood loss and less operative time than did scalpel excisions. Although the laser excisions involved less blood loss than the electrocautery, this difference was not impressive. The electrocautery, on the other hand, usually required less operative time than did the laser; however, this difference was also unimpressive. Evaluation is being continued.

Cryosurgery
Liquid nitrogen
Laser
Escharotomy
Excision
Eschar
Humans

EXCISION OF THE ESCHAR IN BURN SOLDIERS: THE USE OF A CARBON DIOXIDE LASER IN THE DEBRIDEMENT OF THIRD DEGREE BURN ESCHAR

Surgical excision of third degree burns and immediate grafting offers an attractive means of removing the burn eschar and the possibility of a shortened convalescence. The major problems with this technique are twofold: (1) It requires a surgical operation under anesthesia; (2) this procedure involves massive blood loss (up to 40 units of blood for a single patient).

We have been able to minimize such blood loss experimentally by excising the eschar with a continuous wave (10.6 micron wave length) carbon dioxide laser instead of the scalpel; bleeding is minimized because of photo-coagulation of the small blood vessels. Experiments carried out in three month old Hampshire-Landrace pigs indicated that third degree eschars can be excised safely and effectively with 30% of the blood loss incurred with a cold knife excision. Although minimal injury did occur to the underlying tissue, this did not affect the "take" of split thickness skin grafts immediately applied to the freshly lased surface. Preliminary data in patients has suggested that using the laser can effectively reduce blood loss over that incurred by using the scalpel. Graft take appeared to be comparable to that obtained in a similar area undergoing scalpel excision. The purpose of this study is to evaluate the efficacy of the laser for excising third degree burns in patients compared to cold knife and electrocautery excisions of burns of similar depth, area, and location on the same patient.

METHODS

Patients selected for this study have symmetrical burns shown to be third degree by both clinical and histologic evidence. All laser excisions were performed in the operating room under clean conditions. Plate glass spectacles or ordinary corrective lenses have been worn by the surgical and anesthetic teams to protect their eyes from the infrared laser beam. A safety inspection of the laser was made by both the Environmental Hygiene personnel and by the Engineering Corps. A sterile vacuum cleaner nozzle was used in conjunction with the laser to clear away the "plume fragmentation" or smoke caused by photovaporization of the tissue. Signs designating "laser in use" were posted outside the entrance to the Operating Room. Non-explosive anesthetic agents were used.

Preoperative photographs were taken from the areas chosen for both laser and scalpel excisions. During excision, blood loss was monitored by collecting and weighing sponges and towels used to drape the patient. The time required for all excisions was clocked. Immediately following

excision, photographs of the eschar and underlying base were taken. Quantitative bacterial counts were taken both pre- and post-excision from the eschar to be removed (preoperatively) and from the underlying base (postoperatively). Calculation of blood loss in terms of ml per cm square and speed of excision in terms of cm square per minute were made.

RESULTS

Comparisons of laser versus scalpel excisions have been made in four patients. Table I includes the data concerning operative time and blood loss for these excisions. The scalpel excisions entailed from 3.3 to 5.3 times as much blood loss as did the laser excisions. Although this is not statistically significant, because of the small number of patients studied, it does suggest that the laser excision is associated with considerably less blood loss than scalpel excision. Operative time was 1.3 to 2.0 times longer when the scalpel was used.

Table II lists the data on blood loss and operative time when the laser was compared to the electrocautery in six patients. In general, blood loss was less when the laser was used. Statistical analysis of this data suggests that the electrocautery blood loss was roughly 1.5 times greater than laser blood loss within 95% confidence limits. Although this is a significant reduction in blood loss, it is not dramatic. Regression curve analysis of operative time, indicates that electrosurgical excisions proceeded 1.36 times as rapidly as did laser excisions, within 95% confidence limits. This difference, likewise, is not very impressive.

When split thickness skin grafts were applied to the freshly lased surfaces, graft take was excellent, and entirely comparable to that achieved with either scalpel or electrocautery excisions. In many cases, "biologic dressings" were used to cover the freshly lased surface. The adherence of such dressings was excellent.

In our opinion, the optics, and beam-delivery system of the American Optical laser are excellent. The focal point is extremely fine (less than 1 mm in diameter) and the power of the laser beam is very adequate. On larger planar surfaces, the laser handles well, and the speed of excision appears to be limited only by the surgeon's capacity to control the laser beam. Damage to the underlying tissue always appeared to be minimal. However, most of our excisions involved long, cylindrical surfaces, such as the arms or legs. In working in small crevices or on the undersurfaces of these structures, the surgical arm of the laser was quite awkward to handle and is primarily responsible for the reduced operative speed of laser excision. We feel that the design of the surgical arm is in need of improvement.

TABLE I

LASER VS. SCALPEL

PATIENT AREA CM^2	#1 Arm 125 cm^2	#2 Legs 3400 cm^2	#3 Arm 64 cm^2	#4 Arm 600 cm^2
LASER BLOOD LOSS	0.6 cc/cm^2	0.072 cc/cm^2	0.25 cc/cm^2	0.16 cc/cm^2
SCALPEL BLOOD LOSS	2.0 cc/cm^2	0.24 cc/cm^2	1.3 cc/cm^2	0.84 cc/cm^2
SPEED LASER	8.3 cm^2/min.	57 cm^2/min.	13 cm^2/min.	20 cm^2/min.
SPEED SCALPEL	6.3 cm^2/min.	37 cm^2/min.	6.5 cm^2/min.	15 cm^2/min.
RATIO BLOOD LOSS	3.33 S > L	3.33 S > L	5.2 S > L	5.3 S > L
RATIO SPEED	1.3 L > S	1.5 L > S	2.0 L > S	1.33 L > S

TABLE II

LASER VS. CUTTING ELECTROCAUTERY

PATIENT AREA CM^2	#1 legs 800 cm^2(L)/ 500 cm^2(R)	#2 legs 3000 cm^2	#3 legs 1232 cm^2	#4 legs 600 cm^2	#5 arms 300 cm^2	#6 legs 3040 cm^2
LASER BLOOD LOSS	0.77 cc/cm^2	0.57 cc/cm^2	0.049 cc/cm^2	0.13 cc/cm^2	0.17 cc/cm^2	0.10 cc/cm^2
ELECTROCAUTERY BLOOD LOSS	1.0 cc/cm^2	1.12 cc/cm^2	0.18 cc/cm^2	0.16 cc/cm^2	0.22 cc/cm^2	0.062 cc/cm^2
SPEED LASER	12.5 cm^2/min.	33.3 cm^2/min.	20.5 cm^2/min.	18 cm^2/min.	12 cm^2/min.	50 cm^2/min.
SPEED ELECTRO-CAUTERY	16.6 cm^2/min.	50 cm^2/min.	30 cm^2/ min.	18 cm^2/min.	20 cm^2/min.	67 cm^2/min.
RATIO BLOOD LOSS	1.3 B > L	2.0 B > L	3.7 B > L	1.2 B > L	1.3 B > L	0.6 B > L
RATIO SPEED	1.3 B > L	1.5 B > L	1.5 B > L	1.0 B = L	1.7 B > L	1.33 B > L

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CONCLUSIONS

Our data suggest that the carbon dioxide laser may offer a substantial advantage over the cold knife for surgical excisions. Blood loss with the laser is less than one-third of that which occurs when the scalpel is used. Operative time likewise appears to be less with laser excisions than with scalpel excisions. Comparison of the laser to the cutting electrocautery suggests that, at present, there is no substantial advantage of using the laser rather than the electrocautery. Although laser excision caused less blood loss than did the electrocautery, this difference was not dramatic. The electrocautery, on the other hand, usually required less operative time than the laser; however, this likewise was significant but not impressive. Our experience suggests that the awkward surgical arm of the laser is at present the limiting factor in developing this as a clearly useful clinical technique. It is suggested that this surgical arm be redesigned. Evaluation of the laser is being continued.

REFERENCES

1. Levine, NS, Stellar, S, Ger, R, and Levenson, SM: Use of a carbon dioxide laser for the debridement of third degree burns. Ann Surg 179: 246-252, 1974.

PUBLICATIONS AND/OR PRESENTATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0E 6393	74 07 01	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
73 07 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	081
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) Continued Evaluation of Split-Thickness Cutaneous Xenograft as a Temporary Biologic Wound Cover For Use 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
71 07	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 74	.3	7
FISCAL YEAR CURRENT 75	.3	5

19. RESPONSIBLE DOD ORGANIZATION
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NAME: Roger E Salisbury, MAJ, MC DA

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Cutaneous xenograft; (U) Wound cover; (U) Laboratory animals; (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) The purpose of this study is to evaluate different forms of cutaneous xenograft and artificial wound covers which would be less expensive than currently available forms, assuredly sterile, indefinitely shelf-storable, and still effective as temporary biological wound cover for burned combat casualties.

24. (U) Recent laboratory data suggests that many of the beneficial effects of cutaneous allograft are related to the structural characteristics of skin and not to its viability per se. Such evidence includes (1) our demonstration that vascular ingrowth can be achieved into artificial "membranes", (2) that coverage of the wound with a variety of "membranes" promotes survival of rats with large area excisions over survival in animals excised but not covered, and (3) the reversal of intraperitoneal pseudomonas mortality in burned rats by excising the eschars and covering the underlying tissue with formalin-fixed skin (nonviable). An approach has been developed toward producing an artificial skin with such membrane characteristics.

25. (U) 73 07 - 74 06 Extensive testing of materials for a bi-laminate "artificial skin" have been performed. A suitable structure for the "inner layer" of this laminate has been found. Suitable membranes for the "outer layer" are being tested. Appropriate animal test models for the in vivo evaluation of such a product has been developed.

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*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

ABSTRACT

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: CONTINUED EVALUATION OF SPLIT-THICKNESS CUTANEOUS XENOGRAFT AND SYNTHETIC MATERIALS AS TEMPORARY BIOLOGIC WOUND COVERS FOR BURNED SOLDIERS

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

Period covered in this report: 1 July 1973 - 30 June 1974

Investigators: Norman S. Levine, M.D., Major, MC
Roger E. Salisbury, M.D, Major, MC
Hugh D. Peterson, D.D.S., M.D., Colonel, MC

Reports Control Symbol MEDDH-288(R1)

Recent laboratory data suggest that many of the beneficial effects of cutaneous allograft are related to the structural characteristics of skin and not to viability per se. Such evidence includes our demonstration (1) that fibroblastic tissue will grow into artificial "membranes;" (2) that coverage of an excised wound with a variety of "membranes" promotes survival of rats with large area excision over survival of rats excised but not covered; and (3) that reversal of intraperitoneal Pseudomonas mortality in burned rats occurs by excising the burn wound and covering the underlying tissue with formalin-fixed skin, which is non-viable. Technics have been developed to aid in evaluating an artificial skin with the beneficial effects of cutaneous allograft. Extensive testing of materials for a bilaminate "artificial skin" has been performed. Many of the commercially available products were found to be unsuitable. An adequate structure for the "inner layer" of this laminate has been found. Membranes for the "outer layer" are being tested. Appropriate animal test models for the in vivo evaluation of such a bilaminate have been developed.

Cutaneous xenograft
Wound cover
Laboratory animals
Humans

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CONTINUED EVALUATION OF SPLIT-THICKNESS CUTANEOUS XENOGRAFT AND SYNTHETIC MATERIALS AS TEMPORARY BIOLOGIC WOUND COVERS FOR BURNED SOLDIERS

The purpose of this study is to evaluate different forms of cutaneous xenograft and artificial wound covers which would be less expensive than the currently available forms, guaranteed sterile, indefinitely shelf storable, and still effective as temporary biological wound covers for combat burned casualties.

Recent laboratory data suggest that many of the beneficial effects of cutaneous allograft are related to the structural characteristics of skin and not to its viability per se. Such evidence includes our demonstrations (1) that fibroblastic ingrowth can be achieved into artificial "membranes;" (2) that coverage of the wound with a variety of "membranes" promotes survival of rats with large area excisions over survival of rats excised but not covered; and (3) that intraperitoneal Pseudomonas mortality is reversed in burned rats by excising the eschars and covering the underlying tissue with formalin-fixed skin.

Technics have been developed for evaluating an artificial skin substitute which permits a closed adherence to the wound, fibroblastic ingrowth, and which has the desirable membrane characteristics of fresh cadaver allograft. Our approach has been twofold: first, we evaluated a large number of commercially available products, and described the advantages and drawbacks of each; second, we evaluated components for a bilaminate artificial skin with an "inner layer" to permit fibroblastic ingrowth and an "outer layer" to provide the desirable membrane characteristics. The results of our testing are presented in this report.

METHODS

All materials were tested on 180 to 200 g Sprague-Dawley albino rats anesthetized with Pentathol with 20% dorsal wounds excised to the deep fascia. All dressings were maintained in place with metallic clips, although in some cases this was not necessary. Evaluation of all dressings was made in terms of: (1) initial adherence; (2) conformation to the wound; (3) adherence at 5 days: (4) seperation underneath the membrane; and (5) fibroblastic ingrowth. In addition, those materials which showed promise as inner layers were tested for the allowance of fibroblastic ingrowth by placing the thinnest possible layer of this material on a freshly excised fascial surface and covering this layer with multiple layers of coarse mesh gauze, to absorb wound drainage. Finally, the most promising of these "inner layers" was tested in the form of multiple layers to determine the optimal thickness of a "inner layer" or scaffolding surface for the artificial skin.

In addition, a number of membranes were tested with regard to their behavior as possible "outer layers" for an artificial skin. Only gas-permeable and water-impermeable membranes were tested. These were tested

as bacterial filters. Each membrane was gas-sterilized and placed on a sterile petri dish. To this a broth culture was applied containing beta hemolytic streptococci, Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella aerobacter, and Providencia stuartii. Each organism was present in a density per cc of broth of 10^8 organisms per ml. Each membrane was tested twice: once with 2 cc of this organism mixture, and once with 2 cc of the organism mixture and 2 cc of human serum, as a wetting agent. The petri dishes were evaluated for growth or no growth of organisms, thus reflecting the efficacy of these membranes as bacterial filters, at one atmosphere pressure.

RESULTS

1. Initial testing of artificial skin.

Table I list our findings for the artificial skin components. The following paragraphs contain a more detailed description of the materials evaluated.

The first material evaluated was the Edwards silastic-gauze dressing which has been proposed as a synthetic wound cover. This dressing consists of an outer layer of silastic and an inner layer of coarse mesh gauze. The initial adherence of this material was excellent. It conformed well to the wound and did not require sutures to keep the dressing in place. At the end of 5 days, however, though the adherence was still fair, there was considerable pocketing of serum between the fascial surface and the silastic membrane. These pockets did not appear to be infected. Similarly, there was very little tissue response to the material. Although there were copious amounts of serum underneath the dressing, there was no evidence of suppuration underneath the dressings. There was, likewise, no evidence of fibroblastic ingrowth.

The second group of artificial dressings tested consisted of two different thicknesses of collagen membrane, provided with and without an inert "outer layer" membrane. All of these were quite similar in behavior, and therefore no distinction between these four dressings will be made in summarizing the report. They are listed in Table I as Ph-a, Ph-b, Ph-c, and Ph-d. Their initial adherence to the wound was poor. They did not conform well to the wound. Each dressing required metal clips to hold it in place. At 5 days the adherence remained poor. These dressings evoked a marked tissue response which created a sheet of pus underneath the membranes. There was no evidence of fibroblastic ingrowth.

The next material tested consisted of strips of polytetrafluoroethylene of 40 and 80 mils thickness having a "poker-chipped" cross sectional appearance. These materials are being used for the production of small caliber artificial vessels. The initial adherence of these materials was poor and, although their conformation to the wound was good, there was little evidence of adherence at five days. Because these

TABLE - I INITIAL TESTING OF ARTIFICIAL SKIN COMPONENTS

MATERIAL	INITIAL ADHERENCE	CONFORMATION TO WOUND	ADHERENCE AT 5 DAYS	SUB-"GRAFT" SUPPURATION	FIBROBLASTIC INGROWTH
Edwards Silastic Gauze	Excellent	Excellent	Fair: Pocketing of Serum	Serum Pockets	None
Ph-A	Poor	Poor	Poor	Pus	None
Ph-B	Poor	Poor	Poor	Pus	None
Ph-C	Poor	Poor	Poor	Pus	None
Ph-D	Poor	Poor	Poor	Pus	None
G-710	Poor	Good	Poor	___	None
G-711	Poor	Good	Poor	___	None
*G-810236 (0.1 micron)	Excellent	Excellent	Good	Clot	None
G-81012f (20 micron)	Excellent	Excellent	Partial Incorp. In Scab	Clot	None
G-810148 (40 micron)	Excellent	Excellent	Partial Incorp. In Scab	Clot	None
Goretex Weave	Poor	Poor	Poor	Pus	None
Nylon Stocking (Loose Knit single layer)	Excellent	Excellent	Incorp. In Scab	Scab	Fair
Fine Mesh Gauze (Single Layer)	Excellent	Good	Incorp. In Scab	Scab	Fair
Coarse Mesh Gauze (Single Layer)	Excellent	Excellent	Incorp. In Scab	Scab	Fair

materials were tested in strips, there was no submembrane suppuration. There was no fibroblastic ingrowth at 5 days. These materials are referred to in Table I as G-710 and G-711.

Sample G-S10236 consisted of a polytetrafluoroethylene (PTFE) membrane of 1 mil thickness and a nominal void size of 0.1 microns. Initial adherence of this membrane was excellent, conformation to the wound was excellent; and adherence at 5 days was good, although it had stiffened considerably. There was no evidence of pus underneath the membrane and there was no evidence of serum pocketing, suggesting that the membrane permitted adequate "breathing." However, there was a considerable clot underneath the membrane and no evidence of fibroblastic ingrowth present.

A PTFE membrane of 20 microns void size and 1 mil thickness was also tested. This is referred to in Table I as G-S10128. Conformation to the wound and initial adherence was excellent. No sutures were needed to secure the membrane to the open wound. Free egress of water was noted at the time of application. After 5 days, the membrane itself was nearly completely obscured by a scab which had formed above and below the membrane. There was no submembrane suppuration, but there was considerable clot underneath the membrane. There was no evidence of fibroblastic ingrowth.

G-S10148 was a PTFE membrane with a "spider-web" structure and a nominal void size of 40 microns, with a 1 mil thickness. The performance of this membrane was very similar to that of G-S10128. Its initial adherence and conformation to the wound were excellent, however, at 5 days it was partially incorporated into a scab which had formed on both the inner and outer surface of the membrane. There was no evidence of subgraft suppuration, but numerous red cells were seen in a clot below the membrane. This clot had lifted the membrane from the wound surface. There was no evidence of fibroblastic ingrowth.

A Gortex Weave was tested to allow for a void size even greater that 40 microns. This weave was quite stiff, and therefore its initial adherence and wound conformation were quite poor. Its adherence at 5 days was poor and there was considerable submembrane supuration with no evidence of fibroblastic ingrowth.

Next, a single layer of loosely knit nylon stocking (#15 denier) was evaluated. Its initial adherence and conformation to the wound was excellent. No sutures were needed to hold the membrane in place. At 5 days, this membrane was incorporated into a scab, both above and below the membrane. There is evidence that the scab had partially "lifted" the nylon stocking from the underlying fascia. However, in areas where this "lifting" did not occur, there was some evidence of fibroblastic ingrowth.

Because of the fact that nylon stocking, when tested in a single

layer, lifted from the wound, both fine mesh gauze and coarse mesh gauze were tested as inner layers. Although the initial adherence of both materials was excellent and conformation to the wound was good, both fine and coarse mesh gauze, when tested as a single layer, became incorporated in a scab and partially lifted from the wound. There was evidence of minimal fibroblastic ingrowth only when these dressings were tested in a single layer.

II. The testing of promising "inner layers" under 24 layers of coarse mesh gauze.

Because in the testing of even fine and coarse mesh gauze in a single layer, there was evidence of clot formation beneath the gauze with subsequent lifting of the gauze from the underlying surface, it was decided to evaluate the most promising "inner layers" under 24 layers of gauze. The idea of placing 24 layers of coarse mesh gauze over the membrane under evaluation was to draw out some of the fluid, protein, and cells, and avoid the "scabbing" seen when such membranes were tested in a single layer.

The Gortex Weave showed initial poor adherence and, in spite of the gauze overlay, showed considerable subgraft suppuration with no evidence of fibroblastic ingrowth. The PTFE membranes of 20 and 40 micron void size (G-S10128 and G-S10148) again showed excellent adherence at 5 days. However, even with the gauze overlayering, there was evidence of clot underneath each of these membranes. Fibroblastic ingrowth was not present in G-S10128 and was present only to a very modest extent in G-S10148.

However, when fine mesh gauze and coarse mesh gauze were tested with 24 layers of coarse mesh gauze as a cover, there was no evidence of clot formation underneath the gauze and fibroblastic ingrowth occured when either was used. Similarly, the nylon stocking exhibited excellent adherence at 5 days, no evidence of subgraft sub-inner layer seperation and excellent fibroblastic ingrowth.

The nylon stocking was chosen over either fine mesh or coarse mesh gauze for the following reasons: (1) it consists of an inert, nonfragmenting material; (2) it is made of a much finer denier, potentially allowing multiple layers to be put together with minimal thickness; (3) it is stretchable in two dimensions and therefore conforms more readily to irregular surfaces; (4) histologic sections show far less foreign body response to the nylon stocking than to either fine mesh or coarse mesh gauze.

III. Determination of optimal thickness requirements for an inner layer of nylon stockings.

This experiment utilized 12 rats, 3 in each group 15% dorsal fascial excisions were covered with either 1, 2, 4, or 8 layers of nylon stocking

TABLE II - TESTING OF PROMISING "INNER LAYERS" UNDER 24 LAYERS OF GAUZE

MATERIAL	ADHERENCE AT 5 DAYS	SUB-"INNER LAYER" SUPPURATION	FIBROBLASTIC INGROWTH
Gortex Weave	Poor	Yes	None
G-S10128	Good	Clot	None
G-S10148	Good	Clot	Minimal
Fine Mesh Gauze	Excellent	No	Yes
Coarse Mesh Gauze	Excellent	No	Yes
Nylon Stocking	Excellent	No	Yes

TABLE III - DETERMINATION OF THICKNESS REQUIREMENTS FOR "INNER LAYER" NYLON STOCKING.

NO. OF LAYERS	NYLON - FASCIAL APPROXIMATION (GROSS AND MICROSCOPIC)
1	Some - "Lifting" Usually
2	Better - Some "Lifting" Present
4	Frequent - Some "Lifting" Present
8	Always Good

(#15 denier loosely knit). Each group was examined at the end of 5 days for evidence of fibroblastic ingrowth. One layer of nylon stocking, as indicated in Table III showed some clot formation under the stocking with subsequent lifting of the nylon stocking. Two layers were better: there was more evidence of fibroblastic ingrowth but considerable "lifting" was still present. Four layers of nylon stocking were superior with only rare "lifting" being present. Fibroblastic ingrowth always occurred. Fibroblastic ingrowth likewise occurred with 8 layers of nylon stocking.

IV. Testing of possible "outer layers" as bacterial filters at one atmosphere of pressure.

Table 4 indicates the test performed on G-S10128, G-S10235, and G-S10236 as bacterial filters. Because of the charge-structure of all of these membranes, all are impermeable to water alone, in spite of their large pore size. However, when tested in conjunction with broth or serum, only one membrane, G-S10236, was found to be impermeable to bacteria inoculated with a wetting agent.

DISCUSSION

Desirable features of an "artificial skin" preparation include:

1. That it be impermeable to bacteria;
2. That it adhere well to a large, irregular surface;
3. That it conform closely to the wound;
4. That it permit fibroblastic ingrowth from the tissue bed to which it is opposed.

Further, it is thought desirable to have this artificial membrane as thin as possible, to minimize the dead space between the underlying tissue bed and the outer surface of the membrane, thus leaving as small a space as possible for the accumulation of tissue fluids and pus. Our empirical tests suggest that the "outer layer" of a composite membrane must be somewhat permeable to fluids and gases, thereby avoiding the protein accumulation seen with the Edwards silastic-gauze membrane. The experiments with the collagen membrane suggest that it is the architectural characteristics rather than the material per se which is important. Although collagen is biodegradeable, the closely spaced void in the collagen membrane was tested did not allow for fibroblastic ingrowth. In testing structures for an "inner layer" a certain degree of thickness or "layering" is necessary to avoid sub-lattice clot formation with subsequent lifting of a single layer of material. Testing of multi-layered material was performed to find the minimum thickness required to consistently allow for fibroblastic ingrowth. Our experiments suggest that this can best be achieved with 4 to 8 layers of a loosely knit, fine

TABLE IV - TESTING OF POSSIBLE "OUTER LAYERS"
BACTERIAL FILTERS (01 ATM PRESSURE)

MEMBRANE	VEHICLE	BACTERIAL GROWTH ON PETRI DISH BELOW MEMBRANE
G-S10128 (20 micron Pore Size)	Broth	Yes
	Broth and Serum	Yes
G-S10235 (5 Micron Pore Size)	Broth	Yes
	Broth and Serum	Yes
G-S10236 (0.1 Micron Pore Size)	Broth	No
	Broth and Serum	No

denier nylon stocking. Of the outer membranes tested, the most satisfactory was the 0.1 micron void size, 1 mil thick, PTFE membrane. This caused no submembrane pus or serum accumulation, yet served as an adequate bacterial filter.

SUMMARY

A series of tests were performed with the purpose of developing a satisfactory bilaminate "artificial skin" as a substitute for human cadaver allograft. Principles concerning the nature of the needed components have emerged from this investigation. A first order approximation of what is required in an artificial skin has evolved. This structure will require further and more detailed investigation.

PUBLICATIONS AND/OR PRESENTATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OE 6957	74 07 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
73 07 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 (Procode with Security Classification Code): (U) Immunity in Burned Animals - A Laboratory Model of Changes Occurring in Burned Troops (44)

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13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
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c. TYPE — d. AMOUNT
e. KIND OF AWARD — f. CUM. AMT.

18. RESOURCES ESTIMATE	PRECEDING	a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
FISCAL YEAR	74	.2	2
	CURRENT 75	.2	3

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21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Provide EACH with Security Classification Code)
(U) Burns; (U) Bacterial infection; (U) Viral infection; (U) Rats; (U) Mice

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

23. (U) The objectives of this pilot project were three-fold. (1) To determine if removal of the eschar (with or without grafting) can alter survival in animals with 60% burns, (2) to determine if removing the burn wound and primarily homografting the underlying tissue will alter the susceptibility of rats to infection elsewhere, and (3) to determine if burned mice showed an increased susceptibility to viral infections as do burned soldiers.

24. (U) Models of 60% burns in rats have been established, and the effect of excision of the burn wound with and without grafting on mortality has been studied. Rats with 30% dorsal burns were challenged intraperitoneally with Pseudomonas aeruginosa strain 1244 and a dose which was consistently lethal in over 50% but not all of the burned rats was found. The effect of excision with primary grafting on the mortality of such rats was investigated. A model demonstrating increased susceptibility to the Moloney sarcoma virus was developed in burned mice and the effects of burning, steroids, metyrapone, and vitamin A were studied in these animals.

25. (U) 73 07 - 74 06 Excision and allografting without burn did not alter survival. Burned rats show an increased susceptibility to Pseudomonas aeruginosa. This susceptibility can be reversed by excising the burn immediately after burning and covering the exposed fascia with either fresh allograft or formalin-fixed allograft. Such studies suggest that it is the absence of normal skin, or a suitable substitute, which accounts for the increased susceptibility to infection seen after burning, and that suitable autograft substitutes need not be viable. The development of tumors in mice following leg injection with the Moloney sarcoma virus was accelerated by burning or the administration of exogenous steroids. Both metyrapone and vitamin A inhibited tumor development in the normal mouse. These agents however, did not inhibit the increased tumor development in burned mice.

* Available to contractors upon request

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

ABSTRACT

PROJECT NO: 3A161101A91C-00, IN HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: IMMUNITY IN BURNED ANIMALS - A LABORATORY MODEL OF CHANGES OCCURRING IN BURNED TROOPS. PART I. THE EFFECT OF BURNING ON VIRAL ONCOGENESIS IN THE MOUSE; EFFECTS OF EXOGENOUS STEROIDS, GLUCOCORTICOID BLOCKAGE, BETA ADRENERGIC BLOCKADE AND VITAMIN A.

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

Period covered in this report: 1 July 1973 - 30 June 1974

Investigators: Norman S. Levine, MD, Major, MC
Roger E. Salisbury, MD, Major, MC
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The effect of experimental burns on tumor oncogenesis induced by the Moloney sarcoma virus in mice was studied. Twenty-five per cent third degree burns caused a significant increase in tumor size and a delay in tumor regression. Exogenous administration of glucocorticoids likewise promoted tumor development. Metyrapone inhibited tumor growth in normal mice, but had no effect on the increased tumor growth in burned mice. Propanolol, a beta-adrenergic blocker, had no effect on tumor growth in burned or unburned mice. Vitamin A inhibited tumor development in unburned, but not in burned animals. Our data are compatible with the hypothesis that glucocorticoids play a permissive role in viral oncogenesis, and that this can be inhibited by 11-hydroxylase blockage with metyrapone; although increased glucocorticoid production is a likely cause of the increased tumor growth in burned animals, this cannot be reversed with metyrapone.

Burns
Bacterial infection
Viral infection
Rats
Mice

IMMUNITY IN BURNED ANIMALS - A LABORATORY MODEL OF CHANGES OCCURRING IN BURNED TROOPS (PART I)

THE EFFECT OF BURNING ON VIRAL ONCOGENESIS IN THE MOUSE; EFFECTS OF EXOGENOUS STEROIDS, GLUCOCORTICOID BLOCKAGE, BETA ADRENERGIC BLOCKADE AND VITAMIN A

INTRODUCTION

The principal cause of death in burned patients is infection. The origin of this infection is usually the burn wound which serves as an excellent culture medium in which microorganisms can proliferate. Studies of both polymorphonuclear cell function and lymphoid cell function have shown that host defense mechanisms are, to a degree, impaired after burning. The combination of the burn wound serving as a source of sepsis and the impairment of host defense mechanisms often lead to death from infection.

In studying the clinical course of infection in either a patient or a laboratory animal, it is of advantage to be able to separate the infection from the burn wound. However, any disseminated infection which can kill the animal will, in a certain percentage of animals or patients, also be found in the burn wound. Laboratory studies of the Moloney sarcoma virus indicate that this virus causes a very localized tumor which is generally free from metastases. Although no burn patient ever dies from the Moloney sarcoma virus, the experimental studies of tumors caused by this infectious agent offer an *in vivo* method of studying host response to one viral agent for which the disease entity is entirely separate from the burn wound. The study of such an agent is advantageous for initial investigations of the response of burned animals to viral infections, and the effects of hormonal manipulations on this response.

METHODS

Seven week old male CBA mice of 20 to 25 g weight were used for the entirety of these experiments. Standardized burns were applied to the dorsal surface of these animals by using brass blocks with a 2 x 5 cm surface, heating these brass blocks to 98.5°C and placing the animal in contact with the surface for 4 seconds. Two contact burns (each 10 cm square) were placed on the right and left dorsal surfaces of each mouse, resulting in a 20 square cm surface area burn, which is a burn of 25% of the total body surface. These burns were determined to be third degree, and depth of burn was confirmed histologically at 24 and 72 hours post burn. Sham burning was performed by placing unheated brass blocks on anesthetized animals for the same period of time.

Forty-eight hours after burning, 0.1 cc of a uniform inoculum of Moloney sarcoma virus was administered intramuscularly to the medial

aspect of the mouse's right thigh. Tumor size was graded every 4 days after inoculation until 32 and sometimes 40 days after inoculation. Tumor grading was performed according to the scale of Blumenschein and Moloney (Journal of the National Cancer Institute, V 42, Part 1, p 123, 1969). A grade 1 tumor indicated swelling, clearly palpable in the thigh muscle at the site of inoculation. A grade 2 tumor corresponded to a palpable mass involving the entire thigh muscle. A grade 3 tumor indicated extension of the tumor over the pelvic brim into the abdomen. A grade 4 tumor involved the entire leg and had extension across the midline of the abdomen.

At the conclusion of the experiment, the animals were killed. Animals were stored 10 per cage. Because of extensive cannibalism in these mice, it was impossible to obtain autopsies on animals which died during the course of the experiment.

RESULTS

Experiment #1: The Effect of 25% Burns on Tumor Development in Mice

The purpose of this experiment was to see if burning substantially changed the susceptibility of mice to the development of a tumor following inoculation with Moloney sarcoma virus. Twenty mice were given 25% BSA thermal contact burns and a second group of 20 mice sham burned. Figure 1 plots the mean tumor score versus time after inoculation. It is clear that, from day 12 onward, the burned animals developed larger tumors than did the sham burned animals. Tumor regression likewise appears to be slower in the burned animals. Statistical comparison was made using an extended Chi-square test over the ordinal tumor scores. This was performed because mean tumor size, although useful graphically, in actuality has no meaning. Such analysis indicates that there was a significant increase in tumor size in the burned group (p less than 0.001 from day 12 onward). This experiment was repeated several times (infra vide) and the direction of the results was always the same.

Experiment #2. The Effect of Exogenous Glucocorticoid Administration on Tumor Development in Unburned Mice.

The purpose of this experiment was to see if the administration of exogenous glucocorticoids could produce an increase in tumor development comparable to that seen in burned animals. Mice were divided into two groups of 30 animals each. The experimental group received 1 mg of Depo-Medrol in 0.5 cc of saline given subcutaneously in the abdomen. The control group received 0.5 cc of saline given subcutaneously in the abdomen. The results of this experiment are presented graphically in Figure 2. Tumor size was larger and persisted longer in the animals given glucocorticoids. Extended Chi-square analysis of tumor score indicates that the difference between the two groups is significant from day 8 onward (p less than 0.001).

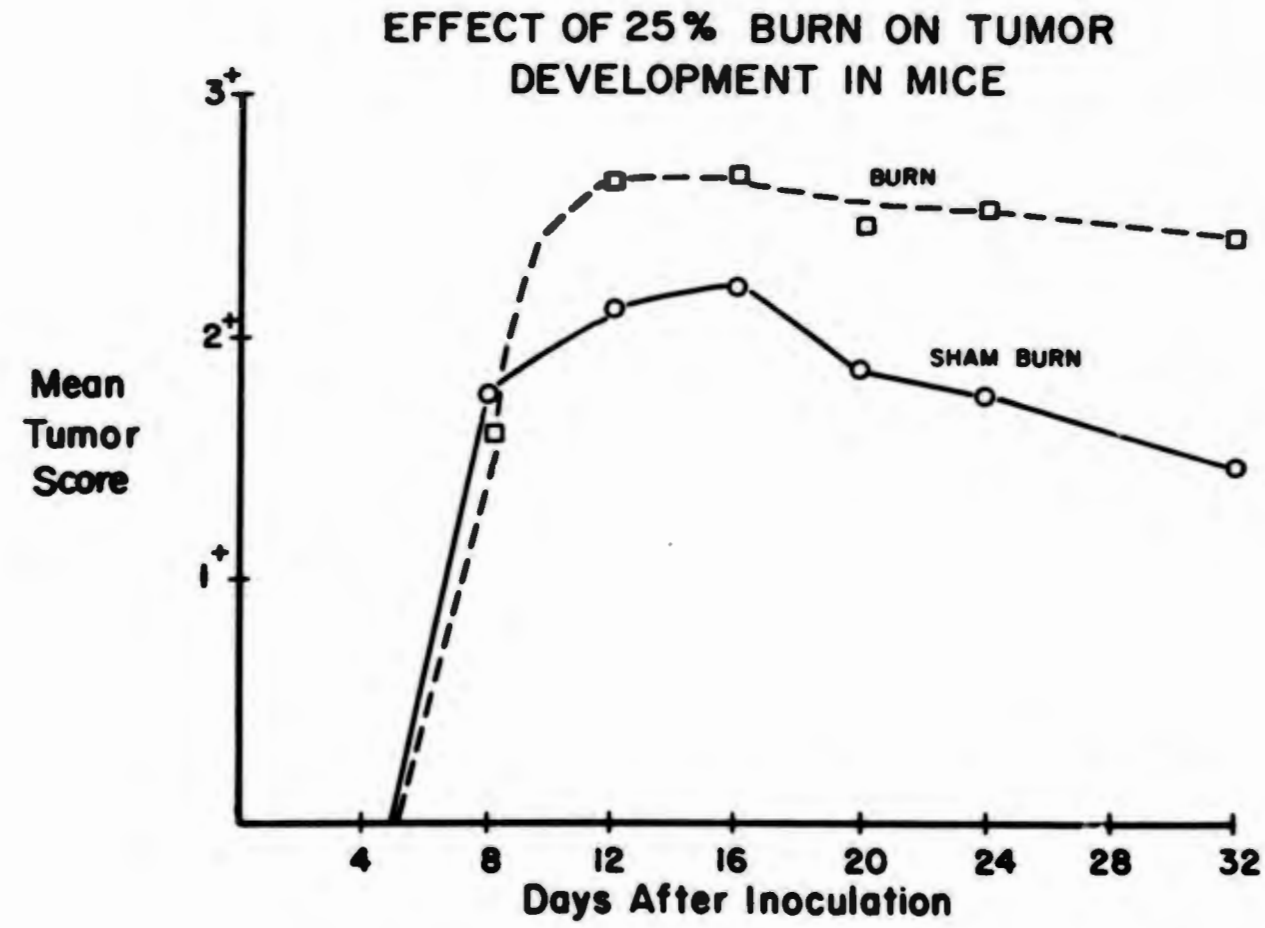

EFFECT OF 25% BURN ON TUMOR DEVELOPMENT IN MICE
Mean Tumor Score
3+
2+
1+
BURN
SHAM BURN
4
8
12
16
20
24
28
32
Days After Inoculation

Figure 1

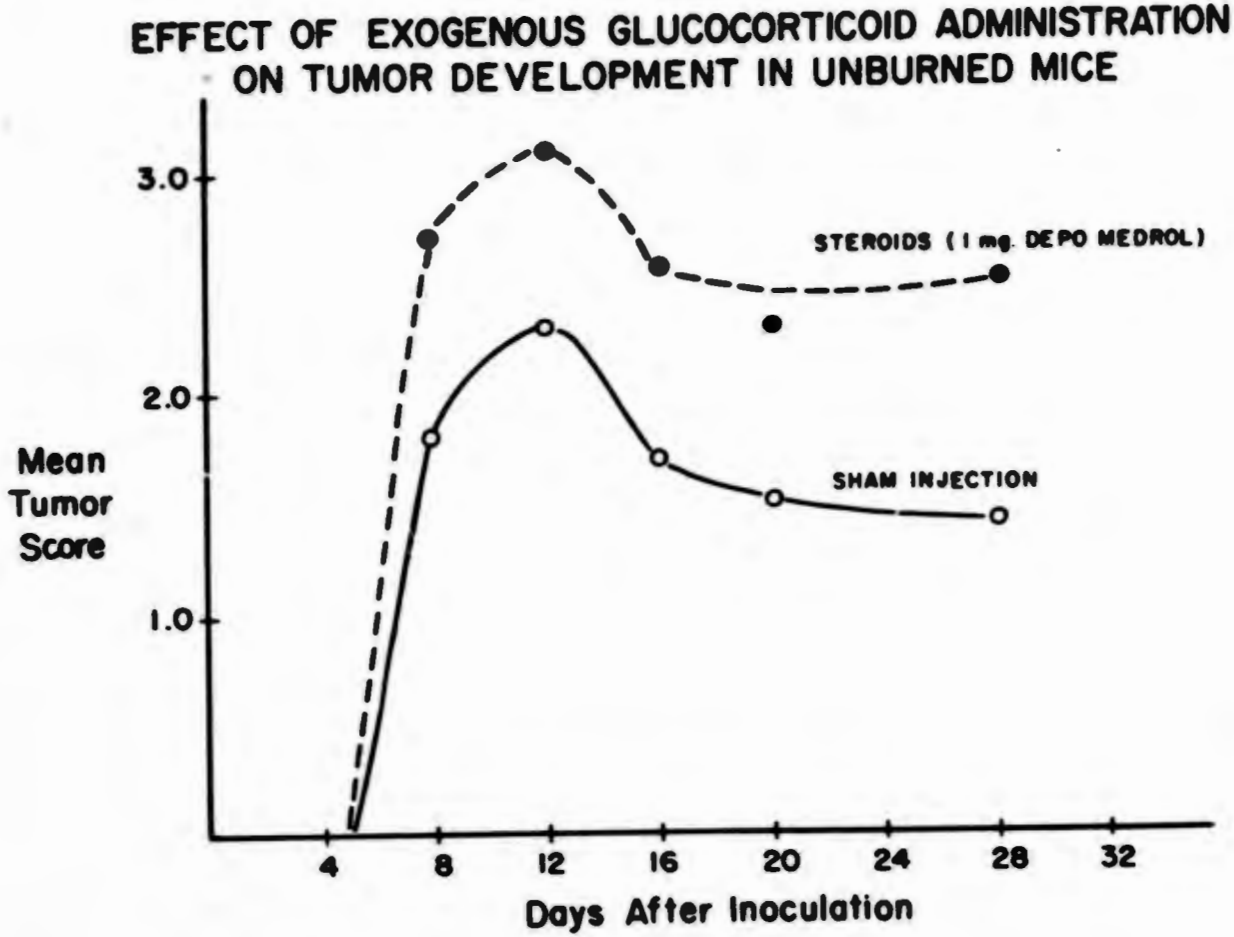

EFFECT OF EXOGENOUS GLUCOCORTICOID ADMINISTRATION
ON TUMOR DEVELOPMENT IN UNBURNED MICE
Mean
Tumor
Score
3.0
2.0
1.0
STEROIDS (1 mg. DEPO MEDROL)
SHAM INJECTION
4
8
12
16
20
24
28
32
Days After Inoculation

Figure 2

Experiment #3: Effect of Metyrapone on Tumor Development in Unburned Mice.

Animals were divided into two groups of 20 mice each. One group was given tap water to drink, the second group was given metyrapone 1 mg per cc drinking water. The drinking water bottles containing metyrapone were covered with brown paper to avoid decomposition of the drug on light exposure. Forty-eight hours after commencement of the drug, the animals were given a uniform inoculum of the Moloney sarcoma virus. Tumor scores were graded every four days thereafter. From day 16 onward, the tumors were significantly smaller (p less than 0.25) in the metyrapone treated animals than were the tumors in the controls. Figure 3 plots mean tumor score versus time. Extended Chi-square analysis reveals that the difference between the two groups was significant (p less than 0.025) from day 16 onward.

Experiment #4: The Effect of Metyrapone on Tumor Development in Mice with 25% Burns.

Burned mice were divided into two groups of 40 each and given either water or metyrapone 2 mg per cc in water immediately after burning. Forty-eight hours after burning, they were inoculated with standardized dose of Moloney sarcoma virus in the right thigh.

Figure 4 graphically presents the mean tumor score versus time after inoculation. It is evident that there was no significant difference between animals who were burned and received water without drug and animals who were burned and received metyrapone. Extended Chi-square analysis confirms the same findings.

Figure 5 is a graphic representation of mean tumor score in burned and unburned animals treated with and without metyrapone plotted against time after inoculation with the Moloney sarcoma virus. It is evident from Figure 5 that, although metyrapone caused an accelerated regression of the tumor from day 12 onward in the unburned animals, it had no effect upon burned animals. Burned animals again showed a significant increase in tumor size (p less than 0.001) over unburned animals.

Experiment #5: The Effect of Vitamin A on Tumor Development in Burned and Unburned Mice.

Mice were divided into 4 groups of animals. Group 1 (20 animals) were sham burned and given ordinary drinking water in their feeding bottles. Group 2 was also sham burned, but given vitamin A, 150 units per cc, in the drinking water. Group 3 was given a 25% dorsal surface burn and 150 units of vitamin A per cc of drinking water. Animals in all groups were given a uniform inoculum of Moloney sarcoma virus in the right thigh 48 hours after burning or sham burning.

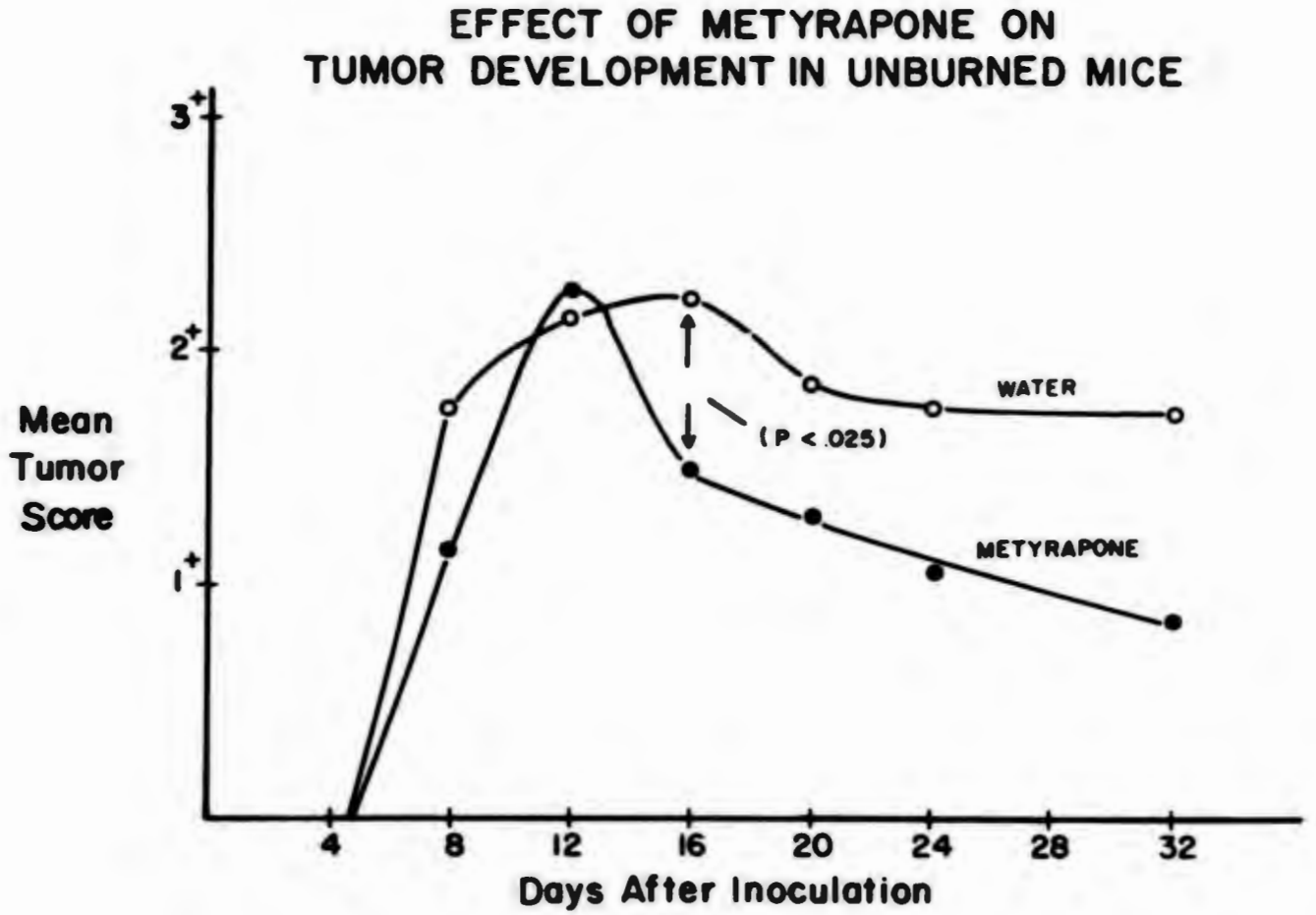

EFFECT OF METYRAPONE ON
TUMOR DEVELOPMENT IN UNBURNED MICE
Mean
Tumor
Score
3+
2+
1+
WATER
(P < .025)
METYRAPONE
4
8
12
16
20
24
28
32
Days After Inoculation

Figure 3

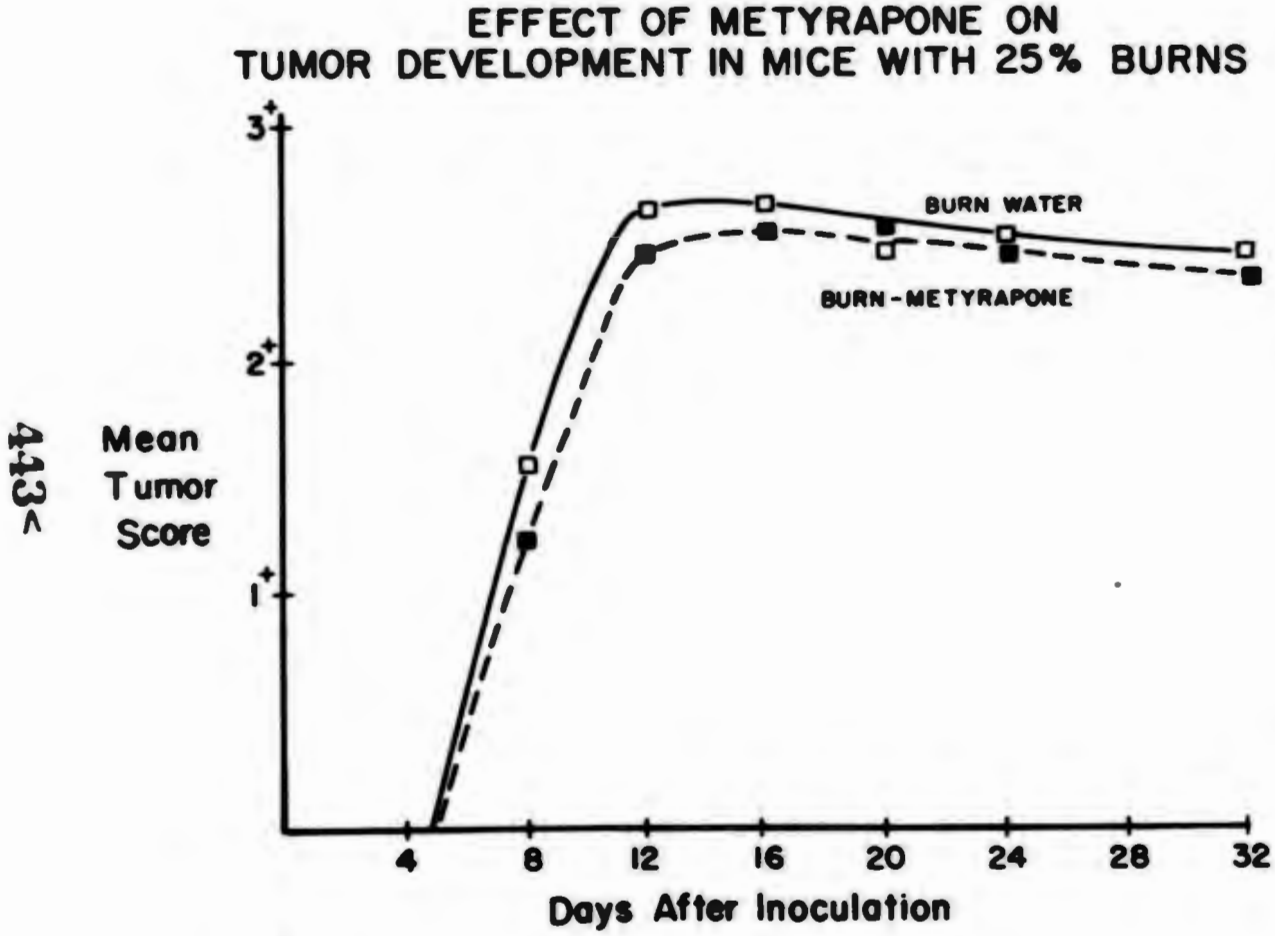

EFFECT OF METYRAPONE ON
TUMOR DEVELOPMENT IN MICE WITH 25% BURNS
Mean Tumor Score
3+
2+
1+
BURN WATER
BURN-METYRAPONE
4
8
12
16
20
24
28
32
Days After Inoculation

Figure 4

EFFECT OF METYRAPONE ON TUMOR DEVELOPMENT IN 25% BURNED AND UNBURNED ANIMALS

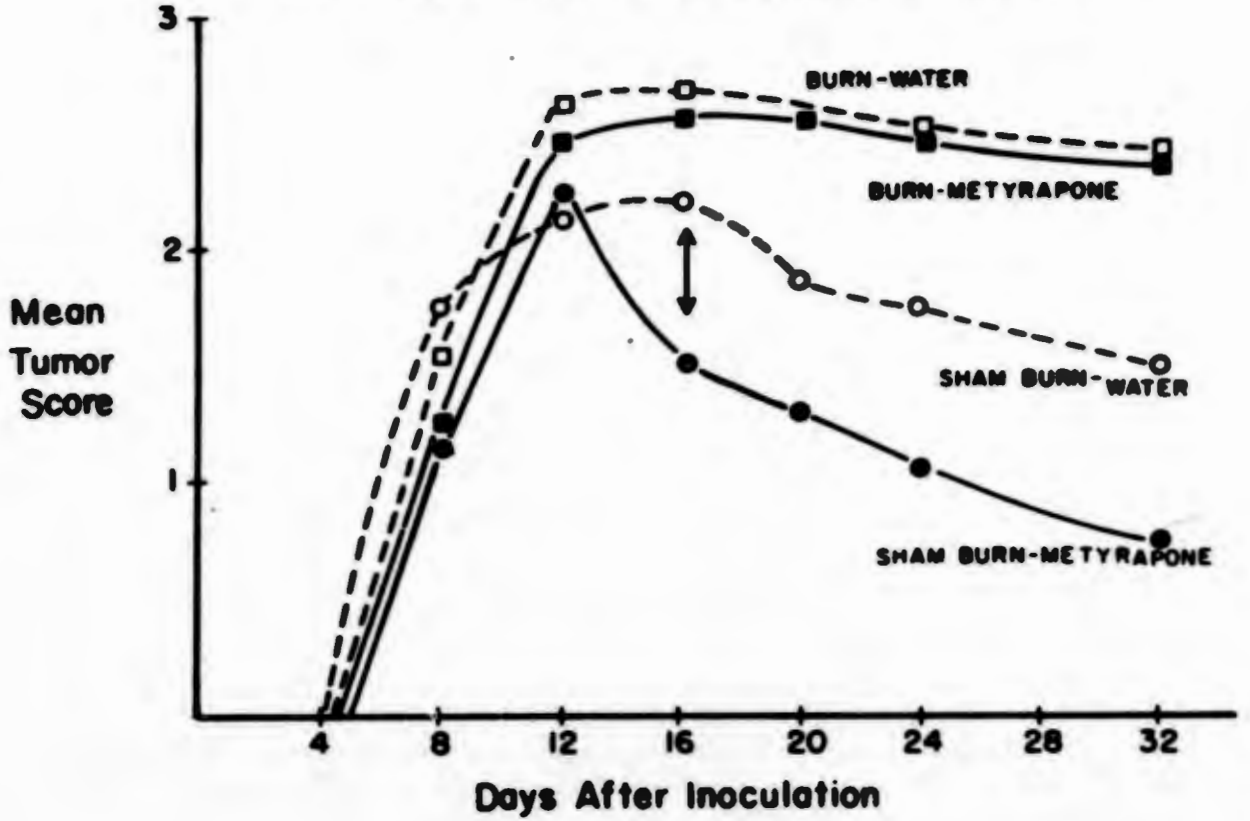


Figure 5

444<

Figure 6 is a graphic representative of a mean tumor score versus time following inoculation. Extended Chi-square analysis reveals that vitamin A treated unburned mice had a lower tumor score which was significant from day 12 onward (p less than 0.025). There was no significant difference between burned animals who received vitamin A and burned animals who received ordinary drinking water.

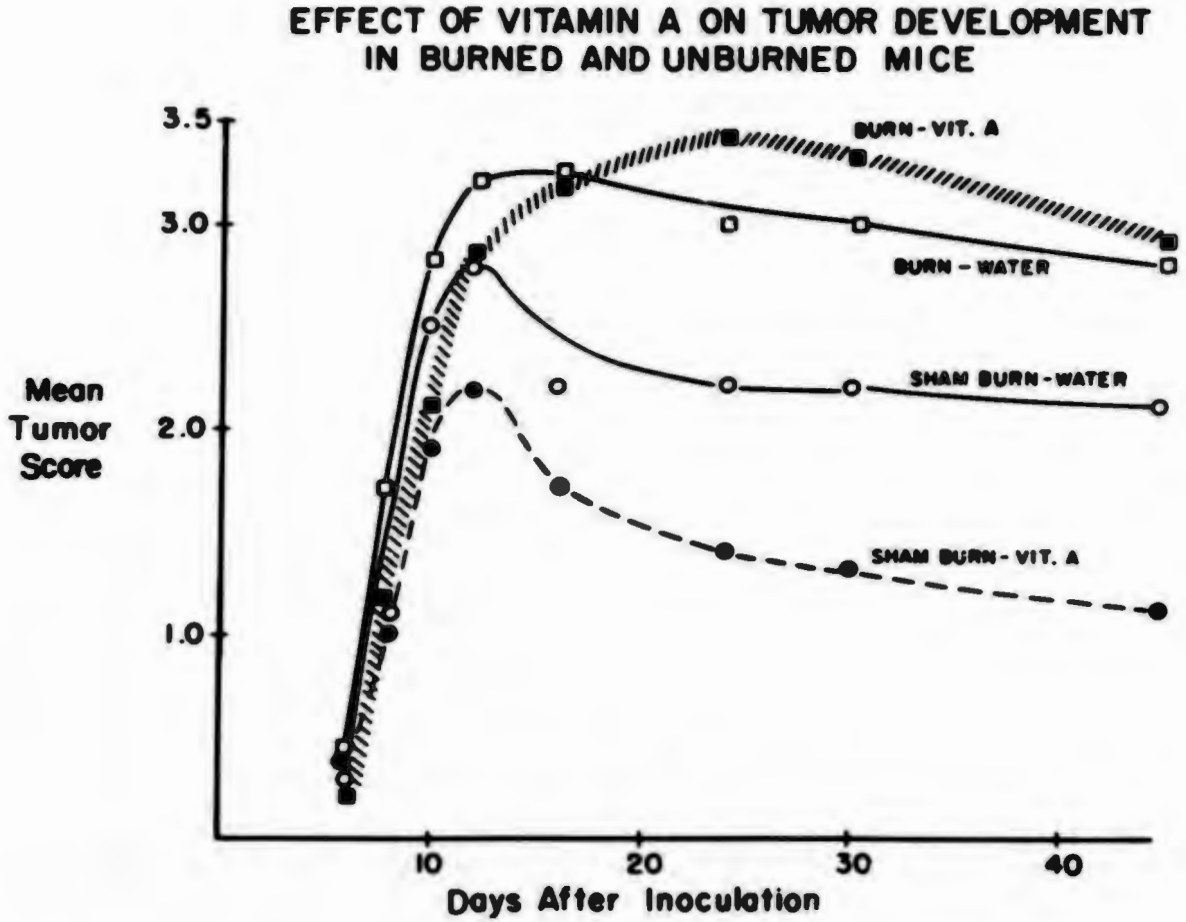
Experiment #6: The Effect of Vitamin A on Tumor Development in Mice Following a Single Dose of Depo-Medrol.

Because of recent evidence suggesting that vitamin A can antagonize the effects of steroids on wound healing and because of the evidence that steroids increase the susceptibility of our mice to tumor oncogenesis, the effects of vitamin A on tumor development in mice following a single dose of Depo-medrol were studied. Experimental mice were divided into two groups of 30 mice each. Both groups were given 1 mg of Depo-Medrol subcutaneously 48 hours prior to viral inoculation. One group of animals was given vitamin A 150 units per cc in the drinking water and the other group was given ordinary drinking water. Forty-eight hours after commencement of vitamin A, the animals received a uniform inoculum of Moloney sarcoma virus in the right thigh. Tumor score was graded every 4 days thereafter. Figure 7 is a graphic presentation of mean tumor score versus days after inoculation. It is evident from the graph and also documented by extended Chi-square analysis that there is no significant difference in the tumor scores between the two groups.

Experiment #7: Effect of Propanol on Tumor Development in Burned and Unburned Mice.

Seven week old male CBA mice were divided into four groups. Group 1 (20 animals with sham burn and fed normal ground Purina chow). Group 2 was sham burned and fed propanol 100 mg per kg of ground chow. Group 3 (40 animals) was given a 25% dorsal surface burn and fed ground Purina chow. Group 4 (40 animals) was given a 25% dorsal burn and fed ground Purina chow containing propanol 100 mg per kg of chow. Tumor score was graded every 4 days following inoculation. Figure 8 is a graphic representation of mean tumor score versus time following inoculation. Inoculations were performed two days after burning and commencement of a regulated diet.

There was no significant difference in the tumor size of either burned or unburned animals receiving either ground chow or propanol in chow. There was again a very significant difference between animals who were burned and animals who were not burned. All unburned animals receiving propanol survived. At 28 days, 21 out of 40 burned animals survived. At 28 days, only 11 out of 40 burned animals receiving propanol survived.


EFFECT OF VITAMIN A ON TUMOR DEVELOPMENT
IN BURNED AND UNBURNED MICE
Mean Tumor Score
3.5
3.0
2.0
1.0
BURN - VIT. A
BURN - WATER
SHAM BURN - WATER
SHAM BURN - VIT. A
10
20
30
40
Days After Inoculation

446<

Figure 6

EFFECT OF VITAMIN A ON TUMOR DEVELOPMENT
IN MICE FOLLOWING SINGLE DOSE DEPO-MEDROL

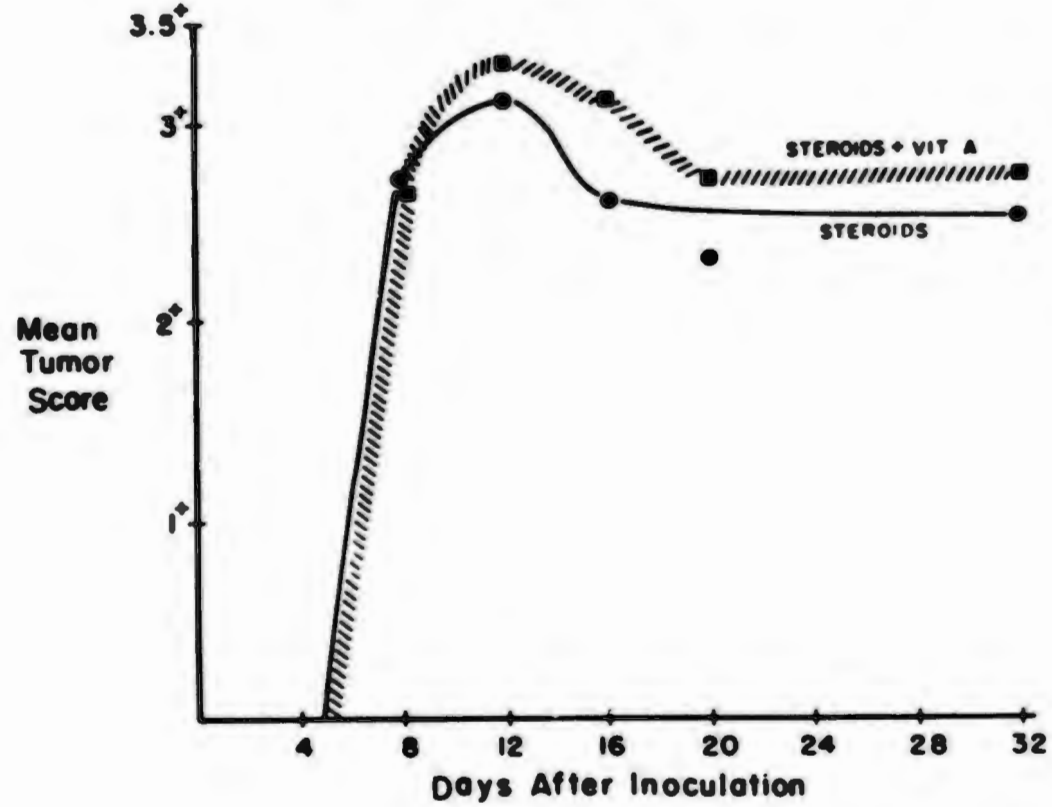


Figure 7

EFFECT OF PROPANOLOL ON TUMOR DEVELOPMENT IN BURNED AND UNBURNED MICE

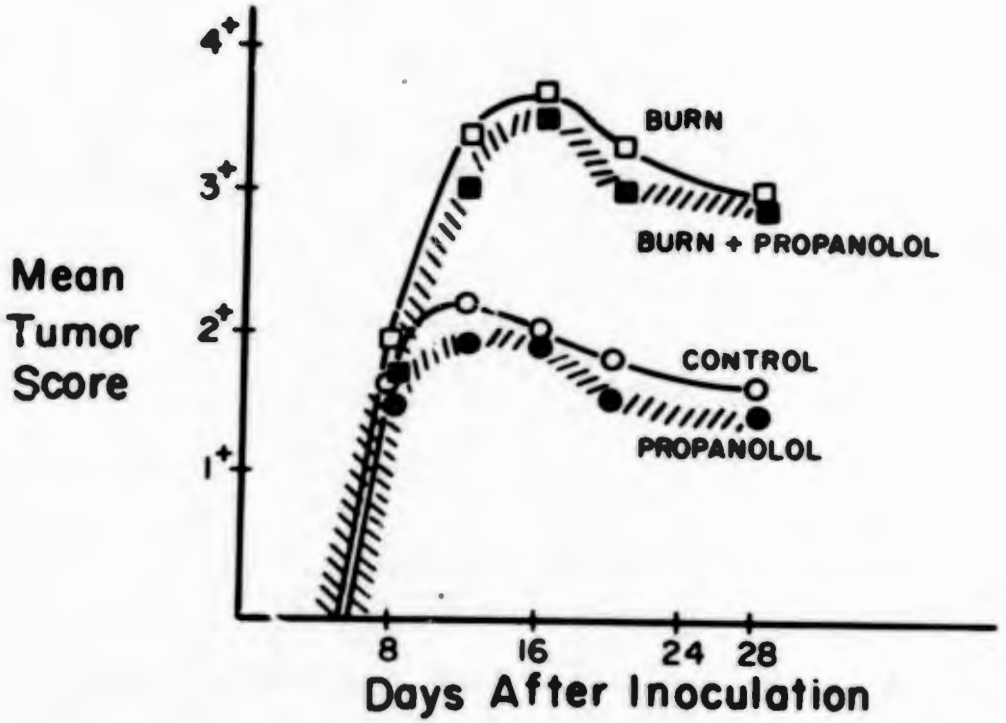


Figure 8

4.18<

Experiment #8: Corticosterone Levels in Burned Rats Treated with Metyrapone.

170-190 g Sprague-Dawley albino rats were given 10 cc saline or 10 cc saline plus 20 mg metyrapone immediately prior to anesthesia and 30% burning or sham-burning. Six hours after anesthesia, blood samples were drawn for serum corticosterone levels. The results of our data are included in Figure 9. The mean serum corticosterone level for unburned animals was 24.1 ug/ml and in burned animals it was 69.9 micrograms/ml. There was no overlap between the two groups. The mean corticosterone level for burned animals receiving 20 mg metyrapone was 61.8 micrograms/ml and not significantly different from that of burned animals receiving saline. Administration of higher doses of metyrapone (40 mg) was lethal to even unburned rats. These data suggest that metyrapone is ineffective in blocking 11-hydroxylation in rats with burns of 30% of the total body surface area. Similar data in mice was unreliable, because simple cardiac puncture could not be readily performed and other methods of "blood-letting" involved considerable "stress" to the animals.

DISCUSSION

From the experiments herein presented, the following conclusions can be drawn:

1. Burning causes a definite increase in the susceptibility to Moloney sarcoma virus. This is reflected in an increased tumor size in burned animals compared to unburned animals and a delayed regression of the tumor in burned animals.

2. The administration of a single dose of glucocorticoids (1 mg of Depo-Medrol subcutaneously) causes a significant increase in tumor size and delay in tumor regression in mice so treated.

3. The oral administration of metyrapone to unburned mice inhibited the development and accelerated the regression of viral oncogenesis.

4. The oral administration of metyrapone to burned mice had no effect on tumor development in animals which were burned.

5. The results of such experiments are compatible with the hypothesis that basal levels of glucocorticoids in the normal animal have a permissive effect on viral oncogenesis, and perhaps other viral or fungal diseases. This is suggested by the fact that metyrapone, by inhabiting 11-hydroxylation and therefore limiting glucocorticoid production in the normal animal causes a decrease in tumor size. Mice with endogenous increases in glucocorticoid production secondary to burning or mice with exogenous steroid administration show increased tumor size and delayed tumor regression. Although metyrapone effectively inhibited viral oncogenesis in unburned animals, it was ineffective in inhibiting

FIGURE 9

EFFECT OF BURNING ON CORTICOSTERONE LEVELS IN RATS GIVEN SALINE AND METYRAPONE

	SHAM BURN (Micrograms/cc)	30% BURN & SALINE (Micrograms/cc)	30% BURN & METYRAPONE (Micrograms/cc)
1	23	60	54
2	14	76	62
3	17	70	72
4	34	76	69
5	25	77	58
6	19	63	69
7	17	67	58
8	44	--	50
9	--	--	64
MEAN	24.1 (Micrograms/cc)	69.9 (Micrograms/cc)	61.8 (Micrograms/cc)

the increased tumor development seen in the burned animal. This is compatible with the suggestion that following thermal injury, the stimulus for increased glucocorticoid production overrides blockage of 11-hydroxylation. Steroid levels in rats following burning and following burning and intraperitoneal metyrapone treatment are compatible with this hypothesis (Table 9).

Our experiments with vitamin A administration support the following conclusions. Vitamin A, like metyrapone, has a significant effect on tumor development in unburned animals. Animals given 150 units of vitamin A per cc of drinking water develop smaller tumors which regress faster than the tumors in untreated animals. Vitamin A administration to burned animals had no effect. This suggests that the requirement for vitamin A by the mouse may be greater than that which is provided by standard chow. No effect of vitamin A was seen in the burned animals.

Our conclusions based on our experiments with propanol suggest the following conclusion: The administration of 100 mg of propanol per kg of chow had no effect on tumor development in either burned or unburned animals. Our data suggest that this level of propanol did not cause death in any unburned animals, but was associated with a higher rate of death in the burned animals.

SUMMARY

The effect of experimental burns on tumor oncogenesis induced by the Moloney sarcoma virus in mice was studied. Twenty-five per cent third degree burns caused a significant increase in tumor size and a delay in tumor regression. Exogenous administration of glucocorticoids likewise promoted tumor development. Metyrapone inhibited tumor growth in normal mice, but had no effect on the increased tumor growth in burned mice. Propanolol, a beta-adrenergic blocker, had no effect on tumor growth in burned or unburned mice. Vitamin A inhibited tumor development in unburned, but not in burned animals. Our data are compatible with the hypothesis that glucocorticoids play a permissive role in viral oncogenesis, that this can be inhibited by 11-hydroxylase blockade with metyrapone; although increased glucocorticoid production is a likely cause of the increased tumor growth in burned animals, this cannot be reversed with metyrapone.

REFERENCE

1. Blumenschein GR, Moloney JB: Quantitative dose response relationships of murine sarcoma virus (Moloney) in BALB/C mice. J Natl Cancer Inst 42:123-133, 1969.

PRESENTATIONS AND/OR PUBLICATIONS

None

ABSTRACT

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: IMMUNITY IN BURNED ANIMALS - A LABORATORY MODEL OF CHANGES OCCURRING IN BURNED TROOPS. PART II. THE EFFECT OF EARLY SURGICAL EXCISION AND ALLOGRAFTING ON SURVIVAL IN BURNED AND BURNED-INFECTED RATS

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

Period covered in this report: 1 July 1973 - 30 June 1974

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Harrel L. Walker, MS

Reports Control Symbol MEDDH-288(R1)

The effect of early surgical excision and allografting on survival in burned, uninfected rats and in burned rats infected with Pseudomonas aeruginosa intraperitoneally has been studied. Surgical excision and allograft coverage had no effect on survival in the uninfected animals with burns of 30 to 60% of the total body surface. Survival in animals treated with surgical excision and no coverage was significantly worse than in the animals who were simply burned. Immediate excision of the burn wound, combined with prompt allograft coverage, resulted in a partial reversal of the mortality asscciated with intraperitoneal injection of Pseudomonas organisms. When the excisions were delayed until 48 hours after thermal injury or 24 hours after intraperitoneal infection, survival appeared to be the same or possibly worse than in burned, unexcised animals. The beneficial effects of surgical excisions and immediate graft coverage could be achieved with formalin fixed allograft as well as with fresh allograft. Neurovascular isolation of the eschar was of no benefit in reversing intraperitoneal Pseudomonas mortality.

Burns
Bacterial infection
Viral infection
Rats
Mice

IMMUNITY IN BURNED ANIMALS - A LABORATORY MODEL OF CHANGES OCCURRING IN BURNED TROOPS (PART II)

THE EFFECT OF EARLY SURGICAL EXCISION AND ALLOGRAFTING ON SURVIVAL IN BURNED AND BURNED-INFECTED RATS

The role of early surgical excision of the burn wound has remained controversial. It is generally agreed that, in patients with limited size third degree burns, early surgical excision of the burn wound combined with autograft coverage can reduce hospital time and hasten recovery of function. However, large area excisions in patients with massive thermal injury have met with limited success. Although many centers have reported encouraging results with this approach, only a limited number of cases have been reported, and survival has not been shown to be appreciably changed using this technique.

Although large scale excision removes the burn wound as a source for infection, patients so treated do not always remain free from systemic infection, and many died from pneumonia or invasive infection in unexcised areas. The purpose of this study was: 1. to examine the effects of surgical excision on survival in experimental animals, where factors such as burn size and wound infection can be controlled; 2. to determine if surgical excision of the burn wound with allograft coverage can radically alter the nature of "burn disease", using the systemic susceptibility to infection as an index of this disease; 3. to determine when excision should ideally be performed and what the critical factors in wound coverage are.

METHODS

Sprague-Dawley rats weighing 170 to 190 g were anesthetized with pentabarbitol (20 mg, i.p.) and 30% dorsal third degree burns were produced by immersion in water heated to 90.5° C for 10 seconds, by the method of Walker. To produce a 60% burn, the ventral surface of the animal was also immersed for 2.5 seconds. Excisions were always performed at the level of the deep fascia. Allografts were harvested from the donor rats of the same breed using the Brown dermatome. These grafts rejected an average of 20 days post application. Formalinized grafts were prepared by fixing freshly cropped allograft in 10% formalin for 7 days, and washing under running tap water for 24 hours immediately prior to use. Grafts were secured to the adjacent tissue by using metal clips.

At 24 hours after burning, the infected animals were injected with a 1 cc. inoculum of Pseudomonas aeruginosa, strain 12-4-4, made by diluting an 18 hour tripticase soy broth culture in 0.85% saline. Quantitative bacterial analysis was performed on all inoculi, revealing from 10^5 to 10^7 organisms per cc. Animals were housed in individual wire cages and fed water and Purina cat chow. Autopsies were performed on all

animals that died.

RESULTS

Experiment #1 - The effect of excision, with and without skin coverage, on survival.

In this study, the survival of untreated animals with 30 and 60% burns was compared to that of animals with 30 and 60% burns treated with immediate excision with and without allograft coverage. Our results are tabulated in Figure 1 and demonstrate that:

1. Neither a 30 or 60% burn was lethal to the rats;

2. All 30 and 60% burns treated with immediate surgical excision and allograft coverage also survived;

3. Excision without skin coverage resulted in a 40% mortality when 30% burns were excised and a 90% mortality when 60% burns were excised.

Experiment #2 - Comparison of intraperitoneal Pseudomonas mortality in the 30% burned and unburned rat.

Figure 2 plots the survival in unburned anesthetized animals and in animals anesthetized and given burns of 30% of the body surface area following an intraperitoneal injection of Pseudomonas aeruginosa given 24 hours after burning or anesthesia. Ten animals were included in each group. Survival in the burned animals was less than in the unburned animals at all three dosage levels. From this information, it was possible to select a dose of Pseudomonas that will kill most, but not all, of the burned animals and none of the unburned controls. An approximation of this inoculum was used for the ensuing experiments. In this as well as in the following experiments, death generally occurred between 6 and 15 days after burning and was associated with hemorrhagic pneumonitis in over 90% of the animals. Cultures of these lungs grew out Pseudomonas aeruginosa. The burn wounds generally remained free from infection.

Experiment #3 - Effect of immediate surgical excision plus allografting on intraperitoneal Pseudomonas mortality.

This experiment compares the ability of burned rats versus burned, excised, and allografted rats to survive an intraperitoneal challenge with Pseudomonas aeruginosa given 24 hours after burning. The results of a single such experiment are presented graphically in Figure 3. All sham burned, anesthetized controls survived. In this particular experiment, all burned animals died. Survival in the burned, excised, allografted group, 8 out of 15 animals, was better than that in the burned group, but not as good as in the unburned animals.

EFFECT OF BURN WOUND EXCISION AND EXCISION PLUS ALLOGRAFTING ON RAT SURVIVAL

	MORTALITY		
% BURN	BURN	BURN, EXCISED NOT GRAFTED	BURN, EXCISED ALLOGRAFTED
30%	0%	60%	0%
60%	0%	90%	0%

Figure 1

RAT SURVIVAL FOLLOWING I. P. INJECTION OF PSEUDOMONAS AERUGINOSA AT 24 HOURS POSTBURN OR ANESTHESIA

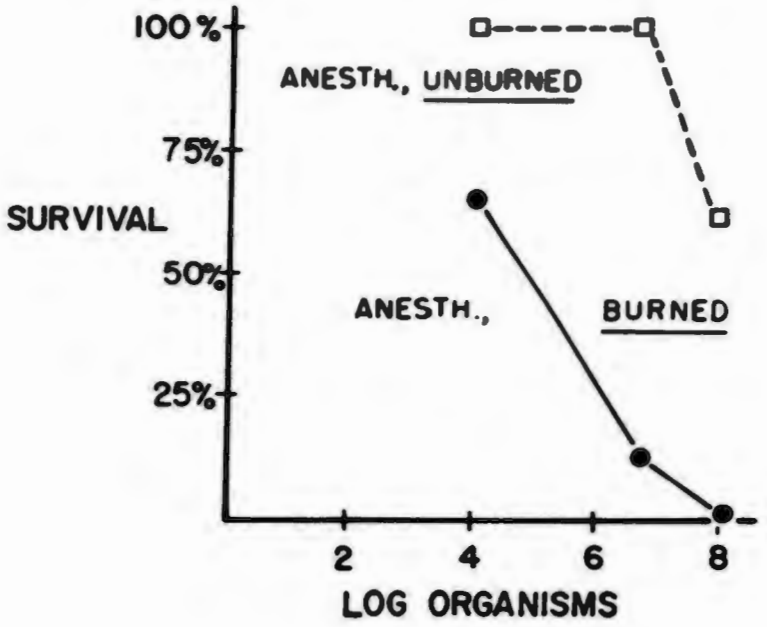

Figure 2

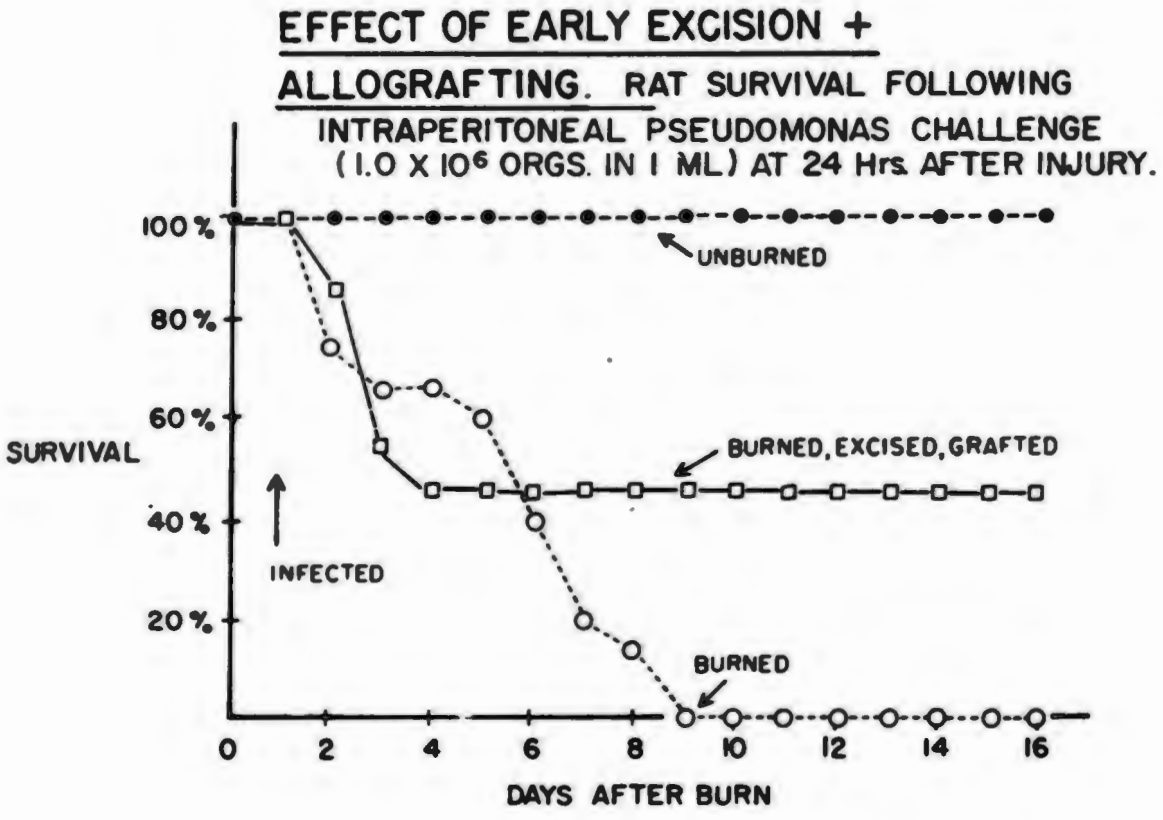
EFFECT OF EARLY EXCISION +
ALLOGRAFTING. RAT SURVIVAL FOLLOWING
INTRAPERITONEAL PSEUDOMONAS CHALLENGE
(1.0 X 10⁶ ORGS. IN 1 ML) AT 24 Hrs. AFTER INJURY.
100 %
80 %
60 %
40 %
20 %
SURVIVAL
UNBURNED
BURNED, EXCISED, GRAFTED
INFECTED
BURNED
0
2
4
6
8
10
12
14
16
DAYS AFTER BURN

Figure 3

This experiment was repeated four times (Figure 4). In each case, survival in the burned, excised, and allografted animals was better than that in the burned untreated animals. In our model, immediate excision with allografting could significantly (p less than 0.001) reduce the systemic liability to Pseudomonas aeruginosa caused by burning.

Experiment #4 - The effect of delayed excision and allografting on intraperitoneal Pseudomonas mortality.

In this experiment, the excisions were delayed until 48 hours after burning, or 24 hours after intraperitoneal inoculation with Pseudomonas aeruginosa. Figure 5 is a graphic presentation of our data. Unlike immediate excision, delayed excision and allografting did not favorably affect survival. Survival following delayed excision was actually less than that observed in the burned, unexcised animals (Figure 6), though this finding was not significant statistically in the two experiments performed.

Experiment #5 - Effect of immediate excision with application of formalinized cutaneous allografts on intraperitoneal Pseudomonas mortality.

An experiment (Figure 7) was performed in which the survival following intraperitoneal Pseudomonas challenge of burned, excised rats grafted with formalinized allograft was compared with that of rats grafted with fresh allograft. Formalinized allograft was used to test the effect of a cover which has the mechanical properties of skin, but which is not viable.

In this experiment, survival of both groups was similar. The survival of both groups was better than that of burned and unexcised rats. The experiment was repeated and the statistical analysis (Figure 8) of the two experiments suggests that there is no difference between survival in excised rats receiving formalinized allografts and excised rats receiving fresh allografts. Both of these two groups had a significantly (p less than 0.01) greater survival than did the burned untreated control group.

Experiment #6 - The effect of neuro-vascular isolation of the burn wound on intraperitoneal Pseudomonas mortality.

In this experiment, the burn wounds of experimental rats were immediately excised, then clipped back in place. These animals, together with burned controls, were subjected to an intraperitoneal Pseudomonas challenge at 24 hours postburn. The data in Figure 9 indicates that this "neurovascular isolation" of the burn wound did not favorably affect survival. Repetition of this experiment (Figure 10) confirms this, and suggests that survival following excising the burn wound, then putting it back in place, is worse than burning without this manipulation.

EXCISION AND ALLOGRAFT

SURVIVAL OF RATS FOLLOWING INTRAPERITONEAL CHALLENGE WITH PSEUDOMONAS AERUGINOSA (STRAIN 12-4-4) GIVEN 24 HOURS AFTER BURNING

	BURNED	BURNED, EXCISED ALLOGRAFTED	
EXPT. # 1	0/15	7/15	B < BEA
EXPT, # 2	7/15	10/15	
EXPT. # 3	3/15	5/15	$X^2_{(1)} = 12.31$
EXPT. # 4	6/15	13/15	$p < 0.001$
	16/60 = 26.7%	35/60 = 58.3%	

Figure 4

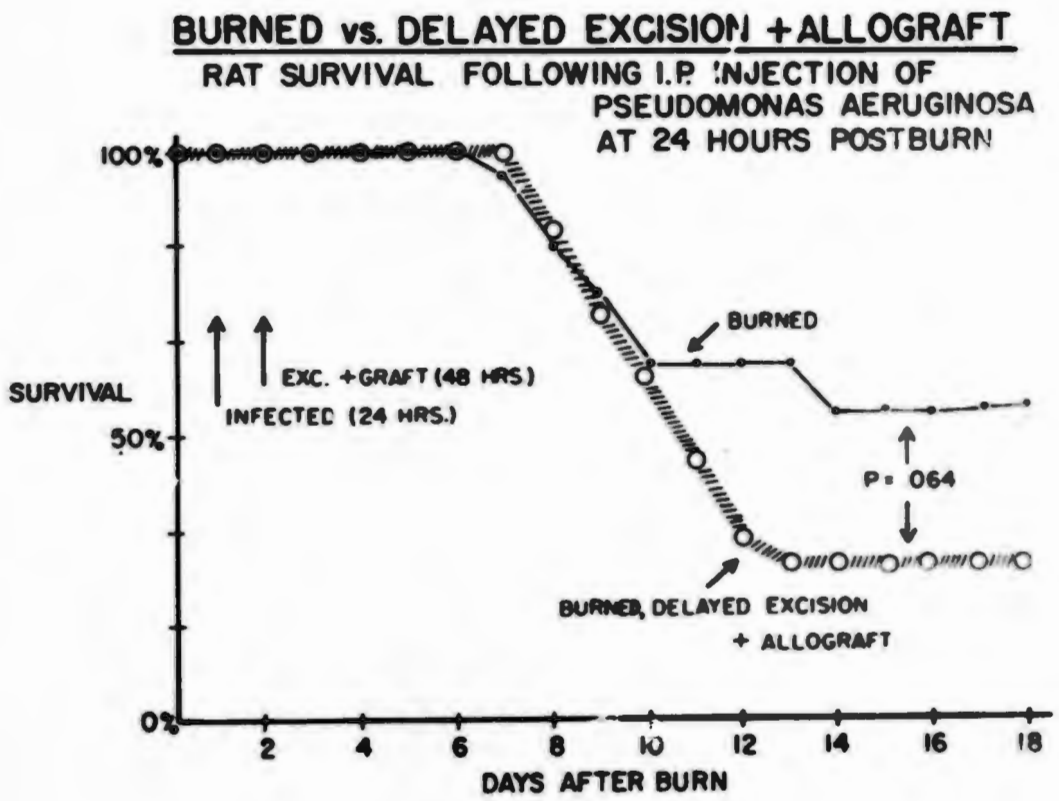
BURNED vs. DELAYED EXCISION +ALLOGRAFT
RAT SURVIVAL FOLLOWING I.P. INJECTION OF
PSEUDOMONAS AERUGINOSA
AT 24 HOURS POSTBURN
100%
SURVIVAL
50%
0%
EXC. +GRAFT (48 HRS.)
INFECTED (24 HRS.)
BURNED
P = 064
BURNED, DELAYED EXCISION
+ ALLOGRAFT
2
4
6
8
10
12
14
16
18
DAYS AFTER BURN

Figure 5

460<

DELAYED EXCISION

SURVIVAL OF RATS FOLLOWING INTRAPERITONEAL CHALLENGE WITH PSEUDOMONAS AERUGINOSA STRAIN 12-4-4.

	ALIVE	DEAD	
BURNED INFECTED I.P. (24 HRS)	13	11	p=0.0642
BURNED INFECTED I.P. (24 HRS) DELAYED (48 HRS) EXCISION + ALLOGRAFT	6	16	

Figure 6

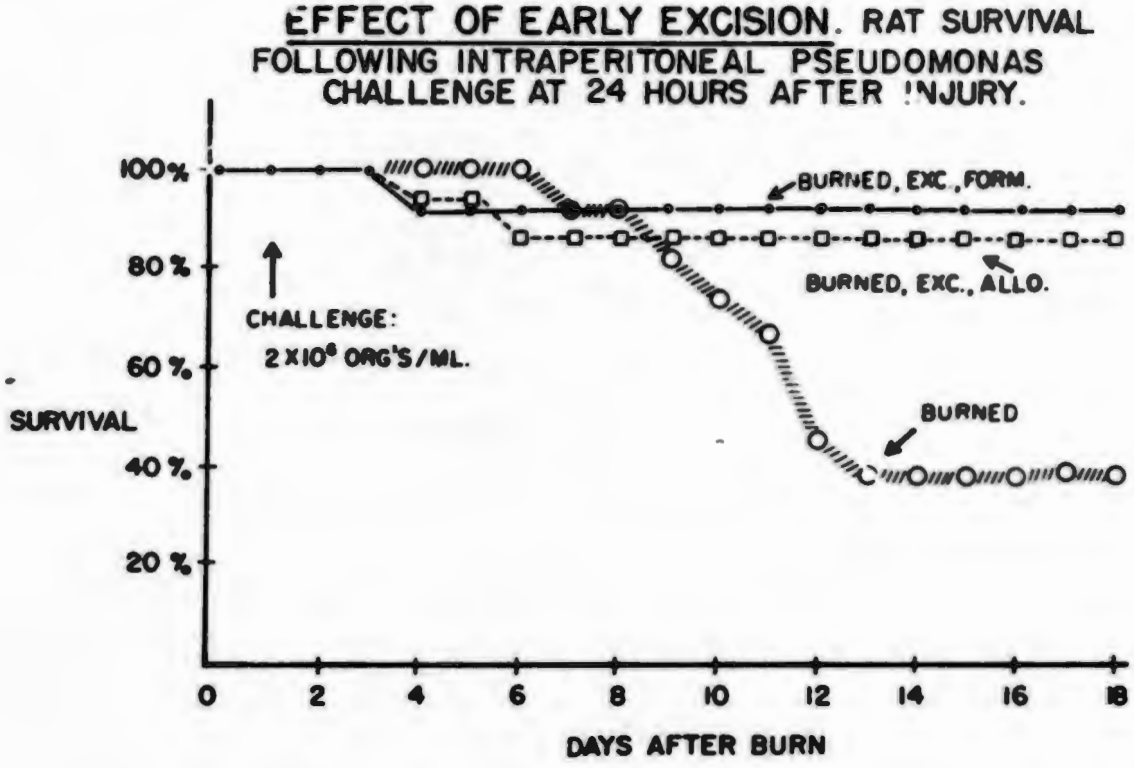
EFFECT OF EARLY EXCISION. RAT SURVIVAL
FOLLOWING INTRAPERITONEAL PSEUDOMONAS
CHALLENGE AT 24 HOURS AFTER INJURY.
100%
80%
60%
40%
20%
SURVIVAL
BURNED, EXC., FORM.
BURNED, EXC., ALLO.
BURNED
CHALLENGE:
2X10⁶ ORG'S/ML.
0
2
4
6
8
10
12
14
16
18
DAYS AFTER BURN

Figure 7

FORMALINIZED ALLOGRAFT

SURVIVAL OF RATS FOLLOWING INTRAPERITONEAL CHALLENGE WITH PSEUDOMONAS AERUGINOSA (STRAIN 12-4-4) GIVEN 24 HOURS FOLLOWING BURNING

463<

$X^2_{(1)} = 9.88$, $p < 0.01$

	EXPT # 1	EXPT # 2
BURNED	3/15	5/13
BURNED, EXCISED, ALLOGRAFTED	5/15	13/15
BURNED, EXCISED, FORMALIN GRAFTS ($X^2 = 0.6$, NS)	7/15	12/13

42-11

Figure 8

EFFECT OF IMMEDIATE NEUROVASCULAR DISRUPTION OF THE ESCHAR ON SURVIVAL FOLLOWING INTRAPERITONEAL CHALLENGE c̄ 4×10^6 Ps. AERUGINOSA

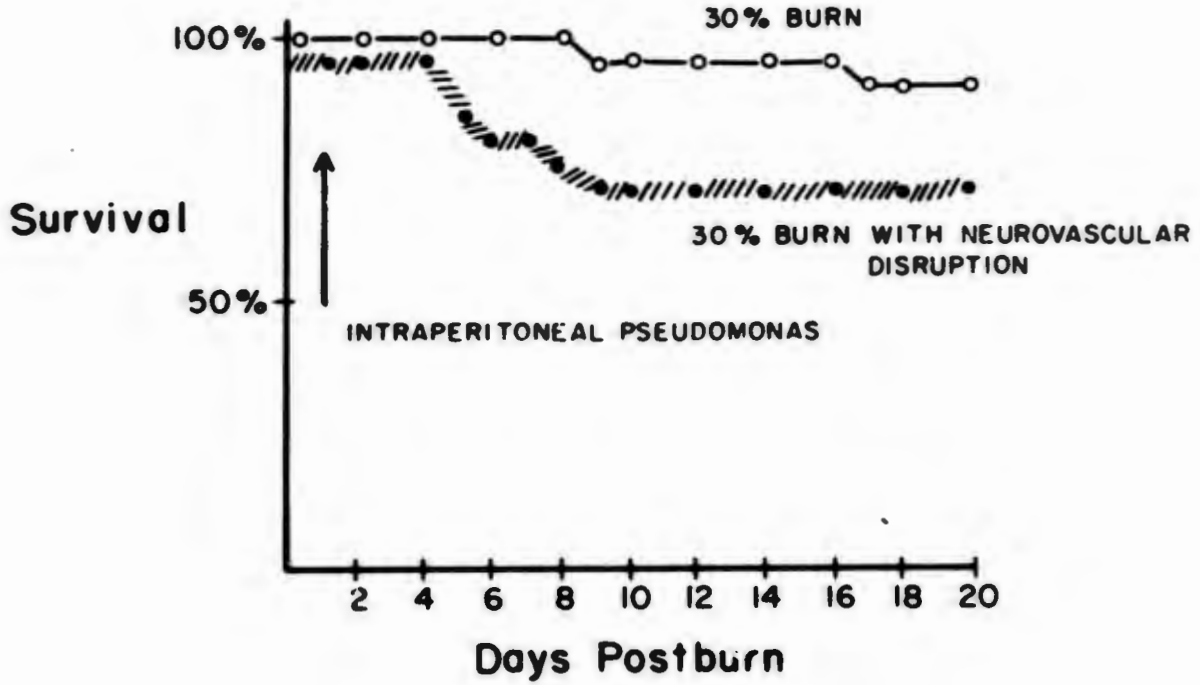

Figure 9

42-12

FIGURE 10

SURVIVAL OF RATS FOLLOWING INTRAPERITONEAL CHALLENGE WITH PSEUDOMONAS AERUGINOSA GIVEN 24 HOURS AFTER BURNING

	BURNED	BURNED + IMMEDIATE NEUROVASCULAR ISOLATION OF ESCHAR
EXPT #1	18/20	13/20
EXPT #2	15/17	16/20
	89%	73%

$p < 0.10$ by extended X^2

DISCUSSION

The principal cause of death in patients with large area burns is infection. We can attribute this liability to infection to two interrelated factors. First, the burn wound, consisting of dead tissue and proteinaceous exudate, forms a good culture medium in which microorganisms can proliferate, providing a local portal for systemic sepsis. Second, thermal injury has been shown to evoke a systemic response in which host defense mechanisms fail, resulting in an increased systemic susceptibility to infections. The combination of both factors may lead to systemic sepsis and death of the patient.

The rationale behind large area excisions has been early removal of the local site for bacterial proliferation. The intention of this study was to determine whether early removal of the burn, plus allograft coverage, could radically alter the nature of "burn disease" by reversing the increased systemic susceptibility to infection caused by burning.

Our initial experiment, comparing survival in uninfected burned rats versus that in burned excised rats, with and without allograft coverage emphasizes several points. First, the uninfected rat is quite resistant to thermal injury: even 60% third degree burns do not kill these animals. Indeed, these animals form dry, nonsuppurative eschars which separated in a single layer. These rats did not require skin grafts and healed these massive third degree burns by a combination of epithelialization and contraction. Although survival in animals with 60% burns treated by excision and allografting was also 100%, this was no improvement over survival in the uninfected animals. However, excision of the burn wound without allograft coverage resulted in a high mortality for 30% burns and a devastating mortality for 60% burns. This suggests that the absence of skin is more detrimental to the animal than the presence of burn. It also stresses the need for prompt, effective coverage of large denuded areas.

Our second experiment confirms the findings of others [1,2,3] who noted that 30% burns cause an increased systemic susceptibility to intraperitoneal Pseudomonas aeruginosa, when this organism is given within the first 24 hours after burning. Our third experiment demonstrates that surgical excision of the burn wound, plus allografting can, in part, reverse this susceptibility to intraperitoneal Pseudomonas aeruginosa. That this is a reversal of systemic susceptibility to infection rather than merely a removal of a site (the burn wound) in which the organisms may lie is suggested by the following two factors: First, infection in the burn wound occurred in less than 20% of the animals inoculated intraperitoneally; second, experiment #4, which involves removal of the burn wound at 48 hours after thermal injury, did not favorably alter survival and appeared to worsen survival. The lack of benefit seen with delayed excisions likewise suggests that, to favorably alter the susceptibility to infection, burn wound excision should be done as early as possible.

In clinical situations, "early" excisions are usually performed at 48 to 72 hours following thermal injury, when the patient has been adequately resuscitated. Our experiments suggest that it might be even more beneficial to perform massive excisions within the first 24 hours after thermal injury, at a time when the patient is still being actively resuscitated.

Our first experiments with the uninfected animals emphasize the importance of a wound cover in conjunction with surgical excision of the burn wound. Experiments #3 and #4 demonstrate the beneficial effects of fresh allografts, combined with prompt surgical excision, using the reversal of mortality due to intraperitoneal injection of Pseudomonas organisms as an index of a "beneficial systemic effect." Experiment #5 demonstrates that formalinized allograft, could be used in conjunction with surgical excision and fresh allograft coverage. This suggests that at least some of the beneficial effects of "biologic dressings" are related to their mechanical properties and that such dressings do not have to be viable.

It has been suggested by many physicians that "toxic" products from the burn wound are released into the systemic circulation and may, in part, be responsible for this increased susceptibility to gram negative infection. Likewise, it is possible that this effect is neurologically mediated. If either of these hypotheses is true, then a "neurovascular isolation" of the burn wound might be expected to be of benefit to these animals. Experiment #6 attempted to accomplish this by excising the burn wound and clipping it back in place on the area of excision. This manipulation, however, appeared to have no beneficial effect on the animals and appeared to make survival worse: p less than 0.10. This experiment does not clearly refute either the concept of a blood born toxin or neurologically mediated toxic effects of the burn wound. However, it lends no supportive evidence for either theory.

SUMMARY

The effect of early surgical excision and allografting on survival in burned, uninfected rats and in burned rats infected with Pseudomonas aeruginosa intraperitoneally has been studied. Surgical excision and allograft coverage had no effect on survival in the uninfected animals burned to 30% or 60%. Survival in animals treated with surgical excision and no coverage was significantly worse than in the animals who were simply burned. Immediate excision of the burn wound, combined with prompt allograft coverage, resulted in a partial reversal of intraperitoneal Pseudomonas mortality. When the excisions were delayed until 48 hours after thermal injury or 24 hours after intraperitoneal infection, survival appeared to be the same or possibly worse than in burned, unexcised animals. The beneficial effects of surgical excisions and immediate graft coverage could be achieved with formalin fixed allograft as well as with fresh allograft. Neurovascular isolation of the eschar was of no

benefit in reversing mortality following intraperitoneal injection of Pseudomonas organisms in this model.

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1. Liedberg CF: Effect of intraperitoneal infection on survival and frequency of septicemia: An experimental study in the guinea pig. Acta Chir Scand 121:351-358, 1961.

2. McRipley RJ, Garrison DW: Increased susceptibility of burned rats to Pseudomonas aeruginosa. Proc Soc Exp Biol Med 99:89-104, 1962.

3. Alexander JW: Effect of thermal injury upon the early resistance to infection. J Surg Res 8:128-136, 1968.

PRESENTATIONS

Levine NS. Reversal of Intraperitoneal Pseudomonas Mortality in the Rat by Surgical Excision and Allografting. Amer Soc of Plastic and Reconstructive Surgeons, Hollywood, Fla, Oct 1973.

PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0E 6389	74 07 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
73 07 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	61102A	3A161102B71P	01	021
b. CONTRIBUTING	61102A	3A161102B71P	08	
c. CONTRIBUTING				

11. TITLE (Procede with Security Classification Code) (U) Laboratory Evaluation of Artificial Tendons and Homografts for Use in Military Personnel with Severe Flexor Tendon Injury (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS
003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
71 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 74	.8	20
FISCAL YEAR CURRENT 75	.3	7

19. RESPONSIBLE DOD ORGANIZATION
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21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Provide EACH with Security Classification Code)
(U) Artificial tendon; (U) Flexor tendon injuries; (U) Neosheaths; (U) Chickens

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

23. (U) To study the feasibility of inducing the formation of a new tendon sheath from undifferentiated connective tissue around an artificial tendon prosthesis, and then transfer that neosheath, with a tendon graft, into an injured finger to improve function.

24. (U) Twenty chickens, 12 weeks old, were used in this experiment. In 10 chickens, the flexor sublimis tendon was excised from the third toe of the right foot, the osseous floor scraped, and the flexor profundus cut in "no man's land" and repaired, using the Verdan technique. In 10 other chickens, an artificial tendon was placed into the soft tissue of the back, and one month later the long toe of the right foot was traumatized in the same manner as the control animals were. Before the flexor profundus was repaired, however, the animal's backs were explored, and a neosheath which had formed around the artificial tendon was dissected out. This neosheath was slid over the damaged flexor profundus after the anastomosis was performed. All animals were splinted for three weeks, and then evaluated for range of motion.

25. (U) 73 07 - 74 06 The control group had very poor function, only one out of 10 animals being able to flex the PIP joint of the repaired toe and none being able to flex the DIP joint. The neosheath transfer group, however, regained excellent function, eight out of 10 animals being able to flex the PIP joint, and four out of 10 being able to flex the DIP joint also.

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*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

ABSTRACT

PROJECT NO. 3A161102B71P-01, BASIC RESEARCH IN SUPPORT OF MILITARY MEDICINE

REPORT TITLE: LABORATORY EVALUATION OF ARTIFICIAL TENDONS AND HOMOGRAFTS FOR USE IN MILITARY PERSONNEL WITH SEVERE FLEXOR TENDON INJURY

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

Period covered in this report: 1 July 1973 - 30 June 1974

Investigators: Roger E. Salisbury, MD, Major, MC
Norman S. Levine, MD, Major, MC
Hugh D. Peterson, DDS, MD, Colonel, MC
Arthur D. Mason, Jr., MD
Basil A. Pruitt, Jr., MD, Colonel, MC

Flexor tendon injuries of the hand in "no-man's land" are often not amenable to successful primary repair or tendon grafting. Although implantation of a silastic prosthesis to recreate the synovial sheath followed by tendon grafting is often successful, it does require two major procedures and much time is lost from work. The purpose of this study was to see if synovial sheaths could be preformed in the back, harvested and transferred with a tendon graft to produce good function with only one hand operation.

In 10 chickens, under local anesthesia, an artificial tendon was implanted in the back. Ten weeks later, the tendon with a new sheath around it was dissected out. The synovial sheath and sublimis tendon were excised from the long toe, the profundus tendon cut in "no man's land," and the osseous floor scraped. The neosheath was positioned over the distal profundus stump, and, following anastomosis of the tendon, was slid over the length of the shaft. In 10 control animals, the sublimis was excised, the osseous floor scraped, and the profundus simply cut and reanastomosed. After three weeks of immobilization, function was evaluated weekly for one month and then all tendon systems were examined grossly and microscopically.

Function was clearly superior in the neosheath group, eight out of 10 animals being able to flex the PIP and four out of 10 the DIP joints as opposed to one out of 10 in the control group. Gross dissection and light microscopy revealed an intact sheath that was

partially adherent to the tendon. The controls had dense scar adherent to the tendon, restricting motion.

This study suggests a possible new application for a tendon prosthesis in those patients in whom preoperative evaluation for reconstructive surgery reveals their injury to be too severe for conventional tendon repair.

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Artificial tendon
Flexor tendon injuries
Neosheaths
Chickens

LABORATORY EVALUATION OF ARTIFICIAL TENDONS AND HOMOGRAFTS FOR USE IN MILITARY PERSONNEL WITH SEVERE FLEXOR TENDON INJURY

Flexor tendon injuries of the hand in "no-man's land" are often not amenable to successful primary repair or tendon grafting. Although implantation of a silastic prosthesis to recreate the synovial sheath, followed by tendon grafting, is often successful, it does require two major procedures and much time is lost from work. The purpose of this study was to see if synovial sheaths could be preformed in the back, harvested, and transferred, with a tendon graft, to produce good function with only one hand procedure.

METHOD

In 10 chickens, 12 weeks old, an artificial tendon was implanted in the soft tissue of the back, using local anesthesia. Ten weeks later, the artificial tendon, with a neosheath around it, was dissected out and placed in saline. The synovial sheath and sublimis tendon were then excised from the long toe, the profundus tendon cut in "no-man's land," and the osseous floor scraped with a scalpel. Having created this severe injury, the neosheath was then carefully slid over the distal profundus stump, and, following the anastomosis of the tendon with 6-0 monofilament suture, the sheath was then slid over the length of the tendon shaft. At this time, in 10 control animals, the sublimis tendon was excised from the long toe. The osseous floor of the toe was scraped, and the profundus tendon cut and reanastomosed with 6-0 monofilament suture. All injured extremities were immobilized for three weeks in plaster casts, and then allowed unlimited motion. Function was evaluated weekly for one month by testing the animals ability to perch. All animals were then sacrificed and the tendon systems examined grossly and microscopically.

RESULTS

Function was clearly superior in the group of animals that was treated with neosheath transfer and tendon grafting. Eight of 10 of these animals were able to flex their proximal interphalangeal joints, and four out of 10 animals were also able to flex their distal interphalangeal joints. In the control group, however, only one animal was able to flex his proximal interphalangeal joints, and none was able to flex the distal interphalangeal joint. The animals that regained function did so within two weeks of mobilization from their plaster cast. No late gains in function were noted. Dissection of the injured toes in the control group revealed a very dense scar adherent to the tendon and the underlying osseous canal. No definable planes were noted between the tendon and surrounding scar and soft tissue.

In the neosheath transfer group, however, the sheath was very much an intact organ, and clearly demarcated from the surrounding soft tissue. The tendon was adherent to the sheath in multiple places, but the adhesions were loose, and the tendon could be easily freed. Light microscopy confirmed the gross examination, and revealed a well-defined fibrous sheath around the autogenous tendon, and fine adhesions connecting tendon to sheath in several places. The normal anatomy and relationship between sheath and tendon were completely destroyed in the control group.

DISCUSSION

In spite of improved surgical technique, the results of tendon grafting for flexor tendon injuries of the hand in "no man's land" have not improved in the last 20 years (Boyes JH, Stark HH, J Bone Joint Surg 53A:1332-1342, 1971).[1] The use of a two-stage repair (Hunter JM, Salisbury RE, J Bone Joint Surg 53A:829-858, 1971),[2] with an artificial tendon prosthesis, has become popular because of the improved results that can be obtained with the most severely damaged fingers. The philosophy of the two-stage technique is sound in that it seeks to recreate the normal anatomy in the finger before tendon grafting is performed. Since previous work had shown that a neosheath would form around an artificial tendon prosthesis in the undifferentiated connective tissue of the back, it seemed logical to investigate the possibility of transferring the sheath as one would do with skin, tendon, or bone. Because the sheath is so thin, it should be quickly vascularized and maintain its viability.

Postmortem examination clearly showed why the group treated with neosheath transfer and tendon grafting had superior results to the control group. In the control group, dense adhesions grew from the damaged osseous floor to the tendon, encasing it completely in a bed of scar. Although the injury was exactly the same in the other group, the interposition of a neosheath prevented uncontrolled adhesion formation between the tendon and the surrounding bed. The neosheath was still a well-defined structure of longitudinally oriented connective tissue. Adhesions were dense to the outside of the sheath, but the adhesions found between the sheath and tendon itself were, for the most part, fine and vascular.

The successful use of a neosheath transfer in the laboratory animal model is encouraging, and perhaps warrants a controlled clinical evaluation. In patients with severe flexor tendon injuries of the fingers, who would be candidates for the two-stage technique, the surgeon could insert an artifical tendon prosthesis into the soft tissue of the thigh or back, under local anesthesia in the office. One month later, the injured finger could be explored, the damaged tendon

system excised, and a neosheath and tendon graft transferred simultaneously. If this technique did not work, the surgeon still would have the option of doing a conventional two-stage tendon repair. If successful, however, the patient would have been saved one hospitalization and an operating room procedure, as well as extra time lost from work.

PRESENTATIONS

Salisbury RE: "Laboratory Evaluation of Artificial Tendons and Homografts for use in Military Personnel with Severe Flexor Tendon Injury," presented at the Sixth International Biomaterial Symposium, Clemson University, Clemson, South Carolina, 22 April 1974.

PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0E 6963	74 07 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	9. SPECIFIC DATA CONTRACTOR ACCESS	10. LEVEL OF SUM
73 07 01	K. COMPLETION	U	U	NA	NL	XX YES ☐ NO	A. WORK UNIT

10. NO. CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A161102B71P	08	065
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Include with Security Classification Code): (U) Abnormalities of Liver Function Following Injury in Burned Military Personnel (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
73 05	74 06	DA	C. In-House

17. CONTRACT/GRANT: NOT APPLICABLE
a. DATE(S)/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	74	.7	22
FISCAL YEAR: CURRENT			

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21. GENERAL USE: FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Provide each with Security Classification Code): (U) Liver dysfunction; (U) Thermal injury; (U) Burned soldier; (U) Jaundice

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

23. (U) To define the incidence, nature, and clinical significance of liver dysfunction in the thermally injured soldier in the immediate postburn and convalescent period. The data will allow identification of hepatic abnormalities that may direct future more specific investigative efforts.

24. (U) One hundred consecutive adult burn patients will have liver function studies and Australia antigen determined upon admission to the ISR Unit. Patients with abnormal liver function studies on admission will have the studies repeated (with the exception of Australia antigen) twice weekly for two weeks and then weekly until studies normalize. Patients with normal initial studies will be surveyed every two weeks. Nonspecific abnormalities will be better defined with alkaline phosphatase isoenzymes, SGOT:SGPT ratios, and BSP retention. Menghini liver biopsy will be performed only when histologic diagnosis would directly alter therapy. The enzyme abnormalities will be correlated with clinical course, surgery, anesthesia, and medications, and studies will be drawn during periods of sepsis, hypoxia, hypotension, and surgery.

25. (U) 73 07 - 74 06 Seventy-seven soldiers with burns greater than 25% TBS were evaluated prospectively. Liver tissue from 34 autopsied patients demonstrated the injury. Clinical and laboratory evidence of hepatic dysfunction was present in 84% of patient soon after major thermal injury. Enzyme patterns consistent with acute hemolysis and hepatocellular necrosis characterized the early postburn period. A cholestatic pattern evolved later in 32 patients, reflecting hepatic regeneration, fat infiltration, or reaction to septicemia. Progression of the early hepatic injury was associated with postburn complications such as sepsis, hypoxia, hypotension, and hemorrhage. All but one jaundiced patient died, although liver failure never directly resulted in death.

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ABSTRACT

PROJECT NO. 3A161102B71P-08, BASIC RESEARCH IN SUPPORT OF MILITARY MEDICINE

REPORT TITLE: ABNORMALITIES OF LIVER FUNCTION FOLLOWING INJURY IN BURNED MILITARY PERSONNEL

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

Period covered in this report: 1 July 1973 - 30 June 1974

Investigators: Albert J. Czaja, MD, Major, MC
William R. Smith, Jr., Captain, MSC

Reports Control Symbol MEDDH-288(R1)

To determine the incidence, clinical characteristics, and natural history of acute liver damage following thermal injury, 81 consecutive, nonpediatric, burn patients were evaluated prospectively for clinical and laboratory evidence of hepatic injury. SMA-12, SGPT, and Australia antigen determinations were made within one week postburn. Enzyme studies were repeated twice weekly while abnormal; otherwise, serum enzymes were screened weekly. Alkaline phosphatase isoenzymes, clotting and hemolysis studies, liver scans, and biliary tract x-rays more specifically implicated hepatic disease. Liver tissue from 34 autopsied patients documented the injury.

Clinical and laboratory evidence of acute liver injury was present within the first week postburn in 47 patients (58%). Acute liver damage appeared to be related to the severity of the thermal injury. Patients with acute liver injury had a larger mean burn size (57.9% TBS vs. 30% TBS) and higher mortality (74.5% vs. 8.8%) than patients admitted with normal liver function. The magnitude of the initial enzyme derangements did not distinguish the survivors from among the patients with large burns. The appearance of jaundice, however, was associated with a poor prognosis; 17 of the 19 jaundiced patients (90%) died. Patients who developed jaundice late in their hospitalization suffered episodes of hypoxia or septicemia, and their liver function and postmortem histology suggested a "reactive hepatitis." Hepatic injury could be present in patients without clinical signs of shock. As patients improved clinically, their liver function also improved, although alkaline phosphatase levels tended to rise late and remain abnormal after the other enzymes had normalized. An increased risk for septic complications in the burn

patient with liver disease could not be determined in this study. Liver histology did not correlate with the degree of enzyme derangements manifested clinically.

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Thermal injury
Burned soldier
Liver dysfunction
Jaundice

ABNORMALITIES OF LIVER FUNCTION FOLLOWING INJURY IN BURNED MILITARY PERSONNEL

The incidence, clinical characteristics, and natural history of acute liver damage following thermal injury have not been fully described. Postmortem (Wilson WC, MacGregor AR, Stewart CP, Brit J Surg 25:826, 1938; Teplitz C, In The Treatment of Burns, edited by CP Artz and JA Moncrief, London, Saunders, p 70)[1,2] and laboratory studies (Gilmore JP, Fozzard HA, Amer J Physiol 198:491, 1960; Talaat SM, Beheri GE, Zaki MS, El-Bolkainy MN, Brit J Plast Surg 26:132-139, 1973)[3,4] have recognized congestion, fatty degeneration, and focal necrosis of the liver as early pathological consequences of a major cutaneous burn. Although prospective clinical studies have documented that impairment of liver function can occur immediately postburn (James GW, Purnell OJ, Evans EL, J Clin Invest 30:191, 1951; Wolff WA, Elkinton JR, Rhoads JE, Ann Surg 112:158, 1940),[5,6] these surveys have not characterized the nature of the clinical course of this dysfunction. In order to determine the incidence, clinical features, and natural history of acute hepatic disease developing after thermal injury, early and serial liver function studies were performed in 81 consecutive burn patients admitted to the US Army Institute of Surgical Research.

METHODS

All nonpediatric patients with burns sustained within one week of admission were evaluated prospectively for clinical and laboratory evidence of hepatic injury. Serum SGOT, SGPT, alkaline phosphatase, lactic dehydrogenase, total and conjugated bilirubin, and albumin:globulin ratios were determined by the multichannel auto-analyzer within the first week postburn. These studies were repeated twice weekly while abnormal; otherwise, serum enzymes were screened weekly. Australia antigen was assayed by the radioimmunological technique. Alkaline phosphatase isoenzymes (separated by cellulose acetate gel electrophoresis), clotting and hemolysis studies, liver scans and biliary tract x-rays were utilized whenever indicated to more specifically define the hepatic involvement.

Each patient was examined daily by the investigator. All medications, procedures, and changes in the patient's clinical status were recorded. Each attending physician managed his patient independently. Resuscitation fluids, systemic and topical antibiotics, vitamins, analgesics, and nutritional support varied with the clinical situation.

Because of the tissue nonspecificity of serum transaminase (SGOT and SGPT), lactic dehydrogenase and alkaline phosphatase, liver dysfunction was diagnosed clinically only in patients with hepatomegaly who had either elevations of both SGOT and SGPT greater than twice normal or abnormal elevations of all serum enzymes, including the liver isoenzyme of

alkaline phosphatase. Hemolysis was suspected by a transient unconjugated hyperbilirubinemia accompanied by an isolated elevation of lactic dehydrogenase. Liver tissue obtained at postmortem examination documented the hepatic disease.

RESULTS

During the eight month study period between May and December of 1973, 81 consecutive thermally injured patients were evaluated. The mean number of serum enzyme profiles obtained on each patient was 4.9 (397 determinations in the 81 patients). Sixty-three patients were male. Ages ranged from 14 to 86 years (mean age 33.9 years); burn sizes ranged from 4% to 96% of the total body surface (mean burn size 46.8% TBS). The mortality of this study population was 47% (38 of the 81 patients). Liver tissue was available from the 34 patients who were autopsied.

ACUTE LIVER DISEASE

Clinical and laboratory evidence of acute liver injury was present within the first week postburn in 47 patients (58%). Physical examination and biochemical studies suggested that liver damage could occur as early as 24 hours postburn. The mean age of patients in this group was 32.7 years (range 14 to 74 years), and the mean burn size was 57.9% of the total body surface (range 14 to 96% TBS). Mortality was 74.5% (35 of 47 patients).

Four patterns of liver function abnormalities were recognized:

1) Acute Hepatocellular Necrosis without Hyperbilirubinemia

Twenty-two patients (27.2%) with a mean burn size of 55% of their total body surface (TBS) had hepatomegaly and SGOT and SGPT elevations above twice normal. In one case, these enzymes were elevated over 40 times normal by the second day postburn. Lactic dehydrogenase levels ranged from normal to seven times above normal. Alkaline phosphatase was usually normal during the first week postburn; total serum bilirubin remained less than 1.5 mg%. Serum albumin ranged from 1.1 to 4.2 g% (mean 2.47 g%) within the first week postinjury. Five patients with a mean burn size of 71.3% TBS had serum albumin levels less than 2 g% within eight days after admission. Globulin levels ranged from 1.3 to 4.8 g% (mean 2.56 g%), and the albumin:globulin ratio was inverted in eight cases. Assay for Australia antigen was negative in all patients.

Fourteen patients (64% of this group) died. Ten patients had clinical signs of shock (systolic blood pressure less than 90 mmHg with oliguria) on admission, and six of these also required early mechanical

ventilation to maintain adequate oxygenation. All ten patients died within the first week postburn. Four patients survived into the second and third week postinjury. Three of these developed intractable shock as a result of a myocardial infarction or pseudomonas septicemia; the other succumbed to a necrotizing pneumonia.

Clinical signs of shock were never apparent in the eight surviving patients, although the initial derangement in their liver function was comparable to that of the nonsurvivors. Liver function improved in these patients coincident with their successful convalescence. Abnormal elevations of serum transaminases usually resolved during the second and third week of hospitalization. In each patient, serum alkaline phosphatase increased up to twice normal levels as the other enzyme elevations resolved.

The degree of the initial abnormalities in liver function did not correlate with ultimate survival. Prognosis appeared to be related to burn size. Survivors had a mean burn size of 39.8% TBS compared to a mean burn size of 63.6% TBS in nonsurvivors.

II) Acute Hepatocellular Necrosis with Early Hyperbilirubinemia

Six patients (7.4%) with a mean burn size of 58.4% TBS were clinically and biochemically similar to Group I patients except that their total serum bilirubin levels were above 1.5 mg% within the first week postburn. None of these patients became jaundiced. Total serum bilirubin levels ranged from 1.5 to 2.3 mg% (mean 1.81 mg%) and was elevated as early as the second day postburn. In three of these patients, unconjugated bilirubin was the predominant component of this elevation, and all patients had received blood transfusions. In four patients, the bilirubin level returned to normal within five days. Abnormal elevations of lactic dehydrogenase did not distinguish this group of patients from Group I.

Only one patient demonstrated clinical signs of shock on admission, and three required early mechanical ventilation. All patients survived into the second week postburn, although four patients did subsequently die of septic complications. In the two surviving patients, the alkaline phosphatase level became abnormally elevated during the second week postinjury and was the last enzyme abnormality to resolve.

III) Acute Hepatocellular Necrosis with Early Jaundice

Eight patients (9.9%) with a mean burn size of 67.4% TBS were jaundiced within the first week postburn. Total serum bilirubin levels during this early period ranged from 2.1 to 9.7 mg% (mean 4.65 mg%),

increasing to as high as 21 mg% by the second week postburn. The magnitude of the initial derangement of serum transaminases and alkaline phosphatase did not differentiate this group of patients from the other groups. Lactic dehydrogenase levels were all abnormally elevated within three days postburn, with five patients having LDH levels at least twice normal. This enzyme elevation was associated with an unconjugated hyperbilirubinemia in four of the patients. Bilirubin levels could be as high as 9.7 mg% as early as the third day postburn, and tended to increase with the duration of survival and the frequency of blood transfusions.

Seven patients died. Shock was present on admission in only three of these patients, and two succumbed within three days after admission. Five patients survived into the second week postburn, only to die of respiratory failure or septicemia. A conjugated hyperbilirubinemia emerged in all patients and was accompanied by a rising alkaline phosphatase. Although bilirubin levels did diminish in five patients prior to death, enzyme levels remained unimproved. Transfusion requirements had diminished in each of these patients.

IV) Acute Hepatocellular Necrosis with Late Onset of Jaundice

Eleven patients (13.6%) with a mean burn size of 56.6% TBS developed a conjugated hyperbilirubinemia in the second week postburn. Bilirubin levels ranged from 2.6 to 28.5 mg% (mean 8.1 mg%) during this period. Nine patients had clinical and laboratory evidence of acute hepatic dysfunction within the first week postburn. In the other two patients, initially normal liver function deteriorated following episodes of congestive heart failure and cardiogenic shock. All patients in this group became jaundiced as their clinical course was complicated by pneumonia, septicemia, respiratory insufficiency, or cardiovascular instability. A cholestatic picture emerged in these patients and was characterized by a conjugated hyperbilirubinemia and elevation of alkaline phosphatase (up to four times normal) out of proportion to changes in the other enzyme levels.

Ten of the 11 patients died from complications developing late in their postburn course. Death attributable to fulminant hepatic failure did not occur. Extrahepatic biliary disease was not encountered.

NORMAL LIVER FUNCTION

Thirty four patients (42%) were considered to have normal liver function. A mean age in this group of 32.9 years was comparable to the mean age of patients with acute liver dysfunction. The mean burn size in this group, however, was only 30% TBS, and mortality was only 8.8% (three of 34 patients). Serum transaminase values were increased

(less than twice normal) in ten of these patients, but hepatomegaly was not present. Isolated, abnormal alkaline phosphatase elevations developed in ten other patients within the first week postburn, but could be explained by bone fractures or orthopedic procedures (amputations, joint pinning, skeletal traction). Six patients developed transient, nonspecific enzyme changes associated with drug reactions, thrombophlebitis, or multiple administrations of anesthesia; four other patients had acute hemolytic episodes immediately postburn. One patient admitted with normal liver function developed an Australia antigen positive hepatitis on the 146th day of hospitalization.

LIVER HISTOLOGY

Liver tissue was available from the 34 patients who came to autopsy. In each case, the liver was enlarged at postmortem examination. Histological abnormalities, however, were usually nonspecific and did not correlate with the degree of enzyme derangement manifested clinically. Vacuolization of centrilobular hepatocytes, sinusoidal congestion, and mild inflammatory infiltration of the portal tracts were commonly present. Fatty changes were mild and centrilobular. All of these changes could be present as early as 48 hours postburn. Infiltration of fat was rarely a predominant histological feature even in patients dying after a prolonged hospital course.

Canalicular cholestasis was present in the liver of jaundiced patients. Acute and chronic inflammation of the portal tracts, inflammatory foci within the hepatic parenchyma and Kupffer cell hyperplasia distinguished the jaundiced patients who were septic. Only one of the patients dying in shock had severe centrilobular necrosis.

DISCUSSION

Clinical and laboratory evidence of hepatic damage was present soon after major thermal injury in 58% of patients evaluated prospectively. Enzyme patterns consistent with acute hemolysis and hepatocellular injury characterized the early postburn period of these patients. Acute liver damage appeared to be related to the severity of thermal injury. Patients manifesting acute liver injury had a larger mean burn size (57.9% TBS vs. 30% TBS) and higher mortality (74.5% vs. 8.8%) than patients admitted with normal liver function.

The magnitude of the initial enzyme derangement did not distinguish the survivors from among the patients with large burns. The appearance of jaundice, however, was associated with a poor prognosis. Seventeen of the 19 patients with jaundice (90%) died. Patients who became

jaundiced within the first week postburn had a larger mean burn size than other patients as well as clinical evidence of hemolysis. Patients who developed jaundice late in their hospitalization were septic or hypoxic. Their liver dysfunction probably reflected the hepatic response to these clinical complications. A conjugated hyperbilirubinemia associated with a predominant alkaline phosphatase elevation suggested a "reactive hepatitis" in these patients (Vermillion SE, Gregg JA, Baggenstoss AH, Bartholomew LG, Arch Int Med 124:611, 1969; Neale G, Caughey DE, Mollin DL, Booth CC, Brit Med J 1:382, 1966).[7,8] Postmortem liver histology supported this diagnosis. The elevated bilirubin level probably reflected the delivery of an increased pigment load (resulting from shortened red blood cell survival and multiple blood transfusions) to a compromised liver. Serum bilirubin levels could vary while enzyme levels remained unchanged.

The etiology of acute hepatic disease following thermal injury is unknown. Animal studies suggest that hepatic blood flow can be significantly diminished immediately after burning (Dobson EL, Warner GF, Circ Res 5:69, 1957).[9] The early appearance of liver injury in patients with large burns suggests the possibility of acute hepatic ischemia occurring at the time of thermal injury. Histological abnormalities of the liver were predominantly centrilobular, although frank centrilobular necrosis indicative of severe hepatic ischemia was rare. Liver disease could be present without clinical signs of shock.

Although the liver is vitally important for the clearance and detoxification of bacteria and endotoxin (Farrar WE Jr, Corwin LM, Ann NY Acad Sci 133:668, 1966),[10] an increased risk for septic complications in the burned patient with liver disease could not be determined in this study. Patients who deteriorated clinically with the development of septicemia, respiratory insufficiency, or cardiovascular instability had a simultaneous decline in liver function. Patients with acute hepatocellular injury who improved clinically resolved their enzyme abnormalities. In these patients, the serum alkaline phosphatase levels tended to rise up to twice normal levels and persist after the other enzymes had normalized. This could be a reflection of hepatic regeneration, as has been described in patients recovering from acute viral or alcoholic hepatitis (Ross RS, Iber FL, Harvey AM, Amer J Med 39:850, 1956).[11]

Although florid hepatocellular necrosis was not usually encountered, the metabolic functions of the liver could have been severely impaired in all of these patients. Experimental studies substantiate this possibility (Cajone F, Ragnotti G, Bernelli-Zazzera A, Bassi M, Exper Molecular Path 14:392, 1971).[12] A better understanding of the etiology of acute

liver dysfunction in the burned patient may lead to better methods of maintaining and improving hepatic function. All of the ill effects of impaired liver function upon the convalescent course of the thermally injured patient may not be appreciated until these methods are developed.

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PUBLICATIONS AND/OR PRESENTATIONS:

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0E 6962	74 07 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
73 07 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	61102A	3A161102B71P	01	022
b. CONTRIBUTING	61102A	3A161102B71P	08	
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) Use of the Hypoxia - Induced Polycythemic Mouse in the Assay of Erythropoietin 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
73 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 74	.8	22
FISCAL YEAR CURRENT 75	.6	15

19. RESPONSIBLE DOD ORGANIZATION
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NAME: Willard A Andes, MAJ, MC
NAME: John W Beason, SP4 DA

21. GENERAL USE: FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code): (U) Erythropoietin; (U) Anemia; (U) Burned Soldiers (U) Renin-angiotensin

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

23. (U) To set up an assay to measure erythropoietin excretion in patients with thermal injury, trauma or renal failure. This assay procedure will be used in the evaluation of the anemia of the thermally injured patient and the relationship of erythropoietin to renin in the traumatized soldier.

24. (U) Polycythemia is induced by placing Swiss-Webster mice in a 0.4 atmosphere chamber for approximately 100 hours. Concentrated urine is injected intraperitoneally; then radioiron given. The percent iron utilization by red cells is calculated and compared to Erythropoietin B standard obtained from the World Health Organization.

25. (U) 73 07 - 74 06 The use of the hypoxia induced polycythemic mouse in the bioassay of erythropoietin has been very successful. A dose response curve comparable to that of other investigators has been obtained and data collected from normal personnel has been within the normal range, consistent and reproducible. Five patients with thermal injury ranging from 15-65% total body surface burns have been studied thus far with concurrent evaluation of the renin angiotensin system, glomeruar filtration rate, sodium excretion, bone marrow morphology, red cell mass, plasma volume, and total blood volume as determined by the chromium$_{51}$ red blood cell technique. Careful documentation of blood loss as well as transfusions have been obtained in the patient studies. Sufficient data has not been accumulated, however, to make definite conclusions at the time of this report.

*Available to contractors upon originator's approval.

485<

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.

ABSTRACT

PROJECT NO. 3A161102B71P-01, BASIC RESEARCH IN SUPPORT OF MILITARY MEDICINE

REPORT TITLE: USE OF THE HYPOXIA - INDUCED POLYCYTHEMIC MOUSE IN THE ASSAY OF ERYTHROPOIETIN IN BURNED SOLDIERS

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

Period covered in this report: 1 July 1973 - 30 June 1974

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Reports Control Symbol MEDDH-288(R1)

The measurement of human urinary erythropoietin (erythron stimulating factor - ESF) as the stimulus for erythrocyte production has only recently been attempted in the burned patient. Bleeding, hemolysis, a hypercatabolic state, infection, surgery and a variety of complications related to the burn have made the evaluation of anemia in burn patients difficult in most instances. Measurement of human ESF was performed with an in-vivo bioassay utilizing hypoxia-induced polycythemic mice. A dose-response curve was established which paralleled that previously reported for this bioassay. Urinary ESF excretion from normal volunteers was assayed. In addition, urinary ESF was assayed in five thermally injured patients with a spectrum of burn sizes. Our studies reveal that, in contrast to reports by other investigators, urinary ESF excretion is frequently elevated in the burned patient. Patients with iron deficiency, polycystic renal disease, and erythroid aplasia have also been studied. It would appear that a reproducible and clinically useful assay for the measurement of ESF has been established and is now available for use in the clinical investigation of anemia of thermally injured patients.

Erythropoietin
Anemia
Burned soldiers
Renin-angiotensin

USE OF THE HYPOXIA INDUCED POLYCYTHEMIC MOUSE IN THE ASSAY OF ERYTHROPOIETIN IN BURNED SOLDIERS

The relationship of erythropoietin (erythron stimulating factor -ESF) to the anemia and postburn course of the thermally injured patient has not been studied in detail. Although one investigator noted what was felt to be a deficiency of ESF in the burned patient, those studies were unaccompanied by certain pertinent facts necessary to completely evaluate the data (Robinson H, Monafo WW, Saver SM, Gallagher NI: Ann Surg 178:565-572, 1973)[1]. We have established a bioassay for human ESF utilizing the hypoxia-induced polycythemic mouse (Adamson JW, Alexanian R, Martinez C, Finch CA:Blood 28: 254-264, 1966;[2] Adamson JW:Blood 32: 597-609, 1968).[3] This in-vivo assay estimates functional ESF by measuring the incorporation of radioactive ^{59}Fe into the erythrocytes of the polycythemic mouse. The utilization of this bioassay and other diagnostic aids should allow us to define some of the causes for anemia in the thermally injured patient.

MATERIALS AND METHODS

Swiss Webster mice 11-17 weeks old at the time of assay were obtained from the Carworth Corporation, Division of Becton-Dickinson. All mice received 4 mg iron dextran* by intramuscular injection just prior to the institution of hypoxia. The animals were then acclimitized to a 0.4 atmosphere hypobaric chamber over a period of a few days and then subjected to approximately 200 hours at 0.4 atmospheres intermittently for twelve hours a day for 2-3 weeks. Hematocrits at the time of removal from the chamber were generally greater than 50 (mean 62) and any animal with an hematocrit less than 51 was not used in the assay. A regular laboratory diet was maintained in the chamber and for 48 hours following cessation of hypoxia. The animals were then divided into groups of 4 or 5 and given a protein depletion diet**.

All human urines were concentrated against dry carbowax.± The concentrated urine was assayed for potassium. Those urines with a markedly elevated final potassium were diluted appropriately to insure survival of the mice. Urines were concentrated to a final volume of 50 to 150 ml. One ml injections of concentrated urine were given intraperitoneally to each mouse every 12 hours for 4 days.

Twelve hours after the last urine concentrate injection, 0.8 -2 μc of ^{59}Fe as ferric citrate in normal saline was injected intraperitoneally. Forty-eight hours after the injection of radioiron,

*Imferon,(R) Lakeside Laboratory, Wisconsin.
** Nutritional Biochemical Inc, Ohio
± Union Carbide Corp, Seattle, Washington

each animal was weighed and sacrificed under ether anesthesia. Blood (0.5 ml) was obtained from the axillary artery for counting and duplicate microhematocrit determinations. The erythrocytes were washed three times with heparinized saline and pipetted into a counting tube for counting on a Packard Model 3002 scintillation spectrometer. A standard was prepared from the ferric citrate solution, and counted in triplicate. Percent utilization of iron for hemoglobin synthesis in each animal was calculated by the following formula:

$$\% \text{ Utilization} = \frac{(\text{RBC counts})(\text{Animal weight in grams})\ (15)}{\text{Injected Activity}}$$

The factor 15 was used to relate radioactivity present in the sample of red cells to the total blood volume by multiplying by 2.0 (conversion of 0.5 ml blood to 1 ml) and 7.5 (the blood volume of the polycythemic mouse expressed as percent of body weight).

In calculating urinary ESF excretion the mean percent of radioiron utilization in each group of mice was used. This was converted to units of ESF injected by reading the total number of units injected per mouse from a standard dose response curve established in our laboratory (Adamson JW, Alexanian R, Martinez C, Finch CA: Blood 28: 254-264, 1966)[2]. In establishing this curve, known doses of erythropoietin standard B** in saline were injected. (Cotes PM, Bangham DR: Bull. Wld Hlth Org 35:751-760, 1966)[4]. This dose response curve, confidence limits, and coefficient of information (r^2) are given in Figure 1. The 12 hour erythropoietin excretion was calculated by first dividing the estimate of total units injected per mouse by 8. This figure (units ESF/ml) was then multiplied by the total volume of the concentrated urine specimen to give the number of units excreted in the time period being assayed. A sample calculation and a summary of such calculations is shown below.

CALCULATION OF URINARY ESF

1) Mean ^{59}Fe incorporation - saline control = (A).

2) For (A), read dose administered from Dose-Response curve = (B)

3) Divide (B) by 8 = Injected units/ml = (C).

**Kindly provided by the National Institute for Medical Research, London, England

≠ See Table II

4) Twelve hour urinary ESF = (C) X (final concentrated urine volume, ml)

D.J., 10/02/73 (Study IX)

% Incorporation = 10.96

B = 1.00 Unit

C = 1.0/8 - 0.125

Final urine concentrate volume = 92

12 hour ESF excretion = 0.125 X 92 = 11.5 units

RESULTS

The dose response curve shown in Figure 1 was used to calculate all subsequent erythropoietin excretion rates. The erythropoietin excretion for a twelve hour period (1800-0800) by 6 healthy laboratory volunteers is shown in Table I.

The values calculated in five burn patients at various days post-burn as well as the values in a few other patients with medical illnesses are given in Table II. Table III displays duplicate determinations on the same sample assayed at different times.

DISCUSSION

From these results it appears that a satisfactory bioassay for the measurement of human erythropoietin as described by Adamson has been established. Our dose response curve and normal controls are comparable to his measurements. The reproducibility of our assay was evaluated during different assay periods and is shown in Table III.

The erythropoietin excretion values in the burn patient were markedly elevated in some cases. The significance of such elevations remains to be determined. Certainly, these patients seem to have responded to a stimulus for accelerated erythrocyte production by increasing the amount of measurable erythropoietin excreted daily. Fairly wide variations (Table II) in ESF excretion in 12 hour urine collections are well known (Adamson JW, Alexanian R, Martinez C, Finch CA:Blood 28: 254-264, 1966)[2]. Values given in Table II for patients with other illnesses are comparable to those measured in similar situations by other investigators (Alexanian R:J Lab Clin Med 82: 438-445, 1973)[5].

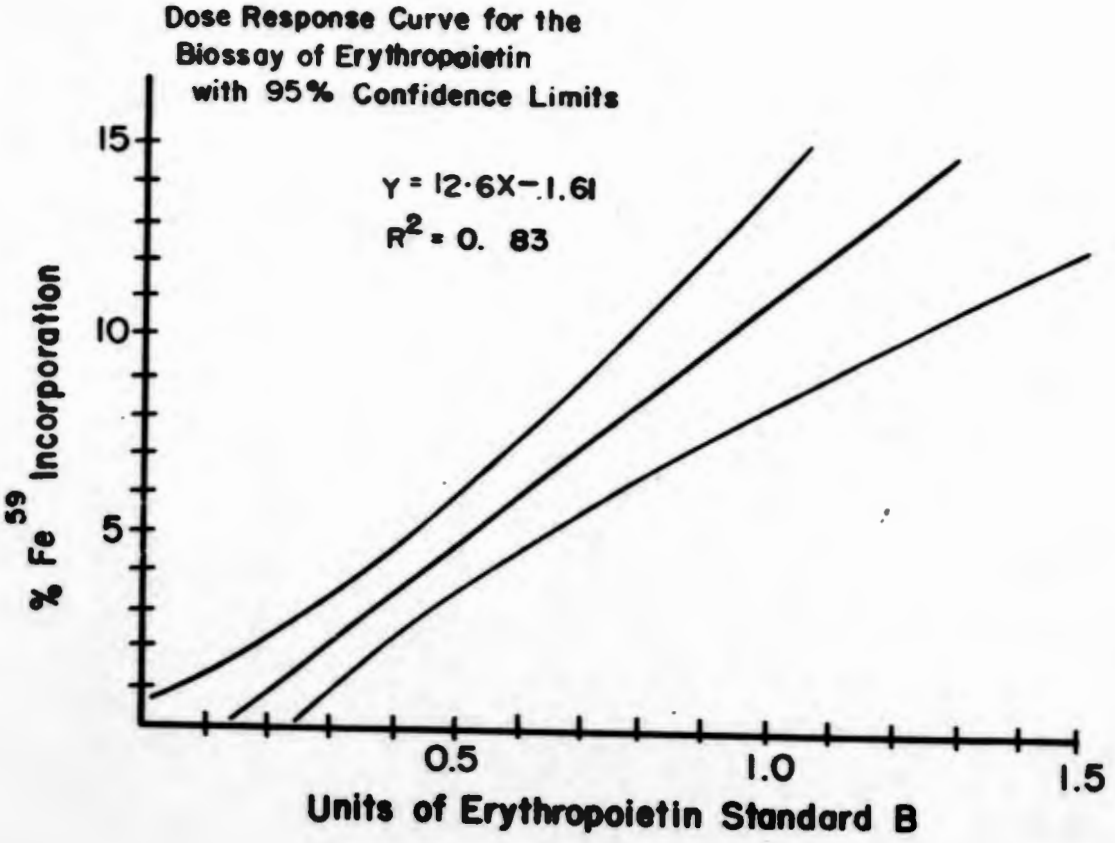
Dose Response Curve for the
Biossay of Erythropoietin
with 95% Confidence Limits
Y = 12·6X−1.61
R^2 = 0. 83
15
10
5
% Fe^{59} Incorporation
0.5
1.0
1.5
Units of Erythropoietin Standard B

Figure 1

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TABLE I

THE ERYTHROPOIETIN EXCRETION FOR A TWELVE HOUR PERIOD USING SIX HEALTHY LABORATORY VOLUNTEERS

NAME	ERYTHROPOIETIN UNITS/12 HOURS
B.E.	1.16
B.E.	1.32
S.K.	1.65
M.C.	1.01
B.A.	2.28
B.A.	2.06
H.E.R.	1.70
E.L.R.	1.31

MEAN = 1.56

S.D. = 0.44

TABLE II

RESULTS OF URINARY ESF EXCRETION IN PATIENTS WITH A VARIETY OF ILLNESSES

PATIENT	AGE	DIAGNOSIS	DAY POST BURN	HCT	UNITS STANDARD B ESF EXCRETION/12 HOURS
D.J.	17	57% TBS Burn	2	47	7.6
			3	37	11.5
			4	37	12.3
			5 AM	34	8.2
			PM		28.0
			6	32	9.2
			7	36	17.9
			8	34	6.6
			9	34	17.2
			19 AM	36	7.2
			PM	36	15.0
			20	36	
			29 AM	36	4.5
			PM		9.8
			45	37	1.2
W.T.	27	65% TBS Burn	4	36	17.6
			5	32	0.08 u/ml
			7	32	12.7
E.A.	29	47% TBS Burns	2 AM	39	2.9
			PM		5.4
			11 AM	34	4.4
			PM		24.8
			29	35	2.3
			30	32	2.7
			50	32	6.3
			56	39	5.1
			57	35	3.2
R.M.	16	14.5% TBS Burns	2	46	4.9
			3	45	7.3
			7 AM	38	5.6
			PM		6.0
			8 AM	37	4.7
			PM		3.3
			23 AM	35	3.7
			PM		1.6
			52		2.0

TABLE II
(Continued)

PATIENT	DIAGNOSIS	DAY POSTBURN	HCT	UNITS STANDARD 8 ESF EXCRETION/24 HOURS
R.N.	30% TBS Burns	3	48	4.1
		10	30	62.6
		23	32	20.8
		56	37	4.6
R.W.	Erythroid Aplasia		22	11.2
E.H.	Iron Deficiency Anemia		24	99.0
A.B.	Iron Deficiency Anemia		28	33.0

TABLE III

RESULTS OF DUPLICATE SAMPLE DETERMINATION OF URINARY ESF

PATIENT	ASSAY GROUP NUMBER	STANDARD B UNITS ESF/12 HOURS
1	XII	11.2
	XIII	10.4
2	XII	3.0
	XII	3.0
3	XII	13.2
	XIII	15.3
4	XII	4.3
	XV	3.8

45-8

Thus, the anemia of chronic renal failure is often associated with low or absent erythropoietin excretion while patients with aplastic anemia or iron deficiency may elaborate large quantities of biologically active protein. Future studies will involve the use of this assay in determining the erythropoietin excretion in specific clinical situations in the burn patient. Definitive conclusions await the completion of assays and related studies now underway in this laboratory.

REFERENCES:

1. Robinson H, Monafo WW, Saver SM, Gallagher NI: The role of erythropoietin in the anemia of thermal injury. Ann Surg 178: 565-572, 1973.

2. Adamson JW, Alexanian R, Martinez C, Finch CA: Erythropoietin excretion in normal man. Blood 28: 254-264, 1966.

3. Adamson JW: The erythropoietin/Hematocrit relationship in normal and polycythemic man: Implication of marrow regulation. Blood 32: 597-609, 1968.

4. Cotes PM, Bangham DR: The international reference preparation of erythropoietin. Bull Wld Hlth Org 35: 751-760, 1966.

5. Alexanian R: Erythropoietin excretion in bone marrow failure and hemolytic anemia. J Lab Clin Med 82: 438-445, 1973.

PUBLICATIONS AND/OR PRESENTATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0E 6970	74 07 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
73 07 01	K. COMPLETION	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62110A	3A162110A821	00	107
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Provide with Security Classification Code): (U) The Effect of Ketamine on Stress-Induced Ulcerations in the Rat - A Model of Thermal Injury in Troops (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
72 12	73 12	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	PRECEDING/CURRENT	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	74	.4	14

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ASSOCIATE INVESTIGATORS
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NAME: DA

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Provide EACH with Security Classification Code)
(U) Ketamine; (U) Stress ulcer; (U) Rats

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

23. (U) To evaluate the effect of ketamine on the incidence of stress ulcers in the rat as a model of thermally injured troops.

24. (U) Using a standard technique of rat restraint for producing gastric stress ulcers, ketamine will be given to evaluate its effect on this incidence relative to the work of others and our own controls.

25. (U) 73 07 - 73 12 At the completion of this study, it was determined that ketamine significantly increases the incidence and severity of ulcers utilizing the restraint technique. In addition, manipulations with pharmacologic agents known to influence the incidence and severity of ulcers in this model have demonstrated that this potentiation is related to the effect of ketamine on vascular tone and integrity.

496<

*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

ABSTRACT

PROJECT NO. 3A162110A821-00, COMBAT SURGERY

REPORT TITLE: THE EFFECT OF KETAMINE ON STRESS-INDUCED ULCERATIONS IN THE RAT - A MODEL OF THERMAL INJURY IN TROOPS

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

Period covered in this report: 1 July 1973 - 30 June 1974

Investigators: David H. Cheney, MD, Major, MC*
Stephen Slogoff, MD, Major, MC
Gary W. Allen, MD, Major, MC

Restraint of rats in wire mesh for four hours produces ulcers histologically similar to human stress ulcers. Ketamine, alone, was incapable of producing ulcers. However, when given to rats prior to restraint, ketamine increased the incidence of ulcers from 35 per cent (restraint alone) to 98 per cent, and the lesions produced were more severe. Phenoxybenzamine and halothane blocked the potentiation of restraint-induced ulcers by ketamine, suggesting that the action of ketamine is mediated by vasoconstriction. Antihistamine pre-treatment with promethazine was without effect. Since analysis of the authors' large experience in burned patients receiving ketamine and review of the literature revealed no data suggesting human counterpart for this phenomenon, no absolute guidelines for alteration of clinical usage should be invoked without further investigation.

*From the Department of Anesthesiology, Brooke Army Medical Center, Fort Sam Houston, Texas 78234

Ketamine
Stress ulcer
Rats

THE EFFECT OF KETAMINE ON STRESS-INDUCED ULCERATIONS IN THE RAT - A MODEL OF THERMAL INJURY IN TROOPS

Acute ulcerations of the stomach or duodenum associated with thermal injury were first described by Swan in 1823 (Swan J, Edinburgh Med J 19:344, 1823).[1] Stress ulcers were recognized by Curling in ten burned patients in 1842 (Curling TB, Medico-Chir Trans (London) 25:260, 1842),[2] and are now recognized as the major gastrointestinal cause of morbidity and mortality in patients with burns. Pruitt, Foley, and Moncrief (Pruitt BA Jr, Foley FD, Moncrief JA, Ann Surg 172:523, 1970)[3] reported the development of such ulcers in 11.7 per cent of 2,772 thermally injured patients. In a smaller series of 98 patients with ulcers, they observed 70 per cent mortality from this cause (Pruitt BA Jr, Foley FD, Moncrief JA, Ann Surg 172:523, 1970).[3]

Historically, at the United States Army Institute of Surgical Research, Curling's ulcers have appeared in clusters, separated by relatively long quiescent intervals only to recur again. In 1972, such an outbreak developed coincident with a marked increase in the usage of the dissociative anesthetic, Ketamine. Although ketamine has significant autonomic properties, a review of the literature revealed no substantive data concerning its effects on gastrointestinal physiology.

The rat restraint model first described by Rossi (Rossi G, Bonfils S, Lieffoogh F, et al, CR Soc Biol (Paris) 150:2124, 1956)[4] allows study of a reproducible lesion in the glandular portion of the murine stomach histologically similar to the human stress ulcer. The incidence of ulcer formation produced by this model may be selected by varying age, weight, and duration of restraint, and by drugs or surgical manipulations known to influence human ulcer formation. Several reported studies have shown that this technique yields reasonable reproducible results (Hanson HM, Brodie DA, J Appl Physiol 15:291, 1960; Brodie DA, Hanson HM, Gastroenterology 38:353, 1960; Menguy R, Am J Digest Dis 5:911, 1960; Brodie DA, Marshall RW, Moreno OM, Am J Physiol 202:812, 1962).[5-8]

A study was initiated using this model to investigate the influence of ketamine on the formation of stress ulcers, and, if such an effect existed, to delineate the mechanism of action.

METHODS

Sprague-Dawley rats, weighing 150 to 175 g, were restrained with wire screen mesh for four hours to produce stress ulcers. This was preceded by 48 hours of food deprivation, with water available ad lib, a maneuver which has shown to decrease the duration of restraint required to produce lesions. All unrestrained controls underwent 48 hours of food deprivation and were totally starved during the four-hour period during which they would have been restrained.

Ten different experiments were performed. These can be separated into three series for ease of understanding (Table I). Series I included three groups: A, untreated and unrestrained; B, ketamine and unrestrained; C, untreated and restrained. Series II included only rats treated with ketamine prior to restraint (Group D) to evaluate the influence of the drug on ulcerogenesis. Series III was performed to evaluate the effect of various pharmacologic agents on ulcer formation both in restrained animals without ketamine and in animals treated with ketamine prior to restraint. This series included Groups E, halothane and restraint; F, halothane, ketamine, and restraint; G, phenoxybenzamine and restraint; H, phenoxybenzamine, ketamine, and restraint; I, promethazine and restraint; J, promethazine, ketamine, and restraint. Each matched pair in Series III was studied in conjunction with rats from Groups C and D to provide controls for statistical analysis. In addition, each experiment was performed at least twice in different populations of rats to confirm the reliability and reproducibility of the technique.

Ketamine (75 mg/kg) was administered intraperitoneally, followed by restraint after the onset of unconsciousness (3-5 minutes). Halothane (1 per cent in oxygen) was given continuously via a rat anesthesia box, starting immediately after restraint and continuing to the conclusion of the four-hour study period. Phenoxybenzamine (1 mg/kg intravenously) and promethazine (20 mg/kg intraperitoneally) were injected one hour before initiation of restraint.

After the four-hour control or restraint period, the animals were sacrificed with an intrathoracic overdose of pentobarbital sodium. The stomachs were removed and labelled with suture material to maintain a coding system. The organs were inflated with 0.9 per cent sodium chloride solution and dipped in 0.4 per cent formaldehyde solution for 30 seconds before they were opened. Two investigators, working separately and blinded to the manipulations performed, examined all coded stomachs at the conclusion of each experimental period. Each stomach was evaluated for the presence or absence of ulcers. A subjective estimate of severity of the lesions was made in each group.

The statistical significance of differences between the incidences of ulcers in control and treatment groups was determined for each separate experiment using the four fold table test (Diems K, Lentner C, Scientific Tables, Ciba-Geigy Limited, Basle, Switzerland, 1970 p 123).[9] The same groups from different experiments were then combined for clarity in presentation of the results. Per cent incidence and 95 per cent confidence limits (Diems K, Lentner C, Scientific Tables, Ciba-Geigy Limited, Basle, Switzerland, 1970, pp 85-97)[10] were used to reveal the substantial differences which existed among the various groups.

Table 1.

EXPERIMENTAL DESIGN AND RESULTS

	Restraint	Ketamine	Halothane	Phenoxy-benzamine	Prometh-azine	Number	Per Cent Incidence
Series I							
Group A						46	2.1 (0.06-11.53)*
Group B		X				20	0 (0.00-16.84)
Group C	X					97	35 (25.64-45.41)
Series II							
Group D	X	X				66	98.4 (91.84-99.96)
Series III							
Group E	X		X			20	0 (0.00-16.84)
Group F	X	X	X			20	0 (0.00-16.84)
Group G	X			X		22	36.4 (17.20-59.34)
Group H	X	X		X		21	33.3 (14.59-56.97)
Group I	X				X	33	36.4 (20.40-54.88)
Group J	X	X			X	21	85.7 (63.66-96.95)

*95 per cent confidence limits.

RESULTS

The data reported represent the 366 of 380 rats which survived the four-hour restraint or control periods. Mortality in the 14 nonsurvivors was attributed to either drug overdose or respiratory embarassment from the restraint device.

Series I

Group A (those rats receiving no drugs or restraint) yielded one animal with ulcers in 46 trials (2.1 per cent). Group B, ketamine without restraint, developed no ulcers (n=20). In Group C, restraint only, ulcers developed in 34 of 97 rats (35 per cent) (Table 1).

Series II

Rats undergoing restraint after ketamine pretreatment (Group D) had an incidence of ulcers of 98.4 per cent in 66 rats (Table 1). Ketamine significantly increased the incidence of ulcers in restrained rats ($P < .002$). Animals receiving ketamine were unconscious for approximately an hour of the restraint period and appeared more agitated than the restraint-only animals for about 30 minutes after emergence.

Series III

In order to elucidate the mechanism of ketamine potentiation of stress ulcers, halothane was administered to restrained animals (Group E) and to rats receiving ketamine prior to restraint (Group F). No death occurred in animals receiving both halothane and ketamine. No ulceration was found in either of these two groups, and the differences between these and the control groups, C and D, were significant ($P < .05$). Because halothane has known vasodilating properties, it was decided to evaluate the effect of phenoxybenzamine, an alpha-adrenergic blocking agent, on the formation of these lesions. After phenoxybenzamine pretreatment, ulcers occurred in 36.4 per cent of restrained animals, an incidence not significantly different from that found after restraint alone. Following phenoxybenzamine pretreatment, however, restraint and ketamine produced an incidence of ulcers (33.6 per cent) significantly lower than that found with restraint and ketamine alone ($P < .01$). Promethazine was given to two other groups of rats to determine whether the effect of phenoxybenzamine might be related to its antihistaminic properties. Promethazine, however, produced no significant difference in ulcer formation in either the restraint-only or the ketamine-restraint group (Table 1).

According to severity or intensity of ulceration, the rats fell into two distinct populations. Ulcers found with ketamine and restraint (Group D) and with promethazine, ketamine, and restraint (Group J) were multiple, large, and associated with large amounts of blood in the

stomach and with massive small intestinal ileus. Ulcers in the other groups were small, with minimal blood in the stomach and no ileus.

DISCUSSION

Brodie and Hanson have demonstrated that ulcer development in the model used in the present study depends on age, weight, duration of restraint, and period of food deprivation prior to restraint (Brodie DA, Hanson HM, Gastroenterology 38:353, 1960).[6] In rats with a mean weight of 185 g, they found a 69-75 per cent incidence of ulcers after 48 hours of food deprivation and six to seven hours of restraint (Brodie DA, Hanson HM, Gastroenterology 38:353, 1960).[6] Using smaller rats (50-125 g), a similar period of food deprivation and four hours of restraint resulted in a 69 per cent incidence of lesions (Hanson HM, Brodie DA, J Appl Physiol 15:291, 1960).[5] In the present study, rats weighing 150-175 g were restrained for four hours to provide an ulcer incidence of 35 per cent. This model could therefore be used to show significant effects of the pharmacologic intervention upon ulcer formation.

Although ketamine alone does not produce ulcers in the unrestrained rat, significant potentiation by ketamine of the effect of restraint is evident.

Several conclusions can be drawn from these findings regarding the mechanism of action of restraint and ketamine in producing ulcers. Halothane blocks the formation of ulcers induced by restraint. To determine whether this effect was related to anesthetic relief of stress or to vasodilation produced by halothane, a series of rats received the alpha-adrenergic blocking agent, phenoxybenzamine, prior to restraint. Phenoxybenzamine did not inhibit ulcer formation in the awake, restrained rat. Therefore, vasoconstriction does not appear to participate in formation of restraint-induced ulcers. Moreover, these findings suggest that halothane may prevent ulcer formation in restrained rats by blockade of conscious response to stress.

The potentiating effect of ketamine in the restrained rat was blocked by phenoxybenzamine, suggesting that this action of ketamine is mediated by splanchnic vasoconstriction. Although phenoxybenzamine has sedative properties, no distortion of behavioral response to restraint or to ketamine combined with restraint was apparent when this drug was used. Sethbhakdi, et al (Sethbhakdi S, Roth JLA, Pfeiffer CJ, Am J Digest Dis 15:1055, 1970),[11] have shown that intraperitoneal injection of epinephrine in the unrestrained rat can produce ulcers and that this effect can be blocked by phenoxybenzamine. Goodman (Goodman AA, Osborne MP, Surg Gynec Obstet 134:563, 1972[12])has also produced stress ulcers in piglets by inducing hemorrhagic shock.

Franco-Browder (Franco-Browder S, Masson GMC, Corcoran AC, J Allergy 30:1, 1959)[13] has shown that phenoxybenzamine can block the effects of the potent histamine liberator, polymyxin B, in producing ulcers. Graham (Graham JOP, Lewis GP, Br J Pharmacol 8:54, 1953)[14] has also demonstrated antihistaminic properties of phenoxybenzamine. Therefore, the antihistamine, promethazine, which is as effective as phenoxybenzamine in inhibiting histamine-induced ulcers (Franco-Browder S, Masson GMC, Corcoran AC, J Allergy 30:1, 1959)[13] was evaluated in our model. Promethazine, however, had no effect on the incidence of restraint- or ketamine-potentiated restraint-induced ulcers.

It can, therefore, be concluded that ketamine potentiates the effect of restraint-induced stress in producing murine gastric ulcers, and that this effect is probably mediated by the vasoactive properties of the anesthetic.

Evaluation of the patient population during the outbreak of Curling's ulcers in 1972 showed that of 39 patients with significant hemorrhage or perforation, five had received ketamine within three days of the onset of the complication. All five patients had burns covering more than 50 per cent of the body surface area, and four were septic at the time of hemorrhage, factors known to predispose to the formation of stress ulcers (Pruitt BA Jr, Foley FD, Moncrief JA, Ann Surg 172:723, 1970).[3] Since, therefore, no real evidence exists that in man ketamine can cause stress ulcers or potentiate known ulcerogenic conditions, no absolute clinical guidelines can be drawn. However, ketamine is currently being withheld from any patient with a known stress ulcer at our institution. More investigation of the effects of this drug on gastric physiology is necessary since it is so widely used for thermally injured patients, in whom Curling's ulcers are a significant complication.

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PRESENTATIONS

None

PUBLICATIONS

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0E 5967	74 07 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
73 07 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	62110A	3A162110A821	00	108
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) Evaluation of Steroids in the Management of Inhalation Injury of Military Personnel (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS:
003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
73 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 74	.5	8
	CURRENT 75	.5	12

19. RESPONSIBLE DOD ORGANIZATION
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NAME: James M Long, MAJ, MC DA

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Burns; (U) Inhalation Injury; (U) Steroids; (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) Pulmonary injury due to the inhalation of products of incomplete combustion or toxic fumes may be quite lethal by itself and when this injury occurs in association with a cutaneous thermal injury the mortality is very high. The incidence of inhalation injury in a large series of burns by standard clinical criteria has been reported to be about 3%. The diagnosis of inhalation injury with the use of the 133Xenon lung scan has proven to be a far more accurate diagnostic tool than if clinical criteria are used alone. The objective of this study is to evaluate the use of systemically administered steroids as a means of treating inhalation injury in military personnel.

24. (U) All patients between the ages of 15-40 years who have sustained burns within 48 hours of admission will be randomized for double-blind administration of steroids or a plasebo as a means to evaluate them in the treatment of inhalation injury. The 133 Xenon lung scan will be used to detect the presence of absence of an inhalation injury.

25. (U) 73 07 - 74 06 Five patients have been entered into the study but the drug code has not been broken and therefore no conclusions can be drawn at this time.

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*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

ABSTRACT

PROJECT NO.3A162110A821-00, COMBAT SURGERY

REPORT TITLE: EVALUATION OF STEROIDS IN THE MANAGEMENT OF INHALATION 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 July 1973 - 30 June 1974

Investigators: John L. Hunt, MD, Lieutenant Colonel, MC
Glenn D. Warden, MD, Major, MC
Peter A. Petroff, Jr., MD, Major, MC
Robert J. Lull, MD, Lieutenant Colonel, MC*

Reports Control Symbol MEDDH-288(R1)

This project is designed to study the effect of steroids on patients sustaining inhalation injury as a complication of their burn. The criterion for the diagnosis of inhalation injury is an abnormal ^{133}Xenon lung scan. Solu-Medrol is the steroid used in a "double blind" manner. The assessment of effectiveness of steroids in the management of inhalation injury will be based on the development of pneumonia within the first seven days post burn.

To date five patients have been entered in the study. No conclusions can be drawn from the study at this point in time.

Burns
Inhalation Injury
Steroids
Burn Patients

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EVALUATION OF STEROIDS IN THE MANAGEMENT OF INHALATION INJURY IN BURNED SOLDIERS

With the advent of topical antibiotics and subsequent improved control of burn wound sepsis the respiratory tract has become a prominent site of infection in patients with thermal burns. Prior to the use of xenon scan for early evaluation of inhalation injury, the diagnosis was based solely on clinical impressions namely, 1) flame burns involving the face 2) singed nasal vibrissae, 3) burns sustained in a closed space, 4) pulmonary physical findings of respiratory distress - hypoxemia, rales, wheezes, and 5) bronchorrhea and carbon tinged sputum. The use of the ^{133}Xenon lung scan has facilitated the early diagnosis of inhalation injury[4].

Steroids have been used for many years in the management of respiratory injuries whether they be due to burns or toxic materials. However, the beneficial effect of steroids in inhalation injury is unknown due to the multiple variables involved in patient care. Many investigators feel that the beneficial action of steroid administration is due to the anti-inflammatory effect and that early administration is essential to achieve this effect. With the use of the ^{133}Xenon scan and clinical impression, the evaluation of steroids in the management of inhalation injury will be investigated in a prospective clinical study.

All patients admitted to the ISR within 48 hours of injury will be evaluated for inhalation injury. The criterion for the diagnosis of respiratory burn is an abnormal xenon scan with the retention of xenon for more than 90 seconds post injection. All patients with a history of chronic pulmonary disease or tuberculosis as determined by the attending physician will be excluded. Only burn patients 15-40 years old will be included in the study group.

After the diagnosis of inhalation injury has been made all patients in the study will receive a standard treatment regimen. The administration of the steroids (Solu-Medrol, 30 mg/kg, i.v. every six hours for 3 days) will be in a "double-blind" manner. Patients will be followed until discharge or death and the hospital course evaluated. The criteria for effectiveness of steroids in the management of inhalation injuries will be primarily based on the development of pneumonia within the first seven days of burn.

RESULTS

Five patients have been entered in the study so far. The ages have ranged from 16 to 49 with a mean age of 29 years. The mean burn size was 52%. All patients were admitted within 24 hours of injury. Three patients were injured in a closed space and two in an open space. Chest roentgenograms on admission were clear and all patients had a positive xenon scan. Four of the five patients died. In three the major cause

of death was direct inhalation injury or complication of the injury. All but one patient received the complete course of drug therapy. One individual was in the study only for 24 hours at which time a moderate upper gastrointestinal bleed resulted and it was elected to discontinue the "drug" therapy.

DISCUSSION

The study is still in progress and the code has not been broken. Consequently, too few patients have been studied to draw any definite conclusions.

SUMMARY

Five patients have been entered in a prospective double blind study to evaluate the effect of Solu-Medrol on inhalation injury. The effectiveness of therapy at this time cannot be ascertained.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0E 6968	74 07 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
73 07 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	62110A	3A162110A821	00	110
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) Fibrinogen-Fibrin Degradation Products in the Thermally Injured Animal: A Model of the Burned Soldier (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
72 10	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 74	.6	13
	CURRENT 75	.6	15

19. RESPONSIBLE DOD ORGANIZATION
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NAME: Joseph P. Baron, SP4 DA

21. GENERAL USE: FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code): (U) Fibrinogen; (U) Antiplasmins; (U) Rats; (U) Thermal injury

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

23. (U) To study the responses in fibrinogen, fibrin-degradation products, fibrinolytic activity, and other coagulation parameters in the thermally injured rat model of the burned soldier.

24. (U) Rats are given a 30% scald burn followed by resuscitation. Half of the animals' burn wounds are then seeded with Pseudomonas aeruginosa, ISR strain 8-28-3. They are sacrificed at one and four hours, one, two, three, six, and ten days postburn for study.

25. (U) 73 07 - 74 06 Infected animals were sacrificed or died within six days postburn while the burned uninfected animals survived. Mean fibrinogen levels in the burned-infected animals were higher than in the burned animals after day 1 postburn. Plasminogen fell dramatically three days postburn in the infected animals but remained normal in the burned animals. Antiplasmin, but not antiactivator, activity was extremely elevated in the infected animals 6 days postburn. These studies indicate that fibrinolysis was markedly impaired by burn wound infection in the rat and may be related to their demise.

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*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

ABSTRACT

PROJECT NO. 3A162110A821-00, COMBAT SURGERY

REPORT TITLE: FIBRINOGEN-FIBRIN DEGRADATION PRODUCTS IN THE THERMALLY INJURED ANIMAL: A MODEL OF THE BURNED SOLDIER

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

Period covered in this report: 1 July 1973 - 30 June 1973

Investigators: Willard A. Andes, MD
Robert B. Lindberg, PhD
Joseph P. Baron, SP4

Reports Control Symbol MEDDH-288(R1)

Hyperfibrinolysis occurring in the thermally injured animal or soldier is a well known phenomenon. The significance, mechanisms, and clinical implications of such hyperfibrinolysis are unknown. Infection alone may also induce a state of hyperfibrinolysis. This study compared changes induced in a group of male Sprague-Dawley rats given a 30% dorsal scald burn with the changes in a similar group of rats which was burned and immediately seeded with Pseudomonas ISR strain 8-28-3. Studies performed since the last report have shown that plasminogen values fell moderately three days postburn and precipitiously ($P < .01$) six days postburn in the infected animals.

Mechanisms underlying this fall were then investigated. Serum from infected animals prevented lysis of clots formed in normal rat plasma by various methods. Potent antiplasmin activity was demonstrated in the infected animals especially 6 days postburn. Euglobulin lysis times were prolonged in both groups of animals postburn but markedly so in the infected animals six days postburn. Antiactivator content was elevated only at three days postburn in the infected animals. These studies indicate that fibrinolysis in the infected animals may be markedly impaired by the development of antiplasmin activity. The identity of this antiplasmin, further observations of its effects and significance await further investigation.

Fibrinogen
Antiplasmins
Rats
Thermal injury

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FIBRINOGEN-FIBRIN DEGRADATION PRODUCTS IN THE THERMALLY INJURED ANIMAL

Previous reports from this institute regarding changes in the extent of fibrinolysis and other hematologic indices in the thermally injured and thermally injured-infected rat and human have pointed out that certain similarities and differences may occur. Inasmuch as the parameters previously studied in the burned and burned-infected rat model (USAISR Annual Report 1973) have shown surprisingly few differences, continued observations were conducted. Identical methods of burning, infecting, and caring for the animals were used in the studies to be detailed below.

Plasminogen as measured by the caseinolytic method of Remmert and Sherry was lower in the infected animal three days postburn (mean 2.3 casein units per ml) than in the burned animal (normal 4.3-4.7). Six days postburn values in the burned-infected animal were even lower (mean 1.1 units per ml) while values in uninfected animals remained normal throughout the study.

Serum was obtained from the animals by cardiac puncture and transfer of the blood to glass tubes. Clotting was allowed to proceed for one to two hours at room temperature. The serum was then separated and stored on ice until being used the same day or frozen at -70° centigrade. Antiplasmin, antiactivator and euglobulin lysis time tests were performed by the method of von Kaulla (Thromb. Diath Haemorr Vol 22: 251-262, 1969) with minor modifications. In the antiplasmin test 0.10 ml of serum from normal, burned, or burned-infected animals was added to the test system. Blank studies were also done using buffered saline. Fibrinolysin was prepared in a 2.4 mg/ml solution in barbital-buffered saline and otherwise used as in von Kaulla's method. In the antiactivator study normal euglobulins were obtained from healthy normal rats by cardiac puncture in syringes containing 3.8% sodium citrate. In this assay, 0.05 ml of the appropriate sera were added to the clot system. All tubes were incubated in a 37° water bath (after clotting) until complete clot lysis had occurred. Each of these studies was done at least twice on the day the animals were sacrificed. Serum was pooled from 3 to 4 rats in each group each day. Euglobulin lysis times were done as before with no additions.

When 0.05 ml serum (1% of the total volume) from the infected animals 6 days postburn was added to the plasminogen assay, a 16% reduction was noted in the amount of casein lysed as compared with the same sample assayed without additions. When 0.1 ml was added a 32% reduction ensued. Normal serum induced no plasmin inhibition when added to the test.

Antiplasmin activity rose dramatically 6 days postburn in the infected animals with a mean lysis time of more than 1400 minutes with a control of 17 minutes. Burned animals had no increased antiplasmin activity. Euglobulin lysis times were slightly prolonged in each group on days 1 and 3 but markedly prolonged 6 days postburn in the infected animals. Antiactivator activity was increased slightly 3 days postburn in the infected animals (280 minutes, control 40 minutes) but had returned to normal by 6 days postburn.

These findings would seem to indicate that although small differences may be detected in the fibrinogen, platelet, or split product titer in the burned or burned-infected rat, some of the most distinctive and perhaps important differences in their responses may be found in the areas of fibrinolysis and its controlling mechanisms.

Further studies will involve identifying the antiplasmin and/or antiactivator compounds found. Both α_1 and α_2 globulins have been variously credited or absolved of antiplasmin activity. Although intimately associated with the animal's demise, the physiological and pathological effects of these compounds must be further studied to determine their importance. Furthermore similar techniques might be utilized to investigate fibrinolysis control mechanisms in the thermally injured and/or infected soldier. That such antiplasmins can be effectively counteracted by drugs in vitro has previously been shown by others and represents a potential form of therapy either in the rat model or the burned soldier.

REFERENCES:

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PRESENTATIONS AND/OR PUBLICATIONS

Andes WA, Lindberg RA, McEuen DD: Hematologic Findings in the Burned and Burned-Infected Rat. Clin Res (Abs) 22: 24A, 1974.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0E 6964	74 07 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DOD'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
73 07 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	62110A	3A162110A821	00	106
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)

(U) The Efficacy of Parenteral Fat Emulsion 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
73 02	Cont		

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 74	.6	17
	CURRENT 75	.4	8

19. RESPONSIBLE DOD ORGANIZATION

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21. GENERAL USE

FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede each with Security Classification Code) (U) Intravenous Fat Emulsion; (U) Intralipid; (U) Crystalline Amino Acid Solution; (U)Hypertonic Glucose; (U)Parenteral Nutrition; (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 determine the efficacy of intravenous 10% soybean oil emulstion (Intralipid) when compared to intravenous glucose as a source of calories for total parenteral nutrition in thermally injured military personnel.

24. (U) Three isocaloric, isonitrogenous diets with different compositions of fat and carbohydrate were evaluated in 4 studies to determine the relative protein sparing effect of intravenously administered fat emulsion and glucose.

25. (U) 73 07 - 74 06 In each of four studies, thermally injured patients received three different diets in which the total calories and nitrogen were kept constant while the fat and carbohydrate content of the diet was varied. The intravenous fat emulsion (Intralipid) was shown to be safe and efficacious as a calorie source for thermally injured patients in the previous report period. When fed the high carbohydrate diet and the intermediate diet, the patients excreted less urea (6.7 and 7.1 grams per square meter per day, respectively) than with the low carbohydrate, high fat diet (9.1 grams per square meter per day). Although the intravenous fat emulsion is an excellent source of calories, the high-fat, low carbohydrate diet showed less protein sparing effect than the isocaloric, intermediate and high carbohydrate diets.

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*Available to contractors upon originator's approval.

ABSTRACT

PROJECT NO. 3A162110A821-00, COMBAT SURGERY

REPORT TITLE: THE EFFICACY OF PARENTERAL FAT EMULSION IN THERMALLY INJURED SOLDIERS

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

Period covered in this report: 1 July 1973 - 1 June 1974

Investigators: James M. Long, III, M.D., Major, MC
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Reports Control Symbol MEDDH-288(R1)

Three isocaloric, isonitrogenous diets with different composition of fat and carbohydrate were evaluated in 4 studies to determine the relative protein sparing effect of intravenously administered fat emulsion and glucose. The high-fat diet contained 1108 Cal/m^2/day as modified soybean oil emulsion (Intralipid) and 350 Cal/m^2/day as glucose. The intermediate diet contained 583 Cal/m^2/day as fat and 875 Cal/m^2/day as glucose. The high-carbohydrate diet contained no fat and 1458 Cal/m^2/day as glucose. The nitrogen source for each diet was an 8.5 per cent crystalline amino acid solution (FreAmine). The patients were mildly hypermetabolic, but stable, after thermal injury. Each patient received 3 diets for 3 days each. The order in which a particular patient received the diets was randomly selected. Blood glucose, BUN, plasma insulin and urine urea nitrogen were measured, and the values for the third day of each diet were considered to represent the effects of that particular diet.

Excretion of urea nitrogen was significantly decreased in the patients on the high-carbohydrate diet (6.7 g/m^2/day, $p<0.01$) and the intermediate diet (7.1 g/m^2/day, $p<0.05$), when compared to the high-fat diet (9.1 g/m^2/day). There was no significant difference between the intermediate and high-carbohydrate diets. To determine if the effect was primarily carbohydrate addition, fat intake was kept constant at 1108 Cal/m^2/day and 3 different levels of glucose were infused for three days each (350, 875, and 1458 Cal/m^2/day, respectively). Nitrogen excretion decreased from 10.0 to 7.5 to 4.7 g/m^2/day, respectively, in response to increasing carbohydrate. Plasma insulin levels increased from 29.5 to 34.7 to 52uU/ml as glucose in the diet increased.

The data suggest that carbohydrate exerts the predominent impact on protein sparing during total intravenous feeding of isonitrogenous diets. The effect of fat and total non-protein calorie intake on nitrogen sparing

is being further investigated. The availability of intravenous fat emulsion (Intralipid) has significantly increased the capacity of the surgeon to provide for the accelerated energy demands of the severely injured soldier. Further investigation is presently underway to elucidate the optimal mixture of non-protein calorie sources that will decrease or prevent the extensive tissue and weight loss which results after major thermal injury.

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Intravenous Fat Emulsion
Intralipid
Crystalline Amino Acid Solution
Hypertonic Glucose
Parenteral Nutrition
Burn Patients

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OE 6966	74 07 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. ORG'N HIST'N	8b. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
73 07 01	D. COMPLETION	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO. CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62110A	3A162110A821	00	109
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) Evaluation of Digital Escharotomy in Thermally Injured Hands of Military Personnel

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
73 02	74 06	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	PRECEDING FISCAL YEAR 74	CURRENT FISCAL YEAR	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
	$		.6	15

19. RESPONSIBLE DOD ORGANIZATION
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21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Digital escharotomy; (U) Hands; (U) Thermally injured soldiers; (U) Ischemia

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

23. (U) To study the effects of escharotomy on increasing tissue survival in burned fingers of military personnel.

24. (U) Among all patients with upper extremity thermal injury requiring escharotomy, one-half will also have digital escharotomy performed. The two groups will be compared to see if there is any difference in the number of phalanges lost.

25. (U) 73 07 - 74 06 In the extremities treated with digital escharotomy, a total of 13 phalanges, or 7.1 per cent of all possible phalanges, became necrotic. In the control group, a total of 38 phalanges or 20.8 per cent were destroyed; thus, almost three times as many phalanges became necrotic in the control group. Digital escharotomy incisions healed uneventfully in survivors, and there was no incidence of hand infections in either group.

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*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

ABSTRACT

PROJECT NO. 3A162110A821-00, COMBAT SURGERY

REPORT TITLE: EVALUATION OF DIGITAL ESCHAROTOMY IN THERMALLY INJURED HANDS OF MILITARY PERSONNEL

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

Period covered in this report: 1 July 1973 - 30 June 1974

Investigators: Roger E. Salisbury, MD, Major, MC
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Reports Control Symbol MEDDH-288(R1)

Circumferential burns of the upper extremity, with progressive edema formation under an unyielding eschar, may result in vascular compromise and finally ischemic necrosis. Careful physical examination, use of the Doppler flowmeter, and judicious escharotomy and fasciotomy have reduced the incidence of loss of limb from this complication. Because the finger has thin skin, minimal soft tissue protection, and is supplied by two digital arteries with negligible collateral circulation, it is particularly at risk to vascular embarrassment with circumferential burns. Although the loss of some phalanges may be due to the severity of the initial thermal insult, extended digital escharotomy might salvage fingers with marginal blood supply that conventional escharotomy would not preserve.

Twenty-six circumferentially burned extremities with vascular compromise requiring escharotomy were included in this study. By random selection, digital escharotomy was also performed on all the burned fingers in one-half of the upper extremities (13) at the time the limb escharotomy was performed. Topical Sulfamylon^{R} was applied to all extremities and the usual treatment of upper extremity burns was followed. Records of phalangeal or complete digital loss, as well as incidence of hand infection, were made.

In the extremities treated with digital escharotomy, a total of 13 phalanges, or 7.1% of all possible phalanges, were lost. In the control group, a total of 38 phalanges, or 20.8%, were destroyed.

There was no incidence of hand infection in either group. Thus, digital escharotomy resulted in an increased survival of tissue without increasing the incidence of infection and should be considered for circumferential burns of the upper extremity and fingers with evidence of vascular compromise.

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Hands
Thermally injured soldier
Digital escharotomy
Ischemia

EVALUATION OF DIGITAL ESCHAROTOMY IN THERMALLY INJURED HANDS OF MILITARY PERSONNEL

Circumferential burns of the upper extremity, with progressive edema formation under an unyielding eschar may result in vascular compromise and finally ischemic necrosis. Careful physical examination, use of the Doppler flowmeter, and judicious escharotomy and fasciotomy have reduced the incidence of loss of limb from this complication. Because the finger has thin skin, minimal soft tissue protection, and negligible collateral circulation, it is particularly at risk to vascular embarrassment with deep circumferential burns. Appreciating that the loss of some phalanges occurs immediately, due to the severity of the initial thermal insult, this study was undertaken to determine if extended digital escharotomy might salvage those fingers with marginal blood supply that conventional escharotomy would not preserve.

METHODS

Fifteen patients, having 26 circumferentially burned upper extremities with completely burned fingers, and absent peripheral pulses, were included in this study. The patient population ranged from five years to 57 years old, with a mean age of 27.2. In all 26 extremities, evaluation with the Doppler flowmeter revealed absence of palmar arch flow during the first 24 hours postburn, necessitating escharotomy. By random selection, the patients were divided into two groups In the control group, 13 extremities had a conventional forearm and wrist escharotomy, but the other 13 extremities also had extended digital escharotomy. Digital escharotomy was performed through a midlateral incision along one side of the finger into the fat. Some patients required supplemental intravenous morphine before the procedure was performed. Hemostasis was achieved with pressure and the electrocautery. Sulfamylon^{R} was applied to all extremities, and the usual ISR management of extremity burns followed, emphasizing elevation, no dressings, and unlimited exercise. The occurrence of phalangeal necrosis and the incidence of hand infection were recorded. Although the physicians on the research protocol performed the escharotomies, most of the patients were cared for by other members of the staff to minimize physician bias. Hands that were obviously charred on admission were excluded from the study. Significantly, the two groups being studied were comparable, total body burn in the control group averaging 67.6% as opposed to 61.9% in the group with digital escharotomy, total extremity burns averaging 17.2% of total body surface as opposed to 17% of total body surface, second and third degree components being comparable.

RESULTS

In the extremities treated with digital escharotomy, a total of 13 phalanges or 7.1% of all possible phalanges became necrotic. In the control group, a total of 38 phalanges, or 20.8%, were destroyed. Thus, almost three times as many phalanges became necrotic in the control group. Digital escharotomy incisions healed uneventfully in survivors. There was no incidence of hand infections in either group. No bacterial or fungal invasion of the incisions occurred, and the flora of the incisions was controlled with Sulfamylon[R] topical chemotherapy.

DISCUSSION

Although the importance of extremity escharotomy has been properly emphasized in the literature, surprisingly little mention has been made of the fingers, considering their importance. Meade, in 1958 (Meade RJ, Plast Reconstr Surg 21:263-271, 1958),[1] reported four upper extremity burns requiring escharotomy and commented that radical finger incisions might have saved the distal phalanges of one bad burn. Kaplan and White, in 1961 (Kaplan I, White WL, Plast Reconstr Surg 28:609-617, 1961),[2] advised that incisions be made the length of the burn and that a midlateral incision was preferable for the fingers. Thompson (Patman R, Thompson JE, Arch Surg 101:663-672, 1970),[3] in reviewing the indications for fasciotomy in peripheral vascular injuries, noted that, in burned patients, "skinotomy may have to be extended to include the hand and fingers." No author, however, conducted any studies to determine the efficacy of digital escharotomy in salvaging fingers.

The incidence of vascular insufficiency of the upper extremity secondary to thermal injury is significant, and, in the past year, 15% of 300 admissions required upper extremity escharotomies, a total of 46 upper extremities. After escharotomy, the return of the superficial palmar arch flow is indicative of adequate perfusion of the palm, but the vascular status of the fingers is less certain. Moylan (Moylan JA Jr, Inge W, Pruitt BA Jr, J Trauma 11:763-770, 1971)[4] noted that in using the Doppler flowmeter to measure digital artery pressure and record flow, it took up to 24 hours to detect return of flow in the patients with escharotomy and up to 36 hours in the non-escharotomized group. Thus, the early failure to detect digital flow with the Doppler does not necessarily mean nonviability, nor does the initial presence of flow guarantee continued vessel patency through the period of maximum edema, the first 48 hours. The other clinical criteria of ischemia - skin pallor, coolness, and poor capillary filling are imprecise signs in a thermally injured patient who may

have decreased cardiac output and poor peripheral perfusion responsible for the same signs. To date, there is no foolproof clinical test to verify digital ischemia during the first 24 hours postinjury.

The vascular anatomy of the finger makes it particularly susceptible to ischemia in thermal injury. There are no deep venae comitantes accompanying the digital arteries in the fingers, and venous return is dependent on very small deep veins unrelated to the arteries and mostly to superficial dorsal digital veins. Since dorsal burns are so common, these veins are often directly injured or compressed by edema, markedly reducing the venous flow from the fingers and increasing ischemia.

The arterial inflow consists of two proper volar digital arteries from the superficial arch and dorsal arterial branches from the dorsal metacarpal arteries. These are small end vessels with minimal collateral circulation and are easily compromised.

The investing fascia of the vessels may also help promote ischemia. The common digital artery bifurcates in the palm into proper digital arteries under the interdigital ligament in the interdigital fold. Cleland's and Grayson's ligaments form a fibrous tube around the vessel in the finger, extending to the DIP joint. In severe thermal injury, with increased capillary permeability, it is possible that the vessels could become compressed by the overlying fascia.

Although conventional escharotomy, extending down from the forearm and across the wrist, has been shown to improve the circulation of the palm, there is no proof that it improves digital survival. In fact, it may be harmful. Decompressing the hand may increase flow into the fingers in the high pressure arterial system without a corresponding increase in outflow in the low pressure venous system, thus leading to more edema and decreased digital blood flow.

In the present series, the difference in phalangeal loss between the two groups is marked, almost three to one. Significantly, all digital escharotomies were done in the first 24 hours postburn, before irreversible ischemic changes occurred and perhaps account for the success of the procedure. It is important to emphasize that the procedure has no salutary effect on charred fingers.

The surgeon must consider that, since 20.8% of all phalanges in the control group became necrotic, almost four-fifths of the phalanges would survive without surgical intervention. The procedure is justified because the risk is negligible and any conservation of viable

tissue may be of great significance in ultimate functional results, and in planning the reconstruction of the hand.

In conclusion, a controlled study of digital escharotomy revealed a significant increase in the number of salvagable phalanges. This procedure has minimal risk and should be considered in patients with vascular compromise of the upper extremity requiring escharotomy who also have circumferentially burned phalanges.

REFERENCES

1. Meade RJ: The prevention of secondary tissue destruction in burns. Plast Reconstr Surg 21:263-271, 1958.

2. Kaplan I, White WL: Incisional decompression of circumferential burns. Plast Reconstr Surg 28:609-617, 1961.

3. Patman R, Thompson JE: Fasciotomy in peripheral vascular surgery. Arch Surg 101:663-672, 1970.

4. Moylan JA Jr, Inge, W, Pruitt BA Jr: Circulatory changes following circumferential extremity burns evaluated by the ultrasonic flowmeter. J Trauma 11:763-770, 1971.

PUBLICATIONS: None

PRESENTATIONS

Salisbury RE: Evaluation of Extended Digital Escharotomy in Thermally Injured Hands. Military Association of Plastic Surgeons, Washington, D. C., January 1974.

Salisbury RE: Evaluation of Extended Digital Escharotomy in Thermally Injured Hands. American Burn Association, Cincinnati, Ohio, April 1974.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OE 6983	74 07 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
74 03 27	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	62110A	3A162110A821	00	113
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Procede with Security Classification Code): (U) Evaluation of Enzymatic Debridement in Burned Hands of Soldiers 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
74 01	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 74	.4	10
FISCAL YEAR CURRENT 75	.3	6

19. RESPONSIBLE DOD ORGANIZATION
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21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Travase; (U) Sutilains; (U) Burned Hands; (U) Grafting; (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 efficacy of enzymatic debridement of burned hands with an assessment of grafting, early active motion, and final functional result in soldiers with thermal injury.

24. (U) Comparable hand burns, seen within the first 72 hours, will be treated with b.i.d. dressing changes and an active range of motion, with the dressing kept continually moist with saline. The enzyme-treated hands will be covered b.i.d. with Travase. The nonenzyme-treated hands will be covered with either the base of the Travase minus the enzyme or saline soaks. No topical chemotherapy will be used. The wound will be monitored for burn wound sepsis with both biopsy and surface culture. Any evidence of deterioration of the wound will result in cessation of enzymatic debridement and application of a chemotherapeutic agent.

25. (U) 74 01 - 74 06 At this time, only one set of symmetrical hand burns have been evaluated. There was earlier separation of the eschar on the enzymatically debrided side at 48 hours as compared to 96 plus on the saline soaked hand. During the time of the debridement, there was greater active range of motion in the enzyme-treated hand. However, the final function was excellent in both hands, and no grafting was required.

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*Available to contractors upon originator's approval.

ABSTRACT

PROJECT NO. 3A162110A821-00, COMBAT SURGERY

REPORT TITLE: EVALUATION OF ENZYMATIC DEBRIDEMENT IN BURNED HANDS OF SOLDIERS WITH THERMAL INJURY

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

Period covered in this report: 1 July 1973 - 30 June 1974

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Jamis C. Townsend, Captain, AMSC

Reports Control Symbol MEDDH-288(R1)

It is generally agreed that the long term problem with burned hands is loss of function. This is related not only to the depth of burn but to prolonged immobilization. Prolonged immobilization is due to delayed eschar separation, limitation of function during the period of separation due to hard unyielding eschar and lastly pain. Delay in eschar separation, and delay in grafting are probably of more functional significance in the hand than anywhere else in the body. One remedy for this is the early surgical excision and grafting. This has several shortcomings. With surgical excision viable tissue is always removed, graft takes are not always excellent and the patient is not always a candidate for a prolonged surgical procedure when it is indicated. It is appealing to think that with use of enzymatic debridement the eschar could be separated and the wound grafted at an earlier date. During the eschar separation the eschar would be softer, allowing a greater range of motion with earlier separation, grafting could be accomplished sooner and there would be a shorter period of immobility and a better functional result could be anticipated. Enzymatic debridement has not found wide acceptance at the Institute of Surgical Research for several reasons. It has been noted that after eschar separation a graftable bed was not achieved, secondly, that the macerated eschar provides an excellent portal of entry for bacteria and thirdly that the vehicle in which the enzyme was prepared may interfere with topical antibiotic therapy. These factors militate against the use of enzymatic debridement for large areas of eschar, especially in areas subject to maceration. Enzymes may however be particularly useful for maintaining function while debriding burned hands. This study will compare the time to eschar separation, time to grafting and final hand function on patients with similar burns on both hands one treated with an enzymatic debriding agent and the other with saline soaks.

To date three patients have been studied and in all three the eschar separated more rapidly from the Travase treated hand. The eschar treated with Travase softens very rapidly and is essentially gone with

72 hours. However, in our initial patients we have not achieved a graftable bed any more rapidly with Travase than we have with saline soaks. The Travase treated eschar is removed leaving a relatively slimy base of deep dermis which is very tenacious and does not separate any faster than it does when treated with saline soaks. In the two patients that survived to leave the hospital grafting was not accomplished any earlier in the enzyme treated hand than the saline soaked hand and at the time of discharge there was no difference in function, both hands being the same. At this time it appears as if enzymatic debridement with Travase does not provide a graftable base any earlier than saline soaks and there is no difference in final function.

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Travase
Sutilains
Burned hands
Grafting
Humans

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OE 6984	74 07 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
74 03 27	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	62110A	3A162110A821	00	114
B. CONTRIBUTING				
C. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) A Prospective Comparison Study of Sulfamylon and Silver Sulfadiazine in The Treatment of Burned Troops (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
73 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 74	.5	14
	CURRENT 75	.5	14

19a. RESPONSIBLE DOD ORGANIZATION
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21. GENERAL USE: FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code): (U) Topical therapy; (U) Burn injury; (U) Sulfamylon (mafenide); (U) Silvadene (silver sulfadiazine); (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 compare silver sulfadiazine with Sulfamylon in six groups of burned soldiers two groups by age 16 to 40, and greater than 40, and three by burn size - 30 to 50 per cent, to to 70 per cent, and greater than 70 per cent. The parameters to be evaluated are survival, acid base balance, pain of application, bacteriology of the burn wound, pulmonary function, and clinical status of the injured troops.

24. (U) Burns greater than 30 per cent, seen within the first 72 hours, will be randomized by pairs in the aforementioned groups and placed in one of the two agents. The pulmonary functions, acid base balance, and wound bacteriology will then be followed meticulously until the eschar is entirely separated or until the patient has expired.

25. (U) 73 12 - 74 06 To date, 18 patients have been studied or are being studied. As yet, no clear pattern of mortality or wound bacteriology has become evident. The silver sulfadiazine is markedly more comfortable for the patient, and seems to cause less hyperventilation than the Sulfamylon. Any other observations will await a larger group of patients.

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*Available to contractors upon originator's approval.

DD, FORM 1498, MAR 68 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

ABSTRACT

PROJECT NO. 3A162110A821-00, COMBAT SURGERY

REPORT TITLE: A PROSPECTIVE COMPARISON STUDY OF SULFAMYLON AND SILVER SULFADIAZINE IN THE TREATMENT OF BURNED TROOPS

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

Period covered in this report: 12 December 1973 - 30 June 1974

Investigators: Hugh D. Peterson, D.D.S., M.D., Colonel, MC
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Arthur D. Mason, Jr., M.D.
Robert B. Lindberg, Ph.D.
Basil A. Pruitt, Jr., M.D., Colonel, MC

Reports Control Symbol MEDDH-288(R1)

Since 1964 topical chemotherapy of the burn wound with Sulfamylon or silver nitrate has been an accepted component of burn therapy. The incidence of burn wound sepsis especially in children has been markedly reduced by these topical agents. Silver sulfadiazine has recently been employed in several burn treatment centers and its purported advantages are: 1) absence of carbonic anhydrase inhibition, 2) absence of post-application pain, and 3) possible antifungal (Candida) activity. The proposed study is to compare 200 patients with burns greater than 30% divided into three age groups, those being 0-15, 15-40 and over 40 and two burn sizes 30-50 per cent and greater than 50 per cent. The patients are randomized by pairs in the six groups and followed by monitoring resuscitation, acid-base balance, and pulmonary functions as well as pulmonary complications, bacteriology of the burn wound and clinical signs and symptoms. The indices to be evaluated are: 1) overall mortality, 2) acid-base balance, 3) bacteriology of the burn wound, 4) pain on application, 5) pulmonary function and pulmonary complications and 6) clinical signs such as onset of GI function, orientation and appetite.

Topical therapy
Burn injury
Sulfamylon (mafenide)
Silvadene (silver sulfadiazine)
Humans

527<

A PROSPECTIVE COMPARISON STUDY OF SULFAMYLON AND SILVER SULFADIAZINE IN THE TREATMENT OF BURNED TROOPS

The intent of this study is to provide a prospective comparison study in six groups randomized by pairs with two burn sizes in three groups as to their overall response to topical therapy with Silvadene and Sulfamylon.

The study commenced in mid-December 1973 and by 15 June 1974 78 patients had been studied. The crude survival of the patients with Silvadene was 54%. The crude survival of patients with Sulfamylon was 24% and if patients were omitted that were switched to Silvadene because of severe respiratory problems, this survival rate drops to 22%. Also of interest the mean burn size of the patients dying with Silvadene was 67% with a mean survival of 24 days, the mean burn size at death with Sulfamylon was 64% with a mean survival of 10 days. Acid-base balance evaluation revealed that unless sepsis intervened the patients treated with Silvadene demonstrated no significant abnormalities in their acid base balance. The patients with Sulfamylon again demonstrated the previously recognized early base deficit and metabolic acidosis. Wound bacteriology awaits complete evaluation but it must be stated at this time that the predominant organism in the Silvadene treated group is Enterobacter cloacae which seems to grow in great profusion on a very benign appearing eschar, being found routinely in counts as high as 10^7 organisms per gram of tissue. No significant Staphylococcal infections occurred nor was there any significant fungal colonization of the Silvadene treated wounds. The Sulfamylon treated wounds had Staphylococcus sp and fungi as predominant organism. Enterobacter sp were noted in these wounds but less frequently than in the Silvadene treated wounds. Pseudomonas sp during the period of this study were not a problem in wounds treated with either agent, there being one instance of Pseudomonas burn wound infection in wounds treated with each agent and a third in a patient being treated with saline soaks following eschar separation. On the topic of pain it can be said unequivocally that Silvadene is essentially free of pain and many times the patients state that the wound feels much better when it is covered with Silvadene. With Sulfamylon there is intense pain post application especially on second degree wounds which lasts from 15 minutes to 1 hour and on several patients required administration of an analgesia. Pulmonary function in the patient treated with Silvadene is essentially the same as in patients treated at this Unit earlier with 0.5% silver nitrate soaks with no instances of the marked hyperventilation seen in the Sulfamylon treated patients. More importantly there were five cases of patients treated with Sulfamylon that demonstrated early "white out" of the lungs and this was not seen in any of the patients treated with Silvadene. As far as clinical signs only general statements can be made, however, the patients in Silvadene had their n.g. tubes removed earlier, tolerated a regular diet at an

earlier time and manifested less confusion, but these are only very gross indices of well being. In spite of the great discrepancy in overall survival of patients treated with the two agents, this difference only approaches statistical significance and further comparison may be required.

PRESENTATIONS AND/OR PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0E 6965	74 07 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	9. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
73 07 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	62110A	3A162110A821	00	105
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) The Uses of Biologic Dressings in Burned Soldiers: Tests of Four Null Hypotheses (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
73 05	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 74	.5	9
	CURRENT 75	.4	9

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21. GENERAL USE: FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code): (U) Homograft; (U) Pig skin; (U) Biologic dressing; (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) The efficacy of biologic dressings in debriding "untidy" wounds, (2) reducing infection on granulating areas, (3) promoting healing of second degree wounds, and (4) as a cover for mesh autograft will be evaluated in burned soldiers.

24. (U) The effect of homograft, porcine xenograft, and formalin-fixed xenograft were studied on the 4 types of wounds described above and tested against the null hypotheses that neither is more effective than coarse mesh gauze treatment. Friedman two-way analysis was used to permit comparison of each treatment regimen with gauze dressings. Intercomparison between the biologic dressings was not made in this study.

25. (U) 73 07 - 74 06 Twenty separate small wounds in burned soldiers were studied under the null hypotheses. Such studies revealed: (1) That three times daily changes of coarse mesh gauze was more effective than any of the biologic dressings in debriding "untidy" wounds; and (2) 24-hour applications of biologic dressings did not reduce bacterial populations of granulating areas any more than did frequent changes of coarse mesh gauze dressings. A few patients were studied to evaluate the effect of biologic dressings on the promotion of healing of second degree burn wounds and as a cover for mesh autograft. It was determined, however, that the end points involved in such studies were difficult to appraise and accordingly these latter studies were abandoned. This type of study has provided us with a technic for examining the effects of biologic dressings on patients. Accordingly, it will be continued, with slight modifications, for the evaluation of synthetic skin products which are currently in phase 2 testing.

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*Available to contractors upon originator's approval.

ABSTRACT

PROJECT NO. 3A162110A821-00, COMBAT SURGERY

REPORT TITLE: THE USES OF BIOLOGIC DRESSING IN BURNED SOLDIERS: A COMPARISON OF COARSE MESH GAUZE VERSUS "BIOLOGIC DRESSINGS" ON GRANULATING WOUNDS

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

Period covered in this report: 1 May 1973 - 20 June 1974

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Twenty patients with large area granulating wounds were selected for the study. On each patient, a small area of uniform appearing burn wound was divided into four subareas, and each subarea was randomly assigned treatment with a single twenty-four hour application of cadaver allograft, fresh porcine xenograft, formalinized xenograft, or "wet-to-dry" applications of coarse mesh gauze changed three times daily. At 24 hours, all four areas were uncovered. The subareas were ranked in terms of appearance on a "best," "second best," "third best," and "worst" scale by experienced paramedical personnel who are not told which area received which treatment. Contact sponge quantitative microbiology was performed on each subarea before and after treatment on 17 of the 20 patients. The results of these rankings suggest that coarse mesh gauze, changed three times daily, was significantly better at improving wound appearance than any of the three "biologic dressings." Among the "biologic dressings," formalinized xenograft was significantly the worst. There was no significant difference between cadaver allograft and porcine xenograft. Analysis of quantitative cultures was limited by the problems of applying statistical methods to series of paired cultures whose initial values are quite different. Within the framework of such limitations and a 24 hour study, there was no significant change in surface colonization when either coarse mesh gauze or biologic dressings were used. As a debriding agent, gauze was superior to any of the biologic dressings tested.

Burn patients
Homograft
Pig skin
Biologic dressing

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THE USES OF BIOLOGIC DRESSINGS IN BURNED SOLDIERS: A COMPARISON OF COARSE MESH GAUZE VERSUS "BIOLOGIC DRESSINGS" ON GRANULATING WOUNDS

The use of human cadaver allograft and other "biologic dressings" has a time honored place in the treatment of burned patients. Such dressings are used as autograft substitutes, whenever donor site limitations or patient considerations make autografting inadvisable. Initially, cadaver allograft was used as a long term dressing, and left in place until rejected by the patient. The problem of allograft rejection was circumvented by changing the "biologic dressing" every three to four days. Prudent physicians also felt that autograft take in any large area could be insured by first "testing" the area with cadaver allograft.

The use of frequent changes of biologic dressings immediately prior to autografting led to the clinical impression that such dressings somehow "prepared" the granulating surface for the acceptance of autograft. At least two factors are involved in the preparation of a granulating surface for graft acceptance. The wound surface must be debrided of non-viable particulate matter and the bacterial density should be below 10^5 organisms per gram.

The purpose of this study was to determine if cadaver allograft and other biologic dressings improve wound appearance and surface bacteriology better than frequent changes of coarse mesh gauze.

METHODS

Twenty patients with large area granulating wounds were selected for the study. On each patient, a small area of uniform appearing burn wound was divided into four subareas, and each subarea was randomly assigned treatment with a single 24 hour application of cadaver allograft, fresh porcine xenograft, formalinized xenograft or "wet-to-dry" applications of coarse mesh gauze changed three times daily. At 24 hours, all four areas were uncovered. The subareas were ranked in terms of appearance on a "best," "second best," "third best," and "worst" scale by experienced paramedical personnel who were not told which area received which treatment. Contact sponge quantitative microbiology was performed on each subarea before and after treatment on 17 of the 20 patients. Four square centimeter gauze sponges were applied to each area for five seconds. The sponges were then shakened in five milliliters of trypticase soy broth, from which serial log dilutions were cultured on trypticase soy agar.

RESULTS

The results of the clinical rankings are summarized in Table I. The areas treated with "wet-to-dry" changes of coarse-mesh gauze were ranked "best" 19 out of 20 times. Cadaver allograft was ranked "second best" most frequently, and formalinized porcine xen graft was ranked "worst" on 14 occasions. Applying Friedman analysis of variance to the

rankings (Table II), coarse mesh gauze was significantly better at improving wound appearance than any of the three "biologic dressings." Among the "biologic dressings," formalinized xenograft was significantly the "worst". There was no significant difference between cadaver allograft and porcine xenograft.

Initial cultures showed counts of from 0 to 6 x 10^4 organisms per milliliter. Staphylococcus aureus, Pseudomonas aeruginosa, non-hemolytic Streptococci, Providencia stuartii, and Corynebacteria were the bacteria most frequently encountered. Forty three per cent of the time, the second culture was within one log of the first culture (Table III). In 29% there was a one log or greater decrease, but in 28% there was a one log or greater increase. None of the four forms of treatment significantly increased or decreased the number of organisms in the underlying tissue.

Although several wounds in each subgroup appeared sterile after treatment, over half of these showed no growth prior to treatment (Table IV). Only seven initially infected subareas (Table V) showed no growth after treatment.

It is important to stress the limitations of applying statistical methods to series of paired cultures whose initial values are quite different. Within the framework of such limitations, there was no significant change in the quantitative cultures following any of the dressings.

DISCUSSION

The intent of this study is not to decry the use of cadaver allograft or "biologic dressings." Such "autograft substitutes" may and should be used whenever patient considerations or limited donor sites make autografting inadviseable. Patients with large area surgical excisions, patients with limited donor sites, patients too sick to undergo a formal grafting procedure, and patients with large areas of clean granulation tissue who are awaiting debridement of contiguous areas all can benefit from "biologic dressings". Such dressings have been demonstrated to decrease fluid and heat loss, limit bacterial growth, decrease wound pain, and facilitate movement and physical therapy. In patients with large open areas of granulation tissue, the "take" of biologic dressings may be used as a test of autograft "take".

However, this study indicates that biologic dressings offer no advantage of coarse mesh gauze in preparing small areas of granulation tissue for graft acceptance. Within the framework of our study, there was no significant change in surface colonization when either coarse mesh gauze or "biologic dressings" were used. As a debriding agent, gauze was superior to any of the biologic dressings tested.

SUMMARY

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Twenty patients with large area granulating wounds were selected

TABLE I

CLINICAL APPEARANCE (RANKING) OF AREAS TREATED FOR 24 HOURS

RANKING	DRESSING			
	CADAVER ALLOGRAFT	PORCINE XENOGRAFT	FORMALINIZED XENOGRAFT	MESH GAUZE
1 - "BEST"	0	1	0	19
2 - "SECOND BEST"	11	4	5	0
3 - "THIRD BEST"	4	14	1	1
4 - "WORST"	5	1	14	0

TABLE II

RANK TOTALS FOR CLINICAL APPEARANCE

COARSE MESH GAUZE	22	"BEST" ($p<.0005$)*
CADAVER ALLOGRAFT	52	N.S.
PORCINE XENOGRAFT	55	N.S.
FORMALINIZED XENOGRAFT	69	"WORST" ($p<.025$)**

* $X^2_{(2)}$ by Friedman analysis of variance

** $X^2_{(2)}$ " " " " "

TABLE III

SIGNIFICANT (1 LOG OF GREATER) CHANGE IN QUANTITATIVE BACTERIOLOGY AFTER DRESSING APPLICATION.

	CADAVER ALLOGRAFT	PORCINE XENOGRAFT	FORMALINIZED XENOGRAFT	MESH GAUZE
DECREASED	4	5	5	6
INCREASED	4	2	6	7
NO SIGNIFICANT CHANGE	9	10	6	4

TABLE IV

NUMBER OF STERILE WOUNDS AT 24 HOURS

CADAVER ALLOGRAFT	PORCINE XENOGRAFT	FORMALINIZED XENOGRAFT	MESH GAUZE
7/17	6/17	2/17	4/17

TABLE V

STERILIZATION BY DRESSING OF PREVIOUSLY INFECTED SITE

CADAVER ALLOGRAFT	PORCINE XENOGRAFT	FORMALINIZED XENOGRAFT	MESH GAUZE
3/17	2/17	1/17	1/17

for the study. On each patient, a small area of uniform appearance was divided into four subareas, and each subarea was randomly assigned treatment with a single 24 hour application of cadaver allograft, fresh porcine xenograft, formalinized xenograft, or "wet-to-dry" applications of coarse mesh gauze changed three times daily. At 24 hours, all four areas were uncovered. The subareas were ranked in terms of appearance on a "best," "second best," "third best," and "worst" scale by experienced paramedical personnel who were not told which area received which treatment. Contact sponge quantitative microbiology was performed on each subarea before and after treatment on 17 of the twenty patients. The results of these rankings suggest that coarse mesh gauze, changed three times daily, was significantly better at improving wound appearance than any of the three "biologic dressings." Among the "biologic dressings" formalinized xenograft was significantly the worst. There was no significant difference between cadaver allograft and porcine xenograft. Analysis of quantitative cultures was limited by the problems of applying statistical methods to series of paired cultures whose initial values are quite different. Within the framework of such limitations and our 24 hour study, there was no significant change in surface colonization when either coarse mesh gauze or biologic dressings were used. As a debriding agent, gauze was superior to any of the biologic dressings tested.

PRESENTATIONS AND/OR PUBLICATIONS

None

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1 July 1973 - 30 June 1974

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