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Brooke Army Medical Center
Fort Sam Houston, Texas

30 June 1973

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13. ABSTRACT

This report documents the clinical and laboratory activities of the USAISR during fiscal 1973. 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. (U)

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

SGRD-US

31 July 1973

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Basil A. Pruitt Jr.

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

ID

FOREWORD

The three princes of Serendip would not be surprised at all by the importance of accidental discovery in clinical research. To effect productive research, however, serendipity must occur in the presence of both a prepared mind and a prepared pocketbook. Clinical experience and continuous patient care responsibility insures so far as is possible, a prepared mind in our investigative staff. With regard to a prepared pocketbook, systematic budgetary planning, essential for continuity of operation and long-term research evolution, unfortunately ignores the importance of capturing the enthusiasm of an investigator with a fresh idea which may erode with time, take second place to other ideas, or be totally lost with the departure of the individual scientist. The ILIR program, however, has provided the seed money and funding flexibility to exploit such scientific leads and is of increasing importance to clinical research at this time of diminishing operating funds.

The success of such opportunistic research is illustrated by the pulmonary studies, metabolic studies, gastroenterology studies, and studies of physiologic dressings described in this report, all of which were initiated in this manner. Each of these fields of investigation has relevance not only to burn patients but to all severely injured patients. Moreover, each represents expansion and diversification of the professional capabilities of our staff which have been translated into improved patient care, the goal of clinical research.

Since the Medical Corps can not compete financially for physicians with the academic community, let alone private practice, flexible funding resources such as are available under the ILIR program will hopefully be increased to counteract that limitation and to maintain the attractiveness of our investigative milieu. Furthermore, the lapse of the physician draft necessitates other means of recruiting enthusiastic highly competent scientists. It is suggested that consideration be given to the establishment of surgical research fellowships to ensure recruitment of investigators with prepared minds and thereby the viability of our research program.

Basil A. Pruitt, Jr.

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

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SECTION NO.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
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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) 72 01 - 72 12 During 1972, 300 patients were admitted to the Institute; 25 patients were evacuated from Viet Nam by way of Okinawa. Attention on early diagnosis and treatment of inhalation injury, re-evaluation of early fluid resuscitation formulae, use of Intralipid as a hyperalimentation supplement 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.

*Available to contractors upon originator's approval

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ANNUAL PROGRESS REPORT

PROJECT NO. 3A161102B71R-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 1972

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ABSTRACT

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

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Three hundred patients with thermal injury were admitted to the Clinical Division of the United States Army Institute of Surgical Research during calendar year 1972. Emphasis has continued to be placed

on providing optimal clinical care to military personnel with major thermal injury. In addition, clinical investigation has continued into the physiological, biochemical and bacteriological aspects of thermal injury. The personnel of this institute have also participated in many educational programs both military and civilian. This report summarizes the activities of the Clinical Division of the U.S. Army Institute of Surgical Research in 1972 and cites the recognizable complications which contributed to mortality in burn patients.

Clinical evaluation of the metabolic requirements of thermally injured soldiers, ketamine analgesia for limited debridement, effectiveness of Intralipid as a caloric source, the use of Sulfamylon-Mycostatin cream, 5% aqueous Sulfamylon dressings, the use of human growth hormone to promote nitrogen sparing, serial measurement of pulmonary function, the management of upper extremity postburn edema, continued evaluation of the ^{133}Xenon scan for the detection of inhalation injury, and the serial measurement of alterations in the coagulation mechanism were specific areas of clinical study.

In 1972 aeromedical evacuation was employed for transfer of 220 (73.5%) of all patients admitted to the Institute of Surgical Research. One hundred and fifty-five Continental United States evacuations for 185 patients set a new record for such evacuations in 1972.

Thermal injury
Topical therapy
Air evacuation
Mortality
Coagulation defect
Metabolic requirements
Biologic dressings
Intralipid
Subanesthetic ketamine
Human growth hormone

CLINICAL OPERATION, CENTER FOR TREATMENT OF BURNED SOLDIERS

The Clinical Division of the US Army Institute of Surgical Research continued through the year 1972 to have as its primary objective the provision of clinical care for thermally injured patients. An increase in the total number of admissions from 268 in 1971 to 300 patients in 1972 was remarkable in that this reflected an increase in both the total of patients and the number of patients admitted early post burn.

In early 1972 burn teams from the Institute of Surgical Research were sent upon request to US Army Hospital, Camp Zama, Japan to prepare thermally injured patients for evacuation and to care for them on their flight from the Far East. On the 1st of June the burn holding area was transferred from Camp Zama, Japan to US Army Medical Center Okinawa. A total of 8 such evacuations for 31 patients were made in the calendar year 1972 from the Far East compared with 13 flights for 93 patients in 1971.

There were 155 Continental United States flights for 185 patients and in addition 4 other flights for 4 patients including 3 flights for 3 patients to Alaska and 1 flight for 1 patient to Puerto Rico. The necessity of having experienced burn team members carry out the aeromedical evacuation of severely burned patients was demonstrated by the fact that none of the 220 patients evacuated by our burn teams to this Institute expired in flight.

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.

Calendar year 1972 completes four years experience with split-thickness porcine cutaneous xenograft as a substitute for fresh human allograft. While porcine xenograft appears to enhance the healing of partial thickness burns the enthusiasm for its use as a biologic dressing has diminished over the past year. Compared to cadaver allograft, human, it is clearly a second choice and fails to prepare a full thickness wound for autografting as well as human cadaver allograft. Several instances occurred in which the porcine xenograft had to be removed from a full thickness wound and topical chemotherapy reinstituted. Lyophilized porcine xenograft appears to be less effective than commercially prepared "fresh" porcine xenograft and its use has been limited. Porcine xenograft continues to offer the advantages of availability, a lack of immune response (take and rejection phenomenon) and does serve as a substitute for cadaver allograft when the availability of cadaver

allograft is limited. Cadaver allograft however continues to be the first choice as a physiologic dressing both for the reduction of evaporative losses, the decrease of infection and the preparation of the wound for autografting.

Ten per cent fat emulsion (Intralipid) is being evaluated for its nitrogen sparing qualities after thermal injury. It offers the advantage that it can be administered through a peripheral vein thus eliminating the necessity for cannulation of a central vein. The occurrence of thrombosis and thrombophlebitis of central veins related to cannulation has been confirmed and the availability of a nutrient material that may be administered through a peripheral vein is clearly advantageous. Three parenteral diets are administered to a single patient with variable portions of fat, carbohydrates and a fixed caloric and nitrogen load. Urine is collected and nitrogen excretion is calculated from the urine urea nitrogen and total urinary nitrogen. No untoward febrile responses, coagulation abnormalities or complications have been noted thus far with use of the fat emulsion.

Evaluation of aqueous Sulfamylon, 5%, has continued during 1972 and it has primarily been used in wet-to-dry dressings, changed every 6 to 8 hours. Such dressings are limited to 20% of the total body surface and are employed for a day or two after the eschar has separated and a superficial, shaggy, pseudoeschar and exudate still remains on full thickness wounds. In addition it has been used to protect freshly applied mesh autograft to prevent desiccation and to reduce the bacterial concentration until vascularization and ingrowth of the interstices has occurred. To date this solution appears to be superior to balanced salt solution used in a similar fashion.

Evaluation of resuscitation regimens has continued throughout the year 1972 and approximately 50 acutely burned patients have been resuscitated using 2-3 cc per kg per per cent burn of a sodium containing solution usually of a concentration of 130 mEq of sodium per liter in the first 24 hours. Blood volume is measured at the end of the first 24 hours post burn and any measured deficit is corrected with a colloid solution, most often plasmanate. Colloid containing solutions have not been administered in the first 24 hours since studies at this Institute indicate that colloid solutions are no more effective than crystalloid solutions in the first 24 hours and apparently leak through the capillary defect with equal ease. However with the repair of the capillary defect occurring during the second 24 hours post burn, colloid given in the second 24 hours post burn appears to be more effective in restoring volume than crystalloid. After 48 hours the fluid administered is primarily dextrose 5% in water to replace evaporative losses and supplementation with potassium maintains normal serum potassium. The volumes of water administered in children have been decreased and they also are resuscitated along isotonic guidelines to avoid rapid shifts in serum sodium, cerebral edema and an untoward weight gain.

In 1972 approximately 60 patients were given 250 (range from 1-13) intramuscular administrations of subanesthetic doses of ketamine for Hubbard tank debridement and dressing changes. A dose of 1.5 mgs per kilogram (15% of the usual anesthetic dose) was selected as an effective analgesic dose. Useful analgesia lasted about 14 minutes and recovery and mental orientation occurred approximately 20 minutes after administration of the drug. Tolerance was observed in virtually all patients receiving more than two exposures, however, for a brief but potentially painful procedure administration of an intramuscular subanesthetic dose of ketamine has proven to be a valuable technique with minimal complications or interference with oral alimentation.

Human growth hormone was administered to 7 burned hypermetabolic patients in 1972 who were also receiving calories and nitrogen to meet their energy demands. No local reaction or anaphylaxis was seen with the administration of this hormone. A reduction of nitrogen excretion occurred in 7 of the 8 study periods with the administration of human growth hormone, 10 I.U. per day for 7 days. In the six patients who demonstrated protein sparing, insulin production appeared to be increased. This effect appeared to be dose related and to require nutrient loading to augment the insulin response.

A study of coagulation abnormalities in thermally injured soldiers was completed with the inclusion of 50 patients whose coagulation factors were serially assayed. The previous findings of marked elevation in fibrinogen and a marked platelet concentration elevation at the 10th post burn day were confirmed. In addition, five patients who demonstrated disseminated intravascular coagulation was marked not only by a thrombocytopenia but by a precipitous fall in the fibrinogen concentration and elevation in fibrin split product titers as determined by the Staphylococcal clumping technique. Depression of the platelet count was also seen in those patients who had invasive infection either bacterial or mycotic, however this thrombocytopenia may not be indicative of disseminated intravascular coagulation unless there is also a concurrent precipitous fall in the fibrinogen concentration and a rise in the fibrin split product titers.

EDUCATION

A prime responsibility of the Institute of Surgical Research has historically been one of education of the military and civilian medical communities in the care of the thermally injured patient. 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, 3 surgical residents from Brooke General Hospital, 3 surgical residents from Fitzsimons

General Hospital and 1 each from William Beaumont, Walter Reed and the Santa Rosa Medical Center in San Antonio, Texas participated as active members of the medical staff for 2-3 month periods as part of their surgical training program. In addition, six medical officers from the U.S. Army Hospital Okinawa, (Far East Burn Center), 3 civilian physicians, 1 US Navy physician, 3 allied officers, 3 reserve officers on annual active duty training, 2 medical students and 2 active duty clinical clerks had from 2 to 6 week assignments with this Institute. Finally, approximately 36 civilians and 123 military physicians, students and paramedical personnel visited this Institute in 1972. Thirty-eight foreign visitors from the following countries: Sweden, France, England, Germany, Brazil, Portugal, Japan, Canada and Australia received extensive briefings on the current status of care of burn patients in general and the Institute of Surgical Research in particular.

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

STATISTICAL RESUME

During the year 1972, 300 thermally injured patients were admitted to the Institute of Surgical Research, 25 (8%) of whom were evacuated from the Republic of Vietnam. There were 301 dispositions during 1972 and the subsequent data will be based on those dispositions. The patients ranged in age from 6 months to 93 years and included 240 males and 61 females. The average age of the patient was 25.8 years with an average total burn of 34.2% (17.5% full thickness injury). The average burn index was 25.6%. Of the 301 dispositions, 235 had full thickness injury (78%). Sixty-five patients were under the age of 15 with an average age of 5.1 years. The average total burn in this pediatric age group was 34% with 19.9% full thickness injury (burn index 26.2). Of the 65 pediatric patients admitted, 57 (87.7%) had some full thickness injury. The overall mortality for the year 1972 was 34.3% or 103 deaths of the 301 dispositions as compared with an overall mortality of 22.6% in 1971. The average age of those patients dying was 28.9 years with an average total burn of 56.7% (36.3% third degree) and an average burn index of 46%. Of the 103 deaths one (1%) was evacuated from the Republic of Vietnam as compared with 102 from the Continental United States. This increase in crude mortality figures most likely results from a shift in emphasis from those patients who are injured in Vietnam and evacuated to this Institute after stabilization to those patients who are injured in the Continental United States and in general are evacuated to the Institute of Surgical Research earlier with more severe and extensive injury. In addition, of the 103 patients who died, 23 (22.3%) were in the pediatric age group. Autopsies were performed on 89 of the 103 patients for an 86.4% postmortem examination rate. The average post burn

day of death was 16.4 days.

Table 1 identifies the source of admission of patients during calendar year 1972. The major area shifted from the Republic of Vietnam and the Far East as in previous years to the Continental United States. Table 2 summarizes the mode of injury in patients from the Continental United States in the calendar year 1972. Table 3 illustrates the effect of age and total body surface burn on mortality. Mortality of burns greater than 60% remains high with only two patients surviving with that degree of injury.

The mortality rates in increments of 10% total body surface burn for the years 1969 through 1972 are tabulated in Table 4. Comparison with 1969 reveals an increase in the mortality for 30-50% total body burn groups.

The survival and mortality data for patients with greater than 30% (1955-1972) are presented in Table 5. No striking change was noted in 1972.

In Table 6 a comparison is presented of burn mortality rates in the pre-Sulfamylon years 1962-63 and the cummulative experience since 1964 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 1972 was 47 hospital days and when convalescent leave was excluded it was 40 hospital days. The average post burn day of admission of all patients to the Institute of Surgical Research was the 7th post burn day. This figure reflects a decrease in average post burn day of admission from 11.2 in 1970 to 9 days in 1971 and 7 days in 1972 which again emphasizes the fact that the current patient population is being admitted earlier since they originate in the Continental United States.

During the year 1972 2,668 operations were performed on 268 patients, an average of 9 operations per patient. There were 602 operations on 191 patients which required a general anesthetic for an average of 2 per patient. A total of 2,057 ward procedures were performed which required no general anesthesia. One hundred and forty-five patients required 363 autograft procedures. In addition, 135 patients had 896 cadaver allograft applications for an average of 3 per patient while 125 patients required 784 porcine xenograft applications for an average of 2.5 per patient. Cadaver homograft was aseptically harvested from 70 donors.

Escharotomies were performed in 75 patients, 25% of 301 dispositions. Thirty-eight patients (13%) required an amputation which includes 31

Table 1. Source of Admission, 1972

Area	A	AD	AF	AFD	N	ND	VAB	Other	TOTAL
1st Army	4	1	0	1	2	2	0	2	12
3rd Army	7	9	2	5	6	3	5	11	48
5th Army	35	25	5	10	4	3	36	46	164
6th Army	1	1	2	2	3	0	5	3	17
MDW	1	0	0	0	0	0	0	0	1
Viet Nam	27	0	0	0	3	0	0	0	30
Germany	5	1	0	1	0	0	0	1	8
Korea	2	0	0	0	0	0	0	0	2
Alaska	1	0	0	1	0	0	1	1	4
Japan	0	0	1	1	0	1	0	0	3
Canal Zone	1	0	0	0	0	0	0	0	1
Mexico	0	0	0	0	0	0	0	2	2
Okinawa	1	0	0	0	5	0	0	0	6
Guam	0	0	0	0	1	0	0	0	1
Thailand	0	0	1	0	0	0	0	0	1
San Salvador	0	0	0	0	0	0	0	1	1
	85	37	11	21	24	9	47	67	301

A - Army
AF - Air Force
D - Dependent
N - Navy, Marine Corps & US Coast Guard
VAB - Veterans Administration Beneficiary
MDW - Military District of Washington
Other: Civilian Emergency (33)
Designee of Secretary of Army (25)
US Public Health Service Beneficiary (8)
Bureau of Employees' Compensation Beneficiary (1)

Table 2. Burn Etiology, 1972 - 301 Dispositions

Causes	Number of Patients	% Disposition	Deaths	% Mortality
Gasoline	74	26%	21	28%
Structural Fires	20	7%	11	55%
Motor Vehicle Accidents	35	12%	17	49%
Aircraft Accidents	21	7%	5	24%
Open Flames	35	12%	13	37%
Electrical	26	9%	8	31%
Hot Liquid	28	9%	7	25%
Chemical	4	1%	0	0%
Others	28	9%	6	21%
Butane, Propane or Natural Gas Exp.	28	9%	13	46%
Welding Accidents	2	0.7%	2	100%
TOTAL	301		103	

Table 3. Age, Body Surface Involvement & Mortality, 1971

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	1	1	1	0	0	0	0	0	0	4	0	0
1-2	3	5	1(1)	2(1)	0	2(1)	0	1(1)	1(1)	0	15	5	33.3
2-3	2	2	1	2(2)	2(2)	0	2(2)	0	0	0	11	6	54.5
3-4	1	0	1	1	0	3(2)	0	0	0	0	6	2	33.3
4-5	0	0	0	0	1	0	0	1(1)	0	0	2	1	50.0
5-10	0	2	3	3(2)	1	0	1(1)	0	0	0	10	3	30.0
10-15	3	2	0	3	2(1)	2	3(3)	1(1)	1(1)	0	17	6	35.3
15-20	2	3	8(2)	4(1)	4	3(3)	2(2)	2(1)	2(2)	1(1)	31	12	38.7
20-30	23	23(1)	16(1)	15(3)	14(3)	10(4)	6(6)	3(3)	2(2)	3(3)	115	26	22.6
30-40	2	6	3	6(1)	2	1(1)	3(3)	4(4)	2(2)	1(1)	30	12	40.0
40-50	5(1)	4	5(1)	4(2)	3(3)	0	4(3)	1(1)	2(2)	0	28	13	46.4
50-60	3	5	3(1)	0	4(4)	2(2)	0	2(2)	0	0	19	9	47.3
60-70	1	2	1(1)	1(1)	3(2)	0	0	0	0	0	8	4	50.0
70-80	0	1(1)	0	0	0	0	1(1)	0	1(1)	0	3	3	100.0
80-90	0	0	0	0	0	0	0	1(1)	0	0	1	1	100.0
90-100	1	0	0	0	0	0	0	0	0	0	1	0	0
Total	47	56	43	42	36	23	22	16	11	5	301		
Deaths	1	2	7	13	15	13	21	15	11	5		103	
% Mortality	2.9	3.6	16.3	31	41.7	56.5	95.4	93.8	100	100			34.2

Note: Deaths shown in parentheses.

Table 4. Per Cent Body Surface Involvement and Mortality, 1969 - 1972

% Burn	0-10	10-20	20-30	30-40	40-50	50-60	60-70	70-80	80-90	90-100	Total
					(1969)						
No. Burned	27	45	56	55	56	26	20	14	6	4	309
Deaths	0	1	1	10	11	14	11	12	6	4	70
% Mortality	0	2.2	1.8	18.2	19.6	53.8	55	85.7	100	100	22.7
					(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	65	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

Table 5. Per Cent Burn Versus Survival, 1955-1972

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

Table 6. Comparison of Burn Mortality Rates, 1962-1963 and 1964-1972

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
1964-72	1405	34	2.4	405	61	15.1	321	87	27.1	187	84	44.9	314	263	83.8

patients with major amputations. Disarticulation of phalanges was the most frequent amputation and was carried out on 17 patients.

Tracheostomy was performed on 60 patients (20%) for specific indications such as prolonged ventilator support or upper airway obstruction. One thousand one hundred and ninety-three blood cultures were drawn from 205 patients with a yield of 391 positive blood cultures. Thirty-nine patients had exploration of previous cutdown sites for a suspected thrombophlebitis which was subsequently proven by clinical, bacteriological, or microscopic evidence in 16 patients for an overall incidence of suppurative thrombophlebitis of 5.5%.

A total of 869,855 cc of blood was administered in 171 patients. Two hundred and ten or 70% of the patients had a cutdown or intravenous line established percutaneously.

Topical Sulfamylon acetate was utilized to control the burn wound flora in 292 patients (97% of dispositions) and silver nitrate in 20 patients (6.5%). A hypersensitivity of varying degree was demonstrated in 20 patients (6.5%) receiving Sulfamylon and usually responded to antihistamine therapy.

One hundred patients (33%) had some type of associated injury on admission. In addition, 7% (21 patients) had chondritis of 22 ears, and 17 patients (5.7%) underwent chondrectomy. Sixty patients (20%) had gastrointestinal bleeding of some type and in 54 patients (18%) Curling's ulcers were diagnosed. Seventeen patients during calendar year 1972 underwent celiotomy and appropriate operation for massive upper GI bleeding or perforation. Eleven patients had a diagnosis of either acute or chronic cholecystitis at postmortem examination, however in no patient was it a cause of death. Thirty-one patients (10%) had a chemical diagnosis of acute pancreatitis and in 30 of these patients this diagnosis was confirmed by postmortem examination. Adrenal hemorrhage was found at postmortem examination in 4 patients and a ruptured spleen in 1 patient. Clinical acute renal failure occurred in 25 (8.5%) of admissions and acute tubular necrosis was histologically documented in 26 (8.5%) of admissions. Hemolydialysis was performed in 7 patients.

Of interest acute myocardial infarction occurred in 7 patients, acute bacterial endocarditis in 9 and congestive heart failure in 5.

Twenty patients were admitted with fractures, one patient developed roentgenographic and clinical evidence of septic arthritis of the glenohumeral joint which was surgically drained and healed without complication. Only one patient developed ectopic calcification about the elbow in contrast to previous years' experience when 6 patients developed such heterotopic calcifications.

Pneumonitis has continued to be a significant problem in 1972. Pneumonia was diagnosed in 94 patients (31%) and inhalation injury was present in 51 patients (17%). In addition, viral pneumonia was diagnosed in two patients, atelectasis in 31 patients (10%), lung abscesses in 11 patients, pulmonary emboli in 19 patients and 92 patients (30%) required mechanical ventilatory support. In addition bacterial or mycotic burn wound sepsis was diagnosed histologically in 48 patients (16%), burn wound cellulitis in 19 patients and septicemia was diagnosed in 97 patients (32%).

SUMMARY

Three hundred patients with thermal injury were admitted to the Clinical Division of the United States Army Institute of Surgical Research during calendar year 1972. Newer modalities of clinical therapy that were evaluated during the year were ketamine analgesia for limited debridement, use of Sulfamylon-Mycostatin cream, 5% aqueous Sulfamylon dressings, continued evaluation of 133Xenon scan for the detection of inhalation injury and continued evaluation of porcine xenograft as a temporary biologic dressing. In addition, evaluation of the metabolic requirements of the thermally injured, effectiveness of Intralipid as a caloric source, serial measurements of pulmonary function and serial measurements of alteration in the coagulation mechanism were studied and have provided a basis for modification and improvement in the management of burn patients.

Again during 1972 the responsibility of the Institute of Surgical Research of education of the military and civilian medical community in the care of the thermally injured patient has been carried out by multiple presentations, publications and participation in medical seminars, in-house training and education of house officers, staff physicians, foreign physicians and paramedical personnel by the staff of this Institute.

Comparisons of mortality data since 1969 has revealed a continued increase in mortality in the 30 to 60% burn group, in addition, the decrease from an average post burn day of admission of 11.2 in 1970 to 7th post burn day in 1972 suggests that the patient population is admitted earlier since they originate primarily in the Continental United States.

The senior author wishes to express his appreciation and gratitude to the entire staff of the Clinical Division of the Institute of Surgical Research who are responsible for the care of severely burned patients, the investigative efforts and the compilation of data that are summarized in this report. This clinical and research ability, devotion to duty, enthusiasm and dedication have made the successful completion of the mission of this unit possible.

Table 7. Causes of Death, 1972

Patient	Age	Sex	% Burn Total	% Burn 3°	PBD Death	Cause of Death
1	23	M	93½	55	9	Pulmonary emboli; Thromboses right subclavian and iliac veins
2	21	M	92	57	0	Cause of death uncertain
3	30	M	91	66	2	Cerebral edema with uncal and tonsillar herniation
4	25	M	90½	61½	2	Pulmonary edema and congestion
5	18	M	90	90	26	Bronchopneumonia (Proteus, Providence); invasive burn wound sepsis (bacterial and mycotic); septicemia (Providence, Pseudomonas)
6	43	F	89	85	2	Acute respiratory obstruction, bronchial
7	36	M	86	77½	4	Acute pulmonary edema; severe inhalation injury
8	46	M	86	15	1	*Cause of death uncertain
9	28	M	85	85	42	Massive aspiration with resultant respiratory and cardiac arrest
10	75	F	85	85	2	Bronchopneumonia (Providence); acute pulmonary edema
11	22	F	85	83	7	Cerebral edema; bilateral Klebsiella pneumonia; septicemia (Klebsiella)
12	1-7/12	M	83½	83½	6	Bronchopneumonia (Klebsiella, Providence, E. coli); invasive (bacterial and mycotic) burn wound sepsis (Providence)
13	17	M	83	45	5	Bronchopneumonia (E. coli, Providence); gram-negative endotoxemia (Klebsiella, Providence)
14	39	M	81	48	5	Myocardial infarction, acute
15	10	M	80	70	3	Gram-negative endotoxemia (Providence, E. coli); acute bilateral interstitial pneumonitis
16	17	F	80	26	7	Bronchopneumonia, acute (Providence, Pseudomonas); septicemia

*Autopsy not performed

Table 7. Causes of Death, 1972

Patient	Age	Sex	% Burn Total	% Burn 3°	PBD Death	Cause of Death
17	1-5/12	F	79	64	11	*Bronchopneumonia, acute, bilateral; septicemia (Providence)
18	12	F	78½	78½	13	Invasive burn wound sepsis (Providence); septicemia (Providence); Bronchopneumonia (Providence, Pseudomonas, Staphylococcus, D. Strep)
19	30	M	78	78	2	Endotoxin shock (E. coli) secondary to infarction and necrosis of intestine
20	56	M	78	57	12	Bronchopneumonia (Providence, Pseudomonas); pulmonary edema
21	4	F	77	33	1	Inhalation injury with acute tracheal obstruction
22	34	M	76	28	9	Bacterial and mycotic burn wound invasion (Pseudomonas, Fusarium); septicemia (Klebsiella, Providence); inhalation injury with pneumonitis (klebsiella, Providence)
23	25	M	76	26½	13	Inhalation injury with necrotizing tracheobronchitis and bronchopneumonia (Providence, Pseudomonas)
24	25	M	75½	57	55	Disseminated visceral mycosis (Candida); acute bacterial endocarditis (Providence, Klebsiella, Enterobacter)
25	53	M	75	70	5	Acute myocardial infarction; gram-negative septicemia (E. coli); hemorrhagic necrotizing enterocolitis; bronchopneumonia (E. coli)
26	31	M	74½	47	135	Gram-negative septicemia (Enterobacter cloacae, Proteus); small bowel infarction secondary to superior mesenteric artery occlusion; generalized peritonitis
27	87	F	73	57	1	*Hypovolemic shock
28	39	M	73	49	7	Lobar pneumonia (Klebsiella, Providence, Pseudomonas)
29	42	F	73	41	10	Septicemic shock, gram-negative (Pseudomonas, Providence); mixed bacterial invasive burn wound infection (Pseudomonas, Proteus); bronchopneumonia, acute (Pseudomonas, Providence)

*Autopsy not performed

Table 7. Causes of Death, 1972

Patient	Age	Sex	% Burn Total	% Burn 3°	PBD Death	Cause of Death
30	27	M	71½	42½	21	*Duodenal ulcer with massive hemorrhage and perforation; bronchopneumonia (Providence, Enterobacter); Gram-negative septicemia (Providence, Serratia, Pseudomonas)
31	19	M	71½	24½	7	Hypokalemic cardiac arrest; inhalation injury; bronchopneumonia (Pseudomonas, Providence)
32	16	M	69	43	13	Bronchopneumonia (Staph aureus, Providence, Klebsiella); invasive burn wound sepsis (Pseudomonas)
33	42	M	69	40	10	Pulmonary edema and hemorrhage; burn wound sepsis (Pseudomonas, Aspergillus)
34	22	F	68	1	24	Invasive burn wound sepsis (Candida and Providence); gram-negative septicemia (Providence); ischemic infarcts of CNS
35	44	M	67	16	11	Bronchopneumonia (Klebsiella, E. Coli, Providence); septicemia, gram-negative (Klebsiella, E. coli)
36	26	M	66	45	5	No definite cause
37	2	M	66	0	1	Bronchopneumonia (Klebsiella); acute tubular necrosis; cerebral edema
38	35	F	65½	18½	10	Bronchopneumonia (Providence, Pseudomonas)
39	27	M	65	54½	8	*Bacterial bronchopneumonia (no culture); septicemia (Klebsiella)
40	38	M	63½	7	6	Bronchopneumonia (Providence); gram-negative septicemia (Providence); inhalation injury
41	11	M	63	40	9	Bronchopneumonia (Enterobacter, Klebsiella); invasive mycotic burn wound sepsis
42	43	M	63	17½	9	Septic shock (Staphylococcus, Providence, E. coli) source undetermined
43	25	M	62½	47	14	Bronchopneumonia (Providence, Pseudomonas); inhalation injury; septicemia (Providence)

*Autopsy not performed

Table 7. Causes of Death, 1972

Patient	Age	Sex	% Burn Total	% Burn 3°	PBD Death	Cause of Death
44	7	F	62½	45	20	Septicemia (Staphylococcus aureus, Providence); bronchopneumonia (Klebsiella, Pseudomonas, Providence)
45	10	M	62	62	3	Pulmonary edema; hypovolemic shock
46	2	M	62	32	4	Septicemia (Providence, Staphylococcus); duodenal ulcer with hemorrhage
47	23	M	61½	19	38	Gram-negative sepsis (Providence); bronchopneumonia (Providence, Pseudomonas, Proteus, Klebsiella); gastric and duodenal ulcers with massive hemorrhage
48	19	M	61	30	0	Inhalation injury and pulmonary edema with bronchiolar obstruction
49	20	M	60½	60	5	Inhalation injury; oculorhinocerebral phycomycosis
50	31	F	60½	43½	42	Perforated gastric ulcer with peritonitis (Candida); septicemia (Salmonella typhimurium, Providencia); ruptured splenic infarct and hepatic fracture; bacterial invasion of burn wound (Pseudomonas, Providence)
51	70	M	60	48	10	Bronchopneumonia (E. coli, Enterobacter cloacae); mycotic burn wound invasion (Alternaria sp.)
52	13	M	60	42	8	Bronchopneumonia (Providence, Staphylococcus)
53	21	M	57½	55	10	Bronchopneumonia (Klebsiella)
54	18	M	56	54	32	Septicemia (Providence, Pseudomonas); Bronchopneumonia (Providence, Pseudomonas) Tracheobronchitis with tracheoesophageal fistula
55	54	M	56	2	10	Bronchopneumonia (Providence, Pseudomonas); Disseminated intravascular coagulation with pulmonary, cerebral and renal infarcts.
56	35	M	55	55	4	Inhalation injury; acute myocardial ischemia; pulmonary edema secondary to congestive heart failure
57	20	M	55	53½	55	Bronchopneumonia (Proteus); septicemia, gram-negative (Proteus, E. Coli)

*Autopsy not performed

Table 7. Causes of Death, 1972

Patient	Age	Sex	% Burn Total	% Burn 3°	PBD Death	Cause of Death
58	18	M	55	36½	20	Bronchopneumonia (Klebsiella, Providence); multiple Curling's ulcers stomach and duodenum with massive hemorrhage; Gram-negative septicemia (Providence, Pseudomonas)
59	1-4/12	M	55	22	16	Pseudomonas burn wound sepsis; septicemia (Pseudomonas); disseminated intravascular coagulation
60	17	M	54	54	27	Bronchopneumonia (Klebsiella); septicemia gram-negative (Klebsiella)
61	27	M	53	6½	7	Bronchopneumonia (Staphylococcus, Pseudomonas)
62	52	M	52	42	21	Bronchopneumonia (Pseudomonas, Providence); adrenal necrosis
63	3	M	51½	33½	5	Bronchopneumonia (Klebsiella, E. coli, Providence, Pseudomonas); gram-negative septicemia (Pseudomonas)
64	12	M	50	35	4	Pulmonary edema secondary to iatrogenic fluid overload
65	3	F	50	35	3	*Bronchopneumonia
66	23	M	48½	0	43	Bronchopneumonia (E. coli); septicemia, gram-negative (E. coli); massive upper gastrointestinal hemorrhage
67	22	M	48	42	60	Gram-negative septicemia (Providence, Pseudomonas, Proteus); massive hepatic necrosis
68	23	M	47½	21	15	Bronchopneumonia (Klebsiella, Providence); septicemia, gram-negative (Klebsiella, Providence)
69	2-6/12	M	47½	13	16	*Septicemia (Staphylococcus); mycotic burn wound sepsis (Aspergillus); bacterial burn wound sepsis (Pseudomonas)
70	43	M	46½	0	3	Bronchopneumonia (Klebsiella); acute myocardial infarction
71	51	M	46	27½	29	*Bronchopneumonia (Klebsiella, Serratia, Staphylococcus); septicemia, gram-negative (Providence, Serratia, Proteus)

*Autopsy not performed

Table 7. Causes of Death, 1972

Patient	Age	Sex	% Burn Total	% Burn 3°	PBD Death	Cause of Death
72	55	M	45½	42	18	Hemorrhage, massive, intra-abdominal and retroperitoneal; septicemia, Staphylococcus, Corynebacterium; bronchopneumonia (Providence, Proteus)
73	51	M	44	38	4	Massive retroperitoneal hemorrhage from perforation left external iliac artery
74	56	M	44	33	2	Acute myocardial infarction and pulmonary edema
75	2-10/12	M	43½	31½	29	Gram-negative septicemia (Providence); perforated Curling's ulcer with resultant peritonitis (Providencia); mycotic burn wound invasion (Fusarium)
76	64	M	40½	17	11	Gram-negative septicemia (Pseudomonas, Providence); pulmonary edema and alveolar hemorrhage
77	65	F	40	40	29	*Gram-negative septicemia (E. coli, Proteus, Pseudomonas); bronchopneumonia (Proteus, Pseudomonas, Klebsiella)
78	46	M	40	32	18	Bronchopneumonia (Pseudomonas, Providence, Proteus); septicemia, gram-negative (Proteus); suppurative thrombophlebitis (Proteus, Enterobacter)
79	41	M	40	11½	15	Bronchopneumonia (Staphylococcus, Providence); severe inhalation injury; disseminated intravascular coagulation
80	23	F	40	0	13	Staphylococcal acute bacterial endocarditis; staphylococcal septicemia; bronchopneumonia (Staphylococcus)
81	5	F	39	39	4	Cerebral edema with uncal and tonsillar herniation
82	5	F	38½	32½	9	Staphylococcal septicemia; staphylococcal meningoencephalitis
83	29	M	35	35	11	Gram-negative septicemia (Providence); necrotizing hemorrhagic enterocolitis; invasive wound sepsis (Providence, Pseudomonas)
84	2	F	35	26½	13	*Bronchopneumonia; gram-negative septicemia (Pseudomonas)
85	30	F	34½	0	3	Acute pulmonary edema; inhalation injury
86	44	F	33	32	29	Pulmonary embolus, massive

*Autopsy was not performed

Table 7. Causes of Death, 1972

Patient	Age	Sex	% Burn Total	% Burn 3°	PBD Death	Cause of Death
87	66	F	33	5	1	Perforated gastric ulcer with massive hemorrhage; inhalation injury
88	21	M	32	18	14	Bronchopneumonia (Pseudomonas) bilateral adrenal hemorrhagic necrosis; gram-negative septicemia (Providence)
89	17	F	31½	22½	21	Bronchopneumonia (Providence, Pseudomonas)
90	2	M	31½	0	34	Pseudomonas invasive burn wound sepsis
91	1	M	31	29	23	Invasive mycotic burn wound infection (Cephalosporium) with dissemination
92	41	M	30	24	42	Acute coronary occlusion
93	24	M	30	15	6	Massive blast injury
94	19	M	29	6	19	Inhalation injury; bronchopneumonia (Pseudomonas)
95	1-4/12	F	25	12½	15	Invasive burn wound sepsis (Pseudomonas); septicemia, gram-negative (Pseudomonas, Providence)
96	26	M	24	24	4	Bronchopneumonia (Aeromonas, Klebsiella, Providence) streptococcal fascitis; gram-negative septicemia (Aeromonas)
97	19	M	24	12	8	*Acute tubular necrosis secondary to hemochromogens after electrical injury
98	68	M	23	23	28	*Bronchopneumonia (Klebsiella); thrombophlebitis, suppurative (Enterobacter, Staphylococcus, Streptococcus)
99	58	M	22½	21½	36	Bronchopneumonia (Providence, Pseudomonas); septicemia (Providence, Pseudomonas
100	46	M	22½	7	1	*Acute pulmonary aspiration
101	23	M	14½	14½	22	Bronchopneumonia (Pseudomonas, Providence)
102	75	M	10	6	54	*Pulmonary embolus

*Autopsy was not performed

Table 7. Causes of Death, 1972

Patient	Age	Sex	% Burn Total	% Burn 3°	PBD Death	Cause of Death
103	41	M	9½	6	90	Bronchopneumonia (Enterobacter, Providence, Proteus, Pseudomonas); septicemia, gram-negative, Serratia; acute pulmonary edema

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PRESENTATIONS

Silverstein P: Enzymatic Debridement of Burn Wound Eschar. Flint Laboratories Symp. Palm Springs, CA, 7 Jan 72.

Pruitt BA Jr: (1) Recommended Procedures for Prevention of Cross-Infection from Septic Cases--The Burn Patient, Special Consideration; (2) Special Laboratory Support Considerations in the Treatment of Burns. Third Symp. on Control of Surgical Infections, ACS, Wash DC 10, 11 Jan 72.

Wilmore DW: Parenteral Nutrition: Indications, Applications and Pitfalls. Portland Surgical Society Mtg, Portland, Ore, 13 Jan 72.

Pruitt BA Jr: (1) Fungal Infections in Surgical Patients; (2) Shock Associated with Thermal Burn. Member, Panel on Surgical Infections

and Panel on Trauma. ACS Sectional Mtg, Miami, FLA 17-19 Jan 72.

Rogers PW: Hypercalcemia: Cause and Therapy. Clinical Nephrology Conf, Brooke Gen Hosp, BAMC, FSHT, 19 Jan 72.

Wilmore DW: Recommendations for Evaluation of Patients. AMA Symp on Total Parenteral Nutrition, Nashville, Tenn, 19 Jan 72.

The following presentations were made to the Global Med Course, USAF Sch of Aerospace Med, Brooks AFB, TX, 20 Jan 72:
Inge WW Jr: Topical Therapy
McManus WF: Complications of Burns
Moylan JA Jr: Fluid Resuscitation and Initial Care
Silverstein P: Biologic Dressings in Burn Wound Care

Allen GW: Anatomy of the Lung. Anesthesia Conf, BGH, BAMC, FSHT 22 Jan 72.

The following presentations were made to the Symposium on Military Plastic Surgery, WRAIR, WRAMC, 24-26 Jan 72:
Pruitt BA Jr: Burn Sepsis
Silverstein P: Treatment of Eyelid Deformities due to Thermal Injury.

McGranahan BG: Staff Development. ANC Chief Nurse Course, MFSS, BAMC, FSHT 26 Jan 72.

Salisbury RE: The evaluation of Orenzyme in the Prevention of Swelling after Hand Surgery. American Soc for Surgery of the Hand Mtg, Wash DC 27 Jan 72.

Johns LA: Psychological Problems of the Burn Patient. Clinical Specialist Course, BGH, BAMC, FSHT. 28 Jan 72

McGranahan BG: Nursing Care of the Burn Patient. Flight Nurses, Sch of Aerospace Med, Brooks AFB, TX 28 Jan 72.

The following presentations were made to Physical Therapy Students, MFSS, BAMC, Fort Sam Houston, TX, 28 Jan 72:
McGranahan BG: Nursing Care of the Burn Patient
Reardon JC: Occupational Therapy for the Burn Patient
O'Brien WJ III: Physical Therapy for the Burn Patient
McManus WF: Medical Management of the Burn Patient

Rogers PW: Bilateral Renal Artery Stenosis. Univ of Texas Med Sch at San Antonio Dept of Med Combined Conf, San Antonio, TX 2 Feb 72.

Silverstein P: Treatment of Burns. Off Basic Course, MFSS, BAMC Fort Sam Houston, TX, 11 Feb 72.

Silverstein P: Trends in Biological Wound Dressings. Univ of Texas Med Sch at San Antonio, Dept of Surg Conf, San Antonio, TX 11 Feb 72

Morris AH: Lung Function Following Nonpulmonary Burns. Washington Univ Sch of Med Staff, St. Louis, Missouri 11 Feb 72.

Hunt JL: The Treatment of Mass Casualties in Thermonuclear Warfare. Medical Aspects of Advanced Warfare Course, Sch of Aerospace Med, Brooks AFB, TX 14 Feb 72.

Pruitt BA Jr: Member, Panel on Infection. Presentation: Epidemiology of Pseudomonas Infections. ACS Sectional Mtg, St Louis, MO 14-16 Feb 72.

Morris AH: Lung Function Following Nonpulmonary Burns. Univ of Colorado Sch of Med Staff, Denver, Colorado 15 Feb 72.

Morris AH: Systems Response Considerations in the Measurement of Dynamic Mechanical Properties of the Lung. Univ of Colorado Sch of Med Staff, Denver, Colorado 16 Feb 72.

Johns LA: (1) Nursing Research in the Army Nurse Corps; (2) Use of Problem-Solving Techniques. MFSS Clinical Head Nurse Course, San Antonio, Texas 16 Feb 72.

Morris AH: Lung Function Following Nonpulmonary Burns. Univ of Arizona Sch of Med Staff, Tucson, Arizona 18 Feb 72.

Morris AH: Systems Response Considerations in the Measurement of Dynamic Mechanical Properties of the Lung. Univ of Ariz Sch of Med Staff, Tucson, Arizona 19 Feb 72.

Rogers PW: Evaluation of Urinary Sediment. BGH Pediatric Residents, BGH, BAMC, Fort Sam Houston, TX 23 Feb 72.

Morris AH: Lung Function Following Nonpulmonary Burns. Univ of New Mexico Sch of Med, Albuquerque, New Mexico, 24 Feb 72.

Johns LA: Emergency Treatment of the Burn Patient. Emergency Medical Technicians (Ambulance), Sch of Paramedical Training, Bexar County Hospital District, San Antonio, Texas 1 Mar 72.

Slogoff S: Basic Respiratory Physiology. Dept of Obstetrics & Gynecology, BGH, BAMC, Fort Sam Houston, Texas 1 Mar 72.

Rogers PW: Idiopathic Nephrotic Syndrome. Renal Conf, Univ of Tex Med Sch at San Antonio, Texas 1 Mar 72.

Wilmore DW: Nutrition in the Surgical Patient. Dept of Surg, Univ of Utah Sch of Med, Salt Lake City, Utah 2 Mar 72.

The following presentations were given to the Brooke General Hospital-Univ of Texas Medical School at San Antonio Symp on Surgical & Orthopaedic Aspects of Trauma, San Antonio, Texas 6 Mar 72:

Inge WW Jr: Frequent Life-Threatening Complications of the Burn Patient

Moylan JA Jr: Early Care of the Burn Patient

Silverstein P: Coverage of the Burn Wound

Wilmore DW: Nutrition of the Injured Patient

Johns LA: Nursing and the Chaplain. Clinical Pastoral Education for Chaplains Course, BGH, BAMC, Fort Sam Houston, Texas 7 Mar 72.

Pruitt BA Jr: Moderator, Panel on Burn Care. Presentation: Fluid Resuscitation of the Burn Patient. Surgical and Orthopaedic Aspects of Trauma, BGH, BAMC, Fort Sam Houston, Texas 6-10 Mar 72

Peche M: Nursing Care of the Burn Patient. Flight Nurses and Medical Technicians, Sch of Aerospace Med, Brooks AFB, TX 14 Mar 72.

Johns LA: Is Gastric Emptying Circadian? 8th Annual Research Conf, American Nurses' Association, Albuquerque, New Mexico 16 Mar 72.

Johns LA: Nursing Care of the Burn Patient. Inservice Program, Bergstrom AFB & Univ of Tex Continuing Edu Prog, Austin, TX 20 Mar 72.

Wilmore DW: Parenteral Nutrition. Dietetic Staff, BGH, BAMC, Fort Sam Houston, TX 22 Mar 72.

Slogoff S: Ketamine Usage in Burn Care. Dept of Anesthesia, Jefferson Medical College, Philadelphia, Pa. 28 Mar 72.

Morris AH: Lung Function Following Severe Nonpulmonary Burn. Pulmonary Conf, Univ of Mich Sch of Med, Ann Arbor, Mich 28 Mar 72.

Warden GD: Biological Dressings. Surgical Grand Rounds, Univ of Utah Medical Center, Salt Lake City, Utah 5 Apr 72.

Warden GD: Fluid Resuscitation. Staff Univ of Utah Medical Center, Salt Lake City, Utah 5 Apr 72.

Warden GD: Treatment of Burns. Medical Students, Univ of Utah Medical Center, Salt Lake City, Utah 5 Apr 72.

The following presentations were made to the American Burn Assn Anl Mtg, San Francisco, California 7-8 Apr 72:

Pruitt BA Jr: Member of panel discussion on "Burn Resuscitation", and discussant of two papers.
Inge WW Jr: Herpetic Infection in the Burn Patient
Wilmore DW: Safety of Parenteral Fat Emulsion as a Caloric Source in Thermally Injured Patients
Morris AH: Lung Function in the Immediate Postburn Period
Silverstein P: Enzymatic Debridement of Burn Wound Eschar
Johns LA: The Hazards of Intravenous Therapy--A Continuing Challenge
Morris AH: Contamination of Inhalation Therapy Equipment in a Burn Unit.
Spitzer M: The Role of Nursing Service in Providing Caloric Support for Burn Patients.
Palm L: A Method for Splinting the Upper Extremity of Thermally Injured Patients.
O'Brien WJ III: Peripheral Neuropathy in the Thermally Injured Patient.

Pruitt BA Jr: Discussion of Stress Ulcers at Surgical Literature Conf, Univ of Texas Med Sch at San Antonio, San Antonio, TX 12 Apr 72.

Silverstein P: Recent Progress in Burn Therapy. Plastic Surg Dept Grand Rounds, Univ of Tex Med Sch at San Antonio, TX 19 Apr 72.

Wilmore DW: Treatment of Burns. MFSS Physician's Assistant Program students, USA ISR, BAMC, Fort Sam Houston, TX 19 Apr 72.

Hall WF: Physical Therapy in the Treatment of the Burn Patient. BGH Physical Therapy Dept, BGH, BAMC Fort Sam Houston, TX 20 Apr 72.

Inge WW Jr: Treatment of Burns. MFSS Physician's Assistant Program students, USA ISR, BAMC, Fort Sam Houston, TX 20 Apr 72.

Hunt JL: Treatment of Burns. MFSS Physician's Assistant Program students, USA ISR, BAMC, Fort Sam Houston, TX 21 Apr 72.

Silverstein P: Biologic Dressings, Dead or Alive. 3M Company, St. Paul, Minnesota, 25 Apr 72.

Welch GW: Treatment of Burns. MFSS Physician's Assistant Program students, USA ISR, BAMC, Fort Sam Houston, TX 26 Apr 72.

Warden GD: Treatment of Burns. MFSS Physician's Assistant Program students, USA ISR, BAMC, Fort Sam Houston, TX 27 Apr 72.

Silverstein P: Treatment of Burns. MFSS Physician's Assistant Program students, USA ISR, BAMC, Fort Sam Houston, TX 28 Apr 72.

Pruitt BA Jr: Co-author of presented paper and discussant of paper on "Infection in Burn Patients". American Surgical Assn Mtg, San Francisco, CA 25-28 Apr 72.

Slogoff S: Shock. Dept of Anesthesia, BGH, BAMC, Ft. Sam Houston, Texas 29 Apr 72.

McGranahan BG: Nursing Care of the Burn Patient. Incarnate Word College nursing students, San Antonio, Texas 3 May 72.

Salisbury RE: Treatment of Burns. MFSS Physician's Assistant Program students, USA ISR, BAMC, Fort Sam Houston, TX 3 May 72

Wilmore DW: Treatment of Burns. MFSS Physician's Assistant Program students, USA ISR, BAMC, Fort Sam Houston, TX 4 May 72.

The following presentations were made on a "Burn Treatment Panel" moderated by L. Palm, Texas State Occupational Therapy Assn Anl Conf, San Antonio, Texas 7 May 72:

Wilmore DW: Medical Management of the Burn Patient
Canfield C: Nursing Care of the Burn Patient
Reardon JC: Occupational Therapy for the Burn Patient
Loveless SD: Physical Therapy for the Burn Patient

Pruitt BA Jr: Synthetic Drape Materials. Symp of ACS Committee on Operating Room Environment, Wash DC 8 May 72.

McGranahan BG: Nursing Care of the Burn Patient. School of Aerospace Med Flight Nurses and Technicians, Brooks AFB TX 9 May 72.

Pruitt BA Jr: Member, Panel on Thermal Injuries; Presentations: (1) Initial Management of the Burned Patient; (2) Metabolic Sequelae of Trauma: (3) Post-traumatic Pulmonary Insufficiency. 16th Annual Postgraduate Course on Fractures and Other Trauma (Chic Comm on Trauma, and ACS), Chicago, IL. 10-13 May 72.

The following presentations were made at the 105th Annual Session, Texas Medical Association, San Antonio, Texas 12 May 72:

Inge WW Jr: Burns--New Concepts in Their Management.
Wilmore DW: Intravenous Hyperalimentation in the Surgical Patient.

Hunt JL: The Treatment of Thermal Injury. Staff USAH Fort Campbell, Kentucky 19 May 72.

The following presentations were made at the Kansas Association of Physical Therapists Mtg, Wichita, Kansas 20-21 May 72:

Inge WW Jr: (1) Initial Care of the Burn Patient; (2) Special Problems of the Burn Patient; (3) Complications of the Thermally Injured.

Canfield C: (1) Physical Plant of the USAISR; (2) Nursing Care of the Burn Patient; (3) Psycho-social Needs of the Burn Patient and His Family.

Loveless SD: (1) Initial Physical Therapy in Burn Patient Care; (2) Physical Therapy Goals and Functional Results in Rehabilitation of the Burn Patient.

Palm L: Role of the Occupational Therapist in Rehabilitation of the Burn Patient; (2) Fabrication of Splints.

Palm L and Loveless SD: Ongoing Research in OT and PT at USAISR.

Hunt JL: Nuclear Weapons Medical Effects-Thermal. Medical Aspects of Adv Warfare Course, Sch of Aerospace Med, Brooks AFB TX 23 May 72.

Pruitt BA Jr: Current Management of Thermal Injury. Annual Mtg of Puerto Rico Medical Assn, San Juan, PR 8 Jun 72.

Pruitt BA Jr: New Opportunistic Surgical Infections. Staff Conf, Dept of Surg, Univ of Puerto Rico Med Sch, San Juan, PR 8 Jun 72.

Canfield C: Nursing Care of the Burn Patient. Flight Nurses and Medical Technicians, Sch of Aerospace Med, Brooks AFB, TX 9 Jun 72.

Pruitt BA Jr: Autografting of Burn Wounds. Postgraduate Course, Care of Thermally Injured, Univ of Tex Southwestern Medical School, Dallas, TX 22-23 Jun 72.

Wilmore DW: Treatment of Burns. Off Adv Course, MFSS, BAMC, Fort Sam Houston, TX 27 Jun 72.

McManus WF: Mission and Function of the Institute of Surgical Research. BGH Interns, BGH, BAMC, Fort Sam Houston, TX 29 Jun 72.

Pruitt BA Jr: The Management of Chest Trauma in Burns. Intl College of Surgeons, Lake George, N.Y. 5-10 Jul 72.

Pruitt BA Jr: (1) Stress Ulcers; (2) Initial Treatment of Acute Burns. Northwestern Univ Med Sch Postgraduate Course, Chicago, IL 27 Jul 72.

McGranahan BG: Nursing Care of Burns. Baptist Memorial Hospital Nursing students, San Antonio, Texas 28 Jul 72.

Hunt JL: The Treatment of Burns. Off Basic Course, MFSS, BAMC, Fort Sam Houston, TX 2 Aug 72.

McManus WF: The Treatment of Burns. Off Basic Course, MFSS, BAMC, Fort Sam Houston, TX 3 Aug 72.

McGranahan BG: Nursing Care of Burns. Flight Nurses and Technicians, Sch of Aerospace Med, Brooks AFB, TX 25 Aug 72.

Salisbury RE: Treatment of Burns. Off Basic Course, MFSS, BAMC, Fort Sam Houston, TX 31 Aug 72.

Wilmore DW: Treatment of Burns. Off Basic Course, MFSS, BAMC, Ft Sam Houston, TX 31 Aug 72.

McGranahan BG and Breault ET: Care and Treatment of Burns. O.R. Nurses, Wilford Hall USAF Hosp, Lackland AFB, TX 7 Sep 72.

The following presentations were made at the International Congress of Nutrition in Mexico City, 4-8 Sep 72:

Long JM III: Management of Renal Failure with Essential Aminoacid and Hypertonic Dextrose Solution.

Wilmore DW: Influence of Parenteral Diet on Serum and Red Cell Fatty Acid Composition Following Thermal Injury.

Pruitt BA Jr: Current Management of Severely Burned Patients. Surgical Grand Rounds, Scott and White Hospital, Temple, TX (1) Pulmonary Complications of Thermal Injury; (2) Gastrointestinal Complications in Burn Patients. Staff Rounds, VA Hosp Temple TX 12 Sep 72.

McManus WF: Advances in Burn Therapy. Postgraduate Course. Univ of Nebraska, Omaha, Neb. 20 Sep 72.

McManus WF: Infectious Diseases, Prevention and Treatment. Emergency Personnel, Lincoln, Neb. 21 Sep 72.

Hunt JL: The Treatment of Burns. Medical Aspects of Adv Warfare Course, Sch of Aerospace Med, Brooks AFB, TX 26 Sep 72.

Levine NS: Treatment of Burns. Off Basic Course, MFSS, BAMC, Fort Sam Houston, TX 27 Sep 72.

The following presentations were made to the Amer Assn for Surgery of Trauma Mtg, San Francisco, CA 28-29 Sep 72:

McManus WF: Disseminated Intravascular Coagulation in Burn Patients

Warden GD: Central Venous Thrombosis.

Warden GD: Thyroid Adaptation. Intl Transplantation Soc Mtg, San Francisco, California 29 Sep 72.

Wilmore DW: Post Traumatic Metabolic Response. Grand Rounds, Univ of Texas Med Sch at San Antonio, San Antonio, TX 29 Sep 72.

Pruitt BA Jr: Seventh Annual Meeting Surgical Biology III Club,

San Francisco, CA 1 Oct 72.

Pruitt BA Jr: Panelist, "Vascular War Wounds," Vietnam Vascular Register Meeting, San Francisco, California. 2 Oct 72.

Slogoff S: The Use of Nitrous Oxide and Ketamine Anesthesia. Amer Soc of Anesthesiologists Mtg, Boston, Mass. 2 Oct 72.

Wilmore DW: Essential Fatty Acid Deficiency in the Red Cell Membrane Following Thermal Injury: Correction with Parenteral Fat Emulsion. Amer Coll of Surgeons Mtg. San Francisco, Calif. 3 Oct 72.

Pruitt BA Jr: Pre- and Postoperative Care Committee Mtg,
Panelist, Plastic & Maxillofacial Surgery Interdisciplinary Panel on, "Burn Wound Management"
Television Program--"Emergency Procedures."
Pre- and Postoperative Care Postgraduate Course on, "Local Treatment of Burns." American College of Surgeons Mtg, San Francisco, CA 2-6 Oct 72.

Warden GD: Treatment of Burns. Surg Staff, Columbia Presbyterian Hospital, New York 8 Oct 72.

Wilmore DW: Nutritional Aspects of Surgical Care. BGH Dietetic Interns and Staff, BGH, BAMC, Fort Sam Houston, TX 11 Oct 72.

Pruitt BA Jr: Current Methods of Burn Care. Tennessee Area Nurses Assn Mtg, Chattanooga, Tennessee 16 Oct 72.

Pruitt BA Jr: Current Methods of Burn Treatment. Tennessee Valley Medical Assembly, Chattanooga, Tenn. 17 Oct 72.

Erickson DR: Treatment of Burns. MFSS Physician's Assistant Program students, USA ISR, BAMC, Ft Sam Houston, TX 17 Oct 72.

Long JM III: Treatment of Burns. MFSS Physician's Assistant Program students, USA ISR, BAMC, Fort Sam Houston, TX 19 Oct 72.

Wilmore DW: Fluid, Electrolyte and Metabolic Problems Following Thermal Injury. Burn Symposium, Univ of La., Baton Rouge, La. 21 Oct 72.

McAlhany JC Jr: Treatment of Burns. MFSS Physician's Assistant Program students, USA ISR, BAMC, Fort Sam Houston, TX 24 Oct 72.

Wilmore DW: Nutrition in the Surgery Patient. BGH Surg Residents, BGH, BAMC, Fort Sam Houston, TX 26 Oct 72.

Agee RN: Treatment of Burns. MFSS Physician's Assistant Program students, USA ISR, BAMC, Fort Sam Houston, TX 27 Oct 72.

Pruitt BA Jr: Visited the Pronto Socorro para Queimaduras (Burn Hospital of Dr. Nelson Picolo), and the Universidade Federal de Goias School of Medicine, Goiania, Brazil. The following presentations were made to the faculty and students attending the course on "The Treatment of Burns", held at the University of Goias Med Sch, Goiania: The Early Resuscitation and Fluid Management of the Seriously Burned Patient. Wound Care, Including Use of Topical Chemotherapy, Physiologic Dressings; Closure of the Burn Wound
Gastrointestinal Complications of the Burn Patient
Diagnosis and Treatment of Inhalation Injury. 22-29 Oct 72.

Erickson DR: Treatment of Burns. Off Basic Course, MFSS, BAMC, Fort Sam Houston, TX 1 Nov 72.

Wilmore DW: Metabolic Response to Injury. BGH Surg Residents, BGH, BAMC, Fort Sam Houston, TX 2 Nov 72.

Long JM III: Parenteral Hyperalimentation Theoretical and Practical Considerations. Dept of Surg, Latter Day Saints Hosp, Salt Lake City UT 10 Nov 72.

Long JM III: Intravenous Techniques. Seminar, Primary Childrens Hospital, Salt Lake City, Utah 10 Nov 72.

Pruitt BA Jr: Attended 4th Annual Symposium on Control of Surgical Infections, ACS and participated as follows:
Co-Secretary for Discussion Group IV; "Summary of Untoward Effects and Dangers of Antibiotics."
Chairman, Discussion Group III, "Assessment of Risk to Infection Related to Treatment and Associated Factors."
Presentation: "Special Considerations and Recommendations on Antibiotic Prophylaxis in Relation to Burns" (with JL Hunt) Wash DC 10-11 Nov 72.

McManus WF: Care of the Burn Patient. Clinical Pastoral Education for Chaplains Course, BGH, Fort Sam Houston, TX 13 Nov 72.

Pruitt BA Jr: Hypernatremia in Burn Patients (co-author); Opportunistic Infections in Surgery. Western Surg Assn Mtg, Rochester, Minnesota 15-18 Nov 72.

The following presentations were made at the Burn Seminar West Cross Timbers Council for Health Development, Tarleton State College, Stephenville, Texas 16-17 Nov 72:
Hunt JL: (1) The Treatment of Burns; (2) Complications of Burns.
Shaffer G: Nursing Care of the Burn Patient.
Loveless SD: Physical Therapy for the Burn Patient.

McGranahan BG: Progressive Patient Care. Health Care Admin Course, MFSS, BAMC, Fort Sam Houston, TX 20 Nov 72.

McGranahan BG: Nursing Care of the Burn Patient. Flight Nurses and Technicians, Sch of Aerospace Med, Brooks AFB, TX 21 Nov 72.

Long JM III: Potential of Total Intravenous Feeding Complications. Food and Drug Admin Board Mtg, Rockville, Md. 22 Nov 72.

McManus WF: Air Evacuation of Burn Patients. 375th Air Evac Wing, Scott AFB, Ill. 27 Nov 72.

Hunt JL: The Treatment of Mass Casualties in Thermonuclear Warfare. School of Aerospace Medicine, Brooks AFB, TX 27 Nov 72.

Long JM III: Treatment of Burns. Off Basic Course, MFSS, BAMC, Fort Sam Houston, TX 1 Dec 72.

Warden GD: Evacuation and Early Treatment of Thermally Injured Patients. Airmen and Flight Crew 507th Med Co (Air Amb) Ft Sam Houston TX 1 Dec 72.

Taylor JW: Burns and Grafting. O.R. Technicians, BGH, BAMC, Fort Sam Houston, TX 4 Dec 72.

Pruitt BA Jr: Panelist, Postgraduate Case on Plastic Surgery & Burns. Facial Burns. 20th Annual Symp on Trauma, Detroit, Mich 7-9 Dec 72.

Reardon JC: Burns - General O.T. Treatment. Occupational Therapy Staff BGH, BAMC, Fort Sam Houston, TX 11 Dec 72.

Pruitt BA Jr: The Institute of Surgical Research as a National Resource. Assn Mil Surgeons Anl Mtg, San Antonio, TX 10-12 Dec 72.

Pruitt BA Jr: What's New in Burn Therapy. Plastic Surgery Grand Rounds, Univ of Tex Med Sch, San Antonio, TX 12 Dec 72.

McManus WF: Advances in Burn Therapy. Medical and Nursing Staff, Jenny Edmundson Hospital, Council Bluffs, Iowa 13 Dec 72.

Wilmore DW: Treatment of Burns. Health Class, Roosevelt High School, San Antonio, Texas 15 Dec 72.

Slogoff S: Shock Lung and Pulmonary Complications of Burns. Dept of Anesthesia Residents, BGH, BAMC, Fort Sam Houston, TX 16 Dec 72.

The following presentations were made to Physical Therapy Students, MFSS, BAMC, Fort Sam Houston, TX 18 Dec 72:

McAlhany JC Jr: Treatment of Burns.
Hall WF: Physical Therapy of Burn Patients.
McGranahan BG: Nursing Care of the Burn Patients.

EXHIBITS

The following exhibits were displayed at the American College of Surgeons Anl Conv, San Francisco, Calif., 2-6 Oct 72:

"Vascular Complications of Thermal Injury"

"Evaluation and Utilization of Biologic Dressings on Thermally Injured Patients"

MOTION PICTURES

The following motion pictures were shown at the American College of Surgeons Anl Conv, San Francisco, Calif., 2-6 Oct 72:

"Heterotopic Calcification of the Elbow in Burn Patients"

"Laboratory and Clinical Evaluation of Porcine Xenograft as a Temporary Burn Wound Cover"

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0A 6983	73 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
72 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	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)
PRECEDING FISCAL YEAR 73	1.1	37
CURRENT FISCAL YEAR 74	1.6	39

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
Surgical Study Branch
ADDRESS: Fort Sam Houston, Tx 78234

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME: Douglas W Wilmore, MAJ, MC
TELEPHONE: 512-221-5712
SOCIAL SECURITY ACCOUNT NUMBER:

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

ASSOCIATE INVESTIGATORS
NAME: Albert J Czaja, MAJ, MC
NAME: Basil A Pruitt, Jr, COL, MC DA

22. KEYWORDS (Precede EACH with Security Classification Code): (U) Trauma; (U) Combat Casualties; (U) Immunity; (U) Laboratory Animals; (U) Pulmonary Function; (U) Joints; (U) Hemodialysis

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) 72 07 - 73 06 This year the Surgical Study Branch was involved in defining the etiology of the post-traumatic metabolic response and the relationships to energy metabolism and ambient conditions. Improved techniques of energy and metabolic support were achieved in seriously traumatized soldiers. In addition, on-going investigation of gastrointestinal and liver function has aided understanding of the pathophysiologic dysfunction of these organs which occurs following injury. In addition, ward officer coverage and gastroenterologic coverage were provided by members of this branch.

*Available to contractors upon originator's approval

ANNUAL PROGRESS REPORT

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

1 January 1972 - 31 December 1972

Investigators:

Douglas W. Wilmore, MD, Major, MC
Albert J. Czaja, MD, Major, MC
Basil A. Pruitt, Jr., MD, Colonel, MC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

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 1972 - 31 December 1972

Investigators: Douglas W. Wilmore, MD, Major, MC
Albert J. Czaja, MD, Major, MC
Basil A. Pruitt, Jr., MD, Colonel, MC

Reports Control Symbol MEDDH-288(R1)

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 the metabolic changes following burn injury 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 the definition of the post-traumatic metabolic response, the neuroendocrine mediators for this response, and the relationship between energy metabolism and ambient conditions. 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 metabolism
Combat casualty
Gastrointestinal function
Liver function

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 burned patients; and 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 metabolism, nutrition, 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) to define the various components of the metabolic response following thermal injury; 2) to modify the afferent stimuli or neuroendocrine response to injury so as to prevent the ongoing loss of lean body mass and body weight. 3) To evaluate the influence of ambient temperature on energy production following injury. 4) To describe the evolution of stress ulcers of the gastric mucosa in burn patients and to interrelate with the observed changes such factors as gastric mucosal blood flow, back diffusion, gastric acid production, bowel reflux, and mucus production. 5) To describe the liver dysfunction which occurs following injury and specifically relate the alterations in bilirubin conjugation to the neuroendocrine response which characterizes the catabolic phase of trauma. 6) To optimize nutritional support of injured troops, particularly to insure optimal energy and nitrogen support by enteral feedings, and safe and effective nutritional support by parenteral feedings.

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 post-traumatic metabolic changes 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

ANNUAL PROGRESS REPORT

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

1 January 1972 - 31 December 1972

Investigators:

Gary W. Allen, MD, Major, MC
Stephen Slogoff, MD, Major, MC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

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 1972 - 31 December 1972

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

Reports Control Symbol MEDDH-288(R1)

In 1972, 183 of 301 patients whose disposition was completed at the United States Institute of Surgical Research were given 575 anesthetics at this institute. Of the anesthetics given, 40.9% were halothane, with or without nitrous oxide and oxygen, 44% were ketamine, with the remainder consisting of nitrous oxide, neuroleptic analgesia, and regional blocks. Of those patients receiving anesthesia at the Institute of Surgical Research, the mean number of anesthetics per patient was 3.14. Five major intraoperative complications occurred during the year and will be discussed in detail in the text. No intraoperative deaths occurred. In addition, approximately 60 patients underwent about 250 exposures to subanesthetic ketamine for debridement or dressing change in the Hubbard tank. This also will be discussed in detail in the text.

Anesthesia

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 fluids 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 serum 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^{R}) or anorectic (Valium^{R}). 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% (40.9) 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 380:512-522, 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 d-tubocurarine and gallamine, both 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, et al, Anesth Analg (Cleveland) 48:768-770, 1969).[4]

3. Ketamine

Ketamine is an intravenous "dissociative" general anesthetic which has been available for clinical use for approximately three years. Approximately one-half (44%) of our anesthetics in the operating room are now administered with this agent. Its use for debridement, skin grafting, various orthopedic procedures, and for certain ward procedures, has been an excellent addition to our anesthetic armamentarium. 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. This fact alone should significantly decrease anesthetic morbidity. However, it must be emphasized that occasionally airway stability is not maintained. One intraoperative complication occurred with ketamine in 1972 and will be discussed in the Case Report Section.

4. Subanesthetic ketamine

Ketamine has been demonstrated to have analgesic effects in subhypnotic doses, both experimentally and in limited clinical trials of postoperative pain relief. This year, 60 patients were given 250 (range 1-13) intramuscular administrations of subanesthetic ketamine for Hubbard-tank debridement and dressing changes consequent to thermal injury. A dose of 1.5 mg/kg (15% of the usual anesthetic dose) was selected after a brief pilot study showed that larger doses produced complete unconsciousness in a high percentage of patients and smaller doses resulted in a high incidence of reaction to surgical stimulation.

Onset of action occurred two to six minutes after injection and was heralded by nystagmus, psychic relaxation, and a blank affect. Useful analgesia lasted an average of 14 minutes, and recovery or orientation occurred approximately 20 minutes after administration of the drug. The mean time from injection to acceptance of oral alimentation was one hour.

During debridement, the patients were observed for signs of inadequate effect. Involuntary movements, a problem commonly found with ketamine anesthesia, occurred in 8% of all exposures; minimal restraint was required in only 2%. Vocalization or screaming in response to pain occurred in 9% of exposures. However, many patients were able to participate in intelligent conversation while denying the sensation of pain. Withdrawal from painful stimuli requiring supplementation did not occur. Amnesia was complete in all exposures.

Tolerance to ketamine, a previously undescribed phenomenon, was observed in virtually all patients receiving more than two exposures. This was manifested by a decreasing duration of effective analgesia despite a progressively increasing amount of ketamine required and administered to achieve the proper level of anesthesia. Complications occurred in only two trials and were easily managed.

In summary, for brief but potentially painful and unpleasant experiences, administration of intramuscular subanesthetic doses of ketamine has proven to be a valuable technique with minimal complications or interference with oral alimentation.

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

Presentation of Tracheo-esophageal Fistula During Anesthesia

This 18 year old white male sustained a 56% total body surface burn in an automobile accident. This burn was complicated by a severe inhalation injury which required intubation and mechanical ventilation. Tracheostomy was performed on the fourth postburn day. His course was complicated by pneumonia due to Pseudomonas aeruginosa, intermittent gastrointestinal hemorrhage, and persistent respiratory failure. On the 20th postburn day, the gastrointestinal hemorrhage increased significantly and an attempt of local epinephrine infusion through the celiac artery produced no diminution in bleeding. He was taken to the operating room for gastrectomy on the 21st postburn day. Anesthesia was induced with ketamine and maintained via the tracheostomy with nitrous

oxide and oxygen, and supplemental ketamine and gallamine. Approximately one and one-half hours into the procedure, it was noted that a gas leak had developed in the trachea, and emergency bronchoscopy confirmed the presence of a tracheo-esophageal fistula approximately 3 cm above the carina at the site of the tracheostomy tube. The operation was completed with the insertion of a Carlens tube for ventilation. The patient tolerated the procedure well. No aspiration was apparent. After the operation, two 6.5 mm nasotracheal tubes were inserted in each mainstem bronchus and the patient was adequately ventilated in the Intensive Care Unit. The patient did well for approximately one week; renal failure then developed, and he died 11 days postoperatively secondary to electrolyte imbalance associated with renal failure.

Comment. This is the first known case of the presentation of a tracheo-esophageal fistula during operation. Management of the airway was as described in one other case in the literature, and it is felt that this complication played a minor, if any, role in the patient's demise.

Case No. 2

Cardiac Arrest During Surgical Debridement

This 19 year old male received a 24% second and third degree electrical burn. Extensive muscle necrosis occurred in the left upper extremity and right lower extremity. Acute tubular necrosis developed on the first postinjury day. On the second postburn day, the patient was taken to the operating room where extensive debridement was performed and terminated after blood loss exceeded 4,000 cc. Replacement was deemed adequate at that time. On the fifth postburn day, the patient was again taken to the operating room for insertion of an AV shunt and further debridement. Anesthesia was induced with halothane and 50% nitrous oxide and oxygen, and intubation was carried out without difficulty. Monitoring was limited to electrocardiogram, central venous pressure, and peripheral pulse.

After two hours and 45 minutes of the procedure, the peripheral pulse suddenly ceased and the electrocardiogram exhibited a straight line. Resuscitation was carried out utilizing sodium bicarbonate, intracardiac epinephrine, calcium chloride, and an isuprel drip. Hear beat returned to a normal sinus rhythm with adequate peripheral perfusion. The patient was returned to the Intensive Care Unit and was placed on a ventilator. No localizing signs were present on neurologic examination; however, the patient was extremely obtunded and would respond only to deep pain. His course went progressively downhill with increasing renal failure, and, on the seventh postburn day, he was

taken to the operating room for tracheostomy and further debridement. Significant hemorrhage occurred, which required large volumes of blood replacement. Hemodialysis was carried out on that day, and the patient demonstrated some electrolyte improvement. On the ninth postburn day, the patient was noted to have fixed and dilated pupils with no response to pain. On that day, blood pressure and pulse fell rapidly, and the patient was pronounced dead.

Comment

Cardiac arrest in the operating room occurred after an extensive debridement with significant blood loss in a patient in renal failure. It is believed that the etiology of the cardiac catastrophe was secondary to hypovolemia and probable hyperkalemia secondary to renal failure. There is no doubt that this complication contributed to the patient's demise; however, considering the patient's preoperative condition and the limited ability to monitor this patient during a prolonged surgical procedure, prevention of such a complication is almost impossible.

Case No. 3

Apnea and Seizures Following Ketamine Induction

This 45 year old white male sustained a 22% total body surface electrical burn when he fell across a power line that was down and was unable to get disengaged. Entry point was at the right side of the neck and exit point in the right ankle. Physical examination at the time of admission demonstrated the electrically induced lesions and an organic brain syndrome. Resuscitation and hospital course were uneventful, and several debridements were carried on in the operating room utilizing halothane anesthesia. On the 20th postburn day, he was taken to the operating room for split-thickness autografting. Induction was carried out utillzing ketamine, 2 mg/kg. The patient became apneic and did not start breathing properly. Diffuse tonic and clonic seizures developed coincidentally. The patient was easy to ventilate manually. Paralysis was induced with gallamine and anesthesia was converted to halothane, nitrous oxide, and oxygen. The rest of the operation and anesthetic was carried out without difficulty. The patient recovered postoperatively with no sequelae.

Comment

Seizure-like activity is being described more frequently with ketamine anesthesia and has been defined in subhuman primates by several investigators. There is some speculation that this is the mechanism of

ketamine anesthesia. Although ketamine is widely used in this institution, we rarely see such complications. In a previous such episode, no sequelae were observed, as was the case here.

Case No. 4

Cardiac Arrest During Bronchoscopy

This 19 month old male sustained an 83.5% total body surface third degree burn secondary to a gas water heater explosion. He was transferred to the Institute of Surgical Research, where resuscitation was extremely difficult and the patient required three to four times what would be predicted by the Brooke Formula. On the second postburn day, massive edema had developed in his burn wounds but serum electrolyte levels were within normal limits. On that day, he developed a respiratory arrest, presumably due to cerebral edema. He was intubated and mechanical ventilation begun using the Bird respirator. He was stable for 24 hours when he experienced an atelectasis of the right upper lobe. He was taken to the operating room for bronchoscopy, which was performed with oxygen administration via the ventilating bronchoscope. Shortly after initiation of the procedure, a cardiac arrest developed, which responded to intubation and good ventilation. The patient left the operating room with a cardiac rate of 30. A tracheostomy was performed on the ward that same day. Repeat chest film demonstrated an 80% pneumothorax on the left and a chest tube was placed. He remained stable throughout the day, but required an isuprel drip to maintain an adequate cardaic output. Renal failure developed rapidly over the next few days, at which time the patient experienced a cardiac arrest and resuscitation was unsuccessful.

Comment

Ventilating bronchoscopes are probably extremely safe in people without significant shunts. In this particular case, with a right upper lobe atelectasis, the combination of a significant intrapulmonary shunt and the somewhat borderline ability to ventilate adequately through the ventilating bronchoscope contributed to this cardiac arrest on the basis of hypoxemia.

Case No. 5

Cardiac Arrest During Debridement and Autografting

This patient was a 31 year old male caucasian, who sustained a 74.5% total body surface burn during a house fire. Bronchopneumonia and a severe tracheobronchitis necessitated a tracheostomy approximately

two weeks postburn, and the patient required positive pressure ventilation intermittently throughout his hospital course. He had required numerous anesthetics for burn wound debridement, various orthopedic procedures, and bronchoscopies prior to the anesthetic in question. On the 64th postburn day, he was brought to the operating room for split-thickness skin grafting of his chest, both upper extremities, and both lower extremities, and for disarticulation of the second, third, and fourth left toes at the DIP joints. Anesthesia was induced with halothane, nitrous oxide, and oxygen, given via the tracheostomy. Blood pressure was not monitored because of the absence of appropriate monitoring site. The patient's status was assessed by the quality of peripheral pulses and by assessment of adequacy of ventilation. Two hours after induction, peripheral pulses were lost and ventilation ceased. External cardiac massage was immediately begun, and the patient was ventilated with oxygen. One milligram of epinephrine and 88 mEq of sodium bicarbonate were given intravenously and volume replacement was begun. Good perfusion, manifested by peripheral pulses and constriction of pupils was maintained throughout the arrest, which lasted approximately five minutes. Pulses returned approximately three minutes after epinephrine administration. The procedure was terminated; two units of whole blood were administered, and the patient was returned to the recovery room where he was noted to be alert and could respond rationally to commands. The patient subsequently died approximately two and one-half months later following superior mesenteric artery occlusion with necrosis of the small and large bowel, with severe suppurative peritonitis and septicemia.

Comment

Cardiac arrest in this case resulted from inadequate volume replacement in a patient whose circulatory status could not be properly evaluated. Blood loss at the time of arrest was estimated at 500 cc. This, combined with peripheral vasodilatation from halothane anesthesia, was poorly tolerated in this seriously ill patient who may have had borderline hypovolemia prior to his operation.

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4. Schaner PJ, et al: Anesth Analg (Cleveland) 48:768-770, 1969.

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WORK COMPLETED

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Cheney DH, Slogoff S, Allen GW: The effect of ketamine on stress-induced ulcerations in the rat.

Table 1. Overall Patient Data, USAISR (1964-1972)

	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/A	Average Per Cent Burn
1964	167	93	55.7	332	1.99	3.57	31
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

Table 2. Nature of Surgery, USAISR

	1970		1971		1972	
Procedure	No.	%	No.	%	No.	%
Debridement and/or Homograft	90	18.1	74	15.5	113	19.7
Autograft	282	56.7	252	52.9	295	51.3
Orthopedics	55	11.1	62	13.0	51	8.9
Ear (Chondrectomy)	21	4.2	19	4.0	18	3.1
Eye and Lid	9	1.8	18	3.8	4	0.7
Intra-abdominal	12	2.4	8	1.7	45	7.8
Tracheostomy & Bronchoscopy	12	2.4	22	4.6	38	6.6
Other	16	3.2	21	4.4	11	1.9
Total	497		476		575	

Table 3. Techniques of Anesthesia, USAISR - 1972

Technique	Number	Per Cent
Halothane	235	40.9
N_2O, O_2	75	13.0
Ketamine	253	44.0
Ketamine alone 171		
Ketamine with N_2O 82		
Neuroleptanalgesia	1	.2
Total General Anesthetics	564	98.1
Local Infiltration & Topical	9	1.6
Axillary Block	1	.2
Spinal Anesthesia	1	.2
Total Local Anesthetics	11	1.9
All Anesthetics	575	100

Table 4. Employment of Anesthetic Agents at ISR, 1964-1972 (In Per Cent)

	1964	1965	1966	1967	1968	1969	1970	1971	1972
Halothane	87.0	68.3	92.9	97.0	99.4	86.9	66.8	47.3	40.9
N_2O, O_2	0.6	3.5	1.3	0	0.3	4.7	8.4	18.7	13.0
Methyoxyflurane	0	20 0	0	0	0.1	0.8	0.4	0.2	0
Cyclopropane	4.8	0.6	0.7	0	0	0	0	0	0
Neuroleptanalgesia	0	0	2.0	3.0	0	1.0	0.4	0	0.2
Ketamine	0	0	0	0	0	4.8	18.7	27.3	44.0
Regional Block & Local	6.0	8.0	1.2	0	0.3	1.8	5.2	5.7	1.9
Other or Unknown	1.6	0.0	1.9	0	0	0	0	0.6	0
Total No. of Anesthetics	332	495	713	670	794	601	497	476	575

Table 5. General Anesthesia Induction Agents, USAISR - 1972

Agent	No. of Inductions	Per Cent of Total
IV Barbiturate	83	14.7
IV Ketamine	244	43.3
IM Ketamine	33	5.9
IV Other	11	2.0
Inhalation	193	34.1
Total	564	100

Table 6. Type of Airway During General Anesthesia, USAISR - 1972

Airway	No. of Anesthetics	% of Total No. of General Anesthetics
Mask	192	34.0
Endotracheal Tube		
Oral	128	22.7
Nasal	26	4.6
Tracheotomy	74	13.1
Natural Airway	144	25.5

Table 7. Use of Muscle Relaxants, USAISR - 1972

Total General Anesthetics	No. of Anesthetics Where Muscle Relaxants Used	dT-Curarine	Gallamine	Pancuronium	Succinylcholine
564	99	21	75	3	5
% of Total Gen. Anesth.	17.6	3.7	13.3	0.5	0.9

	No. of Anesthetics	% of Total General Anesthetics
Muscle Relaxant Used	99	17.6
Used for Intubation	40	7.1
Used for Relaxation	82	14.5

Agent	No. of Anesthetics	No. of Anesthetics Where Muscle Relaxant Used	%
Halothane, N_2O, O_2	235	29	12.3
N_2O, O_2	75	54	72.0
N_2O, Ketamine	82	15	18.3

Table 8. Mortality of Those Receiving Anesthesia, USAISR - 1972

Total Patients	301	Intraoperative deaths - 0
Deaths	103 (34.2%)	Died within 24 hours - 4
Total Given Anesthesia	183	24 hours to 7 days - 32
Deaths	49 (26.8%)	Greater than 7 days - 13

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA 0A 6956	73 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
72 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 (Procede 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 73	3.1	48
	CURRENT 74	2.5	50

19. RESPONSIBLE DOD ORGANIZATION
NAME:* US Army Institute of Surgical Research
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RESPONSIBLE INDIVIDUAL
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TELEPHONE: 512-221-2720

20. PERFORMING ORGANIZATION
NAME:* US Army Institute of Surgical Research
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PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
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TELEPHONE 512-221-4307
SOCIAL SECURITY ACCOUNT NUMBER

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

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Procede EACH with Security Classification Code) (U) Renal Failure; (U) Hemodialysis; (U) Soldiers; (U) Peritoneal Dialysis; (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 care for acute renal failure of varied etiologies and to provide dialysis support for problems concerned with both endogenous and exogenous poisonings. To support clinical research activities, provide measurements of glomerular filtration. rate, and to support the Renal Clinic in the care of military personnel.

24. (U) In addition to acute and chronic cannulation, hemodialysis, peritoneal dialysis, glomerular filtration rates, metabolic balance studies.

25. (U) 72 01 - 72 12 A total of 28 patients underwent hemodialysis in the Renal Unit for a total of 678 patient treatments. Five patients underwent renal transplantation, two patients entered the home training program, seven patients were transferred to the chronic dialysis program at Brooke General Hospital. Two patients were transferred to Centers elsewhere, three patients recovered and nine patients expired.

*Available to contractors upon originator's approval

ANNUAL PROGRESS REPORT

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

1 January 1972 - 30 December 1972

Investigators

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

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

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 January - 30 December 1972

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

A total of 28 patients underwent hemodialysis in the Renal Unit for a total of 678 patients treatments. Five patients undersent renal transplantation elsewhere, two patients entered the home training program, seven patients were transferred to the chronic dialysis program at Brooke General Hospital. Two patients were transferred to Centers elsewhere. three patients recovered and nine patients expired.

Renal Failure
Hemodialysis
Peritoneal Dialysis
Humans

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 three 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 proportioning 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 General Hospital. The USAISR hemodialysis unit now provides backup support when necessary and assists in treatment of cases of acute renal failure occurring at Brooke General Hospital. The USAISR nephrology staff continue 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.

Patients are dialyzed using an external A-V sialastic shunt or an internal A-V fistula to provide access to the circulation. One patient during the reporting period was dialyzed using a Sheldon catheter inserted into the femoral artery with return through a peripheral vein. Also during the reporting period the unit acquired a Vital Assist Unicontrol Pump System which enables one to dialyze using only one venous catheter. In general acutely ill patients are dialyzed using regional heparinization but the patients with chronic renal failure are dialyzed using systemic heparinization. Dialyzers used during the reporting period include the Travenol 145, UF 100, Ultra Flow II, Extracorporeal EX-II, and EX-P.

From 1 January to 31 December 1972 a total of 28 patients were dialyzed for a total of 678 dialyses (Table I).

In addition to the dialysis support provided to the Hospital and the unit, several pilot studies have been initiated. A new technique for measuring blood clotting times on patients receiving regional heparinization while being hemodialyzed is being investigated. This technique(Bason Clotting Test) would provide a faster clotting time thus allowing better control of heparin administration. Also under

TABLE I
PATIENTS RECEIVING HEMODIALYSIS FROM 1 JANUARY 1972 THROUGH 31 DECEMBER 1972

PATIENT NUMBER	TIMES DIALYZED	DIAGNOSIS	DISPOSITION
1	2	Burns 2° and 3° 67% TBS	Expired
2	14	Chronic Glomerulonephritis	Transplant(Living Related) -Survived
3	54	Chronic Glomerulonephritis	Entered Home Training Program
4	23	Chronic Glomerulonephritis	Transplant(Cadaver) - Survived
5	22	Chronic Glomerulonephritis	Chronic Program - BAMC
6	45	Nephrosclerosis	Chronic Program - BAMC
7	24	Nephrosclerosis	Transferred to Dallas TX VA Hospital
8	61	Chronic Glomerulonephritis	Transplant (Cadaver), Expired
9	80	Chronic Pyelonephritis	Chronic Program - BAMC
10	54	Rapidly Progressive Glomerulonephritis	Expired
11	71	Chronic Pyelonephritis	Second Transplant(Cadaver), Expired
12	5	Acute Renal Failure	Resolved
13	45	Polycystic Renal Disease	Chronic Program, BAMC
14	8	ATN secondary to electrical burns	Resolved

TABLE I (CONTINUED)

PATIENT NUMBER	TIMES DIALYZED	DIAGNOSIS	DISPOSITION
15	11	Nephrosclerosis	Transferred to VA Hospital, Houston, TX
16	3	Acute Renal Failure	Expired
17	3	Acute Renal Failure	Expired
18	5	Seminoma	Expired
19	2	Acute Renal Failure	Resolved
20	8	Chronic Glomerulonephritis	Chronic Program BAMC
21	14	Chronic Glomerulonephritis	Transplant(Living Related), Expired
22	2	ATN 2° Electrical Burns	Expired
23	14	Chronic Pyelonephritis	Chronic Program, BAMC
24	27	Nephrotic Syndrome, Cortical Necrosis	Chronic Program, BAMC
25	11	Acute Tubular Necrosis	Expired
26	3	Acute Tubular Necrosis	Expired
27	12	Diabetic Glomerulosclerosis	Expired
28	5	Acute Renal Failure	Expired
TOTAL 28	TOTAL 678		

investigation are the changes in dialysance of creatinine, urea, and Glofil(R) with 50, 100, and 150 mm Hg of pressure applied to cellophane or cuprophane dialyzers via an external cuff around the dialyzer.

PRESENTATIONS:

Rogers PW: Renovascular Hypertension, Grand Rounds, Dept of Med, Brooke Army Medical Center, 21 July 1972

Rogers PW: What is Diabetic Glomerulosclerosis - Combined Renal Conference, University of Texas Southwest Medical School at San Antonio, San Antonio, Texas 78229, 2 August 1972

Rogers PW: Uncontrollable Thirst Associated with Hyperreninemia in a Patient on Chronic Hemodialysis." Southeastern Dialysis & Transplantation Association 7th Annual Meeting, Bolixi, Mississippi, 12 August 1972.

Bristow BF: Renal Diet Exchange System, Food Svc Div, BGH, Fort Sam Houston, Texas, 14 August 1972.

Rogers PW: Evaluation of the Urinary Sediment, Nephrology Teaching Conference, Department of Medicine, BAMC, 13 September 1972

Bristow BF: Dietary Management of Home Dialysis Patients. Kidney Foundation, University of Texas Medical School, San Antonio, Texas September 1972

Rogers PW: A Study of the Renin Angiotensin System in the Thermally Injured Patient, V International Congress of Nephrology, Mexico City Mexico, 12 October 1972

Merrill RM: Anatomy of the Kidney, Nephrology Teaching Conference, Dept of Med, Brooke Army Medical Center, 4 October 1972

Bristow BF: Nutrition in Renal Disease, Calculation of Diets. BGH, Dietetic Interns, BHJ, Fort Sam Houston, Texas 19 October 1972.

Rogers PW: Renal Physiology, Anesthesiology Staff, Brooke Army Medical Center, Fort Sam Houston, Texas, 28 October 1972.

Rogers PW: New Concepts Related to the Renin-Angiotensin-Aldosterone System, Dept Med., BAMC. 6 November 1972.

Rogers PW: Water Metabolism - Renal Physiology Seminar, Dept of Medicine, BAMC, 13 November 1973

Rogers PW: Renal Artery Stenosis, Hypertension, and Hyporeninemia, Combined Renal Conference, UTSW Med School at San Antonio, San Antonio, Texas, 15 November 1973.

Merrill RM: Renal Tubular Acidosis, Nephrology Teaching Conference, Department of Medicine, BGH, 4 December 1972.

Rogers PW: Idiopathic Nephrotic Syndrome, Combined Renal Conference, UTMS at San Antonio, San Antonio, Texas, 10 January 1973.

Rogers PW: Chronic Renal Failure, Nephrology Teaching Conference, Dept of Med, BAMC, 5 February 1973.

Rogers PW: Primary Aldosteronism, Noon Conference Beach Pav., Dept of Med, BAMC, 8 February 1973.

Rogers PW: Consequences of Chronic Renal Failure, Veterans Administration Medical Staff, San Antonio, Texas, 22 February 1973.

Rogers PW: Metabolic Alkalosis, Anesthesiology Staff, BAMC, 3 March 1973.

Rogers PW: Hyperreninemia in the Thermally Injured Patient, American Burn Association Meeting, Dallas, Texas, 3 April 1973.

Rogers PW: Availability of Hemodialysis in 1973, Southwest Kidney Foundation, University of Texas SW Medical School at San Antonio, San Antonio, Texas, 19 April 1973.

ANNUAL PROGRESS REPORT

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

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

DEVELOPMENT OF A DIET MANUAL FOR PATIENTS WITH RENAL FAILURE

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

1 July 1972 - 30 June 1973

Investigators:

Mary E. Spitzer, Captain, AMSC
Barbara F. Bristow, Lieutenant, AMSC
Philip W. Rogers, MD, Major, MC

Report Control Symbol MEDDH-288(R1)

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ABSTRACT

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

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

DEVELOPMENT OF A DIET MANUAL FOR PATIENTS 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 1972 - 30 June 1973

Investigators: Mary E. Spitzer, Captain, AMSC
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Philip W. Rogers, MD, Major, MC

Dietary management is a major part of the treatment for renal disease, requiring attentive and thorough instructions. As a result of advances and improvements in hemodialysis, an extremely restricted diet is not necessary.

The diet manual discussed has been developed for use by both dietitians and patients. Based on a food exchange system, it has greatly simplified the dietitian's work in planning and calculating a meal pattern.

Values were obtained from USDA Handbook #8 with additional values for Na and K+ from analyses performed at the USAISR.

This diet system has been in use for the past 10 months, and has greatly increased the time available for patient instruction.

Dietary management
Food exchange system
Diet manual

DEVELOPMENT OF A DIET MANUAL FOR PATIENTS WITH RENAL FAILURE

The patient with acute or chronic renal failure faces many problems throughout the course of treatment. One such problem is learning to manage a diet with controlled amounts of protein, sodium, potassium and fluid.

In the hospital the patient's diet is calculated exactly each day to within two grams of the prescribed amount of protein, and one milliequivalent of sodium and potassium. This is tedious and requires time that could be spent instructing the patient.

A simple, straight forward system that both the patient and dietitian could use was needed. The outline below gives the major areas of discussion:

I. Introduction

- A. Kidney Function
- B. Purpose of Diet

II. Dietary Constituents

- A. Fluid
- B. Calories
- C. Protein
- D. Sodium
- E. Potassium

III. Renal Diet Exchange System

- A. Explanation of Food Groups
- B. Learning Activities for Patients.

IV. Food Preparation Techniques

- A. Measuring

B. Recipe Selection

C. Buying & Storing Foods

D. Ideas for Seasoning

V. Food Groups and Nutritive Values

A. Summary of Exchange Values

B. Meats I & II

C. Breads & Cereals I & II

D. Fruits & Juices

E. Vegetables

F. Potatoes

G. Dairy

H. Beverage

I. Items that may be used freely

The basis of the diet exchange system consists of the food groups mentioned above. The meat I and bread and cereal I contain less sodium (0.9, 0.24 mEq/serving less than meat II and bread and cereal II which contain 3.6 and 8.6 mEq/per serving respectively.)

Values for calories, protein, sodium, potassium, calcium, and phosphorous were obtained from USDA Handbook #8. Sodium and Potassium values for some foods were calculated from analyses performed at USAISR.

The values for the meat groups are based on 1 ounce (30 gm) cooked-weight portions. Values for the remaining food groups are based on the gram weights of small to average portions (1/4 cup, 1/3 cup, or 1/2 standard serving).

Mean values for calories, protein, sodium, potassium, calcium and phosphorous were calculated for each group. These values were then used to calculate the diets according to a given prescription. Table I is a summary of the exchange values.

TABLE I

SUMMARY OF EXCHANGE VALUES

FOOD LIST	CAL	PROTEIN g	SODIUM mEq	POTASSIUM mEq	CALCIUM mg	PHOSPHOROUS mg
MEATS I	63	8.4	0.92 21 mg	2.90 112 mg	4	70
MEATS II	81	5.6	3.60 83 mg	1.40 55 mg	29	67
BREAD & CEREAL I	103	2.3	0.24 6 mg	1.05 41 mg	11	48
BREAD & CEREAL II	100	2.5	8.60 197 mg	1.20 47 mg	24	40
FRUITS & JUICES	59	0.5	0.11 2 mg	2.75 107 mg	11	11
VEGETABLES	19	1.1	0.37 9 mg	2.89 113 mg	21	23
POTATO	91	1.5	0.50 12 mg	6.05 236 mg	18	42
DAIRY	147	3.7	2.40 55 mg	4.00 156 mg	120	96
BEVERAGE	46	-	0.31 7 mg	0.11 4 mg		

* Coffee and tea from the BEVERAGE LIST contain an average of 3 calories, 0 grams protein, trace of sodium, and 1.4 mEq (55 mg) potassium per 120 ml.

Tables II and III illustrate Breads & Cereals I and Breads and Cereals II.

In using this type of system, it is possible that a patient might select foods with actual values higher than the mean. To evaluate this, a menu plan for 70 grams protein, 50 mEq potassium and 43 mEq sodium was calculated from the exchange values. Using the foods from the hospital menu, the actual value of the foods used was calculated.

The differences between the exchange and actual food values are indicated in Table IV. The differences noted were not significant when patients followed the exchange plan. The range for 14 days was 65.1 - 79.6 grams protein, 50.6 - 58.7 mEq potassium, and 34.8 - 49.5 mEq sodium. These values were random, not from the same day's menu.

Table V shows a sample menu plan for a 70 gm protein, 50 mEq potassium, and 43 mEq sodium diet. This is only one possibility; varied plans may be calculated to suit the individuals needs.

An extensive file of recipes was developed for use by renal patients. These require the use of a special baking mix and high carbohydrate supplement. These recipes can be attached to the diet manual in a separate annex for those patients who need or wish to use them.

The exchange system has considerably reduced the time needed to calculate renal diets, allowing the hospital dietitians to spend their time instructing the patient.

PRESENTATIONS AND PUBLICATIONS:

Dietary Management of Home Dialysis Patients. Kidney Foundation, Univ of TX Med School, San Antonio, Texas September 1972.

Nutrition in Renal Disease, Calculation of Diets. BGH Dietetic Interns, BGH, Fort Sam Houston, Texas, 19 Oct 1972.

No Publications.

TABLE II

FOOD LISTS

BREADS AND CEREALS I

One serving contains an average of 103 calories, 2.3 grams protein, 6 mg sodium, 41 mg potassium, 11 mg calcium, and 48 mg phosphorous.

YOU ARE ALLOWED_____SERVINGS PER DAY

FOOD ITEM	HOUSEHOLD MEASURE	WEIGHT
BREADS:		
White bread, low sodium	1 slice	23
RF White bread without salt	1 slice	
RF Rye bread without salt	1 slice	
TORTILLAS:		
Masa Harina, Quaker	1/3 cup dry or 2 corn tortillas 6" in diameter	
CEREALS:		
Dietetic cornflakes, Van Brode	1 cup	30
Rice, puffed, Quaker	1 1/4 cup	15
Wheat, puffed, Quaker	1 1/3 cup	15
Wheat, shredded, Quaker	2 biscuits	38
Oatmeal, cooked	1/2 cup	
Farina, regular, enriched, cooked	1/2 cup	
Cream of rice, cooked	1/2 cup + 2 Tbsp	
Cream of wheat, cooked	1/2 cup	
Corn grits, cooked	1/2 cup	
Rice, white, cooked	3/4 cup	127
Rice, brown, cooked	1/2 cup	75
Rice, precooked instant	3/4 cup	128
Noodles, enriched, cooked	1/2 cup	80
Macaroni, enriched, cooked	1/2 cup	70
Spaghetti, cooked	1/2 cup	80
RF Coffee cake	1/8 cake	
ADDITIONS:		

TABLE III

BREADS AND CEREALS II

One serving contains an average of 100 calories, 2.5 grams protein, 197 mg sodium, 47 mg potassium, 24 mg calcium, and 40 mg phosphorous.

YOU ARE ALLOWED_____SERVINGS PER DAY

FOOD ITEM	HOUSEHOLD MEASURE	GRAM WEIGHT
Biscuit, home recipe	one, 2" diameter	35
White bread, regular	1 slice	23
Rye bread, American	1 slice	23
Whole wheat bread	1 slice	23
Raisin bread, plain	1 slice	23
Cornbread, home recipe	2" square	45
French or Vienna bread	1 slice	20
Italian bread	1 slice	20
Hamburger bun	1 roll	50
Hot dog bun	1 roll	50
Dinner roll	1 medium	50
Sweet roll	1 medium	50
Masa trigo, wheat tortillas	1/3 cup uncooked 2 tortillas, 6" diameter	
Graham crackers	4 squares	28
Cherrios, General Mills	1 cup	25
Cornflakes, Kelloggs	1 1/3 cup	30
Grapenuts, Post	½ cup	28
Grapenuts flakes, Post	2/3 cup	28
Honeycomb, sweet corn cereal	1 1/3 cup	28
Lucky charms, General Mills	1 cup	26
Pep, Kelloggs	1 cup	30
Rice Krispies, Kelloggs	1 cup	28
Sugar Pops, Kelloggs	1 cup	30
Wheaties or Total, Gen Mills	1 cup	28

ADDITIONS:

TABLE IV

DIET PRESCRIPTION

70 gm Protein

50 mEq Potassium

44 mEq Sodium

	CAL	PRO g	Na mEq	K mEq
Exchange Values	2168	70.5	43.1	52.2
Actual Values (14 Day Mean)	2058	71.8	40.4	54.2

TABLE V

SAMPLE MENU PLAN
Calculated from exchange
70 grams protein
50 mEq potassium
43 mEq sodium

FOOD GROUP	SERV	CAL	gm PRO	mEq SODIUM	mEq POTASSIUM	mg Ca	mg P
Fruit & Juices	1	59	0.5	0.11	2.75	11	11
Bread & Cereal I	1	103	2.3	0.24	1.05	11	48
Bread & Cereal II	1	100	2.5	8.60	1.20	24	30
Meat II	2	162	11.2	7.20	2.80	58	134
Dairy	1	147	3.7	2.40	4.00	120	96
Meat I	2	126	16.8	1.84	5.80	8	140
Potatoe	1	91	1.5	0.50	6.05	18	42
Vegetables	2	38	2.2	0.74	5.78	42	46
Fruit	1	59	0.5	0.11	2.75	11	11
Bread & Cereal II	1	100	2.5	8.60	1.20	24	40
Meat I	2	126	16.8	1.84	5.80	8	140
Bread & Cereal I	1	103	2.3	0.24	1.05	11	48
Vegetable	1	19	1.1	0.37	2.89	21	23
Fruit	2	118	1.0	0.22	5.50	22	22
Bread & Cereal II	1	100	2.5	8.60	1.20	24	40
TOTAL		1451	67.4	41.61	49.82	413	881
Butter, SF 10 tsp	50g	358	0.30	0.23	0.34	10	8
Jelly, 2 Tbsp	30g	81	0.03	0.22	0.58	--	-
Whipping cream	60g	211	1.31	0.83	1.38	45	35
Sugar, 4 tsp	20g	77	-	-	-	-	-
Coffee, 240 cc		-	-	0.05	1.60	2	5
		2178	69.04	42.94	53.72	470	929

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0D 6951	73 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
72 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	084
b. CONTRIBUTING	61101A	3A061101A91C	00	
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 73	.1	5
	CURRENT 74	.2	8

19. RESPONSIBLE DOD ORGANIZATION
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SOCIAL SECURITY ACCOUNT NUMBER:

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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 Limilus amaebocyte lysate reaction. Plasma is extracted with glacial acetic acid then neutralized to pH 6.2 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) 72 07 - 73 06 Sources of error in the present test have been identified and technical modifications have increased the sensitivity and specificity of the reaction. Fifty four patients were tested; of these, 41 had positive blood cultures during their illness. Endotoxin was demonstrated in 15 of these septicemic patients. Out of 19 patients with Staph aureus septicemia, three had endotoxemia; gram-negative infection was also present in these. Out of 19 patients with Providencia in blood culture, eight were proven to have endotoxemia. Other Enterobacteriaceae sp. had associated endotoxemia in half of the patients. This level of detection of endotoxemia is much higher than was previously detected and reflects improvements in technic of the procedure.

*Available to contractors upon originator's approval

ANNUAL PROGRESS REPORT

PROJECT NO. 3A1 61101B71R-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

1 July 1972 - 30 June 1973

Investigators:

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

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ABSTRACT

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Application of Limulus amoebocyte assay to burn patients with bacteremia and/or shock yielded increased numbers of positive reactors over previous trials. Over 30% of bacteremic patients exhibited positive endotoxin reactions. When only gram-negative bacteremia was included, the proportion of positive reactors was still higher. Intermittent negative periods were shown to occur in the presence of bacteremia. A pro-zone phenomenon was discovered to be obscuring positive reactors; dilution of the plasma extract permitted detection of endotoxin in patients negative by the undiluted technic.

Endotoxin
Sepsis
Assay
Humans

DETECTION OF ENDOTOXIN IN BURNED SOLDIERS WITH SEPSIS

The availability of a reasonably simple laboratory procedure for detection of endotoxin in human plasma has aroused intense interest among workers in shock and sepsis. The clinical term "endotoxin shock" is commonly used to connote the spectrum of symptoms that can accompany sepsis due to gram-negative bacilli. The abrupt fall in peripheral pressure, hypothermia, ileus, and other aspects of the shock syndrome are interpreted as due to introduction of endotoxin into the circulation. The presence of gram-negative bacilli, known to be a primary source of endotoxin, would offer a plausible source of this extremely toxic entity. Reliable detection of endotoxin should offer an added means of diagnosing the shock syndrome, and it has been postulated that detection of endotoxin could be a more rapid method than blood culture for detecting gram negative septicemia.

These optimistic projections have not been substantiated by experience with detection of endotoxin in the burned patient, but neither has the test failed to an extent that would justify abandoning efforts in this direction. This report summarizes experience with detection of endotoxin in a series of burned patients who were, at the least, sufficiently ill to merit blood cultures being taken.

METHODS

Heparinized blood was collected in sterile pyrogen-free syringes and placed in sterile pyrogen-free tubes. Samples were iced until processed. (A series of bloods were held and processed at room temperature to test the assumption that cryoglobulins, separating out in the cold, might sequester endotoxin.) Plasma was separated by centrifugation and aspirated with pyrogen-free plastic pipettes. The extraction of endotoxin from plasma was carried out by the pH adjustment method of Reinhold (Reinhold RB. Surg. Forum 21: 1, 1970)[1]. When indications were obtained that the activity of the extract might be altered or masked by variations in pH of the resultant plasma extract, a micro-electrode was used to adjust the pH to 6.25 - 6.3. Parallel tubes were used to titrate the pH, then adjust the parallel aliquot

without exposure to the electrode, which could not, of course, be rendered pyrogen-free.

The Limulus polyphemus amoebocyte lysate had been collected in 1971, (Lindberg RB, Inge WW,Jr, Pruitt BA,Jr, Mason AD, Jr. USA Inst Surg Res Ann. Rpt. FY 1972, Sec.55)[2], titrated by lot, and stored at 4°C. During the subsequent year a major part of the lysate was stored at -70°C in an effort to assure retention of activity of stocks of this unique and valuable material. The ultimate effect of storage at various temperatures remains to be assessed; it is the opinion of informed observers in the field (Levin J. Personal communication)[3] that freezing will maintain activity probably indefinitely, but when thawed, aliquots must be used promptly, since some lots of lysate appear to have a shorter period of stability after freezing.

The test, as originally described, used undiluted plasma extract as the only reactant needed to detect presence of endotoxin. If positive, dilutions could then be run to determine quantity. However, it was observed that in some cases an inhibiting effect was present in undiluted extract, and that this prozone could be eliminated by diluting the extract. This has been done, and higher dilutions as well as undiluted extract were tested after this phenomenon was recognized.

RESULTS

Sixty-one patients who met the criteria of burns of more than 30% of body surface, over 5 years of age, and with physical findings or positive culture to delineate the status of being septic, were tested. A total of 282 tests were carried out. Fourteen patients exhibited positive reactions.Thus, 23% of patients examined had endotoxin present. However, 15 of these patients had no positive blood cultures at any time, and were on further consideration not valid inclusions on the list of plausible sepsis cases. A more valid basis for level of positive reactions is shown in Table 1.

There were 14 cases positive for the Limulus assay, out of 46 who could be listed as plausible prospects for diagnosis of sepsis or septic shock. This level of 30.4% of reactors is higher than has been reported by some investigators, and lower than reported by others. Obviously the test did not

Table 1. Endotoxin Reactions Among Patients with Bacteremia and/or Signs of Sepsis

Blood culture negative, sepsis inconclusive	15
Endotoxin reactions negative	15
Blood culture positive, Endotoxin negative	32
Blood culture positive, Endotoxin positive	13
Blood culture negative, Endotoxin positive (See text for this case)	1
Total patients with endotoxemia	14
% of bacteremic/sepsis cases with positive reactions	30.4

supplant blood culture as a diagnostic tool, but it offers encouragement to continue the study since earlier trials, with less experience in technic of the reaction, gave a positive rate of only 5% (Inge WW, Jr, Lindberg RB, Mason AD, Jr, Pruitt BA, Jr. USA Inst. Surg. Res. Ann. Rpt. FY 72, Sec. 54)[4]. However, the presence of sepsis did not denote continuous presence of demonstrable endotoxin. Many patients had numerous positive blood cultures with negative endotoxin, but they also had positive endotoxin findings at some point in their septic course. There is a strong implication that endotoxemia may be detectable only at intervals; prolonged bacteremia may be accompanied by disappearance of endotoxin from the blood stream.

Negative endotoxin reactors with positive blood cultures are summarized in Table 2. There were 41 patients with negative endotoxin reactions. Thirty-two of these had positive blood cultures. Out of these 32, only 8 had a gram negative bacteremia; 11 had only staphylococcal bacteremia, and 12 had a mixed gram-positive and gram-negative succession of blood cultures. Since staphylococci are described as not giving rise to endotoxemia, these results are consistent with the conclusion that most of the bacteremic cases with negative endotoxemia were bacteriologically not of the category in which endotoxemia would be anticipated.

A detailed summary of positve reactors is shown in Table 3. The succession of bacteria listed in patients with multiple positive blood cultures does not attempt any breakdown of predominance or the time interval between positive culture and presence of detectable endotoxin.

There were 3 patients with only Providencia bacteremia, 2 with Klebsiella, and one with Enterobacter cloacae. All other positive reactors exhibited a succession of more than one species of gram-negative bacillus antemortem.

Patient No. 24 had repeated blood cultures drawn in an attempt to explain his repeated positive endotoxemia. The test was rechecked and carefully controlled; the reactions were unequivocally positive. The patient was afebrile and convalescing for 8 days before his intermittent endotoxemia subsided. The fact that such a condition can exist denotes a potential for

Table 2. Patients with Negative Limulus Lysate Reaction: Clinically Possible Sepsis

Blood Culture	Endotoxin	No. of Patients
Negative	Negative	15
Staph aureus	Negative	11
Prov stuartii	Negative	3
Ps aeruginosa	Negative	1
Klebsiella pneumoniae	Negative	1
Aeromonas sp	Negative	1
Staph, Candida, Providencia	Negative	1
Staph, Candida, Prot mirabilis	Negative	1
Staph, Pseud, Prov, Kleb	Negative	1
Staph, Pseud, E coli, Prov	Negative	1
Staph, Prov	Negative	5
Staph, Entero cloacae	Negative	2
Staph, Serratia	Negative	1
Kleb, Entero cloacae	Negative	1
Prov, E coli	Negative	1
Prov, Candida	Negative	1
Total Patients		47
Staph only		11
Staph plus Gram-negative bacilli		12
Gram-negative bacilli only		8
Gram-negative bacillus, Candida		1

Table 3. Patients with Positive Limulus Lysate Reactions: Correlation with Bacteremia

Patient Nr.	Blood Culture	Limulus Lysate Reaction	Endotoxin ug/ml
36	Providencia stuartii	Positive	0.008
42	Providencia stuartii	Positive	0.01
26	Providencia stuartii	Positive	0.04
10	Prov, Ps aeruginosa	Positive (2)	0.168
			0.168
26	Prov, Klebsiella	Positive	0.02
32	Prov, Klebsiella	Positive (3)	0.64
			0.08
			0.32
35	Prov, Kleb, Staph aureus	Positive (3)	0.008
			0.019
			0.02
14	Klebsiella	Positive	0.168
18	Klebsiella	Positive	0.04
28	Kleb, Entero cloacae, E coli	Positive	0.005
27	Entero cloacae	Positive	0.04
35	E coli, Proteus mirabilis	Positive	0.42
22	E coli, Staph, Pseud	Positive	0.04
	Prov, Kleb	Positive	0.07
24	Negative; No positive	Positive (4)	0.038
	Blood Cultures		0.16
			0.16
			0.16

endotoxemia, or the possible existence of unknown reacting substance that can interfere with the lysate reaction. The plasma from this patient will be assayed by other technics to confirm the identity of the endotoxin found.

Effect of Dilution on Detection of Endotoxin. When negative results were obtained on several patients who exhibited classical endotoxic shock, the technic of the procedure was scrutinized to determine whether a manipulative error could be found. In view of early observations in this laboratory that a prozone pattern of inhibition could be shown in dilution of endotoxin-containing plasma, a pattern of dilution of extract of plasma was set up. Specimens were tested undiluted and at dilutions with pyrogen-free distilled water up to 1: 128. Six out of 10 patients so tested revealed presence of endotoxin when the extract was diluted but not in the undiluted extract. Typical results are illustrated in Table 4.

The effect was clearcut and unequivocal: in many patients, endotoxin would be detected only if the plasma extract were tested at higher dilutions. The explanation of this phenomenon is not yet available. It can be described as diluting out unknown inhibitory substances. In any event, the procedure is much more sensitive when this dilution is made. It makes the reaction more expensive, of course, in terms of lysate used, but the added sensitivity makes it a necessary procedure.

DISCUSSION

The significance of endotoxemia as indicated by the Limulus amoebocyte lysate test has not been elucidated completely by the applications of the test thus far made. Technical details of the procedure remain incompletely clarified. The introduction of a dilution titration in the test may yet uncover presently undetected endotoxin. However, the potential for explaining more fully the real nature of "endotoxic shock" in traumatized patients is very great. Further investigations using this unique and valuable naturally occurring assay substance are planned.

REFERENCES

1. Reinhold RB. Quantitative measurement of circulating endotoxin in shock. Surg. Forum 21: 1, 1970.

Table 4. Reactions of Patients' Plasma with Extract Diluted

Patient	Dilution and Reaction (Lysate)							
	0	2	4	8	16	32	64	128
A	0	0	0	0	1+	1+	4+	4+
B	0	0	0	0	0	1+	4+	4+
	0	0	0	0	0	1+	4+	3+
C	0	0	0	2+	2+	3+	4+	4+

0 = No reaction

1+ = Positive but weak, unstable clot

4+ = Firm clot forms in <1 hour

2. Lindberg RB, Inge WW,Jr, Pruitt BA, Jr, Mason AD, Jr. Detection of endotoxin in burned soldiers with sepsis. II. Variation in sensitivity of Limulus amoebocyte lysate to endotoxin. USA Institute of Surgical Research Annual Rpt FY 1972, BAMC, Ft Sam Houston, Tx. Sec. 55.

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4. Inge WW, Jr, Lindberg RB, Mason AD, Jr, Pruitt BA, Jr. Detection of endotoxin in burned soldiers with sepsis. USA Institute of Surgical Research Annual Rpt FY 72, BAMC, Ft Sam Houston, Tx. Sec. 54.

PRESENTATIONS

Lindberg RB. The significance of natural variations in reactivity of Limulus amoebocyte lysate in raction with endotoxin. Am. Soc. Microbiol. Symposium - "The use of Limulus assay in study of endotoxin", Miami Beach, Fla. May 9, 1972.

PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
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NAME: DA

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22. KEYWORDS (Precede 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) Continued assessment of new antibiotics is necessary in laboratory support of the study of trauma, since in the three major areas of burn therapy, trauma study, and renal study, systemic or local infections with resistant microorganisms pose a constant threat to military burn patients.

24. (U) Tube dilution sensitivity tests determined degree and rate of sensitivity to drugs.

25. (U) 72 07 - 73 06 Totally resistant Providencia stuartii, with strains recovered from wounds, respiratory tract and blood have been recognized; no antibiotic therapy is presently detectable by any recognition technic, and study of new antibiotics, including minocin and two experimental aminoglycosides has been undertaken. Other enteric species, including Klebsiella and Enterobacter, have reverted to a low level of sensitivity, which indicates a drop in resistance-transfer reactions presently occurring. Staph aureus strains were still predominantly type 84, but the level of methcillin-type resistance has stabilized, so that nafcillin at least is relatively useful. Minocin and Clindamycin are under intensive testing as part of the search for more effective antimicrobial therapy in control of burn infections.

* Available to contractors upon originator's approval

ANNUAL PROGRESS REPORT

PROJECT NO. 3A16 1102B71R-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

1 July 1972 - 30 June 1973

Investigators:

Robert B. Lindberg, PhD
Anthony A. Contreras, MS
Harvey O.D. Smith, SP6
Edward C. Plowey, SP5
Arthur D. Mason, Jr., MD

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A1 61102B71R-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 1972 - 30 June 1973

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The major cause of death in burned patients is bacterial sepsis, control of which must rely heavily on use of antibiotics. Since in many instances antibiotics must be chosen prior to completion of testing of offending organisms, a guide to selection of the most suitable antibiotic can be a valuable adjunct in management of burn patients. Ongoing surveillance of bacterial populations in the burn ward has been summarized, with cumulative sensitivity levels presented for Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas aeruginosa, Klebsiella pneumoniae, Enterobacter cloacae, Serratia marcessens, Enterobacter aerogenes, Escherichia coli, Proteus mirabilis, Providencia stuartii, and several minor species. Staph aureus strains included 26% sensitive to Unipen and Lincocin, and 35% sensitive to Gentamycin; 43% were totally resistant. All Providencia strains were totally resistant. A serious problem of resistant strains is present.

Burn wound flora Antibiotic sensitivity Pseudomonas
Providencia Humans

ANTIBIOTIC SENSITIVITY OF CURRENT MILITARY BURN PATIENT FLORA

An increasing population of antibiotic-resistant strains in the burn wound patients has been observed on an annual basis since 1969. This has been observed for staphylococci as well as for members of the enteric group and Pseudomonas. Since sepsis has always been a major component in mortality due to burns in this Institute, a highly resistant microbial flora can present the physician with a problem that at this point is often insuperable. Episomal transfer of antibiotic resistance in gram-negative bacilli has been demonstrated so often that its occurrence is now assumed as the rule rather than the exception (Anderson EO. Ann Rev. Microbiol. 22: 131, 1968)[1]. Recently, DNA-carried resistance factors, transmitted by transduction, have been described as effecting the acquisition of resistance by staphylococci. In each instance, the prospect of increasing cross-resistance of large numbers of opportunistic pathogens looms. The evaluation of such resistance and the status of antibiotic sensitivity of burn wound flora are presented in this report.

TECHNIC AND SOURCE OF STRAINS

Tube-dilution technic has been employed routinely for sensitivity testing at this Institute. To minimize the tendency of dilute solutions of antibiotic to deteriorate even when frozen, a revised procedure for conducting tests was developed. 10 mm plastic tubes containing 1.0 ml of 50 ug/ml of reagent-grade antibiotic were capped and stored. Dilutions of antibiotic in broth were prepared at time of testing to constitute a series of 6 tubes with dilutions ranging from 50 to 1.5 ug/ml. An equal volume of a 4 to 6-hour broth culture containing 2×10^4 organisms/ml was added, to give a final concentration of 10^4 organisms/ml. Incubation for 18 to 20 hours was followed by assessmnt of the MIC, the last amount of antibiotic which would inhibit visible growth as viewed by oblique light against a dark background. When needed, the Minimum Lethal Concentration (MIC) was determined by plating on blood agar after 18 hours incubation in antibiotic and determining the dilution which permitted cells to survive.

Table 1. Sources and Principal Species Tested for MIC of Antibiotic, ISR, 1972

Species	No. Tested	Source and % of All Strains Tested			
		Blood	Lung	Wound	Other
Staph aureus	117	73.5	5.1	16.1	5.3
Strep gp A	11	9.0	9.0	63.6	18.4
Ps aeruginosa	50	42.0	38.0	12.0	8.0
Prov stuartii	84	71.4	22.6	1.1	4.9
Klebsiella sp	43	34.8	39.5	13.9	11.6
Proteus mirabilis	32	62.5	12.5	15.6	9.3
E coli	28	42.8	32.1	10.7	14.4
Enterobacter cloacae	12	50.0	41.6	8.4	0
Serratia sp	11	100.0	0	0	0
Totals Tested	388	232	80	48	26
% of All Strains		60.1	20.6	12.3	6.7

Of the 9 species shown, 388 strains were tested. Sixty per cent of these were from blood cultures; i.e., the presumption of the presence of sepsis was high. Twenty per cent were from sputum and Luken's tube cultures with a strong indication of pneumonia present. Twelve per cent were from wound and biopsy cultures; all other sources together made up less than 7%; i.e., they were of minor importance as far as sepsis was concerned.

Staphylococcus aureus strains and Providencia stuartii were conspicuous as blood stream invaders; over 70% of each species tested were from positive blood cultures. Other numerically important species, including Pseudomonas aeruginosa and Klebsiella (essentially Klebsiella pneumoniae) were significantly less frequent in blood culture. A greater proportion of tested strains of these species were from sputum cultures. The relative invasiveness of these species, at least under conditions of therapy used here, was less than that of Staphylococci and Providencia.

Antibiotic batteries used for gram-positive cocci and for gram-negative bacilli shared some antibiotics and differed on others. The spectra covered in 1972 were as follows:

Symbol	Antibiotic	Gram-positive cocci	Gram-negative bacilli
U	Nafcillin (Unipen)	X	
Ps	Oxacillin (Prostaphlin)	X	
Sc	Methacillin (Staphcillin)	X	
L	Lincocin (Lincomycin)	X	
T	Tetracycline	X	X
K	Kanamycin	X	X
Kf	Keflin (Cephalathin)	X	X
G	Gentamycin (Garamycin)	X	X
Amp	Ampicillin		X
Co	Colymycin (Colistimethate sulfate)		X
Cb	Carbenicillin*		X

* Pseudomonas only tested

Penicillin G was run against strains of Proteus mirabilis, since sensitivity of this species to penicillin has been reported. Sensitive strains were rare. Sequential tests on strains recovered during intensive antibiotic therapy increased the likelihood of recovering strains selected for tolerance of antibiotic.

RESULTS OF SENSITIVITY TESTS

The sensitivity pattern for the major species occurring in severely ill burn patients is presented in terms of cumulative sensitivity. Gram-positive cocci are designated as sensitive if they are inhibited by 6.25 ug/ml or less; gram-negative bacilli, those strains inhibited by 12.5 ug/ml or less (Finland M. Ann Int Med 76: 1009, 1972)[3].

Staph aureus. Sensitivity of 117 strains of Staph aureus are summarized in Table 2. At the sensitive level, the most effective antibiotic inhibited only 35.6% of strains; Lincocin and Unipen were equal at 26.0%; and Oxacillin and Methicillin were progressively less effective at 18.8% and 13.1% of strains respectively. Kelfin was effective against 22.6% of strains, while Kanamycin and Tetracycline were of negligible effect.

This level of resistance was not unexpected in a species that was described in 1971 as constituting a Methicillin-resistant monotype epidemic. It is of real importance, however, to establish the degree of total cross-resistance that occurred in this population. Fifty of the 117 strains that were assayed were regarded as resistant to all antibiotics. They required at least 12.5 ug/ml of antibiotic for inhibition. Twenty-eight of this 50 were even more resistant, and required 25 ug/ml or more of antibiotic for inhibition.

Consideration of the blood stream isolates separate from the total population showed no significant difference in the level of sensitivities. Thus, there did not appear to be a significant sorting effect in the septicemic phenomenon, although the preponderance of blood stream strains would tend to obscure minor differences in the mean level of sensitivity of strains from other sources.

The progression of events regarding the increase in resistance of strains of this major pathogen are shown in detail in Table 3. It is at once

Table 2. Cumulative Inhibitory Levels for 117 Strains of Staph aureus
Institute of Surgical Research, 1972

Antibiotic Level ug/ml	Antibiotic and % of Strains Inhibited at each Level							
	K	L	Ps	Sc	U	T	G	Kf
> 25	100	100	100	100	100	100	100	100
25	23.4	49.5	35.0	44.7	62.6	4.9	66.9	48.0
12.5	15.6	44.3	28.2	29.8	36.5	1.9	53.0	33.0
6.25	6.0	26.0	18.8	13.1	26.0	1.9	35.6	22.6
3.12	3.4	10.4	8.5	7.0	16.5	1.9	11.3	14.7
1.56	0.8	4.3	5.1	0.8	10.4	1.9	2.6	7.8
0.78	0.8	0	3.4	0	5.1	1.9	1.7	2.6
< 0.78	0.8	0	2.5	0	4.3	0.9	0.8	1.7
Total Tested	115	115	117	114	115	101	115	115

Strains sensitive only to 25 µg/ml or greater: 28

Strains sensitive only to 12.5 µg/ml or greater: 22

Total completely resistant strains 50

Table 3. Antibiotic Sensitivity of Staph aureus: Institute of Surgical Research
1967 - 1972

Antibiotic	Year and % Inhibited by 6.25 ug/ml					
	1967	1968	1969	1970	1971	1972
K	23.0	42.8	38.0	2.8	3.8	6.0
L	89.4	64.7	48.5	29.8	28.4	26.0
Ps	94.0	80.0	33.0	22.4	20.1	18.8
Sc	61.1	84.6	25.7	18.0	15.5	13.1
U	94.4	90.0	41.0	33.9	33.0	26.0
T	22.2	38.4	13.0	3.8	2.9	1.9
G	-*	-	52.0	32.0	50.0	35.6
Kf	-*	-	-	-	56.4	22.6

* Antibiotic not used until year indicated

evident that, from a population of relatively susceptible strains in 1967, there has been (with occasional fluctuations) an inexorable progressive emergence of more and more resistant strains. Kanamycin dropped from a high level of effectiveness in 1968 (42.8% of strains sensitive) to 2.8% in 1970; it has essentially lost its value. Tetracycline followed a similar course; it has been replaced by Minocin in the test battery. The synthetic penicillins have each tended to drop in effectiveness; this precipitous fall has possibly levelled out, since the decline in numbers of sensitive strains slowed in 1972. Gentamycin was introduced into the schema in 1969; it may be relatively stable in its level of activity. Keflin, which was very promising in 1971, dropped to 22.6% of strains sensitive in 1972. It will be observed continuously, but the fall in number of sensitive strains is not encouraging. Additional antibiotics are being tested; Clindomycin and Minocin both show promise of being more effective than are antibiotics in the presently employed battery.

Staphylococcus epidermidis. There were 9 strains of Staph epidermidis tested. Five were from blood cultures, 2 from urine and 2 from sputum. Twenty-five per cent of the strains were sensitive to Keflin and Lincocin, one-third to Prostaphlin, Methicillin, and Tetracycline; one-half of them to Unipen, and sensitivity to Keflin occurred in 85% of these strains.

Ps aeruginosa. Ps aeruginosa strains tested totalled 50, of which 42% were blood stream isolates and 38% from sputum. The cumulative sensitivity of these strains is summarized in Table 4. The sensitivity level was even less encouraging than had been seen in previous years. Only Tetracycline, Colymycin, Gentamycin and Carbenicillin offered any inhibitory activity. Of these, Colymycin was slightly more effective than Gentamycin; at the maximum level of 12.5 ug/ml these drugs inhibited 70% of the strains tested. The effective or attainable level of Carbenicillin is much higher, and these results showed that at least a third of the strains might be effectively treated with Carbenicillin. The prospect of antibiotic therapy remains restricted primarily to the aminoglycoside antibiotics and Carbenicillin. A recurring concern has been the presumed continued increase in incidence of totally resistant strains of Ps aeruginosa. A comparison of pertinent antibiotics

Table 4. Pseudomonas aeruginosa: Cumulative Inhibitory Concentrations for 50 Strains, ISR - 1972

Antibiotic Level ug/ml	Antibiotic and % of Strains Inhibited							
	T	K	Kf	Amp	Co	G	Cb	
> 25	100	100	100	100	100	100	> 1250	100
25	34.6	4.1	0	0	72.0	74.0	1250	57.1
12.5	20.4	0	0	0	70.0	66.0	625	51.0
6.25	4.0	0	0	0	60.0	54.0	312	36.7
3.12	0	0	0	0	46.0	26.0	156	34.6
1.56	0	0	0	0	22.0	4.0	78	34.6
0.78	0	0	0	0	0	0	39	28.4
< 0.78	0	0	0	0	0	0	19.5	18.3
							9.5	10.2
							4.5	4.0
No. tested	46	49	49	49	50	50	49	

over the past 4 years is shown in Table 5. It was apparent that little consistent change has occurred. A reciprocal slight increase in sensitivity to Colymycin has appeared at the same time that a comparable slight rise in resistance to Gentamycin has occurred. Tetracycline sensitivity fluctuated over the same period, and Kanamycin and Keflin were never of consequence with this important pathogen. Carbenicillin sensitivity, encouragingly, has not diminished; it was initially effective on approximately 33% of isolates, and is still effective in this range.

Klebsiella pneumoniae was, with the exception of Providencia, the most conspicuous species of the Enterobacteriaceae in burned patients. It was equally prominent in septicemia and in the lung of patients with severe pneumonia. Sensitivity of 43 strains of Klebsiella are summarized in Table 6. Gentamycin, Colymycin, Kanamycin and Tetracycline were antibiotics that were effective often enough to merit their consideration in cases where active infection was suspected. There was no significant change in the sensitivity levels of strains of Klebsiella, in contrast to the often reported increase in numbers of resistant strains of Enterobacteriaceae as a result of dissemination of resistance transfer factors. Indeed, a marked rise in Tetracycline-sensitive strains occurred. There were 11 strains that showed complete cross-resistance.

Enterobacter cloacae, closely related to Klebsiella, was common in sputum, but only 12 strains were tested for sensitivity. Six of these were from blood cultures. Half of the strains tested were sensitive to Gentamycin, Tetracycline and Colymycin. Two were totally resistant to all antibiotics.

Escherichia coli with 28 strains tested, had 12 strains recovered from the blood. Sensitivity at the 12.5 ug/ml level was recorded at the following rates; Gentamycin, 57.1%; Tetracycline, 46.1%; Ampicillin, 32.1%; Colymycin, 53.5%. Keflin and Kanamycin were essentially ineffective.

Proteus mirabilis. Thirty-two strains of Proteus mirabilis were examined. Twenty of these were from blood cultures. As has been the case over the past 4 years, only Keflin, Ampicillin and Gentamycin were effective

Table 5. Antibiotic Sensitivity of Pseudomonas aeruginosa
ISR - 1969 - 1972

Antibiotic	Year and % Sensitive at 12.5 ug/ml			
	1969	1970	1971	1972
Tetracycline	22,8	7.9	12.5	20.4
Kanamycin	12.0	1.5	0	0
Keflin	5.4	0	5.8	0
Colymycin	61	63.4	73.3	70.0
Gentamycin	75.8	71.6	71.4	66.0
Carbenicillin 156 ug/ml	50 (12 strains)	33.9	30.0	34.6
No. tested	65	68	55	50

Table 6. Klebsiella pneumoniae, Cumulative Sensitivity for 43 Strains, ISR - 1972

Antibiotic Level ug/ml	Antibiotic and % Inhibited G	T	Amp	Kf	Co	K
> 25	100	100	100	100	100	100
25	67.4	47.2	4.6	23.2	67.4	39.5
12.5	60.4	30.5	4.6	18.6	48.8	32.5
6.25	51.1	13.8	2.3	2.0	37.2	11.6
3.12	37.2	5.5	0	0	23.2	2.3
1.56	13.9	0	0	0	2.3	0
0.78	2.3	0	0	0	2.3	0
< 0.78	0	0	0	0	2.3	0
Total tested	43	36	43	43	43	43

Total resistance present: 11 strains

to any degree. Comparison of sensitivity levels for the past year is presented in Table 7. The progressive disappearance of Kanamycin-sensitive strains resembles that seen with Ps aeruginosa at the same time. Keflin sensitivity fell drastically; at present only Gentamycin is a promising antibiotic for treatment of Proteus infections. Twelve out of these 32 strains were resistant to all antibiotics. This degree of cross-resistance was spread across strains from all sources. Penicillin G was virtually ineffective against these strains. Our observations have never corroborated accounts of sensitivity of P mirabilis to penicillin.

Table 7. Comparison of Sensitivity of Proteus mirabilis Strains ISR - 1969-1972

Year	Antibiotic and % of Isolates Sensitive to 12.5 ug/ml					
	T	Amp	K	Kf	Co	G
1969-1970	10.1	-	7.0	39.5	0	50
1971	8.3	33.3	8.3	76.4	0	58.8
1972	0	18.7	0	21.8	0	50

Providencia stuartii. As has been pointed out in Table 1, Prov stuartii is the major pathogen presnetly infecting patients in this Institute. While staphylococcal septicemia is also extremely common, it does not carry the connotation of high mortality that is associated with Providencia septicemia. This exotic opportunistic pathogen has colonized patients in this Institute at least since 1963, in a gradually increasing frequency which, in 1969, virtually exploded into a major infection problem. The incidence of Prov stuartii was higher in blood, sputum and wounds in 1972 than it had been previously.

The sensitivity pattern for Prov stuartii in 1972 was complete resistance. The transition from a relatively resistant species to a completely resistant one occurred between 1969 and 1972, as shown in Table 8. There

is no indication that this pattern of complete resistance is in prospect of being ameliorated, at least by any direct effort exerted by the Institute staff. As has been shown, Prov stuartii are not unique to this Institute, and their transmission is most probably secondary to patient care activities. New antibiotics including experimental ones are being tested. The role of Providencia as opportunistic invaders is well established. Its control is not yet achieved.

Table 8. Providencia stuartii: Comparison of Sensitivity to 12.5 ug/ml of Antibiotic, 1969-1972

Antibiotic	Year and % of Strains Inhibited		
	1969-1970	1971	1972
G	23.4	7.3	0
T	17.4	1.4	0
Amp	-	-	0
K	7.8	1.4	0
Co	15.7	0	0
Kf	3.7	0	0
No strains tested	144	68	82

Several species of minor numerical importance were tested, and these results are summarized for completeness. Gram-positive strains included hemolytic streptococci Group A, non-hemolytic streptococci Group D, Corynebacterium sp and Bacillus sp. The number tested and the number sensitive to 6.25 ug/ml or less of antibiotic are shown in Table 9.

The hemolytic streptococci were largely sensitive to the spectrum of antibiotics used. Group D streptococci were resistant to all those tested, as were two-thirds of the Corynebacterium sp and the Bacillus.

Gram negative bacilli in lesser numbers included Serratia, Enterobacter sp, Citrabacter, Salmonella, and Mima. The proportion of these strains which were sensitive to 12.5 ug/ml or less of antibiotic is summarized in Table 10.

Table 9. Streptococci Groups A and D, Corynebacterium and Bacillus sp Sensitive to 6.25 ug/ml of Antibiotic/Total

Species	No. Tested	Antibiotic							
		G	T	L	U	Ps	Sc	K	Kf
Streptococci Gp A	11	8/11	9/11	7/11	7/11	8/11	8/11	6/11	5/11
Streptococci Gp D	7	0/7	0/7	0/7	0/7	0/7	0/7	0/7	0/7
Corynebacterium sp	3	2/3	1/3	0/3	0/3	1/3	1/3	1/3	1/3
Bacillus sp	1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1

Table 10. Less Common Enterobacteriaceae sp and Mima Senstivity Inhibited by 12.5 ug/ml/Total

Species	No. Tested	Antibiotic G	T	Amp	Kf	Co	K
Enterobacter cloacae	12	6/12	6/12	0/12	0/12	5/12	2/12
Enterobacter aerogenes	5	5/5	2/5	0/5	3/5	3/5	1/5
Enterobacter liquefaciens	1	0/1	1/1	0/1	0/1	0/1	0/1
Citrobacter sp	3	3/3	0/3	0/3	0/3	1/3	3/3
Sal typhimurium	1	1/1	0/1	1/1	0/1	1/1	1/1
Serratia sp	11	7/11	2/11	0/11	0/11	1/11	2/11
Mima sp	1	0/1	1/1	1/1	0/1	1/1	0/1

Enterobacter sp were most frequently sensitive to Gentamycin, Tetracycline and Colymycin; Keflin was effective against 3 of the Enterobacter aerogenes strains. Serratia sp strains were relatively resistant; only Gentamycin was a promising agent. Citrobacter strains fell in a similar pattern. The infrequently encountered strains offer little information on probability of sensistivity.

DISCUSSION

Among the numerically important species which colonize burns and which may cause sepsis in burn patients, antibiotic sensitivity displayed no major variations from 1971. Staph aureus antibiotic-resistant strains increased slightly in numbers, but for the synthetic penicillins the change was nominal. The fall in the number sensitive to Gentamycin and especially to Keflin is, however, discouraging. The value of Keflin, based on in vitro data, has fallen sharply. Methicillin-resistance is not complete but is very high in this population against Ps aeruginosa. Colymycin and Gentamycin remain the most effective antibiotics. Interestingly, Carbenicillin resistance which appeared early in the history of this drug, has not increased; about two-thirds of the isolates for each of the past 3 years were resistant.

The role of Klebsiella pneumoniae is of particular concern in pulmonary complications of burn injury. Effective antibiotics are available for 75% of isolates; Gentamycin, Colymycin, Kanamycin, and Tetracycline have remained at a constant rate of effectiveness over the past 3 years.

The major infective agent in most sites on the burn ward population was Providencia stuartii. There is, at the time of this writing, no antibiotic available that controls this organism. It is totally resistant to all agents in routine use. Experimental antibiotics are being investigated.

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PRESENTATION

Lindberg RB. The Microbiology of the Burn Wound. Am. Soc. Microbiology, Pennsylvania Branch: Symposium on Opportunistic Pathogens. November 9, 1972.

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	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
72 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 (Precede with Security Classification Code): (U) Emergence of Methicillin-Resistant Staphylococcus Aureus Type 84 in Burned Military Personnel (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 Clinical Medicine

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69 06	Cont	DA	C. In-House

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a. DATES/EFFECTIVE: EXPIRATION:
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FISCAL YEAR	PRECEDING 73	.4	16
	CURRENT 74	.4	10

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(U) Staphylococcus; (U) Burns; (U) Septicemia; (U) Burn Infection; (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 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) 72 07 - 73 06 Monotype epidemic type 84 continued to predominate on the burn ward. Out of 1155 strains studied, 837 were type 88. Twenty other types, each in small numbers, were recognized. Group 3 staphylococci and some group 1 were observed. Type 84 strains varied in antibiotic sensitivity patterns; they were not homogeneous. No prophylactic measures have reduced its incidence.

*Available to contractors upon originator's approval

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

ANNUAL PROGRESS REPORT

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

REPORT TITLE: EMERGENCE OF METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS TYPE 84 IN BURN PATIENTS

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

1 July 1972 - 30 June 1973

Investigators:

Robert B. Lindberg, PhD
Ruth L. Latta, BS
Basil A. Pruitt, Jr, MD, Colonel, MC
Arthur D. Mason, Jr, MD

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

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Reports Control Symbol MEEDH-288(R1)

The staphylococcal population in the burn wards of the Institute of Surgical Research has been assessed by phage typing and antibiotic sensitivity tests. Since 1967, a heterogeneous population, exhibiting acceptable sensitivity to antibiotic, has been replaced progressively by a type 84 population that constitutes a monotype epidemic. Simultaneously with the incursion of type 84 a progressive drop in sensitivity occurred, so that the methicillin group of antibiotics became relatively ineffective. Keflin, recently added to the spectrum, was highly effective in the first year, but far less so in the second. Minocin shows a high level of effectiveness in late 1972, but as a tetracycline is not the ideal antistaphylococcal drug. There is no present solution to the problem of a burn ward seeded with an antibiotic-resistant Staphylococcus aureus.

Staphylococcus
Burns
Septicemia
Infection

EMERGENCE OF METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS TYPE 84 IN BURN PATIENTS

The role played by Staphylococcus aureus in infection in burned patients was regarded as minor for the period from 1962 through 1968 when control of Pseudomonas burn wound sepsis was regarded as the major bacteriologic problem confronting this Institute. Antibiotic sensitivity during that period was such that antibiotic resistant staphylococci were not common. With the introduction of methicillin and its several analogues, the problem of avoiding the resistance generated by beta-lactamase production was considered solved. During the middle 1960's most Staph aureus strains were of phage Group I, but in the latter part of the decade a series of reports of the change to Group III strains, and of sharply rising rates of antibiotic resistance, appeared (Jessen O, Rosendo K, Bulow C. NEJM 281:677, 1969[1]; Bulo P. Ann NY Acad Sci. 182:21, 1971[2]). In the Institute of Surgical Research a rise in rate of staphylococcal septicemia and a rapid fall in antibiotic sensitivity of staphylococcal strains has necessitated a continuing study of these strains, to clarify the current status of staphylococcal infection in burns and its significance in the pathogenesis of infection in burn patients.

OBSERVATIONS

Bacteremia. The relative number of blood stream infections caused by staphylococci indicates the seriousness with which staphylococcal infection must be viewed. Table 1 sets down the incidence of Staph aureus bacteremia in patients with positive blood cultures at some time during their stay in the Institute of Surgical Research. There are 3 natural divisions into which patients may be sorted: In 1963 topical therapy was non-existent, and it was introduced in 1964. Whether this had a specific effect on reduction of staphylococcal sepsis cannot be answered. The Sulfamylon burn cream was designed and directed toward control of Pseudomonas burn wound sepsis, although its anti-staphylococcal spectrum is on the same level as its anti-Pseudomonas spectrum.

Table 1. Incidence of Staphylococcus aureus Bacteremia in Burn Patients
1963 - 1972

Years	Conditions	No. Patients with: Positive Blood Culture	No. Patients with: Staph aureus Recovered	Incidence of Staph aureus Bacteremia (% of Positive Blood cultures)
1963 & 1964	Prior to full use of topical therapy	114 (57 per yr)	29	25.4
1965 - 1968	Optimal use of topical therapy	117 (29 per yr)	14	11.9
1969 - 1972	Emergence of Methicillin-Resistant Staph aureus type 84	322 (80 per yr)	160	49.6

The years 1965 through 1968 were the period in which topical therapy and the entire therapeutic regimen of burn care produced the optimal results in terms of burn patient survival. These were years when the rate of sepsis and of septicemia reached its lowest level.

The period from 1969 to 1972 coincided with the appearance of methicillin-resistant staphylococci and with a marked increase in the incidence of staphylococcal bacteremia.

Table 1 summarizes the extent of staphylococcal bacteremia in this Institute during the 3 periods noted. The very low level of bacteremia of all kinds, and the correspondingly low level of staphylococcal infection, was obvious in the 1965-1968 interval. The change in 1969 and in the succeeding years was very obvious. More patients exhibited positive blood cultures; from an average of 29 the number rose to 80 per year. The percentage of all blood cultures positive for staphylococci rose from 11.9% in 1965-1968 to 49.6% in 1969-1972. It should be emphasized that these patients did not, in most instances, exhibit staphylococcus as the only organism recovered from the blood. In most instances successive cultures would yield other organisms. Survival of patients with only Staph aureus recovered in culture was far greater than it was in patients who had other species ultimately recovered.

Antibiotic sensitivity of staphylococci in successive years since 1967 is shown in Table 2. It was obvious that the upturn in staphylococcal sepsis coincided with the appearance of more resistant strains of staphylococci. Kantrex and tetracycline were virtually ineffective after 1968; Lincocin and the methicillin group of antibiotics dropped drastically in proportion of sensitive strains, in 1969, and this drop has continued in each year since. Methicillin reached a low point of 13.1% of strains sensitive in 1972; oxacillin and nafcillin were slightly more active. Lincocin and nafcillin were each effective against 26% of strains tested in 1972. Gentamycin was added in 1969 in an effort to improve the armamentarium; its effectiveness has fluctuated on an annual basis. Keflin in 1971 appeared promising but resistant forms increased markedly in 1971. Minocin in 1972 has shown itself active

Table 2. Antibiotic Sensitivity of Staphylococcus aureus:
% of Strains Inhibited by 6.25 ug/ml or less

Year	Antibiotic and % Inhibited by 6.25 ug/ml								
	K	L	Ps	Sc	U	T*	G	Kf	M
1967	23.0	89.4	94.0	61.1	94.4	22.2	-	-	-
1968	42.8	64.7	80.0	84.6	90.0	38.4	-	-	-
1969	38.0	48.5	33.0	25.7	41.0	13.0	52.0	-	-
1970	2.8	29.8	22.4	18.0	33.9	3.8	32.0	-	-
1971	3.8	28.4	20.1	15.5	33.0	2.9	56.0	56.4	-
1972	6.0	26.0	18.8	13.1	26.0	1.9	35.6	22.6	51.5

K = Kanamycin L = Lincocin Ps = Prostaphlin (Oxacillin) Sc = Staphcillin (Methicillin)
U = Unipen (Nafcillin) T = Tetracycline G = Gentamycin Kf = Keflin (Cephalothin)
M = Minocin (Minocycline)

* Minocin included 48 strains collected in 1973. Tetracycline was dropped from the test battery in 1972.

against half of the strains tested, although like all tetracyclines is not a recommended drug for staphylococcal infections.

Complete cross-resistance occurs among antibiotic resistant staphylococci. This attribute was observed in 39.2% of all strains tested, i.e., these strains were not inhibited by any antibiotic. This value has fluctuated; in 1970 it was 40%; in 1971, 24%, and in 1972, it rose again to 39%. This is a high level of complete cross-resistance. Recent reports of transduction as one of the mechanisms by which antibiotic resistance in staphylococci is transmitted offer a plausible mechansim by which the segregation of large numbers of totally resistant strains could be derived. The fact that the predominant strain is a single type of staphylococcus makes such segregating mechanisms more likely.

PHAGE TYPES OF STAPH AUREUS IN BURNED PATIENTS

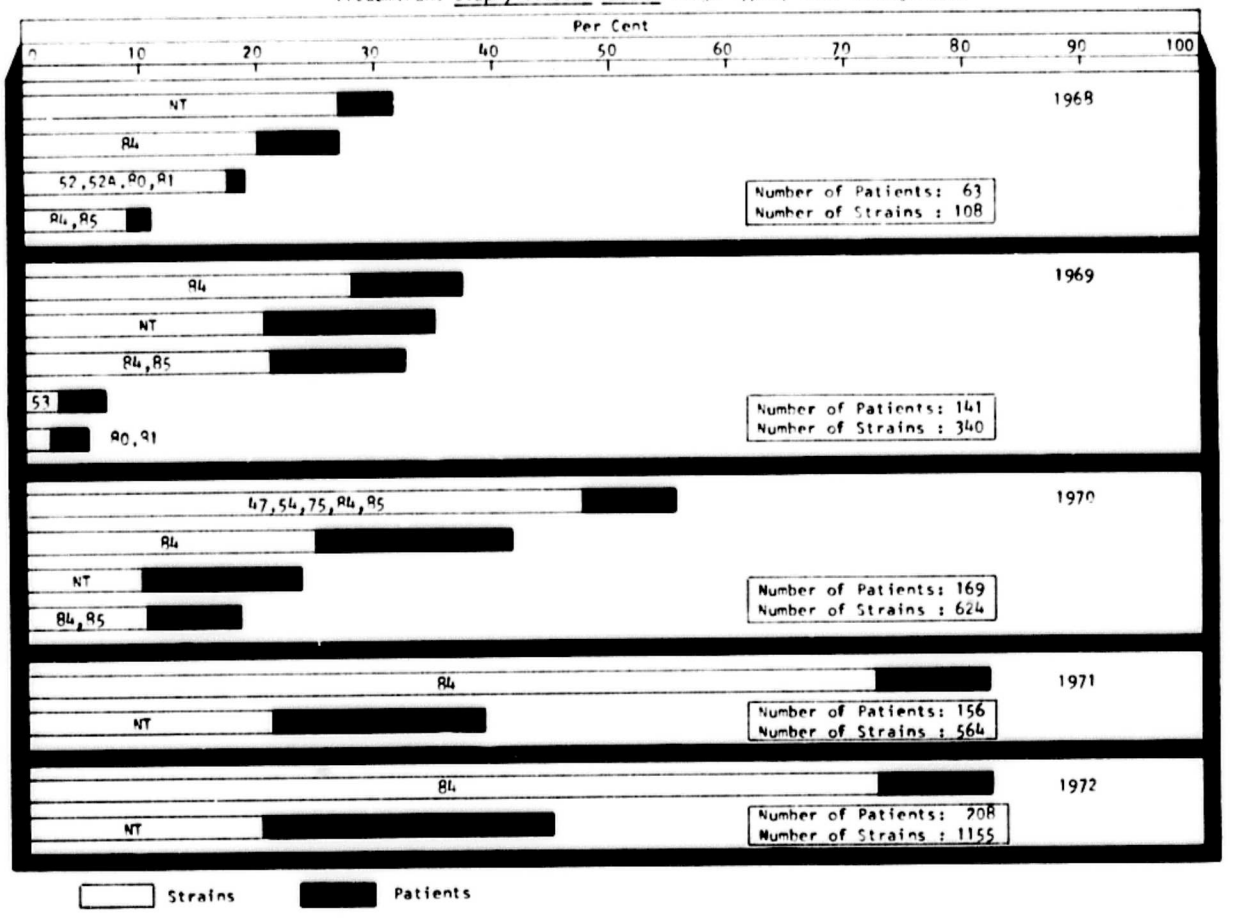
There has been a dramatic rise in the incidence of Staph aureus in burn patients, or at least, many more cultures have been submitted during the past year than in previous years. In 1968, 108 strains were collected for phage typing. In 1972, this figure was 1155.

Predominant phage types and the percentage of nontypable for the period 1968-1972 are shown in the figure. The per cent of each phage type is the proportion of strains of that type to the total number of strains collected for that year. The percentage of patients harboring a certain type is set down in relation to the total number of patients from whom strains were collected in that year.

In 1968, nontypable strains predominated. They were found in 31.7% of the patients cultured and accounted for 26.8% of all strains. Phage type 84 was the most common type; it was found in 27% of the patients and made up 20.4% of all strains. The classic hospital type of the 1960's, 52,52A,80,81, was still found in 1968, but that was its last appearance. 84,85 as an entity distinct from type 84 was present in small numbers. Nontypables and 84 made up 47.2% of all strains.

In 1969, type 84 was the predominant form, being found in 37.6% of the patients and accounting for 28.2% of all strains. Nontypable strains were

Predominant Staphylococcus aureus Phage Types, 1968 through 1972



found in 35% of the patients and made up 20.6% of the strains. These 2 categories made up almost half of all the strains as they had in 1968. Type 84,85 was little changed from its proportion of the population in 1968, and type 53 and 80,81 were present in small numbers.

In 1970, with antibiotic resistance now a conspicuous feature of the staphylococcus population, a complex of closely related types with the designation of 47,54,75,84,85 appeared and predominated for the entire year. Out of 624 strains typed, 47.4% were of this type, and it was harbored by 55.6% of all patients harboring staphylococci. Type 84, which was most closely related to the 47,54..... complex as indicated by the frequency with which it appeared in successions of cultures, was the second most frequently encountered type; it appeared in 41.7% of the patients, and 24.8% of all strains. Nontypable strains and a small population of type 84,85 made up the remainder.

In 1971, the population of staphylococci converted to the unequivocal epidemic of type 84 that has now persisted for 2 years. Eighty-two per cent of the patients harbored type 84, and it made up 72% of all strains. Nontypable strains made up 21% of all strains. The proportion of type 84 in 1972 was exactly the same in patients, and in percentage of all strains. Nontypable strains were still 20% of all strains, although their incidence in patients had increased. The fact that the proportion of nontypable strains was low in proportion to the percentage of patients with such strains is explainable by the fact that 2 new phages were incorporated in the typing set in March 1972. D-11 and WH-1 accounted for a part of the previously untypable population. The total picture of types recovered in 1972 is summarized in Table 3. It is important that all type identities be accounted for but the minor importance of the 6 types that were recorded is apparent in the fact that together they made up only 4.7% of all strains.

PHAGE TYPES OF STAPH AUREUS FROM BLOOD CULTURES, ISR, 1972

The overall picture of staphylococcal epidemiology is based on the type distribution of all strains collected. There remains the question of the existence of virulent or invasive strains that might differ from the

Table 3. Phage Types of *Staphylococcus aureus* in ISR Burn Patients, 1972

Phage Type	Per cent	
	Patients	Strains
84	82.2	72.5
Non-typeable	44.7	19.9
WH-1	3.4	2.1
85	2.9	.8
47,54,75,(81)	2.4	.7
D-11	1.9	.4
83A	1.9	.3
83A,85	1.4	.4

whole population, which includes a numerical predominance of surface and sputum strains. Ninety-six strains of Staph aureus from blood cultures of 50 patients were typed;76% of these strains were type 84, and 23% were nontypable. Their identity exactly followed that of the whole population of staphylococci. Sixty-eight per cent of the patients harbored only type 84, 16% had only nontypable strains, and 16% harbored in succession both nontypable and type 84 strains. One patient harbored type 71.

PHAGE TYPES OF STAPH AUREUS FROM POSTMORTEM LUNG TISSUE OF BURN PATIENTS, 1972

From 21 fatal cases a total of 45 strains of Staph aureus were recovered. The type identity was scrutinized to learn whether any distinctive type pattern existed in patients who developed pneumonia due to staphylococci. The distribution was not significantly different from that of the whole population; 71.1% of the strains were type 84, 26.7% were nontypable, and 2.2% were type 71. Two-thirds of the patients had only type 84, 20% had nontypable strains, and 14% had both type 84 and nontyable strains.

DISCUSSION

The evolution of an exceptionally antibiotic-resistant population of Staph aureus in the burn wards of the Institute of Surgical Research has been documented by MIC determinations on staphylococcal isolates and by phage typing. During the past 5 years a population with many untypable strains and several phage types present has evolved, after passing through a period when an exotic phage pattern was predominent, to a situation which has existed since 1971, i.e., a monotype epidemic, with type 84 vastly predominant over a consistent component of nontypable strains. A high rate of septicemia and staphylococcal sepsis has appeared within the past 2 years, and the antibiotic susceptibility of these strains is markedly reduced. The problem is serious, and no obvious solutions to arrest the spread of this organism in the burn ward are apparent. The mechanisms of transmission in the hospital ward involves a large component of patient-to-patient transfer via attendant personnel. Since with staphylococci a con-

stant shedding from desquamating epidermis occurs, there is introduced a transmission potential that cannot be curbed if patients are to share space physically as is inherent in the structure of the burn ward.

This problem did not become acute until a monotype epidemic appeared. The sorting out of a highly resistant group of strains has increased the likelihood of incoming patients being seeded with staphylococci which can be expected to maintain their position of dominance for some time.

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PRESENTATIONS

Lindberg RB. A Review of Methods for Degerming Burn Wounds. Presented at a Symposium in San Francisco, Calif, 2-3 October 1972.

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 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
72 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	267
b. CONTRIBUTING				
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11. TITLE (Procede with Security Classification Code): (U) Sensitivity of Pseudomonas Aeruginosa Recovered from Burned Soldiers to Sulfamylon (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS:
003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
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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 73	.4	15
FISCAL YEAR CURRENT 74	.5	13

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FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Pseudomonas; (U) Burns; (U) Sulfamylon; (U) Topical Therapy; (U) Humans

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

23. (U) Burned military or civilian personnel represent a major factor in warfare; the control of infection in burns with Sulfamylon has greatly reduced the lethal infection. 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 determined by a drug in agar technic with controlled inoculum.

25. (U) 72 07 - 73 06 Resume of Sulfamylon-sensitivity of Pseudomonas aeruginosa showed periodic fluctuation of minimum inhibitory concentration (MIC) from 67 through 71. Groups of strains, of a single phage type at any given interval, exhibited increased resistance, up to 1.25% MIC, in contrast to a median MIC of .078% to.152%. However, a more extreme epidemic episode in 1972 presented a large group of isolates which required up to 1.25% for suppression. This variation has been accompanied by a small number of cases of burn wound sepsis and by increased Pseudomonas sepsis episodes. The mechanism of resistance is most probably episodal transfer; presence of such resistance factors is being sought. Study of possible treatment refractory strains is being intensified.

*Available to contractors upon originator's approval

ANNUAL PROGRESS REPORT

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

1 July 1972 - 30 June 1973

Investigators:

Robert B. Lindberg, PhD
Virginia C. English, MA
Ruth L. Latta, BS
Basil A. Pruitt, Jr, MD, Colonel, MC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

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Four hundred and sixty-three strains of Pseudomonas aeruginosa, collected from severely burned patients, were tested for sensitivity to Sulfamylon. A marked rise in the proportion of strains with a high Minimum Inhibitory Concentration (MIC) was found. The median sensitivity level rose from 0.125% to 0.316%. 6.3% of all strains showed an MIC of 1.25%, and 45.8% had an MIC of 0.625%. This level of resistance had not previously been seen in annual reviews of sensitivity of Ps aeruginosa to this drug. Resistant strains were concentrated in a group of strains, nontypable by the established phage typing system, which were recognizable as NT-2 strains by use of a pyocin-typing technic using undiluted phage fluids. No increase in Pseudomonas burn wound sepsis nor in complications due to type NT-2 strains was detectable, despite the rise in resistance to Sulfamylon. The strains were amenable to treatment when tested in a burned rat laboratory model. The epidemicologic significance of these resistant strains is not yet known.

Pseudomonas
Burns
Sulfamylon
Topical therapy

SENSITIVITY OF PSEUDOMONAS AERUGINOSA RECOVERED FROM BURNED SOLDIERS TO SULFAMYLON

Monitoring of strains of Pseudomonas aeruginosa for sensitivity to Sulfamylon has been an ongoing project since the adoption of topical Sulfamylon as a primary agent for burn treatment. The history of chemotherapy is replete with examples of therapeutic agents to which resistance appeared among previously sensitive bacterial populations. The phenomenon of persistence of strains of Pseudomonas in the burn ward for long periods of time, with continuous exposure to Sulfamylon, constitutes an obvious milieu for deriving resistant strains. There has been no indication of a marked increase in the incidence of Pseudomonas burn wound sepsis in the past several years, but the organism remains ubiquitous and its frequent appearance in blood cultures of terminally ill patients is a constant reminder of its invasive potential in the compromised individual.

METHODS

The technic for assessing sensitivity has been described (Lindberg RB, Contreras AA, Moncrief JA, Mason AD,Jr. USA Surg Res Unit Ann Rpt FY 1965, BAMC, Ft Sam Houston, Tx. Section 18)[1]. It consists of incorporating doubling increments of Sulfamylon acetate in agar, from 0.019% to 2.5% Sulfamylon acetate, since it is the clinical compound used, is also used for the senstivity assay, although Sulfamylon hydrochloride which was more active against Pseudomonas than is the acetate, was originally used. The inoculum size has been shown to be critical; the optimal seeding number is 1000 cells/drops of inoculum. Heavier inocula result in less sensitive readings.

RESULTS

There were 463 cultures tested in 1972, or 60% more than were tested in 1971. This increase is directly related to the increase in sampling that was occasioned by an unexplained increase in mortality and in morbidity associated with infection in 1972. Changes in the sensitivity of a bacterial population are most readily expressed in a chronologic sequence, and these changes are summarized in Table 1, starting in 1967. It may be seen by

Table 1

Inhibiting Concentrations of Sulfamylon for *Pseudomonas aeruginosa*, 1967-1972

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
1967	0	15	43	28	96	70	145	74	
% of Total (471)	0	3.1	9.1	5.9	20.3	14.8	30.7	15.2	
1968	0	0	12	103	43	94	37	5	
% of Total (294)	0	0	4.0	35.0	14.6	31.7	12.4	1.7	
1969	0	0	13	179	89	74	28	2	
% of Total (385)	0	0	3.4	46.5	23.1	19.2	7.3	0.5	
1970	0	0	0	65	83	83	59	6	
% of Total (296)	0	0	0	21.9	28.0	28.0	19.9	2.03	
1971	0	0	48	41	56	57	65	13	
% of Total (280)	0	0	17.1	14.6	20.0	20.4	23.2	4.7	
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
Total (2189)	0	44	328	462	455	409	371	115	5
% of Total	0	2.0	15.0	21.2	20.8	18.7	16.9	5.2	0.2

inspection that in 1972 a major change occurred in the population of Pseudomonas. Prior to this year, only once, in 1967, had any strains required 1.25% for inhibition. Indeed in 1970 the upper inhibitory limit was 0.312%. However, in 1972, 6% of isolates actually required 1.25% of Sulfamylon for inhibition. Even more disturbing as an indication of increas ng resistance, 45.8% of the strains tested required 0.625% for inhibition. The highest previous proportion with such an MIC had been 17% in 1971. At the opposite end of the spectrum, only 18.9% of strains were inhibited by 0.078% Sulfamylon or less. The lowest figure previously seen for this concentration had been 27.0% of strains, in 1969.

This numerical distribution of sensitivity is illustrated succinctly in the cumulative sensitivity on an annual basis in Table 2. For 1970 and 1971, almost half the strains were inhibited by 0.078% of Sulfamylon; in 1972, only 19% were inhibited at that level. It was necessary to use 0.312%, or 4 times as much Sulfamylon, to inhibit one-half of the strains in 1972. Most of the remainder were inhibited with 0.625%, but never before had such a large proportion of Pseudomonas strains been resistant to the lower concentrations of Sulfamylon. It is not possible to say that the drug would be ineffective against Ps aeruginosa on burns seeded with strains of this degree of resistance; but certainly the change was in a direction that did not bode well for antibacterial chemotherapy against this population.

The median value of sensitivity to Sulfamylon, calculated on an annual basis, shows the trend in response of these strains. Table 3 presents the median values for each year since 1967. The pattern is one of increasing resistance until 1970, with that year being exceptional for its sudden increase in sensitivity. In 1971 the level rose, and in 1972 a marked rise occurred, to an unprecedented level of resistance. The median value of 0.316% is not reassuring as to the future usefulness of Sulfamylon, if this trend continues.

The total incidence of Ps aeruginosa in the burn flora fell during 1972, and there was no increase in cases of Pseudomonas burn wound sepsis that would connote treatment failure. In terms of incidence of disease, frequency

Table 2

CUMULATIVE SENSITIVITY TO SULFAMYLON OF *PSEUDOMONAS AERUGINOSA*
1967-1972

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
1967	471	100	100	96.0	86.9	81.0	60.7	45.9	15.2	-
1968	294	100	100	100	95.4	60.4	45.8	14.1	1.7	-
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

*Concentration in gms.%

Table 3
Median Value of *Pseudomonas aerugirosa* Sensitivity to Sulfamylon

Year	No. of Strains	Median Inhibitory Value (gms.%)
1967	471	0.083
1968	294	0.136
1969	385	0.156
1970	296	0.078
1971	280	0.125
1972	463	0.316
Total of 6 years	2189	0.149

with which high concentrations of Ps aeruginosa were found in burn wounds, and of presence of Ps aeruginosa as the predominant organism in the wound at autopsy, it may be concluded that Sulfamylon remained effective in control of burn wound sepsis. Strains which required 0.625% or 1.25% for inhibition were tested for virulence in the burned rat model, treated with Sulfamylon cream. These strains could be effectively treated in the experimental animal. Nevertheless, the shift of sensitivity of Ps aeruginosa to Sulfamylon remains a matter of concern and of more intensive monitoring, to be sure that any treatment failure due to increased drug resistance will be detected.

Ps aeruginosa strains are differentiated by bacteriophage typing, using the set of typing phages derived by Latta, Brame and Lindberg (Lindberg RB, Latta RL, Brame RE, Moncrief JA. Bact. Proc. 1968, p. 85)[2]. The epidemiology of colonization and of nosocomial infection by strains of this ubiquitous species can be monitored and evaluated by having available a means of differentiating strain identity. Without such a tool, no rational differentiation of strain behavior is possible. In 1972, a marked change took place in phage type identities over that recorded in observations during the preceding decade. A large part of the Pseudomonas population recovered in 1972 was non-reactive with the phage set that had previously been an effective typing system. A new modification was devised, in which the phage nontypables were differentiated using lytic patterns of the pyocin type with undiluted phage typing fluids. Together these technics permitted differentiation and characterization of the type identities of over 95% of all strains examined.

Typable strains from 41 patients are presented in Table 4. These include only the more frequently encountered types; as always, a large number of individual types which occur but rarely are not listed, since their incidence has no bearing on transmission on the burn ward. Type C-26, D-41, and F-21, together with several closely related types in the "D" group, showed a sensitivity level within the limit of inhibition shown by the whole population tested. The majority were inhibited by 0.156% of Sulfamylon. In type F-21, a group of 4 patients, widely separated in time of

Table 4
SULFAMYLON SENSITIVITY REACTION OF PREDOMINANT PHAGE TYPES - 1972

	PATIENT NO.	ISOLATES WITH INHIBITING CONCENTRATIONS* AT 1.25	0.625	0.312	0.156	0.078	0.039	0.019
Type C-26	104			1				
	114				1			
5 patients	142				1			
	134				1	1		
7 isolates	213				2			
Total-each inhibiting strain				1	5	1		
Type F-41	83					1		
	100				1			
7 patients	98			1				
	237				2			
10 isolates	199				2			
Total-each inhibiting strain	78			1				
	247					2		
				2	5	3		
	166							1
Other D groups	278					1		1(<.019)
	253	1						
9 patients	231					1		
	216				1(D-43)		2(D-43)	
12 isolates	167							1
	64					1(D-42)		
Total-each inhibiting strain	63				1(D-42)			
	134						1(D-42)	
		1			2	3	3	
	102			1				
Type F-21	118				2			
	143			2	1			
	63				1			
14 patients	95				1			
	124			1				
	66				1			
	202			1	4			
22 isolates	160				1			
	192				1			
	198				2			
Total-each inhibiting strain	187							1
	186				1			
	194				1			
				5	16			1
Type F-12	194		1					
	202				1			
6 patients	154		1					
	161		1					
6 isolates	124				1			
	162		1					
Total-each inhibiting strain			4		2			

* All concentrations in Gms./100 ml.

admission, all required 0.312% of Sulfamylon for inhibition, and a comparable proportion of D-41 strains showed an MIC of 0.312%. The greater part of these strains were sensitive to 0.156% or less.

Type F-12 was represented by only 6 strains, but the balance of sensitivity was clearly on the resistant side. At 0.625%, this type was more resistant than the average, even in 1972. The existence of resistant types, persisting in the burn ward over at least several months, was again demonstrated.

The nontypable strains were designated as NT-1 through NT-2, depending on the lytic patterns which undiluted typing fluids elicited. Reactions of strains from 143 patients, collected during the entire year, are summarized in Table 5. There was an unequivocal cluster of relatively resistant strains in the group designated NT-2,2a,2b, and 2c. Here, the great majority of strains were relatively resistant to Sulfamylon. One hundred seventy-three out of 183 strains required 0.625% or 1.25% for inhibition. These strains were the most common encountered during 1972; the higher types in numerical order, from NT-3 to NT-20, totalled only 32 strains. Nine of these, in 2 types, required 0.625% for inhibition. The remaining 23 strains fell in an MIC range from 0.312 to less than 0.019.

It was, then, apparent that the predominant types of Ps aeruginosa included a preponderance of relatively resistant strains. This group of organisms did not constitute an overwhelming majority. There were 175 strains in the 0.625% and up bracket of resistance; 104 were inhibited by 0.312% or less, and of these, 74 had an MIC of 0.156% or less.

There was no unusually high rate of wound invasion seen with strains of the NT-2 group. Since they were numerically predominant, it would be expected that they would also be frequently encountered among isolates from blood, tissue and sputum, and this was indeed the case. Eighteen patients yielded NT-2 types in blood cultures; 15 patients had other types. The NT-2 group occurred in proportion to their overall incidence. In lung tissues at autopsy, 18 patients harbored NT-2 types; 29 harbored other types out of 47 patients with Ps aeruginosa present in the lung at autopsy.

Table 5

SULFAMYLON-SENSITIVITY REACTIONS OF VARIOUS NON-TYPABLE STRAINS

Non-typable Strain	No. of Pts.	No. of Strains	1.25 gms. %	0.625 gms.%	0.312 gms.%	0.156 gms.%	0.078 gms%	0.039 gms.%	0.019 gms.%
NT-1	13	19		5	12	1	1		
NT-2	4	6	3	3					
NT-2a	17	48	11	36	1				
NT-2b	24	44	10	31	1	2			
NT-2c	44	85	4	75	1	4			1
NT-3	1	1			1				
NT-5	4	5				5			
NT-6	2	2							2 (≤.019)
NT-7	1	1			1				
NT-8	3	7			1	1	3	2	
NT-11	2	2		2					
NT-13	4	5				2	1	1	1
NT-14	2	2					2		
NT-20	6	7		7					
Other Non-typables	16	33		18	4	3		6	2
TOTAL:	143	267	28	177	22	18	7	9	6

DISCUSSION

After several annual intervals in which it could correctly be stated that no Sulfamylon-resistant forms of Ps aeruginosa had been observed, a shift in identity of strains occurring on the burn wards of this Institute was accompanied by an abrupt increase in tolerance for Pseudomonas to a range of 1.25% MIC. A large part of the total population of Ps aeruginosa belonged to one distinctive group designated as NT-2 on the basis of a pyocin-phage lytic identification technic. It was found that this group of strains accounted for most of the resistant strains found. All NT-2 strains were not resistant but the great majority were. The strains were not refractory to treatment of experimental burn wound sepsis, and the term "resistant" is used to designate their position at the upper end of the scale of sensitivity testing. They appear to be amenable to treatment with 10% Sulfamylon burn cream, and may well represent a transient phenomenon, in view of previous observations on variations in types of Ps aeruginosa populating the burn ward. No previous incursion and persistence of a specifically identifiable type of Pseudmonas has been seen before, and the phenomenon merits further study to be sure that current concepts of topical therapy in burns remain valid.

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PRESENTATIONS

Lindberg RB. Microbiology of the Damaged Epidermis. Presented at Annual Meeting, Soc Ind. Microbiol., Minneapolis, Minn., 31 Aug 1972.

Lindberg RB. A Review of Topical Agents for De-germing the Burn Wound. Presented at Duke Univ School of Med Symposium, San Francisco, Calif. 1 Oct 1972.

PUBLICATIONS

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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) 72 07 - 73 06 The major part of admissions is bacterial flora derived from patients injured in CONUS. Sampling by swab technic resulted in fewer positive samples on admission; the superiority of 100 mm contact plates was reaffirmed. Predominant flora on admission included Klebsiella, E.coli, Staph aureus, Enterobacter cloacae and Pseudomonas aeruginosa. Staph aureus type 84, the predominant type in the ISR, also arrived on incoming patients. Prov stuartii was less common on arriving patients than had previously been the case, but was found in significant numbers. The predominant species included in the Enterobacteriaceae have not thus far been reflected in a rise in clinical disease due to these species.

*Available to contractors upon originator's approval

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ANNUAL PROGRESS REPORT

PROJECT NO. 3A 161102B71R-01, RESEARCH IN BIOMEDICAL SCIENCES

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

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

1 July 1972 - 30 June 1973

Investigators:

Robert B. Lindberg, PhD
Anthony A. Contreras, MS
Virginia C. English, MA
Ruth L. Latta, BS

Reports Control Symbol MEDDH-288(R1)

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ABSTRACT

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Routine swab culture technic was used to sample 139 patients at time of arrival at the Institute of Surgical Research. The technic reflected proportional distribution of species but was less sensitive in detection of small numbers of bacteria. Only 104 patients yielded positive cultures. Eighty-five per cent of the negative cultures were from patients sampled within the first 2 postburn days. Predominant flora on admission included Pseudomonas aeruginosa, Klebsiella pneumoniae, Enterobacter cloacae, Escherichia coli, and Staphylococcus aureus. Pseudomonas was most common after 10 days postburn, as was Klebsiella. Enterobacter and Providencia stuartii were most common at 3 to 5 days postburn; Staph aureus was at its maximum incidence soon after burning (0-2 days) or after 10 days postburn. The incidence of species which predominate throughout the patients' course indicate that a continuous re-seeding of the burn ward population by incoming patients is virtually inevitable. It renders less probable the presence of an unmanageable indigenous flora peculiar to the burn ward.

Burns Microbiology of burns Pseudomonas Providencia Humans

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

With the reduction of forces engaged in ground combat in Vietnam during 1972, the proportion of patients admitted to this Institute from the Far East fell precipitously. This change in the patient population was accompanied by an altered bacterial population on the incoming patient. The flora of the incoming burn patient is important in offering clues as to the source of species and strains of bacteria that colonize the burn wound and which may be the offenders in actual wound infection. When an increase in incidence of a common pathogen, such as Staphylococcus aureus, is believed to have occurred, any control measures must take into consideration the input of this species which occurs as part of the flora of the burned patient on admission. Attempts to eradicate a colonizing species from the burn ward population are fruitless if the organism is to be introduced into the burn ward with new admissions. This report is based on culture data obtained from 139 patients at time of admission to the Institute burn ward.

METHODS

Previous studies have emphasized the importance of contact plates as a technic for collecting the most precise information regarding surface wound flora (Lindberg RB, English VC, Pruitt BA,Jr. USA Inst Surg Res Ann Rpt FY 1969, BAMC, Ft Sam Houston, Tx. Sec 13)[1]. The marked decrease of input of burn patients from the Far East, and altered emphasis on study priorities, led to suspension of this time-consuming technic, but admission cultures on an adequate sample of patients were obtained by using moistened cotton swabs for sampling, with a stable holding medium used for transport of swabs to the laboratory. Unquestionably, this technic produced a smaller yield of positive cultures than did the use of a 100 mm diameter contact plate (Lindberg RB, Switzer WE, Moncrief JA, Mason AD,Jr. USA Surg Res Unit Ann Rpt FY 1968, BAMC, Ft Sam Houston, Tx. Sec 7)[2], but the positive findings still show the likelihood of presence of significant flora. These positive findings, compared with those obtained with contact

plates, indicate the relative distribution of flora on incoming patients in a more conservative manner: if these cultures are positive, then certainly more sensitive detection technics would only increase the number recovered.

RESULTS

The incidence of positive cultures on 139 patients sampled on admission to the Institute of Surgical Research is shown in Table 1. It was evident on inspection that the rate of positive cultures was far below that obtained with the contact plate technic: that procedure yielded virtually no completely sterile plates. However, the proportion of cultures positive for various species was not markedly unlike that obtained in 1970 with contact plates. Since the recovery rate was lowered by a less sensitive technic, a more realistic comparison with contact plate technics was obtained by presenting the incidence rate of species among those patients whose cultures were positive. The predominant species were Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli, Enterobacter cloacae, and Pseudomonas aeruginosa. Among all patients cultured, Providencia stuartii was recovered from 9.3%, or 12.5% of those with positive cultures. Thus, the opportunity for introduction of these species, which include those presenting the greatest problem of sepsis in the burned patient, is high. When more sensitive culturing technics were used, the recovery rates were in many instances much higher.

Since the incidence rate of various opportunistic colonizing or invading species may vary with changes in the antecedent history of patients, and since the sampling technic was less sensitive than that used in earlier years, a comparison was made of the incidence of the major species on an annual basis. These comparisons are presented in Tables 2, 3, and 4. Each group of cultures is presented in terms of proportion of patients with positive cultures who harbored each individual species, over the period from 0 to over 21 days postburn at time of admission.

Pseudomonas aeruginosa: Over the past 5 years, the rate of seeding of the burn with this important pathogen has been relatively low. Prior to 1972, a sharp rise in carrier rate on admission had always been seen.

Table 1. Cultures on 139 Patients on Admission to ISR: Swab Culture of Burn Surface - 1972

Species	% of all Patients Positive	% of Each Species Among Patients Positive
Pseudomonas aeruginosa	14.3	19.2
Klebsiella pneumoniae	27.3	35.5
Enterobacter cloacae	30.9	41.1
Escherichia coli	20.1	26.9
Serratia marcessens	2.8	3.8
Citrobacter freundii	1.4	1.9
Proteus mirabilis	8.6	11.5
Providencia stuartii	9.3	12.5
Mima-Herellea sp	4.3	5.7
Staphylococcus aureus	25.1	33.6
Staphylococcus epidermidis	9.3	12.5
Streptococcus, non-hemolytic	2.8	3.8
Corynebacterium sp	2.8	3.8
Bacillus sp	8.6	11.5
Candida sp	13.5	18.2
No growth on swab culture	25.1	
Total patients cultured:	139	
Patients with positive cultures:	104	

Table 2. Pseudomonas, Klebsiella, and Enterobacter on Burn Wounds at Time of Admission to ISR: 1968 - 1972

Postburn Interval	Species and % of Patients Positive											
	Pseudomonas				Klebsiella				Enterobacter**			
	68	69	70	72	68	69	70	72	68	69	70	72
0 - 2	21	6	7	9	29	25	21	20	40	30	32	38
3 - 5	46	52	54	5	55	47	54	32	48	26	54	64
6 - 10	50	44	4	16	67	53	30	42	37	44	27	37
11- 20	52	51	55	48	51	56	40	52	26	27	21	38
21+	54	76	70	38	45	61	44	62	18	23	15	13

	No. Patients Cultured at Each Interval			
	1968	1969	1970	1972*
0 -2	47	59	28	34
3 - 5	56	19	11	22
6- 10	97	54	26	19
11 - 20	78	66	82	21
21+	11	13	27	8

* This figure in 1972 includes patients with positive cultures; in preceding years, culture technic recovered bacteria from every patient.

** Primarily E. cloacae; on admissions, E. aerogenes was rare.

Table 3. Proteus mirabilis, Providencia stuartii, and Escherichia coli on Burn Wounds at Time of Admission to ISR: 1968 - 1972

Postburn Interval	Species and % of Patients Positive											
	Proteus mirabilis				Providencia stuartii				Escherichia coli			
8	69	70	72	68	69	70	72	68	69	70	72	68	69	70	72
0 - 2				10	10	0	9	6	3	7	9	23	30	18	18
3 - 5				23	31	0	18	7	5	9	27	15	31	36	23
6 - 10				43	38	27	0	17	40	27	5	23	24	38	37
11 - 20				38	57	27	14	32	31	41	14	21	36	28	28
21+				36	46	29	25	27	23	48	0	9	0	29	50

Table 4. Staphylococcus aureus and Staphylococcus epidermidis on Burn Wounds at Time of Admission to ISR: 1968-1972

Postburn Interval	Species and % of Patients Positive							
	Staph aureus				Staph epidermidis			
	68	69	70	72	68	69	70	72
0 - 2	14	20	21	38	48	52	70	20
3 - 5	13	26	36	22	30	26	27	14
6 - 10	24	64	54	21	17	11	15	5
11 - 20	28	43	54	38	30	21	8	5
21+	18	30	48	62	27	23	22	12

It is distinctly possible that with increasing use of topical therapy on the burn at the hospital of initial admission, the general seeding rate is suppressed for a longer period. After the first week postburn, the likelihood of presence of Ps aeruginosa increases somewhat but the rate has not been near the 50% rate recorded for 1968 and 1969. Again, more widespread use of topical therapy could explain this change. However, in the 11 to 20 day postburn period, the seeding rate rises to half of the admissions. It has long been observed (Lindberg RB, Moncrief JA, Switzer WE, Mason AD, Jr. Antimicrobial Agents & Chemotherapy, 1964, p. 708)[3] that the seeding rate with Ps aeruginosa increases during the third postburn week, even with continued topical therapy. This pattern has continued to the present. The unexpectedly low value of 38% of positives after 21 days in 1972 may reflect a relatively small sample of patients.

Klebsiella pneumoniae and Enterobacter cloacae, E coli. The initial high seeding rate with these common enteric forms reflects the ease with which fecal contamination occurs. Klebsiella and E coli are in the same range in the early burn; the higher rate of Enterobacter may reflect a greater affinity of this species for the early wound, since it is not that much more common in the grit. Klebsiella rises to a 50% to 60% level on burns at

admission in later periods, and persists to the latest observations. The Enterobacter incidence stays high for the first week, then tends to drop somewhat. Although it remains relatively common, E coli, unlike the Klebsiella never really reaches a high level of incidence on incoming patients. Its incidence has remained relatively constant over the last 5 years.

Proteus mirabilis and Providencia stuartii are closely related species. Other species of Proteus have appeared on wounds infrequently during hospitalization at this Institute, but have not been found at time of admission. Neither of these species is common on the wound in the first 48 hours, but they are consistently found. Thus, the presumed uniqueness of Providencia in this Institute is refuted by its appearance early in the postburn period. Its incidence was higher in the 3 to 5 day interval in 1972 than it was previously, but its incidence rate has fluctuated widely in subsequent intervals postburn. The lessened incidence in the admissions arriving more than 10 days postburn in 1972 has no immediate explanation; again, less sensitive sampling technic and a smaller sample contribute to such changes. This change was less marked with Proteus, the ubiquitous nature of the latter on older wounds may make its recovery more uniform.

Staphylococci: Staph aureus has appeared as a major lethal factor in various forms of sepsis supervening in burn patients in 1972, and there is a natural desire to eliminate its presence in the burn population. Such control, were it to be achieved, would obviously need to start with diminishing the input of seeded patients as ready sources of new contamination. The record of seeding with Staph aureus on incoming patients is not encouraging in this regard. The carrier rate on arriving patients has fluctuated, but it is obvious that it has never been low enough, nor was it in 1972, to suggest that incoming patients would not soon reexpose the ward population. The decrease in carrier rate in the 6 to 20 day postburn period since 1970 corresponds to the reduction in patients from the Far East late in their postburn course; it is probable that the heterogeneous patient population transported in CONUS offers less opportunity for cross-seeding than occurred in long trans-Pacific flights with groups of patients in close proximity.

The pattern of cultures of Staph epidermidis has been one of initial seeding, presumably from the patients' endogenous flora, with a rapid decrease in the carrier rate after the first week. This species rarely has caused infection in the burned patient.

One-fifth of the Staph aureus strains arriving on incoming patients has been type 84, the epidemic type that has been persistent in the burn population of this Institute for over a year.

DISCUSSION

In view of a current problem which has been described as epidemic presence of Providencia stuartii and of Staph aureus type 84 on the Institute of Surgical Research burn ward, the bacterial flora of incoming burn patients remains of interest. The bacteria on incoming patients represents a source of new seeding of patients already on the wards and describes the natural history of typical burn wound microbiology at progressive intervals postburn. The early postburn wounds continue to exhibit a consistent low level of seeding with Ps aeruginosa, through the first 2 weeks. Normal fecal flora constitutes a major part of the admission flora, especially Klebsiella sp. Providencia stuartii was less common on incoming patients than was the case in 1970, but it still is present on significant numbers of incoming patients. Staph aureus is a major part of the early burn flora on incoming patients; the incidence was lower than in 1970, although this may be due to changes in sampling technics. The incoming patient harbors a flora that may readily be supplanted by resident microepidemic flora, but a completely sterile burn ward would, with such patients, promptly acquire a population of bacterially seeded patients.

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PRESENTATIONS and/or PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

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23. (U) Soldiers in combat incur thermal injury at a high rate; in its treatment, suppression of invasive 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) 72 07 - 73 06 Localized episodes of exotic wound infection have been observed. Sepsis associated with Salmonella typhimurium was detected, as a unique burn wound invader. Intermittent epidemic episodes of Serratia and of Mima-Herellea infection were also differentiated. Staph aureus type 84 continued to present a major epidemic problem, most probably based on intense and perpetuating seeding of the burn ward. Phage types WH-1 and D-11, not previously observed in this population, appeared on several occasions, but did not become established on the burn ward. Providencia stuartii continued to be present in epidemic pattern. Serotyping has been developed; at present a high proportion of a single sero-group (samatic) appears to be present. Control of this opportunistic pathogen has not yet been achieved.

*Available to contractors upon originator's approval

ANNUAL PROGRESS REPORT

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

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

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

1 July 1972 - 30 June 1973

Investigators:

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Reports Control Symbol MEDDH-288(R1)

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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 OF MILITARY PERSONNEL RECEIVING SULFAMYLON TREATMENT

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

Period covered in this report: 1 July 1972 - 30 June 1973

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Sepsis has continued as a major cause of morbidity and death in burned patients, and the role of opportunistic bacteria in this situation is studied by assessing the bacterial population of wound, lung, urine and other pertinent sources in the burn patient. The predominant flora of the wound surface, deeper tissues, viscera, lung, and blood stream included Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae, and Providencia stuartii. The occurrence of Escherichia coli increased slightly, but Proteus mirabilis decreased further in incidence in a pattern now of 2 years' duration. Gram positive cocci tended to prevail in the early post-burn period, only to be replaced by Enterobacteriaceae, with a marked terminal increase in sepsis due to Providencia. Staphylococcal sepsis was more common in 1972 than was the case at any time in the past 6 years. Control of infection with antibiotic was hampered by the high incidence of resistant forms.

Burns Staph aureus Providencia stuartii Sepsis Humans

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

Control of infection in the severely burned patient has been, and continues to be, a major facet of burn care. Topical treatment with Sulfamylon has reduced the incidence of Pseudomonas burn wound sepsis (BWS) to a low level. Those cases of BWS which do occur are, in most instances, late invasive sepsis occurring several weeks after injury in a patient who had surmounted the initial phases of injury through separation of eschar, with subsequent failure to form a granulating wound bed capable of accepting grafts. Such wound surfaces remain a serious threat to survival, and their involvement with a mixed flora of Enterobacteriaceae of several species, together with Pseudomonas and Staphylococcus aureus constitutes an infection problem less clearcut than the classical Pseudomonas burn wound sepsis which occurred essentially as an infection caused by a single species, with onset usually within 10 to 12 days after injury. Changes in the incidence of the principal bacterial species concerned with infection in the burned patient occur, and these patterns of colonization and predominance have been assessed in this Institute with study of the various categories of cultures collected. The totals reported include strains collected in various prospective studies which have been carried out during 1972.

The bacterial flora observed during 1972 includes those species which were observed during colonization of the patient as well as strains recovered from specimens collected in attempts to diagnose and manage episodes of pneumonia and other systemic or local forms of bacterial sepsis in the burn patient.

ANTEMORTEM BACTERIOLOGY IN BURNED PATIENTS

Total Cultures. The bacterial flora recovered from clinical specimens in 1972 is shown in Table 1. The largest numbers of isolates were collected from burn wounds, sputum, and biopsies. The number of blood cultures taken was presented as the total specimens inoculated; 2 blood

Table 1. BACTERIOLOGY OF ANTEMORTEM BURN PATIENTS - 1972

ORGANISM	SOURCE AND NUMBER ISOLATES								
	WOUND Surfaces	STOOL	BLOOD	LUKENS Tube	URINE	CATH. I.V.	TIP Foley	BIOPSY	TOTAL ISOLATES
Staph. coag. pos.	520	3	103	107	26	61	26	78	924
coag. neg.	52	1	4	35	15	12	7	9	135
Alpha hemol. strep	60	-	1	131	3	-	-	-	195
Beta hemol. strep	66	-	1	8	2	1	-	2	80
Group A strep	56	-	1	1	1	2	-	-	63
Non-hemol. strep	78	10	8	20	11	5	8	23	163
Diplococcus pneumoniae	1	-	-	1	-	-	-	-	2
Corynebacterium sp.	49	-	3	6	1	-	1	6	66
Bacillus sp.	22	-	11	5	3	1	1	7	50
Pseudomonas sp.	331	7	45	305	58	32	34	75	887
Mima-Herellea sp.	41	1	1	4	2	2	1	2	54
Aeromonas liquefaciens	3	2	1	5	1	2	-	8	22
Klebsiella sp.	278	57	37	221	37	23	40	80	773
Enterobacter aerogenes	19	2	2	47	5	8	2	8	93
cloacae	108	6	8	52	6	13	12	51	256
hafniae	2	-	-	2	1	-	-	-	5
Serratia sp.	24	-	22	21	4	6	1	10	88
E. coli	239	73	15	113	57	9	56	51	415
Citrobacter sp.	4	3	-	3	1	-	2	5	18
Salmonella typhimurium	6	-	1	-	-	1	-	-	8
Proteus mirabilis	195	22	30	89	53	8	19	25	441
vulgaris	2	1	-	-	1	1	-	-	5
rettgerii	1	-	-	-	-	1	-	-	2
morganii	27	-	2	3	4	1	2	1	40
Providence stuartii	526	10	124	435	125	109	74	149	1552
Neisseria sp.	29	-	-	77	-	-	-	-	106
Candida sp.	70	-	7	23	71	21	31	30	253
No. of Specimens	1478	81	3520	721	528	620	205	362	
No. of Isolates	2809	198	427	1722	490	318	319	622	6696
No. Patients Cultured	224	50	209	122	175	185	116	101	
Total Specimens Received:	7525								

culture bottles were collected in each instance, so that 1760 sets were cultured. The proportion of patients regarded as possible bacteremia suspects obviously increased over the 1971 figure, when 1331 sets were collected. At least 28 species of bacteria were recovered. Predominant were Staph aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae, and Providencia stuartii. These species constituted 61.6% of all isolates recovered. These same species were predominant in 1971. No significant increase in new opportunistic invaders occurred in 1972. The principal alteration from previous years was an absolute increase in the recovery rate of Prov. stuartii.

The proportion of all isolates which the 4 predominant species contribute has remained relatively constant over the past 4 years despite variations in emphasis on various aspects of infection in burn patients which has occurred during that time. The distribution of these species is summarized for this period in Table 2. The proportion of all isolates made up by these 4 species varied from 52 to 61%.

Bacteriology of the Burn Wound Surface. The burn wound surface is inevitably colonized by bacteria, no matter what type of antimicrobial topical therapy is used. Sulfamylon was not designed to prevent colonization, but to control BWS due to Pseudomonas invasion. The massive and prolonged colonization that is present from the first few days of burn to the time of closure by grafting plays a role that is not clear in the pathogenesis of infection in burn patients. In any event, the surface flora is invariably a matter of concern in burn therapy, and it represents the essential milieu for the bacterial population of the burn ward.

There were 26 species (or genera) of bacteria recovered on burn wounds in 1972 (see Table 1). Many of these were numerically inconsequential, but their capability to colonize the burn wound is thus documented. Species of enteric bacilli once considered innocuous opportunists, have today become problem species in burn patients; there is no assurance that this cannot occur with other species.

Predominant species, and those of particular interest as pathogenic

Table 2. Predominant Species Among Clinical Isolates, 1969-1972

Predominant Species	% of All Isolates from Clinical Specimens			
	Year			
	1969	1970	1971	1972
Staph aureus	11.2	12.6	15.0	13.8
Ps aeruginosa	15.0	13.6	12.4	13.2
Kleb pneumoniae	10.8	11.5	9.7	11.5
Prov stuartii	17.2	21.0	15.2	23.1
Percentage of all Isolates	54.2	58.7	52.3	61.6
Total Number of Isolates	4482	3293	3179	6696

agent in wounds are listed in Table 3. Seventy per cent of all patients admitted in 1972 were cultured at least once. This number of patients cultured is a higher proportion of admissions than had previously been recorded; it reflects the increased concern which has developed over the possible contribution of burn wound infection to an increased mortality rate in burn patients in this Institute.

Staph aureus was the most common pathogen recovered on burn wounds. These strains were predominantly phage type 84, and the homogeneity of this population supports the conclusion that this type constituted a monotype epidemic perpetuated by patient-to-patient transfer of strains on the burn ward (Bulow P. Ann NY Acad Sci. 182:21-39, 1971)[1]. The antibiotic resistance level of staphylococci was high.

It should be noted that 30% of patients cultured did not harbor staphylococci. Many of these patients were heavily colonized by gram-negative bacteria. Even staphylococci thus could not be regarded as ubiquitous in this burn population.

Klebsiella pneumoniae increased in incidence in 1972 to 43.8% of all patients cultured, from the 25.2% incidence recorded in 1971. Enterobacter cloacae showed a similar rise, but most of the other enteric species showed little change in incidence from previous years. An exception was Prov. stuartii, which increased from an incidence of 34% in 1971 to 49.5% in 1972.

Streptococci on Burn Wounds. In previous years, group A hemolytic streptococci were relatively rare on burn patients. The predominant streptococcus was a group D organism, essentially Enterococcus (Lindberg RB, Contreras AA, Pruitt BA, Jr, Smith HOD, Jr, Kirchgessner PM. USA Inst Surg Res Ann Rpt FY 72, Sec 19)[2]. However, in 1972 a new picture appeared; group A streptococci appeared in a series of explosive episodes. The relative frequency with which this organism had previously been encountered is shown in Table 4.

In November 1971, a change in prophylactic regimen for incoming patients was instituted. Prior to this date every patient admitted was placed on a 5-day course of Penicillin G. The assumption was that this procedure

Table 3. Predominant Burn Wound Surface Flora in 224 Patients, ISR, 1972

Species	Nr Patients Positive on Burn Surface	% of Patients Positive	Nr. Strains Isolated
Staph aureus	156	69.6	520
Klebsiella pneumoniae	98	43.8	278
Enterobacter cloacae	56	25.0	108
Escherichia coli	74	33.0	239
Proteus mirabilis	45	20.1	195
Providencia stuartii	111	49.5	526
Pseudomonas aeruginosa	90	40.2	331
Streptococcus Group D	56	25.0	78
Streptococcus Group A	29	12.9	56

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

would minimize the introduction of carriers of group A streptococci into the burn wards. On the premise that this constant use of penicillin might exert a predisposing effect toward emergence of a resistant opportunistic flora, the regimen was discontinued. On 30 December 1971, the first of a series of 6 patients was found to harbor group A streptococci on the burn surface. This was the first of 10 chronologically separate episodes, between January and September 1972, in which group A streptococci were recovered from the burn wound. A total of 31 patients yielded 56 strains. None of these patients exhibited major systemic invasion. However, the pathogenic potential of group A streptococci in unprotected burn patients is well documented. If this upsurge of hemolytic streptococci was indeed due to the omission of prophylactic penicillin on admission, then the advisability of this omission merits, at least, careful consideration.

Ps aeruginosa has continued to be a conspicuous part of the burn flora. There appears to be little clinical significance to such colonization in most instances. Actual Pseudomonas burn wound sepsis, as an early, overwhelming, invasive process, was rare, as has been the case since the initiation of topical Sulfamylon treatment of burn wounds. The emergence of a treatment resistant form of the organism is reported elsewhere in this report.

RESPIRATORY TRACT BACTERIAL FLORA IN BURNS

Respiratory tract disease has become the most serious complication of burn injury in patients in the Institute of Surgical Research during the past 4 years. During 1972 the number of samples of sputum and Luken's tube aspirates submitted for culture was 721, from 122 patients. This was a marked increase from the 287 samples from 94 patients recorded in 1971, and may well reflect at least an increase in concern over the etiology and pathogenesis of respiratory disease, if not an absolute increase in the proportion of patients who exhibit symptoms which call for bacteriologic study of the sputum.

In sputum alpha-hemolytic streptococci were recovered from 52% of all patients cultured. This constituted the normal flora of the upper respiratory tract; it was not a pathogenic component of the respiratory problem. The microbial species associated with pneumonia in burn patients in 1972 are summarized in Table 5. The comparable figures for 1969 and 1971 are presented to illustrate the relatively constant incidence of some species and the changes noted with others.

Table 5. Species of Bacteria Recovered from Respiratory Tract of Burned Patients, 1969-1972

Species	% of Patients with Positive Sputum 1969	1971	1972
Ps aeruginosa	52	39	38.5
Klebsiella sp	64	45	58.8
Enterobacter cloacae	-	11	27.0
Proteus mirabilis	45	-	19.0
Prov stuartii	54	33	56.5
E coli	48	27.2	40.9
Staph aureus	42	43	38.5
Patients cultured	112	94	122

The most frequently occurring gram negative species were Klebsiella pneumoniae and Providencia stuartii. Ps aeruginosa has remained relatively constant in incidence in the sputum, and it appeared with the same frequency as did Staph aureus. Gram negative bacilli were present in the most intractable pneumonias, and have continued to constitute the most pathogenic flora in the lung of the burned patient. As noted in the section on antibiotic sensitivity of burn wound flora, these species were relatively resistant to antibiotics. In those patients who failed to survive and in whom pneumonia was a major factor in the lethal outcome, a frequent sequence of events was the emergence of Prov stuartii and Ps aeruginosa as the sputum flora in the last 3 or 4 days of the patients' illness. These species have been found in a high proportion of sputa from fatally ill patients with pneumonia in 1971 and 1972.

SEPTICEMIA AND BACTEREMIA IN BURN PATIENTS

Sepsis has remain a major cause of death in burns, and the identity of bacteria which invade the blood stream is of maximum interest in assessing the pathogenesis of burn mortality. Blood cultures are, of course, collected on some patients who did not show clinical signs of septicemia, and thus the number of patients cultured includes some who were not plausible candidates for bacteremia.

With 209 patients cultured, there were 100 patients with positive blood cultures. This was a positive rate of 33.3% of all admissions, and a 47% rate of positive cultures among those patients sampled. The septicemia rate was thus higher than was recorded in 1971 when 22% of all admissions had a positive blood culture, and over 30% of those cultured were positive. The rate was far higher than was recorded in 1966 and 1967, when septicemia in burn patients was far less common than it has now become. With 1760 sets of cultures collected, there were an average of 11.8 cultures on each patient on whom blood cultures were drawn. The comparable figure for 1971 was 7.5 per patient.

A resume of the bacteriology of blood stream infection in burn patients in 1972 is shown in Table 6. Predominant species recovered included

Table 6. Blood Culture Isolates from 209 Burn Patients - 1972: Relation of species to Mortality in Specimens

ORGANISM	No. Patients Positive	No. Blood Isolates	No. Patients Expired	% Patients with Positive Culture Expired
Staph. coag. pos.	51	102	25	49.0
coag. neg.	3	3	1	
Strep., Alpha hemol.	1	1	1	
Beta hemol.	1	1		
Gp A.	1	1	1	
Non-hemol.	8	8	7	
Corynebacterium sp.	3	3	1	
Bacillus sp.	13	16	7	
Prov. stuartii	50	116	38	76.0
Pseudomonas sp.	31	41	26	83.8
Klebsiella sp.	20	35	15	75.0
Mima-Herellea sp.	1	1		
Enterobacter cloacae	7	9	6	
aerogenes	2	2	2	
E. coli	14	18	12	85.7
Serratia sp.	7	16	5	
Proteus mirabilis	12	32	9	75.0
morganii	2	2	1	
Salmonella typhimurium	1	1	1	
Candida sp.	6	8	4	
Aeromonas liquefaciens	1	1	1	

Staph aureus, Prov stuartii, Ps aeruginosa, Klebsiella pneumoniae, E coli and Proteus mirabilis. The gravity of illness varied with species; with staphylococcal septicemia, 49% of patients in whom a positive staphylococcal blood culture expired. This included, of course, a large number of patients in whom a succession of bacterial species were recovered. Patients in whom Staph aureus was the only species recovered showed the organism to be relatively inocuous. There were 14 patients in whom Staph aureus was the only species recovered from the blood. All 14 of these patients survived. Twelve more patients with blood culture positive for Staph aureus and for one or more other species survived. The other species recovered included gram positive cocci and bacilli; Enterobacteriaceae, Pseudomonas and Candida sp. The most extreme example of multiple positive cultures with survival was one patient who had blood cultures positive for Staph aureus, Providencia, E coli, Proteus mirabilis, and non-hemolytic (group D) streptococci during his hospital course.

The pattern of blood stream invasion was complex, in that a wide spectrum of successive positive cultures for different species was obtained. Blood culture findings on 100 patients with positive cultures are shown in Table 7. About one-third of the patients had one species recovered; from 2 species up to 9 were recovered with decreasing frequency. Twenty-five different combinations with Staph aureus occurred, and 25 with Prov stuartii. Six patients had only Prov stuartii recovered and 4 harbored only Pseudomonas. The multiplicity of combinations of organisms involved in sepsis is a reflection of the opportunistic nature of septicemia in the severely burned. There did not appear to be a specifically lethal sequence, although Providencia and Pseudomonas appeared in patients unduly often in the last 24 to 36 hours of life.

BIOPSY OF BURN WOUNDS: BACTERIAL FLORA

Wound biopsy in burned patients, as a means of obtaining meaningful data about the bacteriologic status of the wound, has become a commonly used procedure. There were 101 patients from whom biopsies were collected, a 24% increase in patients over those sampled in 1971. The major species of

TABLE 7. BLOOD CULTURES POSITIVE IN 100 BURNED PATIENTS
SPECIES RECOVERED FROM INDIVIDUAL PATIENTS - 1972

SPECIES RECOVERED	NO PATIENTS
Staphylococcus aureus	18
Staphylococcus aureus, Prov. stuartii	7
Staph. aureus,Prot. mirabilis, Candida Prot. morganii	1
Staph. aureus, Pseudo	2
Staph. aureus, Prov.,Klebsiella, Hemol-Strep, Gp. A	1
Staph. aureus, Pseudo.,Salmonella	1
Staph. aureus, Staph. coag. neg.	1
Staph. aureus, Bacillus, Candida	1
Staph. aureus, Klebsiella,Prov., E. coli, Bacillus, Entero. aerogenes	1
Staph aureus, Klebsiella, Prov., Bacillus, Non Hemol-Strep.	1
Staph. aureus, Bacillus	2
Staph. aureus, Klebsiella, Prov., Pseudo., Candida	1
Staph. aureus, Corynebacterium, Serratia	1
Staph. aureus, E. coli	1
Staph. aureus, Prov.,Prot. mirabilis, Bacillus, Non Hemol-Strep.,E. coli	1
Staph. aureus, Prov., Bacillus	2
Staph. aureus, Prov.,Pseudo.,Serratia, Prot. mirabilis	1
Staph. aureus. Prov.,Bacillus, Non Hemol-Strep.	1
Staph. aureus, Candida	1
Staph. aureus, Prov.,Pseudo., E. coli Staph. coag. neg.	1
Staph. aureus,Prov.,Pseudo., E. coli	1
Staph. aureus,Klebsiella, Prov. ,	1
Staph. aureus,Prov., Candida	1
Staph. aureus,Klebsiella,Prov.,Pseudo., Prot.mirabilis,Non Hemol-Strep.,E. coli, Ent. aerogenes, Serratia	1
Staph. aureus,Klebsiella,Prov.,Pseudo., Prot. mirabilis, Bacillus	1
Providencia stuartii	6
Prov., Klebsiella	3
Prov., Klebsiella, Pseudo.	1
Prov., Klebsiella, Pseudo.,Ent. cloacae	1
Prov., Pseudo.,Non Hemol-Strep	2
Prov.. Pseudo.,Ent. cloacae	1
Prov., Prot.mirabilis,Prot morganii,E. coli	1
Prov., Pseudo.	6
Prov., Pseudo.,Prot. mirabilis	2
Prov., Klebsiella,Prot.mirabilis,Serratia	1
Prov., Klebsiella,Pseudo.,Non Hemol-Strep., Prov., Pseudo., E. coli	2
Prov., Candida	1
Prov., Pseudo., Serratia	1
Prov., Bacillus	1
Klebsiella	1
Klebsiella, Ent. cloacae	3
Klebsiella, Ent. cloacae, E. coli	1
Klebsiella, Staph. coag. neg.,Candida,E. coli Non Hemol-Strep., Mima, Serratia	

TABLE 7 Continued

SPECIES RECOVERED	NO PATIENTS
Proteus mirabilis	1
Prot. mirabilis, Serratia	1
Prot. mirabilis, Pseudo., E. coli	1
Pseudomonas	4
Alpha-Hemol-Strep.	1
Aeromonas liquefaciens	1
Bacillus	1
Corynebacterium	2
Bacillus, Ent. cloacae, E. coli	1

		% of all Positives
No. of patients with one species recovered	35	35
No. of patients with two species recovered	29	29
No. of patients with three species recovered	19	19
No. of patients with four species recovered	8	8
No. of patients with five species recovered	4	4
No. of patients with six species recovered	3	3
No. of patients with seven species recovered	1	1
No. of patients with nine species recovered	1	1
	100	

bacteria recovered are shown in Table 8, with comparable data for 1969 and 1971 included to provide a perspective on the trends related to species inhibiting the burn wounds.

The 7 species shown were those prominent in all biopsy cultures performed. Staph aureus has been remarkably consistent; for several years, about 40% of the patients biopsied harobor this species. Prov stuartii, which has, in many respects, constituted the most serious bacteriologic problem of this Institute, varied more in its recovery rate; it was, however, undoubtedly the most frequently encountered organism in biopsies. Ps aeruginosa was less common than the 2 preceding organisms; its incidence is now in the range recorded for Klebsiella pneumoniae and E coli. The latter 2 have become more common in biopsies in the past year; they now occur in one-fourth to one-third of all patients biopsied.

The percentage of patients with biopsies positive for a given species who subsequently expired, was determined in an effort to determine whether any species is especially prone to produce sepsis in the burn wound. The information derived is equivocal; each species has varied on a year-to-year basis in the frequency with which its presence antemortem has portended a fatal outcome. Staphylococci have fluctuated; Providencia would appear to connote a more serious prognosis in the past 2 years than it formerly did. Pseudomonas is another species that varies in a manner paralleling the Staphylococci. With Klebsiella and Escherichia, a plausible inference may be made that their presence in biopsies has had a progressively less serious prognosis, although the incidence of such isolates is increasing.

There were 15 other species recovered from biopsied tissues, none in a high proportion of patients. Candida (primarily albicans) was present in 22% of the patients; Enterobacter cloacae was found in 17% and Proteus mirabilis in 14% of all patients biopsied. Micrococci, Group A streptococci, Group D streptococci, non-hemolytic streptococci, Corynebacterium sp, Bacillus sp, Enterobacter aerogenes, Citrobacter sp, Proteus morganii, Serratia marcessens, Mima sp, and Aeromonas liquefaciens were also recovered.

Table 8. Burn Wound Biopsies on 101 Patients
Institute of Surgical Research - 1972

Species	No. Patients Positive	% of Patients Positive			% of Patients Positive Who Expired		
		1969	1971	1972	1969	1971	1972
Staph. aureus	41	42	44	41	22	38	22
Prov. stuartii	56	51	40	56	14	58	36
Ps. aeruginosa	32	30	30	32	39	57	20
Klebsiella sp.	32	20	17	32	50	31	19
E. coli	27	14	19	27	47	33	16
Proteus mirabilis	14	34	13	14	38	40	9
Candida sp.	22	6	22	22	-	53	13

PROVIDENCIA STUARTII IN BURN PATIENTS

There is recurrent evidence of Prov stuartii as a major pathogen in burn wounds at this Institute. Further, this species has presented a uniquely virulent and extensive involvement, in contrast to the less frequent occurrence reported from other burn centers. A resume of the incidence of this species in clinical and autopsy specimens is shown in Table 9. It was a major part of the microbial population in every site cultured. Of particular import was the high proportion of lung samples at autopsy in which Providencia appeared. The incidence of Providencia has been discussed in each area of bacteriologic study, but its role appeared even more prominent when the various sites of culture were considered together.

CATHETER TIP CULTURES

In view of the continued concern over the development of infection at the site of indwelling catheters, cultural surveillance of the catheter tip is maintained. There were 185 patients from whom catheter tips were cultured, with 620 individual catheter tips being sent to the laboratory. The bacterial flora recovered was not simply a reflection of the burn surface flora recovered (Table 3); species and incidence of recovery are shown in Table 10. The preponderance of Staph aureus and especially Prov stuartii are beyond the levels which would reflect simply surface contamination of the tip at the time of its removal. Conversely, the incidence of Ps aeruginosa and Klebsiella pneumoniae were far lower than would be plausible if their incidence were to reflect only the surface seeding of these species. That positive catheter tip cultures indicate, at least to a significant degree, the presence of bacteria on the catheter tip, rather than being surface contamination, is further substantiated by these findings.

DISCUSSION

Sepsis as a complication of burn injury remains a major problem in treating patients with extensive burns. During 1972, the elevated mortality rate suggested that this problem had become more acute. The microbial flora of burn patients, including surface, lung and blood stream, showed the principal species concerned to be Staph aureus, Prov stuartii, Ps aeruginosa,

Table 9. Providencia stuartii: Isolates from Clinical and Autopsy Specimens, 1972

Source	No. Isolates/ Total Specimens	Per Cent Positive	No. Patients Positive/Total Patients Cultured	Per Cent of Cultured Patients Positive
Burn wound, swab, clinical	526/1478	35.6	111/224	49.5
Biopsy, wound	149/362	41.2	56/101	55.4
Blood Culture	124/3520	3.5	50/209	23.9
Sputum (and Lukens)	435/721	60.0	69/122	56.6
Urine (and Foley)	199/743	26.8	126/291	43.3
I.V. catheter tip	109/620	17.6	50/185	27.0
Autopsy: burn	301/485	62.1	66/89	74.2
Autopsy: lung	207/337	61.4	64/85	75.3

Table 10. Bacterial Flora of I.V. Catheter Tips from 185 Burn Patients, ISR - 1972

Species	No. Patients Positive	% of Patients Positive	No. Isolates
Staph aureus	40	21.6	61
Staph epidermidis	12		12
Streptococcus Group D	1		1
Streptococcus Group A	1		1
Non-hemolytic strep	5		5
Bacillus sp	1		1
Klebsiella pneumoniae	19	10.2	23
Enterobacter aerogenes	7		8
Enterobacter cloacae	11		13
Escherichia coli	9		9
Serratia marcessens	6		6
Salmonella typhimurium	1		1
Proteus mirabilis	7		8
Proteus vulgaris	1		1
Proteus morganii	1		1
Providencia stuartii	50	27.0	109
Pseudomonas aeruginosa	18	9.7	32
Aeromonas liquefaciens	2		2
Mima-Herellea gp	2		2
Candida sp	16		21

Klebsiella pneumoniae. Burn wound sepsis due to Pseudomonas was, as in previous years, rare. But the presence of large concentrations of Providencia in viable tissue as revealed by biopsy strongly suggests that this species possesses tissue invading capability. Klebsiella appeared to be more conspicuous than in former years, in pneumonia, septicemia and other septic processes. Invaded tissue and pulmonary involvement appear to be 2 major sources for the sepsis that still occurs in burn patients. The control of these major bacterial species is not entirely effective because of the high levels of antibiotic resistance that are exhibited by these predominant organisms.

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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
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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
72 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
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b. CONTRIBUTING				
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(U) Bacteriophage Types of Pseudomonas Aeruginosa Found in Burned Soldiers (44)

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003500 Clinical Medicine

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

23. (U) Pseudomonas aeruginosa is not only a major lethal threat in burn infection, but an increasing problem in nosocomial hospital infection. In both these areas, military personnel are at risk, and better delineation of infecting strains and cross infecting patterns, which can be precisely achieved with phage typing, permits effective monitoring of therapy, emergence of resistance, and environmental contamination recognition and control.

24. (U) The classicial phage typing system which has been developed and applied for over a decade in this Institute was not effective on the whole Pseudomonas aeruginosa population during 1972. To categorize phage non-typable strains, concentrated typing fluids which embody lytic elements corresponding to bactereocins were applied effectively. These "C" groups were useful in typing the phage non-typable agents.

25. (U) 72 07 - 73 06 Phage-typing yielded over 40% of non-typable strains in 1972. Most of these could be characterized by use of undiluted phage fluids, which behaved in the manner of pyocins. Multiple types were found, with peaks of monotype seeding of the burn ward. A parallel heightened degree of Sulfamylon resistance, with minimum inhibitory concentration up to 1.25% occurred. Treatment of such strains was still effective with Sulfamylon. Typable strains included relatively elaborate phage patterns; the previous predominance of strains with simple reactions of identity had disappeared. The type pattern was one not previously seen during an 11-year period of phage typing.

*Available to contractors upon originator's approval

ANNUAL PROGRESS REPORT

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

1 July 1972 - 30 June 1973

Investigators:

Ruth L. Latta, BS
Robert B. Lindberg, PhD
Basil A. Pruitt, Jr, MD, Colonel, MC
Arthur D. Mason, Jr, MD

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

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Period covered in report: 1 July 1972 - 30 June 1973

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Phage typing procedures, which initially permitted typing over 90% of Pseudomonas aeruginosa strains, have, since 1970, shown a large increase in nontypable strains. To type this group, the phage typing battery of 18 types was assessed for bacteriocin-like activity with undiluted phage fluids. A series of 13 reacting filtrates were demonstrated, and to these were added 5 bacteriocin-fluids from lysogenic strains collected from burned patients. The combination of phage and bacteriocin enabled type identities to be assigned to 1087 Ps aerguinosa isolates from 132 burn patients. Four NT lytic types and 5 phage types made up 64% of all isolates. A major epidemic due to NT-2 was recognized, 43% of all strains were of this type. Former major types which had been prominent until 1971 were present, but in low numbers. The relatively Sulfamylon-resistant NT-2 strains occupied a peak period of 8 months in the burn ward, and at the end of 1972, had abruptly disappeared.

Pseudomonas
Phage typing
Burn wounds
Topical chemotherapy
Humans

BACTERIOPHAGE TYPES OF PSEUDOMONAS AERUGINOSA FOUND IN BURNED SOLDIERS

Despite the continued effectiveness of topical therapy in controlling Pseudomonas burn wound sepsis (BWS), the ubiquitous Pseudomonas aeruginosa has continued to play a major role in the pathogenesis of BWS primarily by exhibiting a marked propensity for colonization and invasion of the respiratory tract, and also by appearing in the blood stream of severely ill patients, often with no detectable antecedent lesion. Nosocomial infections due to Pseudomonas have increased markedly in the past 2 or 3 years, and it has continued to merit a major effort in assessing its role and studying better methods for its control. The phage typing system developed in this laboratory (Lindberg RB, Latta RL, Brame RE, Moncrief JA. Bact Proc 1964, p.81[1]; Latta RL, Lindberg RB, Pruitt BA,Jr. USA Inst Surg Res Ann Rpt FY 1970, BAMC, Ft Sam Houston, Tx, Sec 18[2]; Latta RL, Lindberg RB, Brame RE, Mason AD,Jr, Pruitt BA,Jr. USA Inst Surg Res Ann Rpt FY 1972, BAMC, Ft Sam Houston, Tx, Sec 23[3]) has served as an effective method of differentiating strains of Ps aeruginosa for the period from 1962 through 1970. Beginning in 1971 and increasingly in 1972, a nontypable component of strains appeared. It was necessary to alter the technical approach to typing these strains in order to differentiate them.

METHODS

The incidence of nontypable strains from all sources was 19% in 1970; in 1971 this had doubled to 40% of all strains. In 1972, the nontypable rate was 68%. Obviously, the typing system was being matched against a population too dissimilar to permit its use as an identifying tool.

Use of 100 x RTD. The Routine Test Dilution (not less than 1:1000 dilution) was raised to 100 x RTD. Most strains nontypable at RTD remained untypable. Even at 1:10 dilution, phage lysis failed to appear.

Use of Undiluted Typing Fluids. Undiluted phage fluids may contain pyocin, and so the phage set was used undiluted to use the pyocin effect in lysis. Out of 18 phages in the typing set, 13 exerted a pyocin-type of lysis against test strains. Five phages: 2,7,352,F10 and M4, were inactive

when used undiluted. To replace them, 5 phages derived from lysogenic strains isolated from patients were used. These were designated #6, #7, #9, #25, and #37. The lysis obtained probably was pyocin-activated, but its nature awaits further study. In any event, a usable lytic system for assaying type identity was obtained.

RESULTS OF COMBINED PHAGE-PYOCIN TYPING

Phage types were obtainable on 32% of all strains tested, and these are shown as phage types. The undiluted lytic effect is listed as NT (nontypable undiluted lysis).

Three hundred patients were admitted to the Institute of Surgical Research burn ward in 1972. Of this number, 132 (44%) of these patients had at least one Pseudomonas strain submitted for phage typing; some had 20 or 30 strains, and one patient had as many as 84. For this reason, more importance is placed on the number or per cent of patients with strains of a given type to show the predominant types rather than the total number of strains, since a single patient with a large number of strains, as the one mentioned above and possibly many of the same phage type, could load a particular group and distort the results. One thousand eight-seven Pseudomonas strains derived from many sources including the burn wound, biopsies, sputum, blood, urine, catheters, etc, of 132 burned individuals comprise the 1972 study.

The predominant phage types based on the number of patients having a particular type are listed in order in Table 1. The number of nontypable strains found in 1972 was unprecedented in our 12 year survey of Pseudomonas phage types: an astounding 68%. Using concentrated phage solutions in the typing set up, most of the nontyable strains have been categorized and designated by NT followed by a pattern number: 1,2,3,4,5, etc. identifying these nontyable strains. Many patterns were found even among the nontypable strains using concentrated phage, but the great majority fell into several large categories.

Four categories of nontypable Pseudomonas are shown, separated only by their different reactions with concentrated phage. The predominant

Table 1. Predominant Phage Types of 1,087 Pseudomonas Strains from 132 ISR Burn Ward Patients, 1972

Phage Type Code	Phage Type	Per Cent Each Phage Type	
		Patients	Strains
NT 2	Non-typeable *(21),44,1214,(68),109,(119X),F8	43.2	39.0
F21	31,44,1214,119X,F7	11.4	8.3
NT 1	Non-typeable *21,24,44,1214,68,73,119X,F7,M6	10.6	6.3
D41	21,68	7.6	2.0
C26	16,44,1214,68	5.3	2.3
M 2	119X	5.3	1.9
NT 20	Non-typeable *68	4.5	1.6
F12	31	3.8	1.0
NT 5	Non-typeable *21,24,44,1214,68,119X	3.0	1.5
			63.8

*Phage Type using concentrated phage

category of strains for 1972 were nontypable with the usual phage system. With concentrated phage, they were identified as pattern 2. Forty-three per cent of the patients had strains of this type, and they accounted for 39% of the total strains.

Phage type 31,44,1214,119X,F7 strains were the largest typable group. They appeared in 11.4% of the patients and accounted for 8.3% of the total strains. Only 4 strains of this type, occurring in 2 patients, were found in 1971.

Another group lysed only by undiluted phage fluids, designated NT-1, constituted the third most commonly encountered type. It was recovered from 10.6% of the patients; 6.3% of all strains were type NT-1.

Phage type 21,68 reacted with phages in the typical manner. It was fourth in prevalence, and occurred in 7.6% of the patients. Only 2% of the strains were type 21,68, or pattern D41. This type had, in previous years, been relatively frequent in occurrence, although last year, in 1971, only one strain was found.

The fifth most common forms, which occurred each in 5.3% of patients, were phage type 16,44,1214,68 (pattern C26) and 119X (pattern M2). C26 was a new type, not previously observed, but type 119X has been a common type over several years. It was fourth in frequency in 1971.

The sixth most common type was a phage-resistant strain designated NT-20 (reacting with undiluted phage fluid 68). These strains appeared on 4.5% of all patients, and were 1.6% of all strains. They reacted only with one phage fluid.

Strains of phage type 31 were next in order of occurrence. They were found in 3.8% of patients, and were represented by 1% of all strains. Phage type 31 (F12) were the fifth most common type in 1971.

The eighth most common type was NT5, reacting with undiluted phages 21,24,44,1214,68,119X. This complex pattern was reminiscent of the more extensive phage patterns that originally were observed in this Institute. NT5 appeared in 3% of the patients, and made up 1.5% of all strains typed.

These type categories made up a total of 63.8% of all strains typed. Phage

types which occurred in fewer than 4 patients were not listed. This group included 64 different types, which indicates the vast array of phage types which occur in the burn ward. Despite the preponderance of NT-2, there were still a very large number of distinct strains making up the burn ward patient flora. The diversity of types also included the NT category, lysed only with undiluted phage fluids.

This population represented a marked, unique change in lytic pattern, with loss of reactivity to the formerly effective spectrum of Pseudomonas strains. Previous patterns that had risen and fallen over several years, disappeared from the numerically significant scene in 1972. In 1970, patterns H3, M2, and F12 were present in significant numbers, together with 13 other types which could be distinguished. In 1971, these 3 types plus M4 were still present, although the reacting types were reduced to 7 which occurred in significant numbers. In 1972 only 4 of the previously described phage types occurred in significant numbers; 3 of these were still present H3, M2 and F12. There were 3 other phage types recognized: F21, D41 and C26. The remaining strains were recognizable by the NT system, but not as phage types. Whether this change is permanent cannot yet be determined. One obvious recourse to recognition of these nontypable strains will be to recover new phages which will be reactive. However, the labile nature of Ps aeruginosa prompts a degree of reserve in further search for effective typing phages until the current pattern of lysis has been confirmed for at least another 6 months.

PSEUDOMONAS PHAGE TYPES OF BLOOD CULTURE STRAINS, 1972

Because of the high mortality in burn patients showing a blood culture positive for Pseudomonas, a close surveillance is maintained on these particular strains to detect a particular phage type which might be predominant in this particularly lethal category.

During the year, 43 strains were recovered from 32 patients with positive blood cultures. Phage typing results of this series of strains are given in Table 2. The number of patients and strains for each month of the

Table 2. Phage Types of Pseudomonas Strains from Blood Cultures of ISR Burn Ward Patients, 1972

Month	Patients	Strains	Phage Type Code													
			A71	C26	D41	D42	F21	G22	G 2	I 1	NT 1	NT 2	NT 3	NT14	NT16	NT18
			Patients - Strains													
Jan	2	3										2-3				
Feb																
Mar	1	1											1-1			
Apr	2	2					1-1				1-1					
May	4	5									1-1	3-4				
Jun	2	4		1-1		1-1						1-2				
Jul	1	1										1-1				
Aug	6	7								1-1		5-6				
Sep	3	6										2-4			1-2	
Oct	7	8						1-1	1-1			4-5		1-1		
Nov	1	1			1-1											
Dec	3	5	2-4													1-1
Total	32	43	2-4	1-1	1-1	1-1	1-1	1-1	1-1	1-1	2-2	18-25	1-1	1-1	1-2	1-1

Phage Type Code	Phage Type
A71	2,7,21,24,44,119X,F7,M4
C26	16,44,1214,68
D41	21,68
D42	21,31,68
F21	31,44,1214,119X,F7
G22	44,1214,68,352
G 2	44,68,119X,F7
I 1	68

Phage Type Code	Phage Type
NT 1	Non-typeable; *21,24,44,1214,68,73,119X,F7,M6
NT 2	Non-typeable; *(21),44,1214,(68),109,(119X),F8
NT 3	Non-typeable; *21,24,44,1214,68,73,109,F7,F8
NT14	Non-typeable; *21,31,44,1214,68,109,119X,F8
NT16	Non-typeable; *M7,31,1214,68
NT18	Non-typeable; *21,68
	* Phage type using concentrated phage

year are listed to the left with the incidence of each phage type (Patients-Strains) during each monthly period on the right.

It is readily apparent that NT-2 strains dominate this collection. Eighteen patients had 25 strains of this particular type. Phage Type Code A71 (2,7,21,24,44,119X,F7,M4) and NT-1 each occurred in 2 patients. Except for these, no other phage type appeared in more than a single patient.

As in the case of Pseudomonas strains from all sources, NT-2 strains were predominant in septicemia, occurring in a higher percentage of patients, 56% as compared to 43.2% in all cultures. A somewhat higher percentage of other NT strains occurred in this collection also,with 74.4% as compared to 68% overall. This increase reflected the higher incidence of NT-2 strains. Only 2 patients had multiple types. The high mortality rate in this group of patients is borne out in that only 3 patients survived.

There was little to indicate that a particular septicemic organism was involved in this series. The incidence of Pseudomonas septicemia was higher than in 1971, but this increase was distributed over a total of 14 different types from 32 patients.

PHAGE TYPES OF PSEUDOMONAS FROM POSTMORTEM LUNG TISSUES, 1972

Pneumonia with predominant numbers of Ps aeruginosa present in the sputum has had a high mortality in patients at this Institute, and the nature of these strains has prompted scrutiny of the types of Pseduomonas found in the lung of the burn patient. One hundred and two strains of Pseudomonas from lung tissue of autopsies of 38 patients were studied for phage or lytic type.

The phage types of these particular strains appear in Table 3. On the left is given the number of patients in each month and to the right the phage type code and number of strains. Patients were listed individually to show the multiple types which can occur within a single patient. At times, both a typable and nontypable strain would be recovered. Again, 2 NT patterns might be discerned. The last patient in June had strains typed by NT system which fell into 3 different patterns when concentrated phage was used.

Table 3. Phage Types of *Pseudomonas* from Post Mortem Lung Tissues, 1972

Month	No. of Patients	Phage Type Code - No. of Strains
Jan	1	NT 2-2
Mar	1	NT 1-1
Apr	1	NT 1-4 | NT11-1
	1	NT 1-4
May	1	NT 2-4
	1	NT 2-2 | NT19-1
Jun	1	C26-1
	1	M 2-1 | NT 2-3
	1	NT 1-3
	2	NT 2-7
	1	NT 2-1 | NT19-1 | NT20-1
Jul	1	F21-4
	1	M 2-1 | NT 2-2
	1	NT20-1
Aug	5	NT 2-8
	1	NT 5-4
	1	NT20-1
Sep	1	F21-4
	1	G 2-4
	1	M 4-1
	1	NT 2-4
	1	NT 2-1 | NT17-3
Oct	1	F21-3
	1	G22-1 | NT 6-1 | NT21-1
	1	NT 2-1
	1	NT17-3
Nov	1	G25-3
	2	NT 2-6
	1	NT 5-1
	1	NT25-2
Dec	1	A71-4
	1	NT15-1

Phage Type Code	Phage Type
A71	2,7,21,24,44,119X,F7,M4
C26	16,44,1214,68
F21	31,44,1214,119X,F7
G 2	44,68,119X,F7
G22	44,1214,68,352
G25	44,119X
M 2	119X
M 4	119X,F7

Phage Type Code	Phage Type
NT 1	*21,24,44,1214,68,73,119X,F7,M6
NT 2	*(21),44,1214,(68),109,(119X),F8
NT 5	*21,24,44,1214,68,119X
NT 6	*24,1214,68
NT11	*21,68,73,F7
NT15	*21,68,109,119X,F7,M6
NT17	*68,#9
NT19	*1214,68
NT20	*68
NT21	*68,119X
NT25	*#7,16,21,24,44,1214,73,109,119X,F8

As in the case of strains from all sources, and blood culture strains, NT-2 strains were again predominant in the lung; 47.4% (18) of the patients had 41 strains of this type. Four patients yielded 12 strains of NT-1. Next in frequency were phage type code F21 and NT20, each in 3 patients. Two patients each harbored phage type code M2, NT-5, NT-17 and NT-19. These types accounted for 82 of the 102 strains. Of the remainder, each occurred in only one patient. 73.5% of these strains were nontypable with the standard concentration of phage. This value closely resembled the NT percentage in blood cultures. It was somewhat higher than that of strains from all sources. There was no plausible basis for concluding that there was a Pseudomonas type with a propensity for inducing pneumonia. Although the technic for delineating strains was changed to include a pyocin component, the type distribution pattern was not altered from the essential form that was observed when most strains were phage-susceptible.

CHRONOLOGIC DISTRIBUTION OF PSEUDOMONAS AERUGINOSA PHAGE AND LYTIC TYPES, 1972

With the use of phage typing on susceptible strains and the concentrated phage-pyocin effect on those not typable with phage, typing of Ps aeruginosa was more complete than was the case with only phage typing. The distribution of all strains recovered, on a chronological basis, reveals the pattern of persistence and disappearance of individual types. In view of the abrupt change in phage pattern which Pseudomonas exhibited in 1972, this ecologic information is of particular interest since virulence and increased clinical involvement both suggest the possibility that a major change in the nature of the Pseudomonas problem may have occurred.

Table 4 shows the monthly distribution of Pseudomonas phage and lytic types, including those appearing in at least 2 patients. In previous years the number of types was so great that it was often not feasible to go below types that occurred in at least 4 or 5 patients, or the Table would have become so unwielding that it would be noninformative. The fact that even with patterns appearing in only 2 patients, the total patterns were 26, indicates that the spread of types was smaller than it had been prior

Table 4. Monthly Distribution of *Pseudomonas* Phage Types in ISR Burn Ward Patients, 1972

Phage Type Code	Month												Total Patients-Strains Each Type
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	
A71												[illegible]	3-22
A93			1-6						1-1				2-7
C26		1-5		1-1		3-7			1-1	1-11			7-25
D41				2-2	1-1	2-2		1-6	1-4	3-3	1-3	1-1	10-22
D42			1-5	1-3		1-1							3-9
D43					2-2					1-8	1-1		3-11
F 3	1-4		2-5	1-1									3-10
F12							1-1	2-3	1-6		1-1		5-11
F21			2-6	4-18	2-7	3-15	4-20	1-3	5-13	2-8			15-90
F26				1-7		2-8	1-1						3-16
G22						1-2			1-4	1-4			2-10
H 3	1-4							1-3	1-3				2-10
H 2			1-3		1-1	2-6	2-3		1-8				7-21
H 4							1-1		1-2				2-3
H 8							1-1	1-1					2-2
NT 1	1-4	5-14	5-18	4-13	3-21	1-5							14-68
NT 2	3-11			3-29	7-69	12-72	11-51	14-[illegible]	9-44	17-61	4-12	1-1	57-424
NT 3			1-5	1-2									2-7
NT 5								2-10	1-4		1-2		4-16
NT 6										2-4			2-4
NT 10									1-8	2-24	1-4	1-1	2-37
NT 11				1-1	1-1								2-2
NT 13		1-3	2-6	2-2									3-11
NT 14					1-1					1-1	1-1		2-3
NT 15									1-2	1-7	1-7	1-8	2-24
NT 20					2-3	1-1	1-1		2-6	2-6			6-17
Total Patients-Strains Each Month	7-31	6-25	16-51	20-87	15-119	20-129	14-110	20-135	25-132	20-176	12-46	8-46	-882

to 1972. Using patterns occurring in at least 2 patients accounted for 882 of the 1087 strains. The remaining 205 strains were of types each of which occurred in only one patient.

On the left of Table 4, "Phage Type Code" lists the code either of Phage or the NT-code designation. Each block contains the number of patients and the number of strains (No. Patients_____No. Strains_____) during the month it occurred. The right hand column lists the total of patients who harbored a given type and the number of that type recognized during the year. Since one patient could contribute strains in 2 or more different months, the monthly total of patients may exceed the actual total. Similarly, since one patient could harbor more than one type, the monthly totals of patients in the bottom row may not equal the totals by type. Note that the number of patients in January, February and December was small; Pseudomonas carriers were numerous during the remaining 9 months. The predominant type in each month is denoted by a heavy bordered box; the next most common type(s) if any, by a double lined box.

NT-2: The most common type by far, and present in a proportion exceeding any previous experience with Ps aeruginosa was NT-2. It was predominant (but only on 3 patients) in January, re-emerged as a secondary type in April, then was unequivocally predominant until November, when its numbers fell abruptly. There is no way to tell if this type had previously been present since the NT- system had not been developed. Future surveillance will determine whether NT-2, the "type of 1972" will reappear or be supplanted by other types.

Phage Type F-21: This was the second most common type in 1972. They exhibited no lytic pattern suggesting any relationship to NT-2. They were present for 6 months and predominant from February through April, then disappeared in July.

Phage Type D-41: This was the fourth most frequent strain in 1972. The incidence was never high, although in October it was the second most common type. This type has a long pedigree, both in this Institute and throughout the world. In 1963, type 21,68 (Code D41) was the second most

common type at this Institute. From 1964 through 1966, covering the first 3 years of the use of Sulfamylon burn cream, it was the most commonly encountered type. It seemed to be inherently a prominent and persistent part of the flora. Then in 1967 it was seventh most common, and in 1968 through 1970 was twelfth or thirteenth. In 1971 it was not listed in the table of types occurring. It has now re-emerged, and from its pattern of persistence and past history, will be observed with especial interest.

Types C-26 (16,44,1214,68) and M-2 (119X) were present in equal frequency as the fifth most common types. C26 strains appeared sporadically from February through October but never in large numbers. M2 occurred almost parallel to C26. M2 was recognized as far back as 1964; for the next 3 years it disappeared, only to reappear, especially on soldiers coming in from Vietnam. It has appeared annually since 1968, and in 1970 was the second most common type.

NT-20 was the sixth most common type; it appeared between May and October in small numbers.

The remaining types were present in numbers too small to merit separate discussion. They included the following:

Type	# Patients	# Strains
A-93	2	7
D-42	3	9
F-3	3	11
F-12	5	11
F-26	3	16
G-22	2	10
H-3	2	10
M-4	2	3
M-8	2	2
NT-3	2	7
NT-5	4	16
NT-6	2	4
NT-10	2	37
NT-11	2	2
NT-13	3	11
NT-14	2	3
NT-15	2	24

Type A-71, a previously unknown pattern, was the predominant strain in December, at a time when only 11 other strains were collected in

the entire month.

Type H-3 (1214,68,119X) was found on only 3 patients. This was a very important type until recently. In 1969 and 1970 it was the predominant type, and in those 2 years, 95 patients carried 241 strains. It presented at least 3 cases of treatment-resistant burn wound sepsis, although the strain was sensitive to 0.156% of Sulfamylon or less. The strain was common on Vietnam returnees, and its present fall to a low incidence may reflect its relative scarcity in the United States.

Type F-3 was, in 1972, found in only 3 patients, with 11 strains. In 1971 it was the predominant type; it appeared for the last 7 months of the year and was the first unequivocally "resistant" strain of Ps aeruginosa encountered: 34 out of 38 strains had an MIC of 0.625%. Obviously it disappeared after 1971, and the resistant strains of lytic type NT-2 have no relation, in phage/lysin pattern, to type F-3.

DISCUSSION

The population of Ps aeruginosa did not fall in 1972, but the identification and the entire typing concept, which had been effective in tracing identities of infecting strains for 11 years, had to be changed to enable the laboratory to continue differentiating strains of Ps aeruginosa. The nontypable problem, which had gradually increased in the past 2 years, suddenly assumed proportions that required development of a new technic. Essentially, all strains were phage-typed, and the nontypables were characterized with undiluted phage, or in essence, a pyocin-type of reaction. Selected strains of the standard typing set produced adequate bacteriocins, and several more were collected from lysogenic patient strains. This bacteriocin set permitted identification of the phage-nontypable strains; for the first time, all strains collected could be typed.

The specificity of phage typing appeared to be continued in the bacteriocins and the patterns they produced. The population of pseudomonads did not break down into as many types as were formerly found with phage typing alone. This difference was due to the very large number of strains of NT-2 type, which constituted an epidemic peak of Pseudomonas incidence

never before seen in this Institute. Coupled with a new high in Sulfamylon "resistance", this picture made it clear that continuous monitoring of Ps aeruginosa in a burn unit is essential. The variations in type identity still indicate that new strains are being introduced to the burn ward on a continuing basis.

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PUBLICATION

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
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3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8A. DISP'N INSTR'N	8B. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
72 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
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b. CONTRIBUTING				
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11. TITLE (Provide with Security Classification Code): (U) Bacteriophage Types of Serratia Marcessens from Burn Wounds of Military Personnel (44)

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c. TYPE: d. AMOUNT:
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	CURRENT 74	.5	14

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21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Provide each with Security Classification Code)
(U) Burns; (U) Serratia; (U) Bacteriophage; (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) 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) 72 07 - 73 06 The epidemiology of Serratia in nosocomial infections is not well documented, and so the opportunity to type strains recovered in the Brooke General Hospital was taken. Out of 56 strains, 17 types were identified. Distribution of types was not compatible with existence of an epidemic population. From the burn ward, 109 strains were collected. A predominance of phage type II was recognized, together with other types. The existence of micro-epidemic of single phage types of Serratia is indicated.

*Available to contractors upon originator's approval

ANNUAL PROGRESS REPORT

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

1 July 1972 - 30 June 1973

Investigators:

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

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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One hundred and fifty-seven strains of Serratia marcessens from patients in the Institute of Surgical Research burn ward and from Brooke General Hospital wards were typed with the phage system developed here. Thirteen types were recognized in the burn ward, with one epidemic type, 11, as the major strain. This is the first time an actual monotype epidemic has occurred in 7 years of surveillance. In Brooke General Hospital a presumed epidemic was shown to be a heterogeneous collection of types, with no pattern of transmission to indicate that an epidemic situation was present. The usefulness of phage typing as a tool 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

The importance of gram-negative bacteria, including Enterobacteriaceae sp in burn wound colonization and infections has been made abundantly clear in recent years. The importance of epidemiologic information on the spread of such species is rendered even more important by the increased awareness of such species in nosocomial infections. The intensive cross-contamination and transmission problems that appear in the burn ward may well offer insight which can be applied to the problem of opportunistic enteric flora as a factor in nosocomial infections.

The genus Serratia, essentially represented by a single species, Serratia marcessens, has been observed frequently as an opportunistic infecting agent in burn wards, as well as in general hospital wards. Its incidence in the Institute of Surgical Research has not increased dramatically in the past year, but it continued to appear in episodic fashion. Typically, one patient would be identified with a positive culture, followed by a prompt appearance of Serratia on several other patients, followed by its disappearance from the ward. Seven patients had Serratia septicemia; 5 of these died.

Continued surveillance of this species has been carried out in this laboratory, using a phage typing which was developed in this laboratory (English VC, Latta RL, Brame RE, Lindberg RB. USA Inst Surg Res Ann Rpt FY 68, Section 32)[1].

METHODS

The technics of typing Serratia with bacteriophage are essentially those of Adams (Adams MH. Bacteriophages, NY, Interscience Pub, 1959)[2]. The system has remained specific. No cross-reaction with Klebsiella sp or Enterobacter sp has been observed.

Recognition of strains does not rely on pigment formation. Over 65% of isolates from Institute of Surgical Research patients were achromogenic. Strains are recognized as nonlactose fermenting, and a convenient attribute for detection is formation of a small amount of gas in glucose (inverted tube).

Differentiation from Enterobacter liquefaciens is by fermentation of arabinose and raffinose, which are negative with Serratia.

RESULTS OF PHAGE TYPING

One hundred strains of S marcessens were recovered from patients on the burn wards of this Institute in 1972. An additional 56 strains were received from Brooke General Hospital, where cross-infection or nosocomial infection problem was postulated. Typing of isolates was a major factor in determining whether an outbreak did indeed exist.

Strains were recovered from 27 patients in the Institute of Surgical Research burn wards. The adequacy of the typing system was indicated by the fact that 89 out of 100 were typable. Only 43 out of 57 strains recovered in Brooke General Hospital were typable. The greater typable rate in this Institute's wards may well be due to a more localized transmission pattern, with fewer exotic sources of strains.

Phage types of S marcessens recovered from burn patients are shown in Table 1. The number of patients harboring a strain is, of course, more significant than the number of isolates collected, in terms of the type distribution. There were 13 types; obviously type 11 was the only predominant type in this year. Reactivity to this phage was widespread; 11 of the 12 patterns observed included it. Phage number 11 has continuously been a highly reactive entity, but never before had it been predominant.

Predominant phage types observed over the past 6 years are summarized in Table 2. In 1967, 1969, and 1970 the predominant type was 5,7,9,11,15,18; in 1968 it was one of 3 equally prominent types, and in 1971 it was one of 2 types that occurred in significant numbers. In 1972 it did not appear. Its abrupt disappearance reflects the unequivocal preponderance which one type showed in this year. It would appear that the phage type pattern has grown narrower with the passage of time. The sources of these strains have not been obvious since the cessation of combat in the Far East; incoming microbial flora is far less profuse in CONUS patients, since their arrival time is usually earlier in the postburn period.

Mixed infections with multiple types occurred in 4 patients. The

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	1	1
3,5,7,11,15,18	1	1
3,5,11,15	1	1
3,11,15	1	9
5,7,9,11,15	1	1
5,7,11	1	1
5,7,11 ,15	1	3
5,9,11,18	1	1
7	1	4
7,11	1	1
7,11,15	1	1
9,11	1	2
11	19	63
NT	2	11

Table 2

COMPARSION OF YEARLY PREDOMINANT PHAGE TYPES

Year	Phage Type	Number of Patients	Number of Isolates
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	7	5
	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 prior to 1972

variations are shown in Table 3. Patient 132 harbored 4 types, only 2 of which might plausibly be related. On admission he harbored type 5,7,11,15; this type was found 6 days later in I.V. tip and blood culture, and 29 days later in a wound culture. He had type 7 over a 3-day period one week after admission, twice in blood cultures. Only late in his course, 3 months after admission, was a wound culture positive for type 11.

Patient 242 yielded 2 different phage types from autopsy tissues. No antemortem isolates were recorded for this patient. Patient 268 also harbored 2 types in successive wound samples taken at autopsy. Finally, Patient 247 had a succession of strains of type 3,11,15 over a 3-day period, with a type 11 appearing on the third day. These latter samples were from biopsies; the patient subsequently experienced Pseudomonas septicemia and expired with Providencia sepsis.

The appearance of S marcessens is an episodic pattern, with long periods in which it is not seen, strongly indicates an epidemic potential of spread in an environment well provided with susceptibles. Table 4 shows the chronologic record of type 11 in the Institute of Surgical Research burn ward. The admission dates and dates of first isolation show that in most instances the patient had been on the ward for more than a week before he first was seeded with S marcessens type 11. Further, the initial positive culture dates suggest that episodes of infection occurred during the first week post-admission on July 27, August 4, August 25, September 5 and 15, October 10-17, and November 27 extending into December. This transmission pattern is not one that would be legible if multiple types were present, but the monotype pattern makes it discernible.

Serratia Types in Brooke General Hospital. Typing of Serratia strains collected in the wards of Brooke General Hospital was carried out to determine whether an epidemic of Serratia infections did indeed exist. The pattern of distribution was sufficiently heterogeneous to negate this supposition (Table 5). The sources of strains were varied and no plausible centering of types occurred.

There were 18 phage types recovered among patients at Brooke General

Table 3

Serratia Phage Type in Patients with Multiple Types
ISR - 1972

Patient Number	Admission Date	Phage Type	Date Isolated	Source of Culture
132	6-13-72	5,7,11,15	6-9-10	I.V. tip
			7-17-22	Left arm
			6-19-26	Blood
		7	6-19-10	I.V. tip
			6-20-14	Blood
			6-20-15	Blood
			6-21-29	L.forearm
		7,11,15	6-17-22	L.arm
		11	9-5-8	Drainage, L.shin
242	10-20-72	5,9,11,18	10-30-25	Liver
		9,11	10-26-1	Lukens
			10-30-27	RUL
269	11-24-72	7,11	11-27-8	PM specimen # 1
		11	11-27-8	PM specimen # 1
247	10-27-72	3,11,15	10-23-5 (2 isolates)	L. groin
			10-28-6	L. calf
			10-31-5	L. leg
			10-31-6 (2 isolates)	L. leg
			10-31-16 (2 isolates)	L. leg
			10-31-17	R. leg
		11	10-31-17	R.leg

Table 4
DISTRIBUTION OF THE PREDOMINANT SERRATIA MARCESCENS
TYPE NUMBER 98 IN THE TSR BURN WARD, 1972

Patient Number	Admission Date	Date Isolated	Source
66	3-29-72	7-27-1	Lukens
		7-27-10	Urine
		7-28-5	I.V.tip
		7-31-22	RUL
		(2 isolates)	
		7-31-23	RLL
		7-31-24	LUL
		(2 isolates)	
		7-31-25	LLL
		7-31-26	Spleen
		(2 isolates)	
		7-31-27	Liver
		(2 isolates)	
		7-31-28	PM specimen #1
		7-31-31	PM specimen #8
		7-31-32	PM specimen #10
		(2 isolates)	
118	5-24-72	6-30-7	Lukens
		7-5-18	Lukens
		7-7-2	Lukens
132	6-13-72	9-5-8	Drainage, l.shin
138	6-27-72	7-1-10	I.V. tip
147	7-9-72	R-4-2	Lukens
154	7-19-72	R-25-A	Wound
		9-6-29	Urine
		9-11-11	RUL
		9-11-12	LLL
159	7-27-72	R-2-1	Lukens

Patient Number	Admission Date	Date Isolated	Source
186	8-31-72	9-7-7	L.leg
		(2 isolates)	
		9-19-26	LLL
		9-19-27	PM specimen # 1
		9-19-25	LUL
		9-19-29	PM specimen # 3
		(2 isolates)	
		9-19-30	PM specimen # 4
		9-19-31	PM specimen # 5
		9-19-32	PM specimen # 6
191	9-9-72	9-13-21	LLL
		9-13-23	RLL
		9-13-24	Liver
		9-13-25	Spleen
194	9-9-72	9-17-10	Blood
195	9-9-72	9-13-38	PM specimen # 1
196	9-9-72	9-15-24	PM specimen # 8
199	9-13-72	10-12-4	Urine
204	9-19-72	11-30-10	Blood
		12-11-21	Liver
		12-11-4	LUL
		(2 isolates)	
		12-11-5	LLL
		12-11-6	RUL
		12-11-7	RLL
207	9-21-72	10-12-7	L.leg
208	9-21-72	10-10-2	Liver
215	9-27-72	10-17-22	Liver
		10-17-23	Spleen
		10-17-24	Kidney
		10-17-27	LUL
247	10-27-72	10-31-17	r. leg
268	11-24-72	11-27-8	PM specimen # 1

Table 5

SURVEY OF SERRATIA PHAGE TYPES FOUND ON WARDS OF BROOKE GENERAL HOSPITAL - 1972

Ward	Culture Number	Source	Phage Type
12-A	013417	Urine	Nontypable
	13558	Urine	Nontypable
	1957	Urine	Nontypable
	012558	?	Nontypable
13-A	014795	Urine	5,9,11,15
	01346	Trachea	Nontypable
14-B	3303	Catheter	7,9,11,15
15-A	7198	Vaginal Cuff	11,15
16-B	001923	Nose	11,15
32-A	1623	Trachea	5,9,11
42-A	5379	Foley Cath.	5,7,9,11,15,18
	01424	?	5,7,9,11
	7136	Jugular I.V.tip	5,7,11,15
42-B	006541	Wound	7
	7116	Subclavian cath.	5,9,11,15
42-H	009940	Urine	11
	011794	Urine	15
	7319	CSF	15
	6475	Serous fluid	15
	2354	?	11,15
	5302	Trachea	Nontypable
43-A	11757	L.stump	3,5,7,11,15,18
43-B	000720	L.leg	11
43-C	7936	?	5,7,9,11,15

Ward	Culture Number	Source	Phage Type
43 N/S	011913	Sputum	3,5,7,9,11,15,18
	00025	Sputum	5,9,11,15
43-E	8903	Sputum	5,9,11
	1583	Bladder cath.	15
	6377	Urine	Nontypable
	003094	Sputum	Nontypable
	2312	Trachea	7,11,15
	01453	?	Nontypable
	007730	?	Nontypable
	4712	?	5,7,9,11,15
43-E	001378	Urine	5,7,9,11,15
CCU	016161	Urine	5,7,9,11,15
43-E	001299	Tracheal	
TCU		aspirate	Nontypable
	2967073	Sputum	Nontypable
43-G	012549	Throat	5,9,11
	3873	?	11
43-H	3356	Sputum	11
	009012	Tracheal secretions	15
ER	006506	Sputum	5,7,9,11,15
	001414	Throat	9,11
	21143	Throat	3,9,11,15
CUSC	014535	Urine	5,7,9,11,15
Out-Patient	020136	Throat	Nontypable
GU.CL.	24959	Urine	11,15
	1946	Urine	Nontypable
Others:	Anesthesia,Tab.# 2,651		3,5,7,9,11,15
	Alcohol basin, 636		3,5,7,9,11,15
	Sink, 640		3,5,7,9,11,15
	Emerson Resp. # 3		3,5,7,9,11,15
	Emerson Resp. # 5		3,5,7,9,11,15
	Oral airway, 638		3,5,7,9,11,15

Hospital. These were organized as to sources in terms of wards or clinics (Table 6). It was then quite apparent that the population was entirely diverse and there was no indication of a build-up of an individual type in any ward or location. Such has been the usual pattern in the Institute of Surgical Research burn ward. The presence, in the burn ward in 1972, of an epidemic type, suggests that conditions for transmission of such a strain must have been optimal in contrast to those observed in previous years.

The pattern of distribution in Brooke General Hospital shows the strong likelihood of a succession of outside sources feeding in strains to the wards. Type 11, as an example, was not found adjacent to Ward 14A, in the main hospital, but was recovered from 3 different wards in Beach Pavilion. Had it indeed been carried from the burn ward, it should have appeared in adjacent wards. This did not occur. The typing data ruled out the existence of a Serratia epidemic in the population of Brooke General Hospital.

DISCUSSION

The feasibility of differentiating strains of S marcessens by the typing system devised in this laboratory was affirmed by the testing performed on 157 strains of Serratia in 1972. Two epidemiologic facts were uncovered: the presence of a virtual epidemic strain of S marcessens in the burn ward of the Institute of Surgical Research; and the absence of an epidemic in the hospital wards of Brooke General Hospital. This was the first time that unequivocal epidemic spread had occurred in the burn ward; its implications as far as ward management and patient care are extensive. One must presume a competent reservoir of Serratia to permit this transmission of a single type over a period of time. It is entirely possible that a sequence of seeded patients could account for this phenomenon. As with other tracer methods, the spread of S marcessens in a monotype pattern may be a useful tool for detecting defects in burn ward technic and procedures.

REFERENCES

1. English VC, Latta RL, Brame RE, Lindberg RB. Development of bacteriophage system for organisms of the genus Serratia. USA Inst Surg Res Ann Rpt FY 68, BAMC, Ft Sam Houston, Tx. Section 32.

Table 6

DISTRIBUTION OF 18 PHAGE TYPES ISOLATED FROM WARDS AT ROANOKE GENERAL HOSPITAL - 1972

Ward	Number of Patients	Number of Isolates
3,9,11,15		
ER	1	1
3,5,7,9,11,15		
Anesthesia, Tab # 2, 451		
Alcohol basin, 436		
Sink, 440		
Emerson Resp., # 3		
Emerson Resp. # 5		
Oral Airway, 438		
3,5,7,11,15,18		
43-A	1	1
3,5,7,9,11,15,18		
43-M/S	1	1
5,9,11		
43-C	1	1
43-E	1	1
32-A	1	1
5,7,11,15		
42-A	1	1
5,7,9,11		
42-A	1	1

Ward	Number of Patients	Number of Isolates
5,7,9,11,15		
ER	1	1
43-CCU	2	2
43-C	1	1
CHSC	1	1
5,7,9,11,15,18		
42-A	1	1
5,9,11,15		
43-M/S	1	1
13-A	1	1
42-B	1	1
7		
42-B	1	1
7,11,15		
43-E	1	1
7,9,11,15		
14-A	1	1
9,11		
ER	1	1

Ward	Number of Patients	Number of Isolates
11		
42-H	1	1
43-H	1	1
43-B	1	1
43-C	1	1
11,15		
16-B	1	1
15-A	1	1
GI-CL.	1	1
42-H	1	1
15		
43-E	1	1
43-H	1	1
42-H	2	3
Nontypable		
12-A	4	4
13-A	1	1
43-E	4	4
GI-CL.	1	1
Outpatient	1	1
43-E		
ICU	1	2
42-H	1	1

Phage type

2. Adams MH. Bacteriophages. New York, Interscience Pub., 1959.

PRESENTATION

Lindberg RB. Symposium on Identification of Commonly Encountered Gram-Negative Bacilli held at Univ of Pennsylvania, August 1, 1972.

PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0E 6382	73 07 01	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. ORD'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
72 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)
PRECEDING FISCAL YEAR 73	.4	14
CURRENT FISCAL YEAR 74	.2	5

19. RESPONSIBLE DOD ORGANIZATION
NAME: US Army Institute of Surgical Research
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TELEPHONE: 512-221-2720

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TELEPHONE: 512-221-2943
SOCIAL SECURITY ACCOUNT NUMBER:
ASSOCIATE INVESTIGATORS
NAME: William F McManus, MAJ, MC DA
NAME:

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 granulation, 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) 5% 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 sponges are soaked with the solution periodically and changed completely anywhere from 4 to 6 hours. Since 1 Feb 72, the gauze sponges have been applied soaked with the solution or with normal saline, allowed to dry and removed dry every 6 or 8 hours.

25. (U) 72 07 - 73 06 By using 5% Sulfamylon acetate solution as wet soaks, residual non-viable tissue can be removed by mechanical action as the gauze dressing is changed. A level of bacterial control is maintained within the burn wound by using the solution. Less than 20% of the total body surface should be treated on such dressings at any given time. Allergic reactions manifested as a rash developed in less than 15% of patients studied. No leukopenia or fall in hematocrit was associated with use of the solution. No hyperventilation was attributable to use of the solution. There was no significant change in the burn wound flora and no Pseudomonas burn wound sepsis developed during the study period. Presently quantitative wound cultures are being done to determine the solution's role in suppressing bacteria. Length of treatment utilizing normal saline and the solution is being done to determine whether or not the solution retards the debridement process.

*Available to contractors upon originator's approval

FINAL REPORT

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

1 July 1972 - 30 June 1973

Investigators:

Daryl R. Erickson, MD, Major, MC
John L. Hunt, MD, Major, MC
Basil A. Pruitt, Jr., MD, Colonel, MC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

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 1972 - 30 June 1973

Investigators: Daryl R. Erickson, MD, Major, MC
John L. Hunt, MD, Major, MC
Basil A. Pruitt, Jr., MD, Colonel, 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 soaks have been applied to such wounds in order to facilitate debridement of the residual nonviable tissue by mechanical action of soak changes while maintaining some element of bacterial control within the burn wound.

Burn
Eschar separation
5% Sulfamylon acetate solution

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

Forty additional patients were included in this study, bringing the total number of patients to 120, using 5% aqueous Sulfamylon soaks. The burns have ranged between 5% and 70% of the total body surface with the majority of the wounds being third degree in character. No more than 20% of the body surface is included at one time in a dressing.

The 5% Sulfamylon acetate solution is applied to wounds which have been wrapped with coarse mesh gauze and 4 x 8 inch sponges. The solution is applied to the dressings every 3 to 4 hours and the dressings are changed every 6 to 8 hours. Records are kept of the patient's age, weight, height, the extent of the burn (both total and full thickness) and note has been made of the occurrence of any skin rash indicative of hypersensitivity which is known to occur in approximately 7% of the patients treated with Sulfamylon burn cream. Occurrence of skin rash necessitates treatment with an antihistaminic and if such is ineffective in controlling the rash, Sulfamylon soak therapy is discontinued. Hyperventilation is recorded and a weekly CBC is obtained. The burn wounds are monitored daily and any evidence of bacterial overgrowth recorded and documented. Rapid bacterial proliferation is cause for re-institution of Sulfamylon burn cream therapy.

White blood counts with differentials and hematocrits were followed biweekly during treatment. At no time was the white count depressed. No fall in hematocrit was attributable to use of the 5% Sulfamylon acetate soaks.

Hyperventilation which has been reported in treatment of burns with topical 10% Sulfamylon cream was not noted in any patients treated with the 5% acetate soaks. Strikingly less cutaneous pain has been noted with the 5% soaks as compared to the 10% cream when applied to comparable burns.

Wound cultures were obtained prior to the use of the 5% aqueous Sulfamylon acetate soaks and biweekly thereafter. All wounds had been previously treated with topical 10% Sulfamylon cream. There was no significant change in the bacterial flora of the wounds. Common organisms isolated were Staphylococcus aureus coagulase positive, Providencia stuartii, and Pseudomonas aeruginosa. At no time did Pseudomonas burn wound sepsis develop during treatment. Allergic reactions as manifested by an erythematous rash were noted in 14% of the patients. Anyone known to be allergic to sulfa drugs was excluded from the study. Patients developing a rash characteristic of a sulfa allergy were treated with an antihistaminic such as benadryl which usually controlled this side effect, and if such treatment proved to be unsatisfactory, the drug was discontinued. This unexpectedly high

incidence of apparently related skin rashes is being more closely scrutinized.

SUMMARY

Five per cent Sulfamylon acetate soaks have been found to be a safe, useful variant of topical chemotherapy of the burn wound. A surprisingly high incidence of cutaneous hypersensitivity is of little apparent significance clinically, but is potentially limiting. Debridement of residual nonviable tissue from burn wounds is hastened by use of the soaks after the bulk of the eschar has separated. Bacterial control with the 5% soaks is felt to be less than with the 10% Sulfamylon burn cream, but the bacterial density remains at clinically safe levels. This "limited" bacterial proliferation recommends limitation of soak application to limited surface areas, i.e., no more than 20% of the total body surface at any one time.

PUBLICATIONS AND/OR PRESENTATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0B 6982	73 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
72 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)
PRECEDING FISCAL YEAR 73	.3	10
CURRENT FISCAL YEAR 74	.3	7

19. RESPONSIBLE DOD ORGANIZATION
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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME:
NAME:

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

DA

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) 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 on the prevention of burn wound sepsis using a laboratory model to improve care of thermally injured troops.
24. (U) Scrutiny of strains of Pseudomonas aeruginosa from the extremely rare case of Pseudomonas burn wound sepsis which occurred despite prompt Sulfamylon therapy was carried out in depth, to discern the role of the infecting agent in such episodes.
25. (U) 72 07 - 73 06 Sulfamylon-tolerance in strains of Pseudomonas aeruginosa from the rare case of burn wound sepsis occurring despite Sulfamylon treatment uncovered a new infection pattern which had not been previously recognized. The offending strains were relatively resistant to Sulfamylon in vitro, and were of a phage type currently predominant in the burn ward. They were of relatively weak virulence in the infected rat model, yet did not respond to Sulfamylon treatment. Similar strains from non-invasive colonization showed that Sulfamylon to be effective in the animal model. Thus, a distinctive treatment-resistant factor was recognized, coupled with a low level of animal invasiveness. Further studies on alternate methods of chemotherapy for these unusual strains are in progress.

*Available to contractors upon originator's approval

ANNUAL PROGRESS REPORT

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

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

1 July 1972 - 30 June 1973

Investigators:

Robert B. Lindberg, PhD
Virginia C. English, MA
Ruth L. Latta, BS
Russell E. Brame, MS
Basil A. Pruitt, Jr, MD, Colonel, MC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

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 report: 1 July 1972 - 30 June 1973

Investigators: Robert B. Lindberg, PhD
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Reports Control Symbol MEDDH-288(R1)

Use of 10% Sulfamylon burn cream has markedly reduced the incidence of burn wound sepsis (BWS) due to Pseudomonas aeruginosa. As with all chemotherapeutic regimens directed toward suppression of bacteria, emergence of drug-resistant strains is a potential hazard that is guarded against by continued monitoring of sensitivity by in vitro testing. Although over a period of several years median sensitivity varied in the range from 0.083% to 0.176%, there have been occasional episodes when sensitivity levels rose to 0.625% to 1.25%. During periods when such strains pervaded the burn ward, a small number of classical BWS cases occurred. The offending strains were relatively resistant to Sulfamylon in vitro (MIC 0.625 or greater). They were not highly virulent, but rats seeded with them could not be saved with topical Sulfamylon. Strains of other types, non-epidemic but with MIC of 0.625, were not treatment-resistant. The virulence mechanism differs from the typical Sulfamylon-sensitive strain since treatment with this drug does not alter the outcome.

Burn wounds
Sulfamylon
Pseudomonas
Rats

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

With the use of 10% Sulfamylon cream in topical treatment of severe burns, the incidence of invasive Pseudomonas burn wound sepsis (BWS) has been markedly reduced. The protection afforded by this drug was directed toward Pseudomonas aeruginosa, the only species with which BWS has been created in an experimental model. In any chemotherapeutic antimicrobial system, the appearance of resistant bacterial strains is a possibility, and because of this possibility, surveillance of Ps aeruginosa from patients on the Institute of Surgical Research burn wards has been carried on routinely since 1965. The tests were conducted using dilutions of Sulfamylon in agar, from 0.019% to 2.5%, with seeding of approximately 1000 cells of a 4-hour culture of the strain being tested. Although their incidence was much reduced when Sulfamylon was introduced, cases of BWS still occur despite prompt topical treatment.

The actual number of such cases has been small, but the implication of a treatment-resistant strain of Ps aeruginosa has prompted detailed study of the Sulfamylon-sensitivity and of the invasive, lethal characteristics of strains isolated from such episodes. A distinctive behavior of virulence and response to therapy of isolates from Sulfamylon-treated BWS has been observed. This study reports these results.

The overall susceptibility of Ps aeruginosa for Sulfamylon is indicated by the median level of inhibition as it has been determined on an animal basis. This is defined as the concentration at which 50% of the strains tested, per annum, are inhibited. The median ranged from 0.083% in 1967 up to 0.176% in 1969, then fell to a low value of 0.078% in 1970 before rising to a higher level, 0.125%, in 1971. There is definite indication that the number of relatively resistant strains encountered has increased since 1970 (Lindberg RB, Contreras AA, Mason AD, Jr. USA Institute Surg. Res. Ann Rpt FY 1972, BAMC, Ft Sam Houston, Tx, Section 21)[1].

The cumulative sensitivity of Ps aeruginosa shows the sensitivity pattern for Sulfamylon in more detail (Figure 1). The values are distributed

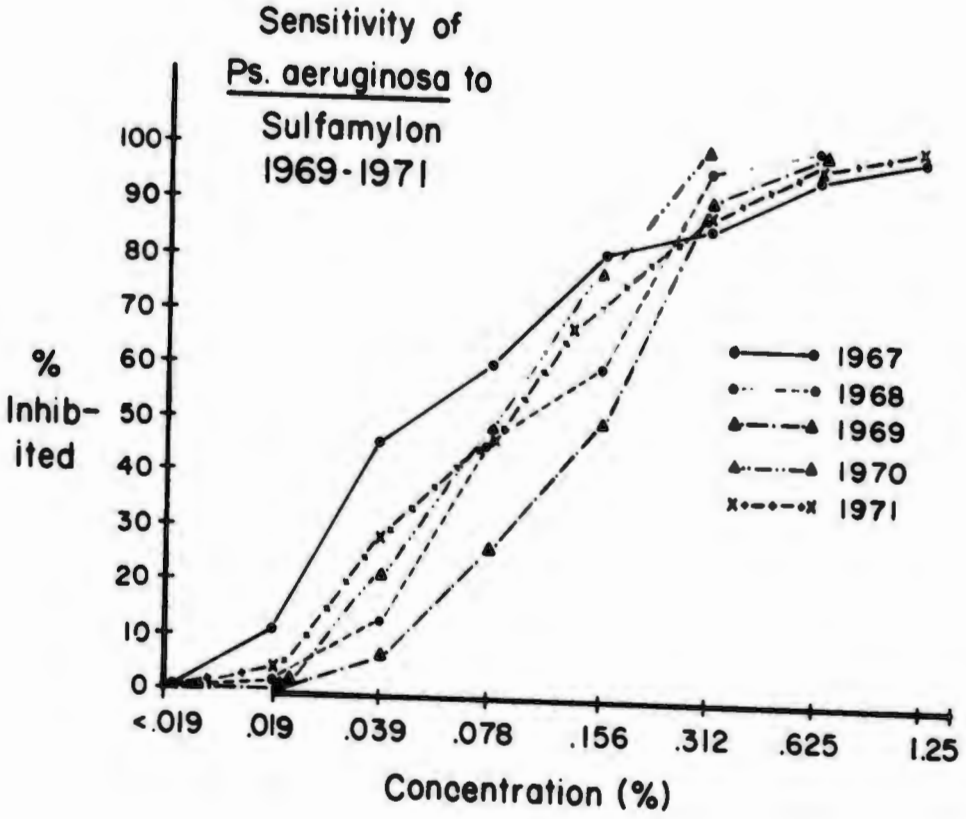
Sensitivity of
Ps. aeruginosa to
Sulfamylon
1969-1971
% Inhibited
100
90
80
70
60
50
40
30
20
10
0
<.019
.019
.039
.078
.156
.312
.625
1.25
Concentration (%)
1967
1968
1969
1970
1971

Figure 1

in a fundamentally sinusoidal curve, and vary from year to year around a mean level with no consistent trend to heightened resistance. The widest range on the curves occurred in 1967 and again in 1971; the narrowest range, an almost straight line increase was observed in 1970. A small number of strains with a Minimum Inhibitory Concentration (MIC) of 0.625% to 1.25%, have appeared as a recurrent pattern.

Strains of Ps aeruginosa recovered from 2 cases of BWS in 1969, 2 in 1971, and 2 in 1972 required from 0.312% to a high of 1.25% of Sulfamylon for inhibition. At the time of each of these episodes, the burn ward was colonized with a phage type of Pseudomonas which showed a higher than average tolerance for Sulfamylon. These monotype populations of Pseudomonas have been characterized as microepidemics; they occur, predominate and then tend to disappear, to be replaced by other phage types differing in sensitivity. However, cases of BWS in Sulfamylon-treated patients occurred during such microepidemics, and the offending strains were of the phage type and Sulfamylon-sensitivity level that characterized the microepidemic. The Pseudomonas strains from such cases of BWS were tested for virulence and response to Sulfamylon treatment in rats scalded over 20% of body surface, and seeded with Ps aeruginosa.

The behavior of these strains was compared with that of typical virulent strains and also with that of isolates collected during the same epidemic period, from other patients who did not develop BWS. As a basis for comparison, typical behavior patterns of 3 virulent strains of Ps aeruginosa on the burned seeded rat are shown in Figure 2,

Mortality rate is presented in increments of repeated experiments to show the fluctuation that occurs with successive tests and the ultimate derivation of a significant median level of lethality and of therapeutic response. The 3 strains shown were all virulent, with kill rates ranging from 85% to 99% of untreated controls. With Sulfamylon treatment, significant survival occurred. Over 90% of treated rats survived with strain 12-4-4, 83% with the more virulent 8-23-3, and 43% with the extremely virulent 3-24-5. These survivals occurred with once-daily treatment; when more frequent treatment was applied, the survival rate with each strain

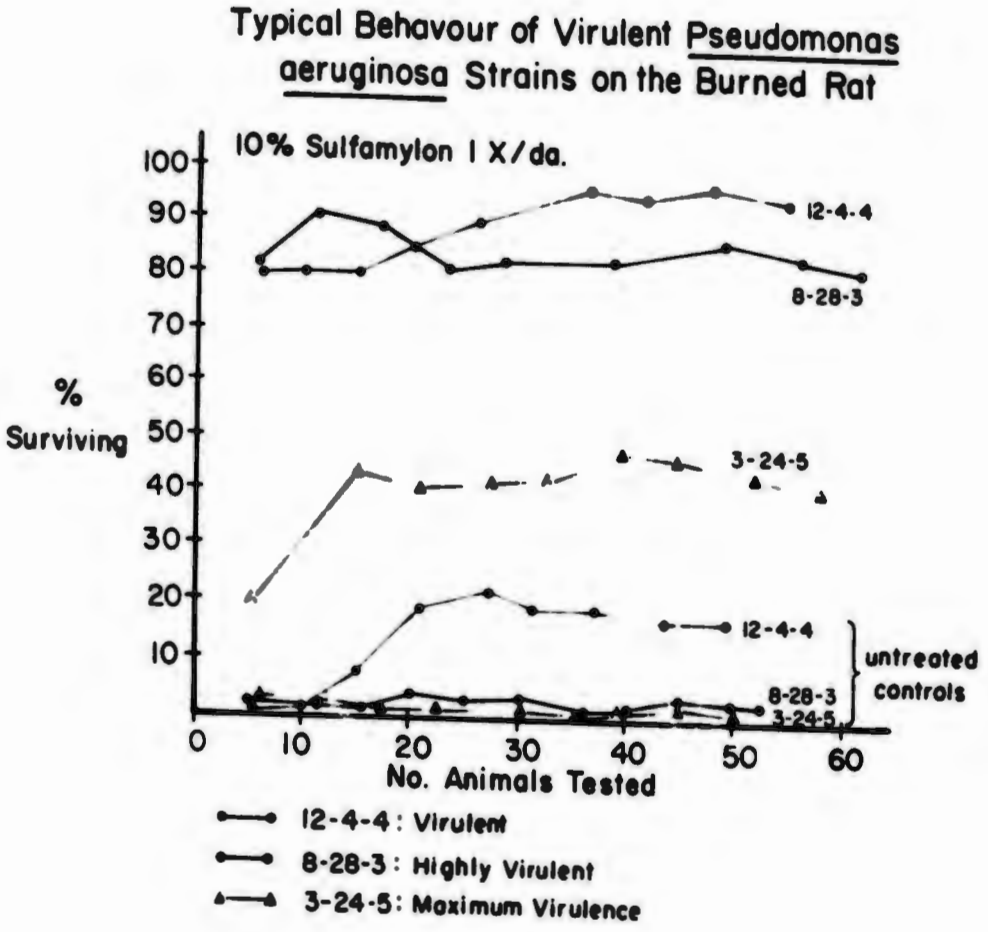
Typical Behavour of Virulent Pseudomonas aeruginosa Strains on the Burned Rat
10% Sulfamylon 1 X/da.
100
90
80
70
60
50
40
30
20
10
%
Surviving
12-4-4
8-28-3
3-24-5
12-4-4
untreated controls
8-28-3
3-24-5
0
10
20
30
40
50
60
No. Animals Tested
12-4-4 : Virulent
8-28-3 : Highly Virulent
3-24-5 : Maximum Virulence

Figure 2

was increased. These levels of lethality and survival rates represent typical behavior of Ps aeruginosa virulent strains, amenable to treatment with Sulfamylon topically. Over 60 strains have been assessed in this manner, with survival response varying with the relative level of virulence. These strains varied from 0.078% to 0.312% MIC with Sulfamylon. Survival rate with Sulfamylon treatment did not correlate with in vitro sensitivity, i.e., highly sensitive strains that were highly virulent could kill a higher percentage of treated rats than could relatively resistant strains of lower virulence.

The strains recovered from patients who developed BWS despite Sulfamylon treatment behaved in a manner quite different from that seen with the preceding typical Pseudomonas strains. Illustrative examples of this difference are shown in the following figures.

Figure 3 shows the behavior of 2 isolates of Ps aeruginosa, one from a wound biopsy and one from the spleen at autopsy. The patient was a 2-year-old boy with a 30% total body surface burn, who developed Pseudomonas BWS despite prompt intensive topical treatment with Sulfamylon. The strains were weak in virulence in the burned rat model. Sixty per cent of animals seeded with strain #6-1-10, and 77% of those with strain #6-9-7, survived. A very high survival rate would have been expected with Sulfamylon treatment of these strains, but the treated animals survived in the case of strain 6-9-7 at exactly the same rate as untreated, while with strain #6-1-10, survival of 75% of seeded treated rats occurred as opposed to 60% for the untreated. There was, thus, no real indication that survival in the experimental animal was affected by topical Sulfamylon. This was true despite the relatively low virulence of the infecting strain.

The behavior of 3 additional isolates of Ps aeruginosa from this case of treatment-refractory BWS is summarized in Figure 4. All 5 of the strains shown required 1.25% of Sulfamylon for inhibition. This placed them at the upper limit of observed Sulfamylon tolerance for strains of Ps aeruginosa. Sulfamylon exerted no effect on the mortality of animals seeded with strain 6-9-11. Strains 6-6-6 and 6-9-12 showed a slight effect of increased survival with Sulfamylon, but the death rate was only reduced by 10% to 15%. Thus,

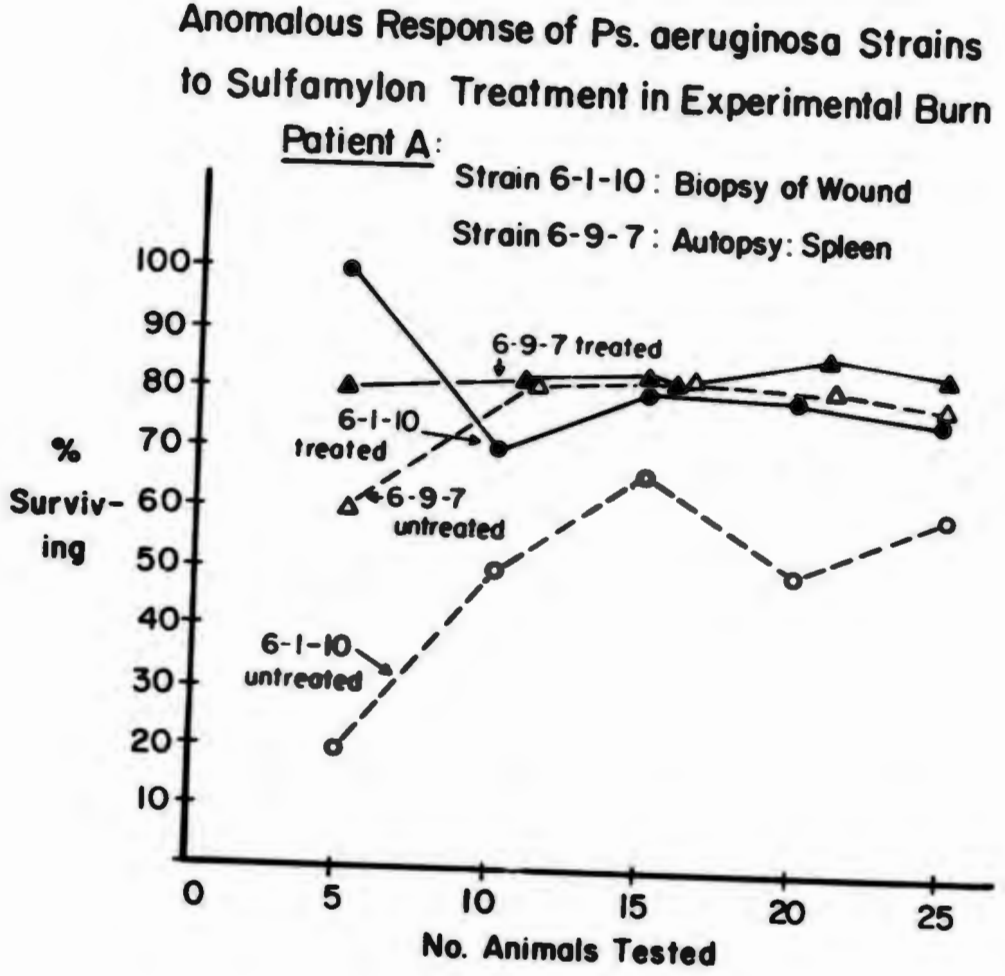
Anomalous Response of Ps. aeruginosa Strains
to Sulfamylon Treatment in Experimental Burn
Patient A:
Strain 6-1-10 : Biopsy of Wound
Strain 6-9-7 : Autopsy: Spleen
% Surviving
100
90
80
70
60
50
40
30
20
10
0
5
10
15
20
25
No. Animals Tested
6-9-7 treated
6-1-10 treated
6-9-7 untreated
6-1-10 untreated

Figure 3

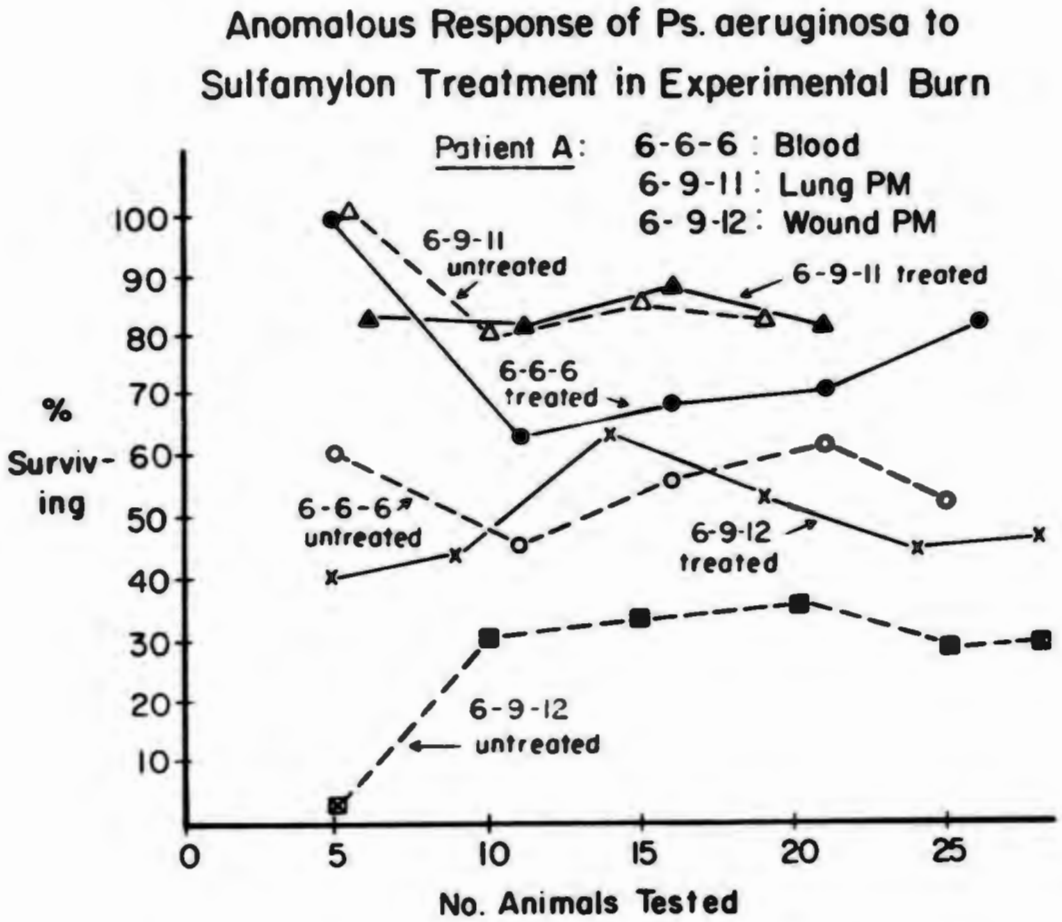
Anomalous Response of Ps. aeruginosa to
Sulfamylon Treatment in Experimental Burn
Patient A: 6-6-6 : Blood
6-9-11 : Lung PM
6-9-12 : Wound PM
6-9-11 untreated
6-9-11 treated
6-6-6 treated
6-6-6 untreated
6-9-12 treated
6-9-12 untreated
% Surviving
100
90
80
70
60
50
40
30
20
10
0
5
10
15
20
25
No. Animals Tested

Figure 4

none of these isolates showed a significant response to Sulfamylon therapy.

The strains from this patient were all of the same phage type, NT-2a. They were part of a large series of isolates all of which were culturally identical, there was a consistent variation in virulence between successive isolates. This difference was not large, but it was reproducible. The common attribute of most concern was the fact that these Pseudomonas strains were capable of causing BWS despite treatment with Sulfamylon. The fact that they were low in virulence made this phenomenon even less explicable.

In a second case of BWS, this time in a 3-year-old with 32% total body surface burn, a response to therapy entirely analogous to that described with the preceding patient occurred. Four strains, 3 from biopsies and one from blood, were very low in virulence, but treatment had little effect on survival. The patient harbored 2 phage types, F-12 and NT-2a during his hospital course. The strains tested were type NT-2a but a sampling of the F-12 strain gave a virulence and a therapeutic response level that was in the same range as that seen here with strain 10-3-11. These strains were slightly more susceptible to Sulfamylon than those from the preceding patient; they required 0.625% for inhibition. This information is depicted in Figure 5.

The strains of Pseudomonas from the preceding patients illustrate an anomalous lack of response to therapy in strains of low virulence. In contrast, the typical pattern of response of low-virulence strains is shown in Figure 6.

These strains, from 2 burn cases in which clinical BWS did not occur, were tested for virulence and therapeutic response. Both strains required 0.625% of Sulfamylon for inhibition. The spread between survival of treated rats and of the untreated controls is significant. While survival was not complete, 85% to 95% of the treated rats survived. This response to therapy is the pattern we have regarded as typical of strains of low to moderate virulence.

The behavior of 3 additional sets of strains, recovered from burn wound sepsis in Sulfamylon-treated patients since 1969, showed a pattern

Patient B: Anomalous Response of Ps. aeruginosa to Sulfamylon Treatment in Experimental Burn

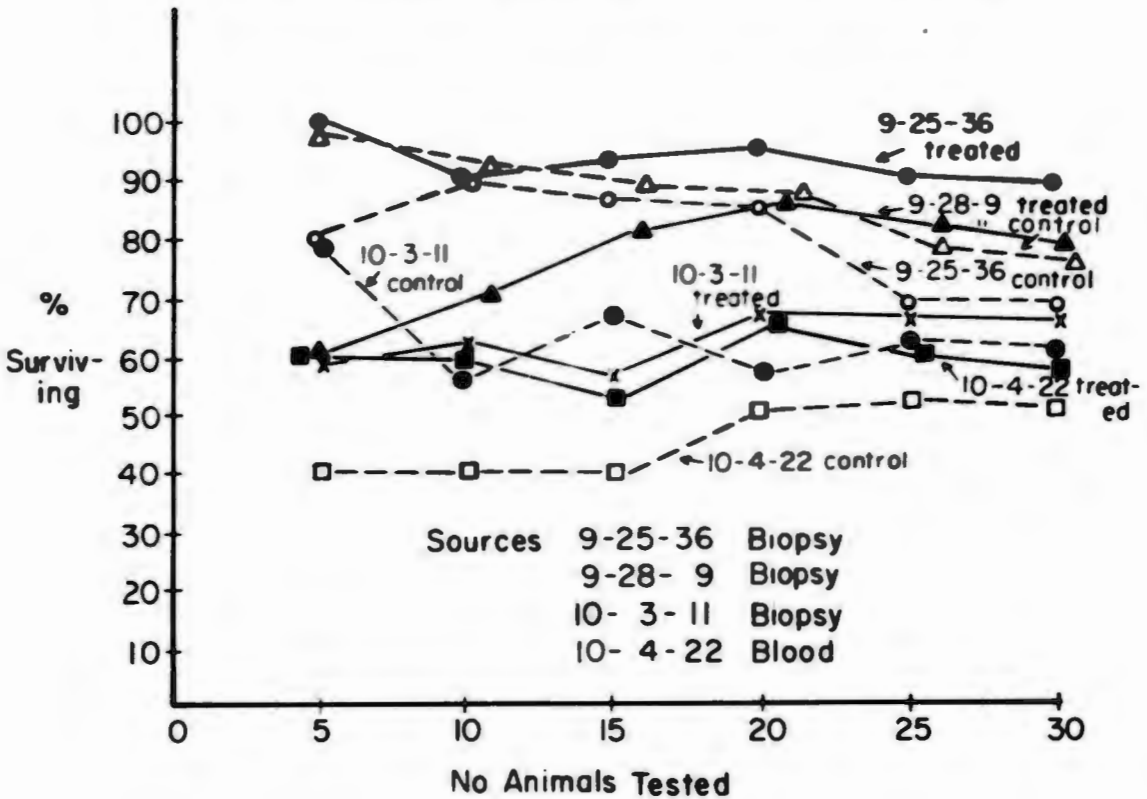

Figure 5

TYPICAL RESPONSE OF LOW-VIRULENCE PSEUDOMONAS AERUGINOSA STRAINS TO SULFAMYLON TREATMENT IN THE EXPERIMENTAL BURN. Two patients.

Patient C : strain 5-28-3 : Blood
Patient D : strain 4-18-9 : Lung, autopsy.

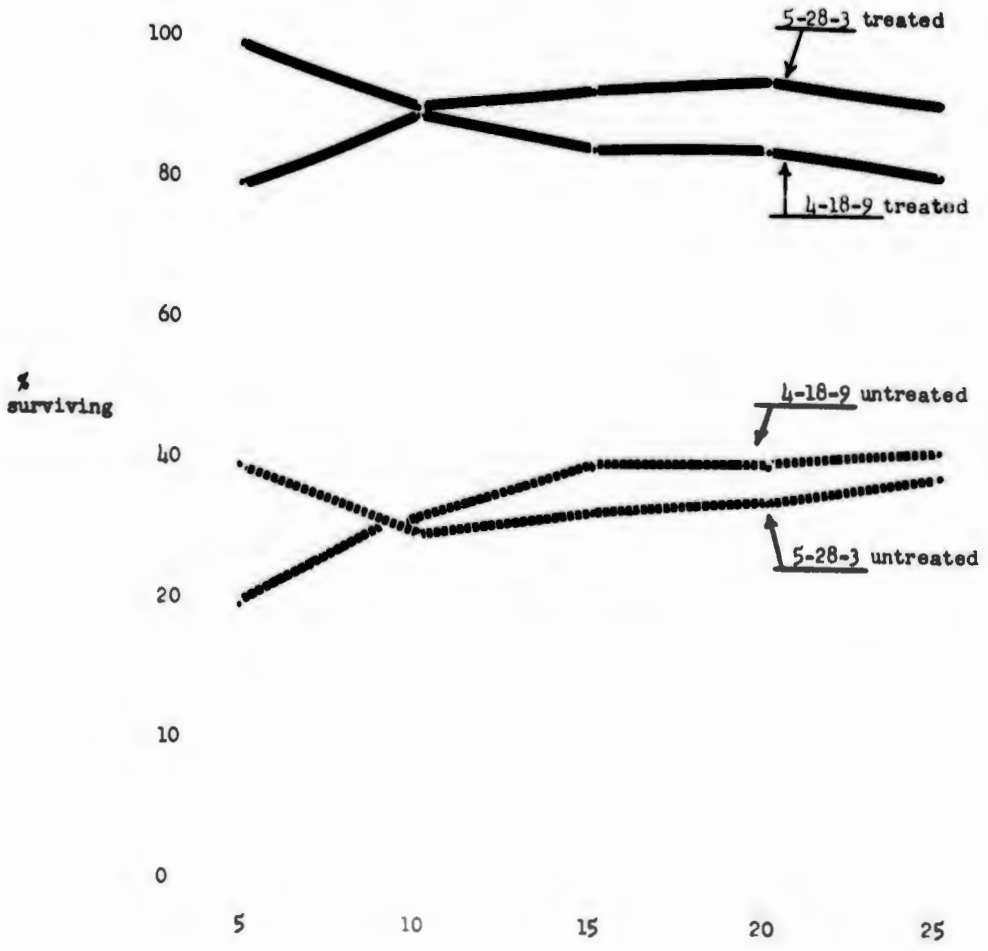

Figure 6

similar to the one shown above of refractoriness to Sulfamylon treatment. This behavior was exceptional and in complete contrast to the typical performance of Ps aeruginosa, in which survival of treated rats is widely separated from the death rate of the untreated controls. This unusual response of failure to respond to Sulfamylon treatment was apparently not due solely to Sulfamylon-resistance, since many strains requiring 0.625% for inhibition have been shown to respond fully to topical Sulfamylon as a means of preventing experimental BWS.

The cultural characteristics of these treatment-resistant strains were scrutinized in detail, thus far with no results that would permit detection of a potentially treatment-refractory strain simply by its biochemical reactions. The only distinctive characteristic that has thus far been recognized is the fact that the treatment-refractory strains have an unusually slow growth rate in the logarithmic phase.

It must be emphasized that most of the low-virulence strains which have been tested respond to treatment with Sulfamylon. Neither Sulfamylon tolerance in vitro nor low-virulence necessarily connoted a treatment-resistant strain in the patient, but if both of these attributes were present, then there was a real possibility that the strain would be resistant to treatment with Sulfamylon. If we were to propose a course of action in which monitoring the strains were to be attempted in order to anticipate this development, then sensitivity testing should be done. When a population of Sulfamylon-tolerant strains, usually of single phage type or of 2 closely related types appears, virulence and therapy response could be assessed. If treatment-refractory strains were found, it would prompt enhanced vigilance to detect the treatment-refractory patient. Presence of such strains on the wound might justify intensive appropriate antibiotic treatment to ward off potential invasive sepsis.

There is, as yet, no evidence that treatment refractory strains can become permanently ensconced as the resident Pseudomonas flora on a burn ward. Their numbers fluctuate as do those of other Pseudomonas phage types.

SUMMARY

The existence of a previously unknown pattern of virulence and invasive potential in Ps aeruginosa has been delineated. The strains tend to be part of a monotype epidemic of Sulfamylon-resistant strains. They are of low to moderate virulence, but do not respond to Sulfamylon therapy in the burned rat model. They have been detected in cases of BWS due to Ps aeruginosa, in which Sulfamylon topical treatment was ineffective in preventing the disease. This attribute offers one possible explanation for the occasional treatment failure, which is signalled by the appearance of Pseudomonas BWS.

REFERENCE

1. Lindberg RB, Contreras AA, Mason AD, Jr. Antibiotic Sensitivity of Current Military Burn Patient Flora. USA Institute Surg Res Ann Rpt FY 1972, BAMC, Ft Sam Houston, Texas. Section 21.

PRESENTATION

Lindberg RB. Sensitivity of Pseudomonas aeruginosa to Sulfamylon and Its Relation to Experimental and Clinical Burn Wound Sepsis. Presented at American Burn Assoc., April 1973, Dallas, Tx.

PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0B 6978	73 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
72 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 (Procede 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. 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 73	.3	10
	CURRENT 74	.4	10

19a. RESPONSIBLE DOD ORGANIZATION
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20. GENERAL USE: FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede 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 (Furnish 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) 72 07 - 73 06 Recovery of fungi from autopsy and biopsy specimens has reached a higher proportion of positive cultures in relation to positive microscopic findings than had previously been achieved. Multiple samples of thin tissue slices have produced this improvement. A marked rise in incidence of Fusarium sp in burn wounds has been observed. This previously infrequent genus has been observed as an active invader of second and third degree burns; this level of pathogenicity had not previously been reported for Fusarium. Attempts to create an animal model with tissue-invading isolates of this genus are in progress. Its behavior is not similar to that of the Phycomycetes. Its high incidence suggests that it plays a potentially significant role in burn pathogenesis.

* Available to contractors upon originator's approval

ANNUAL PROGRESS REPORT

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

1 July 1972 - 30 June 1973

Investigators:

Robert B. Lindberg, PhD
Anthony A. Contreras, MS
Harvey O.D. Smith, Jr, SP6
Peter M. Kirchgessner, SP5
Basil A. Pruitt, Jr, MD, Colonel, MC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A16 1102B71R-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 report: 1 July 1972 - 30 June 1973

Investigators: Robert B. Lindberg, PhD
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Reports Control Symbol MEDDH-288(R1)

Monitoring of autopsy and biopsy specimens for presence of fungi is a necessary continuing procedure. The recovery rate as to numbers of genera and proportion of specimens positive for fungi has remained quite stable; 15 genera were recovered in 1972. Candida sp (primarily albicans) was the most commonly encountered yeast. Fusarium, Cephalosporium, Aspergillus, and Sepedonium were the most frequently encountered fungi. Phycomycetes, which have contributed the major part of severe invasive fungal wound sepsis, were rare; only 3 strains were found in 3 autopsies, and 5 strains of Rhizopus and Mucor in 362 biopsy samples. The role of fungi in burn wound infection was diminished over that seen in previous segments of this study. Continued surveillance is called for, but in 1972, fungi in the burn wound were a heterogeneous population, often of equivocal significance as pathogens.

Fungi Mucor Candida Rhizopus Burns Phycomycosis
Humans

THE ROLE OF FUNGI IN BURN WOUND INFECTION: OBSERVATIONS ON BIOPSY AND AUTOPSY TISSUES FROM SERIOUSLY BURNED SOLDIERS

The role played by fungi and yeasts in burn wound infection has presumably been increasing in recent years, although to some degree the alledged "increase" may reflect more assiduous search for these agents. The unsolved conundrum of the demonstration of hyphal structures in tissues from which no fungi can be recovered in culture has prompted search for improved manipulation that might increase the yield of cultures. There remains the fact that presence of fungi in the burn wound is still an unknown entity as far as clinical significance is concerned. Continued directed study, coordinating clinical, histopathologic and microbiologic technics offers promise of further clarification of this question.

METHODS

Continued use of fresh biopsy samples, collected when possible near the live-dead interface at the margin of the suspect burned area, has been the point of greatest emphasis in recovering fungi from patients. Autopsy samples were collected with similar emphasis. The planting of thin slivers of tissue, preferably removed from the sample with a scalpel, has offered best results in recovery rates (Lindberg RB, Townsend CH, Contreras AA, Pruitt BA,Jr. USA Inst Surg Res Ann Rpt FY 1970, BAMC, Ft Sam Houston, Tx. Sect. 26)[1]. Prompt planting, without refrigeration of the specimen, was emphasized. Sabouraud's agar was the basic substrate and incubation was at 26°C.

RESULTS

Disseminated fungal infection in burn patients is most convincingly demonstrated by recovery of the organism at autopsy. Table 1 lists fungi recovered and their frequency in terms of number of patients with positive visceral or burn wound samples. Parallel information is shown for 1971 and 1972. In all 19 genera of yeasts and fungi were recovered. In 1971, 17 genera were recovered, and in 1972, 15; there were 13 genera that were recovered during both years (Lindberg RB, Contreras AA, Smith HOD,Jr, Kirchgessner PM, Pruitt BA, Jr. USA Inst Surg Res Ann Rpt FY 1971,BAMC,

Table 1. Genera of Fungi Recovered from Viscera (Lung, Liver, Spleen) and Burn Wound at Autopsy, 1971-1972

Genera	Number of Patients Positive			
	Burn Wound		Viscera	
	1971	1972	1971	1972
Mucor	3	0	0	0
Rhizopus	1	1	1	1
Absidia	1	1	0	0
Aspergillus	6	11	3	2
Penicillium	8	1	5	6
Paecilomyces	0	0	1	2
Alternaria	2	3	0	0
Cephalosporium	2	4	0	4
Fusarium	9	30	7	20
Helminthosporium	4	1	0	0
Nigrospora	9	0	3	0
Scopulariopsis	5	3	1	2
Sepedonium	1	5	1	4
Diplosporum	1	2	0	0
Geotrichum	0	0	1	0
Fonsecaea	2	0	2	0
Curvularia	0	5	0	0
Microsporum	0	1	0	0
Candida	13	32	11	25
No. patients positive			46	65
Total patients cultured			61	89
No. genera present			17	16

Ft Sam Houston, Tx. Sec. 30)[2]. Predominant genera in 1972 included Candida, Fusarium, Cephalosporium, Aspergillus and Sepedonium. Yeasts were routinely listed with fungi, but the behavior of Candida sp on the burn wound more resembles that of bacteria as far as spread and incidence of occurrence are concerned. Candida albicans was the predominant species; Candida stutzeri was also recognized in 2 instances. Candida sepsis does occur, and the positive cultures from liver and spleen would be interpreted as indicative of disseminated infection. At least three-fourths of patients with positive Candida cultures at autopsy harbored Candida in the burn wound. Thus, when it does occur in burn patients, a large proportion of burn wound samples tend to be colonized by Candida. The lung was another major site for recovery of Candida in burn autopsies.

Fusarium sp were, in 1972 as in 1971, a predominant genus. In viscera it was most common in the lung, although in cases with probable systemic involvement with this genus, it was present also in liver and spleen. Its prominent position in lung samples suggested that it was a potential factor in pulmonary disease in the burn patient. One patient died with massive, disseminated burn wound sepsis and systemic fungal involvement due to Fusarium. The strain was a borderline one in morphology and was originally designated as Cephalosporium sp.

Mucor and Rhizopus sp have, in former years, each been recovered from fungal invasive burn wound sepsis. In the autopsy samples these genera were rare in 1972. This scarcity was reassuring, since when once established, invasive sepsis caused by these genera is not readily amenable to treatment.

Other genera of relative abundance included Cephalosporium and Penicillium. These became more common in 1971 and this frequency continued in 1972. The overall picture of fungi in burns at this time reached a relatively stable state where a large variety of saprophtic fungi colonize the burn patient, and a small number of this total may achieve systemic invasion. There is no strong indication for the presence of a particularly malign genus abroad among the burn patients.

Biopsy of the burn wound as a diagnostic tool has been more extensively used in the burn ward during the past 2 years than at any previous period. In 1972, 201 patients were submitted to biopsy sampling, with 362 samples collected. The results of these cultures, which reveal the most direct relationship between on-going treatment of the burn and fungal colonization, are summarized in Table 2. The results of burn wound autopsy samples are set down in parallel to show the close correspondence in incidence that existed. The most common organism encountered, by far, was Candida sp, in 13.9% of patients cultured, and in 35.9% of autopsies. Fusarium, as was noted above, was by far the most common genus of fungi in autopsies and in biopsies. Aspergillus, a common contaminant, was fourth in incidence in biopsies and autopsies. Other species were present in small numbers of biopsied patients and in comparable frequency in autopsies. Autopsy tissues harbored the same number of genera as did the biopsied group, but in the less common forms discrepancies occurred. Ten genera appeared in both sources: in addition to Candida, Fusarium and Aspergillus, there were Cephalosporium, Curvularia, Scopulariopsus, Alternaria, Diplosporium, Penicillum and Rhizopus. Mucor, Rhizopus and Absidia the genera most prone to be found in invasive fungal wound sepsis, were represented by 8 strains altogether and none occurred in large numbers. The parallel between species incidence in biopsy and autopsy of the burn wound reinforces the validity of the biopsy sampling technic.

DISCUSSION

Since 1969, the number of fungal genera and of individual strains recovered from biopsies and autopsy samples has remained relatively constant. Predominant genera have changed; in 1969, Geotrichum was found in 59 autopsies; this year, one strain was found in a biopsy. Fusarium has remained the most commonly encountered species, and in 1972, Cephalosporium was also prominent in incidence. These 2 genera are very similar in recognition criteria, and it is probable that some of each genera belonged in the other category No conspicuous new pattern of fungal infection by opportunistic saprophytes was discerned, and the strong probability exists

Table 2. TISSUES POSITIVE FOR GENERA AND FUNGI AND YEASTS - 1972

Genus	Biopsy of Burn Wound		Autopsy of Burn Wound	
	No. Patients Positive	No. Isolates	No. Patients Positive	No. Isolates
Candida	28	46	32	64
Aspergillus	8	11	11	33
Cephalosporium	5	15	4	16
Fusarium	19	33	30	65
Sepedonium	-	-	5	10
Curvularia	3	3	5	8
Scopulariopsus	1	1	3	8
Alternaria	3	7	3	4
Diplosporium	1	1	2	3
Penicillium	1	1	1	3
Helminthosporium	-	-	1	1
Rhizopus	2	3	1	2
Absidia	-	-	1	1
Mycelia sterilia	-	-	3	6
Microsporum	-	-	1	1
Stemphyllium	1	1	-	-
Syncephalastrum	1	2	-	-
Mucor	2	2	-	-
Geotrichum	1	1	-	
No. Patients Cultured	201		89	
No. Tissue Samples	362		485	

Note: Fungi Imperfecti - Hyphae resembling Phycomycetaceae but no fruiting bodies developed.

that opportunistic invasive infection of the burn wound by fungi is now less common in occurrence than was pictured 3 to 4 years ago. Continued surveillance of fungi involved in this syndrome is required. Experimental efforts to establish invasive fungal infection in rats treated with streptozotocin, then burned (a technic which makes the animal susceptible to infection with Mucor and Rhizopus) failed to show that this common species was capable of invading the burn wound. The role of fungi in burn wound infection can be a serious one, but these epidemiologic studies do not indicate an unequivocal relationship between fungi and burn wound infection.

REFERENCES

1. Lindberg RB, Townsend CH, Contreras AA, Pruitt BA,Jr. Role of Fungi in Burn Wound Infections. USA Inst Surg Res Ann Rpt FY 1970, BAMC, Ft Sam Houston,Texas. Section 26.

2. Lindberg RB, Contreras AA, Smith HOD, Kirchgessner PM, Pruitt BA,Jr. The Role of Fungi in Burn Wound Infections: Observations on Biopsy and Autopsy Tissues from Seriously Burned Soldiers. USA Inst Surg Res Ann Rpt FY 1971, BAMC, Ft Sam Houston, Texas. Section 30.

PRESENTATIONS AND/OR PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0E 6952	73 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
72 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	303
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) Prevention of Fungal and Yeast Colonization and Infection of the Burn Wound with Topical Nystatin (Mycostatin) in 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 09	73 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		a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
FISCAL YEAR	PRECEDING 73	.5	17
	CURRENT 74	0	0

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20. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Fungus; (U) Topical Chemotherapy; (U) Topical Nystatin; (U) Burn Wound; (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 ascertain if topical nystatin (Mycostatin) applied to the burn wound would prevent fungal and yeast colonization and infection of the burn wound in burned troops.

24. (U) Patients admitted to the US Army Institute of Surgical Research within 72 hours of injury will be studied and placed in one of three groups according to burn size. Group I less than 30% total body surface burn; Group II 30 to 60 % total body surface and Group III greater than 60% total body surface burn. Periodic burn wound biopsy for histologic and bacteriologic study will be obtained and weekly BUN, WBC, differential, SGOT and urinalysis. Mycostatin Sulfamylon cream with 10,000 units of nystatin per gram of Mafenide will be the study drug.

25. (U) 72 07 - 73 05 Sixteen patients have been admitted to the study, all having burn wound biopsies showing fungal burn wound infection or invasion. No control patients have been studied. The small number of patients which have entered into this study has generated insufficient data to justify the continuation of this study. Therefore, as of 1 May 1973 the study was terminated.

*Available to contractors upon originator's approval.

FINAL REPORT

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

REPORT TITLE: PREVENTION OF FUNGAL AND YEAST COLONIZATION AND INFECTION OF THE BURN WOUND WITH TOPICAL NYSTATIN (MYCOSTATIN^{R}) IN MILITARY PERSONNEL

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

1 July 1972 - 30 June 1973

Investigators:

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Basil A. Pruitt, Jr., MD, Colonel, MC
Robert B. Lindberg, PhD

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

REPORT TITLE: PREVENTION OF FUNGAL AND YEAST COLONIZATION AND INFECTION OF THE BURN WOUND WITH TOPICAL NYSTATIN (MYCOSTATIN^{R}) IN MILITARY PERSONNEL

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

Period covered in this report: 1 July 1972 - 30 June 1973

Investigators: John L. Hunt, M.D., Major, MC
Basil A. Pruitt, Jr., M.D., Colonel, MC
Robert B. Lindberg, PhD

Reports Control Symbol MEDDH-288(R1)

At the present time three additional patients have been added to this study. One patient was in the group between 30 and 60%. The other two patients were in the greater than 60% group. All three had Sulfamylon Mycostatin topical treatment as the initial and only agent used during their burn course. Cause of death of the one patient who was in the greater than 60% group, was pneumonia. Serial determinations of BUN, white blood count, reticulocyte count and urinalysis revealed no abnormalities. A slight initial elevation in the SGOT in all three patients, which soon fell to normal, was noted. In no patient was there evidence bacteriologically or histologically of burn wound invasion by bacteria, fungi, or yeasts.

The small number of patients which have been entered into this study has generated insufficient data to justify the continuation of this study. Therefore as of 1 May 1973 the study will be terminated.

Fungus
Topical chemotherapy
Topical nystatin
Burn wound

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0D 6386	73 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
72 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	117
b. CONTRIBUTING	61102A	3A061102B71P	05	
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) Experimental Fungal Burn Wound Infection as a Model of Burns in Soldiers: Effect of Topical Antifungal Agents (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
69 07	73 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	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 73	.6	19
FISCAL YEAR CURRENT 74	0	0

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21. GENERAL USE: FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code): (U) Burns; (U) Infection; (U) Fungus; (U) Rats; (U) Fungi; (U) Mycotic; (U) Colonization; (U) Invasion; (U) Alloxan-Diabetes

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

23. (U) Spores suspensions of fungi were evaluated for their ability to produce localized infection of the burn wound in rats and evaluate the effects of available topical antifungal preparations applied to the seeded burn wound. This model simulates burn injury on soldiers.

24. (U) Scald wounds of rats seeded with spore suspensions of Rhizopus rhizopodiformis, Aspergillus flavus and Fusarium sp. were left exposed or treated with topical compounds which included standard cream base, amphotericin and nystatin, alone or in combination with mafenide. Wounds were examined histologically and cultured for bacteria and fungi.

25. (U) 72 07 - 73 06 Surface proliferation of Rhizopus is retarded by amphotericin but fungal burn wound infection occurs regardless of treatment. Aspergillus wound infection was limited by amphotericin and nystatin and wound infection with Fusarium appeared enhanced by all topical cream preparations studied. Topical treatment with antifungal compounds resulted in bacterial overgrowth which was not corrected by mafenide-containing compounds at the concentrations used.

*Available to contractors upon originator's approval

FINAL REPORT

PROJECT NO. 3A161102B71R-01 RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: EXPERIMENTAL FUNGAL BURN WOUND INFECTION: EFFECT OF TOPICAL ANTIFUNGAL AGENTS

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

1 July 1972 - 30 June 1973

Investigators:

F. D. Foley, MD
Robert B Lindberg, PhD
Jimmie E Murphy, SSG
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Beverly Worley, BS

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A161102B71R-C1, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: EXPERIMENTAL FUNGAL BURN WOUND INFECTION: EFFECT OF TOPICAL ANTIFUNGAL AGENTS

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

Period covered in this report: 1 July 1972 - 30 June 1973

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Reports Control Symbol MEDDH-288(R1)

Scald wounds of rats were seeded with suspensions of Rhizopus rhizopodiformis, Aspergillus flavus, and Fusarium sp. The wounds were left exposed or treated with topical compounds which included standard cream base, amphotericin and nystatin, alone and in combination with mafenide.

Histologic examination of the wounds revealed fungal infection with Rhizopus despite suppression of surface growth by amphotericin. Aspergillus wound infection was limited by amphotericin and nystatin. Wound infection with Fusarium appeared to be enhanced by the topical creams used.

Topical treatment with antifungal compounds resulted in bacterial overgrowth and the addition of mafenide at the concentration applied (5%) did not suppress bacterial density to the level of untreated rats.

The absence of fungicidal properties and diffusivity of these antifungal creams associated with enhanced bacterial proliferation may preclude their use on burn wounds as compounded herein, particularly since the significance of fungal infection limited to the burn wound is uncertain. Compounds with an effective antibacterial concentration are suggested.

Burns
Infection
Fungus

EXPERIMENTAL FUNGAL BURN WOUND INFECTION: EFFECT OF TOPICAL ANTIFUNGAL AGENTS

Previous studies have demonstrated deeply invasive fungal burn wound infection (FBWI) during the acute phase of alloxan-induced diabetes in burned rats seeded with the phycomycotic organism, Rhizopus rhizopodiformis (Rr) associated with visceral involvement (spleen, kidney, lung, liver) in 30% of animals. Examination of non-alloxanized control rats during this study revealed a limited form of FBWI without visceral lesions. Since the most frequent presentation of FBWI noted clinically occurs without systemic dissemination, the occurrence of limited FBWI in the scalded rat suggested a study of currently available topical antifungal compounds in relation to prophylaxis of these infections.

MATERIALS AND METHODS

All rats (170-190 g) received a 20% scald burn and those surviving the anesthetic and injury were seeded with 1 ml of 10^5/ml spore suspensions within two hours postburn. Organisms studied included Rhizopus rhizopodiformis (Rr), Aspergillus flavus (Af) and Fusarium sp (Fs) isolated and identified by the USAISR Microbiology Branch from burned patients and confirmed by the Mycology Branch, CDC. Crude suspensions were prepared by agitation in distilled water, filtered through mesh gauze and adjusted to 10^5 spores/ml.

Topical antifungal agents included amphotericin B (Fungizone cream(R)), nystatin (Mycostatin cream(R)) and mafenide acetate Sulfamylon(R)). Each of the three inocula were seeded on the wounds of 30 rats which were equally distributed at random among six treatment groups as follows: amphotericin (A), nystatin (N), mafenide-amphotericin (M-A), mafenide-nystatin (M-N), base cream (B) and untreated controls (U). The agents were compounded as below and 4 grams of each were applied to the wounds within two hours post seeding and daily thereafter.

A	=	Base	100 g
		Amphotericin, 3%	100 g
N	=	Base	100 g
		Nystatin, 100,000 units/g	100 g
M-A	=	Mafenide acetate, 10%	100 g
		Amphotericin, 3%	100 g
M-N	=	Mafenide acetate, 10%	100 g
		Nystatin, 100,000 units/g	100 g

The above compounds resulted in halving of the concentration provided by the proprietary agents and all combinations of mafenide therefore were reduced to 5% which is less than the recommended concentration for suppression of bacterial burn wound infection.

All rats were killed 10 days post burn and 6 standard blocks removed from the burn wound for histologic examination. A segment of each wound also was submitted for fungal recovery on Sabouraud's media and quantitative bacteriologic culture.

The presence of FBWI was determined histologically by the occurrence of fungal hyphae or spores beneath or adjacent to the eschar associated with an inflammatory response.

RESULTS

Rhizopus rhizopodiformis

All rats exhibited foci of limited FBWI and Rr was isolated from all wounds post mortem. Although FBWI was not prevented, observations of the magnitude of fungal involvement among the treatment groups indicated suppression in rats treated with amphotericin. These quantitative impressions were based on thickness of the surface fungal mat and amount of fungal proliferation within the center and margins of the burn wound.

Rats in treatment groups U, B, N and M-N generally exhibited thick fungal colonization with central and marginal fungal lesions in contrast to group A which demonstrated a thinner surface growth with only marginal fungal involvement. Compound M-A appeared less effective in suppressing FBWI than A alone.

Observations of histologic density of bacteria correlated with quantitative bacteriologic cultures. Untreated open wounds were less heavily colonized with bacteria than wounds in any other treatment group including those treated with mafenide-containing compounds. Also, wounds covered by antifungal compounds without mafenide became more heavily contaminated with bacteria than those with mafenide (see table).

Aspergillus flavus

The histopathology of fungal involvement of wounds seeded with Af differed from FBWI as defined with Rr. The latter organism was identified with a focal inflammatory or granulomatous response whereas Af, although an avid colonizer associated with eschar penetration of seeded wounds, did not elicit distinct granulomas. Invasion to the subeschar zone by Af was accompanied by augmentation of necrosis and

suppuration in untreated or base coated wounds. Using this endpoint in conjunction with histologic fungal density on the wound surface and within the eschar, it was apparent that treatment with amphotericin or nystatin resulted in suppression of FBWI by Af.

Af was isolated post mortem from the eschars of all rats. Rats in treatment groups U and B exhibited dense colonization and eschar penetration mainly via hair follicles associated with occasional microfoci of fungi, necrosis and suppuration at the junction of eschar and viable subcutaneous tissue. In contrast, surface fungal growth was sparse, stunted and demonstrated altered tinctorial features suggestive of hyphal necrosis in groups A, N, M-A and M-N. Eschar penetration also was limited in these groups and no fungi were found at the subeschar level.

Rats in groups U and B were less heavily colonized with bacteria than other groups. Histologic density of bacteria was greater and quantitative cultures yielded maximum growth in groups A and N. Wounds of groups M-A and M-N showed a variable bacterial component which was apparently less than groups A and N and significantly greater than groups U and B.

Fusarium sp.

Each treatment group seeded with the conidial, suspension of Fs consisted of 4 rather than 5 rats as above. The surface appearance of the untreated burn wounds had a yellow color attributed to Fs pigmentation which differed from the brown appearance of wounds colonized by Rr and Af. Fs was cultured from all eschars post mortem.

The histopathology of FBWI by Fs included colonization and eschar penetration associated with minor foci of follicular and subeschar abscesses which were more extensive in base covered than untreated wounds. Topical antifungal creams did not prevent FBWI although a slight and variable suppressive effect was noted on the wound surface.

Rats in group U demonstrated less surface colonization and FBWI than cream-covered wounds in all other treatment groups. Wounds in treatment groups A and N showed focal diminution in density of surface colonization where a granular precipitate of the antifungal compound was evident histologically, in comparison to B. Elsewhere, colonization was as heavy and FBWI was present regardless of surface effects. One rat in group A exhibited bacterial overgrowth in the eschar and no FBWI. Similar histologic findings in groups M-A and M-N included possible effects on surface growth, no difference in FBWI compared to B, and some increase in magnitude of infection compared to group U.

Bacterial density was augmented in groups A and N and, although appreciably diminished in M-A and M-N, was least apparent in groups U and B.

SUMMARY AND CONCLUSIONS

Representative species of Rhizopus, Aspergillus and Fusarium colonize, penetrate and infect scald wounds of seeded rats and permit comparisons of the effects of topical treatment.

FBWI with Rr is not prevented by treatment with topical amphotericin or nystatin although the former appears to suppress the magnitude of fungal involvement. FBWI with Af was limited by treatment with amphotericin and nystatin. These agents provided no protection against FBWI by Fs despite possible surface effects where antifungal agents were in contact with the organisms. Exposure alone was more effective than any of the topical compounds used in treatment of FBWI with Fs.

Bacterial density was least in untreated wounds. Application of amphotericin or nystatin enhanced bacterial growth histologically, a finding which correlated with the results of quantitative bacterial cultures. Addition of mafenide to the antifungal compounds at the final concentration used (5%) was ineffective in preventing heavy bacterial colonization although some reduction was demonstrated.

Effectiveness of these topical antifungal agents therefore varies with the fungal species. Also, similar to concepts of bacterial infection, the demonstration of suppression in this in vivo system may or may not be reflected in prevention of FBWI. Lack of diffusivity, absence of fungicidal properties and augmentation of bacterial growth appear to preclude use of the agents as compounded herein especially since information regarding the course and consequences of FBWI is limited in forms of morbidity and mortality. An effective concentration of antibacterial agent is required in compounds under consideration for topical antifungal prophylaxis of the burn wound.

PRESENTATIONS AND/OR PUBLICATIONS

None

Table 1. Quantitative Bacteriologic Cultures of Burn Wounds

	Rr	Af	Fs
U	1.0×10^3	7.0×10^4	NG
	Overgrowth (Rr)	2.0×10^2	NG
	Overgrowth (Rr)	2.0×10^6	NG
	1.0×10^2	4.0×10^4	1.0×10^4
	3.5×10^7	NG*	---
B	1.1×10^5	2.5×10^3	NG
	1.0×10^6	7.0×10^4	NG
	2.0×10^8	3.0×10^6	6.0×10^1
	2.0×10^8	4.0×10^4	7.6×10^5
	1.1×10^9	NG	---
N	5.0×10^7	4.9×10^7	4.7×10^5
	4.0×10^8	3.8×10^7	9.8×10^5
	1.0×10^8	1.0×10^8	1.0×10^4
	1.0×10^8	5.7×10^7	1.1×10^6
	4.2×10^7	1.1×10^8	---
M-N	1.5×10^6	1.0×10^4	7.0×10^6
	5.0×10^6	6.4×10^5	NG
	1.5×10^5	1.7×10^7	NG
	1.5×10^5	1.0×10^8	5.0×10^2
	1.2×10^3	9.0×10^6	---
A	6.0×10^8	3.0×10^7	6.3×10^5
	1.0×10^9	3.0×10^8	1.7×10^7
	6.0×10^8	2.0×10^8	1.1×10^3
	---	2.0×10^8	8.4×10^5
	1.6×10^9	5.0×10^8	---
M-A	6.0×10^6	2.0×10^7	NG
	5.0×10^6	1.7×10^7	NG
	1.0×10^6	2.0×10^8	1.1×10^6
	2.0×10^6	6.3×10^7	8.3×10^5
	1.5×10^7	5.8×10^7	---

* No growth

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OE 6384	73 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'N	8a. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
72 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	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	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		g. PROFESSIONAL MAN YRS	h. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 73	.4	13
	CURRENT 74	.1	3

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) 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 DoD 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) 72 01 - 73 06 All control animals exhibited a healed burn wound when sacrificed at one months time. There was no histologic evidence of fungal infection. Seeded animals demonstrated varying degrees of fungal invasion. Rhizopus consistently demonstrated fungal infection and invasion whereas Aspergillus demonstrated only erratic fungal invasion in the animals. No animal exhibited fungal infection or invasion with either Cephalosporium or Fusarium. Histologically animals having invasive fungus disease demonstrated fungi in the burn wound, soft tissues of the back, liver, kidney and spleen. One or more of the above mentioned organs was found to be invaded in each of the inspected animals. The organ invasion was by direct continuity from the burn wound. There was no histologic evidence of hematogenous spread in any animals infected.

*Available to contractors upon originator's approval.

ANNUAL PROGRESS REPORT

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
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1 July 1972 - 30 June 1973

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Reports Control Symbol MEDDH-288(RL)

UNCLASSIFIED

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 1972 - 30 June 1973

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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 in this animal with 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.

Further studies are to be carried out using different fungi as well as other species of the previously tested fungi. Evaluation of the serologic tests useful in detecting systemic infection with Candida will be carried out.

Burns
Fungi

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 the modalities of treatment still 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. Hyperglycemia 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. Animals seeded with Aspergillus, Fusarium, and Cephalosporium exhibited no burn wound infection or visceral involvement at the time of sacrifice.

In conclusion Rhizopus consistently demonstrated the capacity to produce burn wound infection and direct visceral extension in the streptozotocin rat model. Further studies will be carried out with various other fungi in an attempt to evaluate their potential to produce burn wound invasion in a hyperglycemic animal model.

Candida burn wound colonization and infection is commonly noted in thermally injured soldiers, consequently evaluation of serologic tests used to detect systemic candida infection is planned.

REFERENCES

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 0A 6980	73 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
72 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	165
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede 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 73	.8	26
	CURRENT 74	.8	18

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

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Protein; (U) Burn; (U) Trauma; (U) Turnover; (U) Rats

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

23. (U) To 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. The intravascular/extravascular distribution of albumin is being measured by radioimmunoassay using plasmas and extracts of tissues obtained on the sixth day postburn.

25. (U) 72 07 - 73 06 Albumin determinations completed to this date show that the amount of extravascular albumin in the tissues of the injured animals is much greater than that of the control animals at a time when the size of the intravascular albumin pool is much smaller than that of the controls. Most of the increase in extravascular albumin can be accounted for by the increased albumin content of the burn eschar. The results obtained in this study will be coordinated with those obtained from the measurement of C-14 incorporation into serum proteins to aid in their interpretation.

* Available to contractors upon originator's approval

ANNUAL PROGRESS REPORT

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
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1 July 1972 - 30 June 1973

Investigators:

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Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

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 1972 - 30 June 1973

Investigators: Wanda L. Brown, MS
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Reports Control Symbol MEDDH-288(R1)

Measurement of plasma and tissue albumin pool sizes in control, burned, and burned-infected rats has shown that although the plasma albumin pool is reduced in the injured rats, the total body pool size is greater than that of the controls. Carcass and unburned skin albumin levels were not significantly reduced in the injured animals. The greatly increased albumin content of the burn wound eschar is sufficient to account for the increased size of the extravascular pool.

Protein
Burn
Trauma
Turnover
Rats

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

Earlier studies in which the incorporation of [2-^{14}C] glycine into serum proteins of rats was used as a measure of protein synthesis or turnover yielded presumptive evidence that decreased synthesis could not fully explain the marked dysproteinemia which occurs following burn injury (Brown WL, Bowler EG, Mason AD,Jr. USA Inst Surg Res Ann Res Rpt FY 1969)[1]. In order to insure that the observed increased relative specific activity was not due to the entry of the labeled proteins into a smaller protein pool, rather than to a true increase in synthetic rate, we have measured the albumin extracted from the tissues to obtain an estimate of the total body albumin pool. Preliminary results indicated that the total body depletion was not as severe as would be anticipated from the size of the plasma albumin pool (Brown WL, Bowler EG, Mason AD,Jr. USA Inst Surg Res Ann Rpt FY 1972)[2]. This report presents additional documentation of this conclusion.

MATERIALS AND METHODS

Male Sprague-Dawley rats weighing from 180-200 gm were anesthetized with sodium pentobarbital before burns were inflicted by immersing their backs in boiling water for 10 seconds (Group B). A protective mold was used to limit the area of the burn to 20% of the body surface. The burned-infected rats (Group B I) had the burned area immediately seeded with 0.1 ml of a 24-hour culture of Pseudomonas aeruginosa (SRU 12-4-4-(59). All rats were housed in individual cages and given food and water ad libitum until they were sacrificed on the fifth or sixth day postburn. Stock rats of equivalent size and age were used as controls (Group C).

Rat albumin was labeled with ^{125}I by an iodine monochloride procedure (McFarlane AS. J Clin Invest. 42:346, 1963)[3]. Free ^{125}I was removed by passing the solution through a small Amberlite IR-4B column. Albumin concentration of the eluate was determined from its absorbance at 279 nm (Peters T, Jr. J Biol Chem 237: 1181, 1962)[4] before 0.1 volume normal rabbit serum was added to protect the albumin from self radiation damage. The [^{125}I]-albumin migrated with the albumin band when mixed with normal rat serum and was retained in the gamma globulin area when crossover electrophoresis with rabbit anti-rat albumin was performed on cellulose acetate plate.

in triplicate and the samples were maintained at 0-4° C until the final sample was transferred to the counting tube. A solution containing 1 volume normal rabbit serum (NRS) and 9 volumes of 0.05 M borate buffer in 0.1 M NaCl, pH 8.5 (NRS-buffer) was used as diluent for all reagents and samples used in the test.

Samples of the tissue extracts or diluted plasma samples estimated to contain 5-15 ug albumin, and standards containing 4, 6, 8, 12, and 16 ug albumin were placed in 12 x 75 mm polystyrene test tubes and made to 0.2 ml volume with NRS-buffer. Hamilton syringes, equipped with hand operated repeating dispensers, were used to dispense first approximately 15,000 cpm in 0.05 ml of rat [^{125}I] albumin and finally, 0.05 ml antiserum (diluted to precipitate 3 ug albumin) into each tube. A normal serum control containing NRS instead of antiserum, and an antiserum control to which no unlabeled albumin was added were included with each run. After mixing, the tubes were capped and incubated overnight in the refrigerator. The next morning 0.5 ml of a solution prepared by mixing 64 ml of an aqueous solution which was saturated with ammonium sulfate at 4°C with 36 ml cold borate buffer was added to each tube. The contents were mixed and allowed to stand for 30 minutes before they were centrifuged for 30 minutes at 1600 g. Five-tenths ml of each supernate was transferred to counting tubes and the ^{125}I content was counted with a Packard two-channel Auto-Gamma counter which was set to discriminate between ^{125}I and ^{131}I pulses.

Calculations. Various transformations of the data were made in an attempt to extend the linear portion of the standard curve. The best fit was achieved using a logit transformation (Rodbard D, Bridson W, Rayford PL. J Lab Clin Med. 74: 770, 1969)[7], and deriving separate regression equations for the lower and upper half of the curve. These calculations were programmed on magnetic cards for a Hewlett Packard 9810A calculator in such a way that the regression equations for the standard curve were first derived and stored. Upon entry of the number of ^{125}I cpm in the test supernate, the choice of the proper regression equation and the corrections for the control values were made automatically, and the mean albumin content of the tube was printed out. In subsequent steps, entry of the ^{131}I cpm per ml extract, ^{131}I cpm per ml plasma, ug albumin per ml plasma, and gm tissue per ml extract

yielded a value for the ug albumin per gm tissue corrected for residual plasma albumin in the extracts. The final conversions to whole body albumin content and mg albumin per 100 gm rat weight were performed manually.

Additional Procedures. As confirmation that the albumin molecules in the tissue extracts which was measured in the RIA system were essentially intact rather than immunoreactive fragments, some of the extracts were ultrafiltered by centrifugation using Amicon Centriflo™ CF50A membrane ultrafilters which are rated to retain molecules above 50,000 molecular weight. The ultrafiltrates were removed and saved. The material retained on the filters was washed once with 0.15 M NaCl, recentrifuged, and finally was concentrated in Amicon Minicon™ B Concentrators. These have a retention (cut-off) rating of 15,000 molecular weight.

The proteins in the concentrated extracts were separated by electrophoresis in Tris-barbital buffer, pH 8.8 on cellulose acetate plate. Normal rat serum was run on each plate as a control. Duplicate plates were prepared and after the electrophoretic separation was completed, one plate was stained for protein visualization and antiserum was applied to the other for immunodiffusion. When the anti-rat albumin antiserum which was used for RIA was used for the immunodiffusion only one precipitin band appeared with the extracts and the control serum. When anti-rat IgG antiserum was applied a strong precipitin band appeared in both the extracts and control serum also. No attempt was made to quantitate the reaction.

Aliquots of the ultrafiltrates of the extracts were added to the standard RIA system and there was essentially no interaction.

RESULTS

The albumin content of the various tissues from a small group of control and burned rats is shown in Table 1. The mean albumin content of the plasma of the burned rats is lower, while that of the carcass is essentially the same as that of the control rats. The total albumin of the skin of the burned rats is much higher than that of the controls. The percentage reduction of the albumin in the viscera of the burned rats is quite large but because of its small total value, it contributes little to the total albumin pool size.

When the albumin content of these same tissues is compared on a basis of mg albumin per gm wet tissue weight (Table 2) one can see that the elevated

Table 1. Mean Albumin Content of Plasma and Tissues of Control and Burned Rats* (mg albumin/100 gm rat wt)

Sample	Group		Ratio (B/C)
	Control (C)	Burned (B)	
Plasma	117.7	98.1	0.83
Viscera	27.2	17.9	0.66
Carcass**	117.0	113.6	0.97
Unburned skin of burned rat	-	56.9	-
Burn eschar	-	123.9	-
Total skin	88.8	180.7	2.04
Total tissue albumin	233.0	312.2	1.34
Total tissue plus plasma albumin	350.7	410.4	1.17

* Mean of 3 rats per group

** Muscle and bone

Table 2. Mg Albumin per Gram Tissue (wet weight) from Control and Burned Rats*

Sample	Group		Ratio (B/C)	% Total Water**
	Control (C)	Burned (B)		
Viscera	1.28	0.90	0.70	-
Carcass+	2.14	2.10	0.98	-
Unburned skin	4.88	4.73	0.97	62.5
Burn eschar	-	17.50	3.95	70.0
Total skin	4.88	9.59	1.97	-

* Mean of 3 rats each group

** Determined by drying to constant weight

\+ Carcass including muscle and bone

albumin content of the burn eschar is sufficient to account for almost all of the difference between the sizes of the extravascular albumin pools of the 2 groups. The skin from the unburned area of the burned rat has essentially the same albumin content as does the skin from the control animals. The total water content of the skin (determined by drying to constant weight) was 60-65% for unburned skin of both burned and control rats, and 70% for the burn eschar. This indicates that the mean albumin concentration in the tissue fluid in the burn eschar is a minimum of 3 times greater than in unburned skin.

Processing all of the different tissues of each rat separately required so much time that only a small number of animals could be processed during the 2-day interval which had been chosen as the appropriate time for study. Since the primary objective of this study was to compare the total albumin pools of the experimental groups, in the remaining experimental runs each blood-free eviscerated body was processed as a single sample. These results are shown in Table 3.

The plasma albumin pool in the burned animals was reduced to about 88% of normal, while that of the burned-infected animals had dropped to about 33%. This occurred in the presence of tissue albumin pools which were greater than normal in both groups. Plasma volumes were essentially the same for all groups at this time.

This model for burn-infection constitutes a very severe injury. About half of the rats in this group died before the fifth postburn day. The remaining animals had lost about one-third of their preburn body weight. During this period, animals in the burned group gained 5-10 gm and control rats 20-25 gm. The rapid weight loss experienced by the burned-infected rats may bias the interpretation of the results for this group since the values were calculated on the basis of body weight at the time of sacrifice.

DISCUSSION

The rapid movement of water, electrolytes, and protein into the burn wound immediately after burn injury has been attributed to simple passage of plasma out of the capillaries (Cope O, Graham JB, Moore FD, Ball MR. Ann Surg. 128: 1041, 1948)[8] and to increased extravascular osmolarity in the burned area resulting from thermal damage to the cells (Arturson G, Mellander S.

Table 3. Mean Albumin Content of Plasma and Blood-free Eviscerated Body* of Control, Burned, and Burned-infected Rats Determined on the Sixth Day Postburn

Group	Gm Wt Preburn	Wt at Time of Sacrifice	Mg Albumin/100 gm Rat Weight		
			Plasma	Carcass*	Total
Control (C) N = 9		191	125.7 (118-133)**	207.5 (200-215)	333.4 (319-347)
Burned (B) N = 14	186	193	108.2 (101-115)	296.0 (278-314)	404.2 (384-425)
Burned-infected (BI) N = 8	185	134	41.5 (31-53)	257.4 (229-286)	299.4 (270-329)

* Includes muscle, skin and bone

** 95% C.L.

Acta physiol Scand. 62: 457, 1964[9]; Leape LL. J Trauma 10: 488, 1970[10]).

The inability to restore the plasma albumin pool size to normal has been ascribed to decreased synthesis (Kukral JC, Meadows DC. Surg Forum 15: 43, 1964[11]; Rothschild MA, Oratz M, Schreiber SS. N Eng J Med. 286: 816, 1972[12]), increased catabolism and direct loss through the burn wound surface (Davies JWL. Clin Sci 23: 411, 1962[13]; Birke G, Liljedahl SO, Plantin LO, Reizenstein P. Acta chir Scand. 134: 27, 1967[14]; Nylen B, Wallenius G. Acta chir Scand. 122: 97, 1961[15]) and to sequestration of albumin in the burn wound area (Lynch JB, Bray JP, Lewis SR, Blocker TG. J Surg Res. 4: 226, 1964)[16].

When labeled albumin or gamma globulin is injected intravenously, after surgery or at various times during the postburn period, it continues to move from the plasma into the burn wound at an increased rate until the time when wound healing is almost complete (Lynch JP, Bray JP, Lewis SR, Blocker TG. J Surg Res. 4: 226, 1964[16]; Mouridsen HT. Acta chir Scand. 134: 417, 1968[17]). It has also been shown that the concentration of all protein fractions in lymph flowing from the burned area increases rapidly following burn and remains elevated for 7-14 days (Roberts JC, Courtice FC. Aust J exp Biol med Sci. 47: 421, 1969)[18].

Although we found normal albumin levels in the unburned skin and carcass of burned rats on the sixth postburn day, the albumin content of the burn wound area was still more than 3 times normal. This albumin was found by ultrafiltration and electrophoresis to be essentially intact, rather than small immunoreactive fragments. Since albumin continues to move from the plasma into the burn wound at accelerated rates and the albumin content of lymph flowing from the burn wound area is still elevated at this time, it appears that the burn wound albumin pool must be constantly exchanging with the plasma albumin pool as if a new "equilibrium" had been attained.

SUMMARY

Measurement of plasma and tissue albumin pool sizes in control, burned, and burned-infected rats has shown that although the plasma albumin pool is reduced in the injured rats, the total body pool size is greater than that of the controls. Carcass and unburned skin albumin levels were not significantly reduced in the injured animals. The greatly increased albumin content

of the burn wound eschar is sufficient to account for the increased size of the extravascular pool.

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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
DA OE 6971	73 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
	A. NEW	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 (Procede 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	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 73	.5	12
	CURRENT 74	.4	10

19. RESPONSIBLE DOD ORGANIZATION

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Daniel E McEuen, SP4

NAME: 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) 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 incidence of abnormal prothrombin times and the response to vitamin KI in the thermally injured soldier. In some instances this will also serve as an indication 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 or two consecutive days he will be treated with liberal doses of vitamin K and his prothrombin time remeasured.

25. (U) 72 10 - 73 06 Thirty-three patients have been studied for periods of time ranging between one day and five weeks. Seven patients have been treated with vitamin K. Two have shown marked improvement in their prothrombin activity. More patients need to be studied over longer periods of time to more fully attain the objective.

*Available to contractors upon originator's approval

ANNUAL PROGRESS REPORT

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

REPORT TITLE: VITAMIN K DEFICIENCY IN THE THERMALLY INJURED PATIENT

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

1 July 1972 - 30 June 1973

Investigators:

Willard A. Andes, MD, Major, MC
Dwight D. Mceuen, BS, SP4

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

REPORT TITLE: VITAMIN K DEFICIENCY IN THE THERMALLY INJURED PATIENT

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

Period covered in this report: 1 July 1972 - 30 June 1973

Investigators: Willard A. Andes, MD, Major, MC
Dwight D. McEuen, BS SP4

Reports Control Symbol MEDDH-288(RI)

Patients on an experimental vitamin K deficient diet or in an intensive care unit may suffer the onset of hypoprothrombinemia, manifested as a prolonged prothrombin time, within seven days to four months(Frick PG, Riedler G Brogli H.: J Appl Physiol 23: 387, 1967[1]; Ham JM: Med J Aust 2: 716, 1971[2]). Because the thermally injured soldier may be on a similarly inadequate diet or under other conditions favorable to the production of vitamin K deficiency, it seemed necessary to investigate the frequency with which vitamin K-responsive prolongation of the prothrombin time occurred. Weekly blood studies were drawn following thermal injury for four weeks whenever possible. Liberal doses of vitamin K_1 (0.25 mg/kg) were administered parenterally to those patients who suffered from an isolated defect in their prothrombin time. Less than 70% of normal activity as determined by standard dilution curves on at least two consecutive days was required for treatment. Forty-one patients have been studied since the inception of the protocol. Eight patients have fulfilled the criteria for treatment with vitamin K. Three patients have responded as evidenced by shortening of their prothrombin time to normal. Each of these three was on parenteral antibiotics. Two patients were being given intravenous glucose as the sole source of calories. Occurrence of vitamin K deficiency is a preventable consequence of thermal injury. However, the significance of its presence, casual factors, and incidence are not defined at this time.

Thermal injury
Vitamin K
Prothrombin time

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]). Some of these patients develop vitamin K deficiency as evidenced by an abnormal prothrombin time which is corrected by administration of the vitamin. Chronically disabled, elderly patients may also 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 sources of the vitamin are dietary vegetables although synthetic menadiones are available.

In this continuing study we are attempting 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 have been drawn as seemed clinically indicated but at least weekly for four weeks following thermal injury. Specimens were drawn, mixed with anticoagulant, and immediately chilled on ice. Coagulation studies were then performed by methods used previously in this lab within one hour. 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 for the individual patient.

Forty-one patients have been studied to date. There have been 11 survivors. Eight patients have fulfilled the criteria for treatment. Three patients responded to the 0.25 mg/kg dose of vitamin K administered intravenously. The mean burn size of the survivors was 38% and of the nonsurvivors 62%. Of those treated, the mean burn size was 57% and the responsive patients had a mean burn size of 46%. All of the treated patients in whom studies were available had at least one abnormal liver enzyme test, and several were jaundiced. The three patients who responded to vitamin K administration were between 11 and 188 days post burn. Two of the three had been receiving almost continuous intravenous dextrose and intravenous antibiotics. The third was poorly nourished and had been receiving multiple antibiotics for five months because of skin infections. Two patients with active bleeding had clinical improvement with correction of their prothrombin time. Too few patients have been

studied for adequate periods of time to allow any conclusion as to the incidence of vitamin K deficiency. Persistent prolongation of the prothrombin time may be more prevalant than previously suspected, especially when one considers patients with extensive burns with apparent liver dysfunction. Antibiotic therapy and inadequate diet do seem to be important in the group of patients who will respond to Vitamin K.

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PRESENTATIONS AND/OR PRESENTATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0D 6953	73 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
72 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	307
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) The Metabolic State of the Red Cell in Burned Soldiers and in a Laboratory Model (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
71 05	73 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 73	.6	19
FISCAL YEAR CURRENT 74	0	0

19. RESPONSIBLE DOD ORGANIZATION
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SOCIAL SECURITY ACCOUNT NUMBER:
ASSOCIATE INVESTIGATORS
NAME: Jerl P Blackwell, BS
NAME: Douglas W Wilmore, MAJ, MC DA

21. GENERAL USE: FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code): (U) Membrane Adenosine Triphosphatase; (U) Hexokinase; (U) 2,3-Diphosphoglyceric Acid; (U) Glycolysis; (U) Rats; (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 general metabolic state of erythrocytes from burned soldiers will be evaluated in an effort to account for elevated sodium concentrations associated with these cells.

24. (U) Subjects include patients with a variety of burn size. Intracellular sodium and potassium flux of sodium-22, membrane adenosine triphosphatase, and red cell hexokinase were determined in these patients.

25. (U) 72 07 - 73 06 Various aspects of erythrocyte sodium movement have been measured in 48 patients with extensive thermal injury. Intracellular sodium and potassium concentrations were determined and found to be identical to those observed in a group of healthy individuals of comparable age. Flux studies, using sodium-22, revealed no differences between burn patients and controls in the ouabain-sensitive transport of sodium from the cells; only a slight decrease in ouabain-insensitive exit was noted among burn patients. Furthermore, there was no statistically significant difference in sodium influx for the two groups. Consistent with these results were essentially normal values for red cell membrane adenosine triphosphatase activities, including Km and Vmax. Burn patients, however, did demonstrate nearly a two-fold increase in red cell hexokinase activity, and the implications of this finding are discussed with respect to red cell glucose utilization and membrane cation permeability.

*Available to contractors upon originator's approval

FINAL REPORT

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

REPORT TITLE: ERYTHROCYTE SODIUM TRANSPORT AND MEMBRANE ADENOSINE TRIPHOSPHATASE IN PATIENTS WITH THERMAL INJURY

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

1 July 1972 - 30 June 1973

Investigators:

George M. Helmkamp, Jr, Captain, MSC
Jerl P. Blackwell, BS
Douglas W. Wilmore, MD, Major, MC

Reports Control Symbol MEDDH-288(RI)

UNCLASSIFIED

ABSTRACT

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

REPORT TITLE: ERYTHROCYTE SODIUM TRANSPORT AND MEMBRANE ADENOSINE TRIPHOSPHATASE IN PATIENTS 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 1972 - 30 June 1973

Investigators: George M. Helmkamp, Jr., Captain, MSC
Jerl P. Blackwell, BS
Douglas W. Wilmore, MD, Major, MC

Reports Control Symbol MEDDH-288(1)

Various aspects of erythrocyte sodium movement have been measured in 48 patients with extensive thermal injury. Intracellular sodium and potassium concentrations were determined and found to be identical to those observed in a group of healthy individuals of comparable age. Flux studies, using ^{22}Na, revealed no differences between burn patients and controls in the ouabain-sensitive transport of sodium from the cell; only a slight decrease in the ouabain-insensitive exit was noted among burn patients. Furthermore, there was no statistically significant difference in sodium influx for the two groups. Consistent with these results were essentially normal values for red cell membrane adenosine triphosphatase activities, including K_m and V_{max}. Burn patients, however, did demonstrate nearly a two-fold increase in red cell hexokinase activity, and the implications of this finding are discussed with respect to red cell glucose utilization and membrane cation permeability.

Membrane adenosine triphosphatase
Hexokinase
2,3-Diphosphoglyceric acid
Glycolysis
Rats
Humans

ERYTHROCYTE SODIUM TRANSPORT AND MEMBRANE ADENOSINE TRIPHOSPHATASE IN PATIENTS WITH THERMAL INJURY

Following thermal trauma, significant abnormalities in the intracellular sodium concentration of red blood cells have been described (Lantsberg LA, Fed. Proc. 23 (1964) T515[1]; Proctor HJ, Smith EKM, Cole C, Welt LG, Surg. Forum 18 (1967) 69[2]; Curreri PW, Wilmore DW, Mason AD,Jr, Newsome TW, Asch MJ. J Trauma 11 (1971) 390[3]). In general, during the first postinjury month, burn patients demonstrate an elevation in red cell sodium, the magnitude of which is 40-60% above normal; at the same time, intracellular potassium, red cell volume, and plasma electrolytes are essentially normal. This cation imbalance could result from several factors, including a defective active transport system, an altered passive permeability barrier, or changes in the intracellular energy-generating process. We have investigated sodium transport, membrane adenosine triphosphatase activities, and certain aspects of glycolysis in red blood cells from a large population of burn patients. The present studies were designed to allow one to distinguish among these possibilities and, in turn, to provide an explanation for these observations.

METHODS

Red Cell Cation Content: Approximately 10 ml of whole blood was drawn into heparinized plastic syringes and chilled immediately in ice. Following the procedure of Smith and Samuel (Smith EKM, Samuel PD, Clin. Sci. 38 (1970) 49)[4], the blood was centrifuged at 15,000 xg for 60-75 seconds, using a Sorvall RC-2B instrument refrigerated at 4° C. After removing the plasma and buffy coat, the red cells were washed three times in 290 mOsM $MgCl_2$ containing 25 ml/l of isotonic glycylglycine-$MgCO_3$ buffer at pH 7.4. The cells were then suspended in an equal volume of buffer and the exact hematocrit determined. Sodium and potassium analyses were performed with an Instrumentation Laboratories model 143 flame photometer, with the results expressed as mmoles per liter of packed red cells.

Sodium Influx and Efflux: Without modification, the technique of Smith and Samuel (Smith EKM, Samuel PD, Clin. Sci. 38 (1970) 49)[4] was used for the measurement of rate constants for sodium influx and efflux.

^{22}NaCl was obtained from New England Nuclear Corporation, and radioactivity was determined in a Packard model 3002 autogamma spectrometer. When possible red cells from a patient and a control were examined on the same day. Efflux measurements were made by exposing red cells for 3 hours at 37° C to a solution containing isomotic sodium phosphate, pH 7.4, glucose (2.5 mg/ml), and 2.5 uCi of ^{22}NaCl; the loaded cells were then washed six times in 290 mOsM $MgCl_2$ and resuspended at a hematocrit of 0.2 to 0.3% in the following medium: 140 mM; KCl, 5 mM; sodium phosphate buffer, 1.4 mM, pH 7.4; glycylglcyine (27 mM)-$MgCO_3$ (5.3 mM) buffer, pH 7.4; and glucose 1.5 mg/ml. Aliquots were taken periodically over the course of an hour and analyzed for radioactivity and extent of hemolysis in the cell-free incubation medium. Influx rates were measured by analysis of washed cells which had been incubated at 37° C for 30 minutes in the above medium, to which 1.25 uCi of ^{22}NaCl had been added. Aqueous saponin was used to achieve complete hemolysis prior to radioactivity determinations. All flux measurements were performed in duplicate. The rate constants express the fraction of radioactive sodium which crosses the cell membrane per hour. Influx was corrected for the sodium pumped out of the cell during the experiment (Sachs JR, Conrad ME, Am J Physiol. 215 (1968) 795)[5], while efflux was corrected for the extent of hemolysis, generally less than 2% (Sachs JR, Welt LG, J. Clin. Invest. 46 (1967) 65)[6]. Total sodium movement, in mmoles $l^{-1}h^{-1}$, represents the product of the rate constant and the initial sodium concentration. Ouabain (10^{-4}M) was added to inhibit the active component of sodium efflux.

Adenosire Triphosphatase Activity: Red cell membranes were prepared from heparinized blood by washing in 310 mOsM Tris-HCl, pH 7.5, and by lysing in 15 mOsM Tris-HCl, pH 7.5, as described by Rosenberg and Guidotti (Rosenberg SA, Guidotti G, J. Biol. Chem. 243 (1968) 1985).[7]. Stromal protein was determined by the biuret method, using sodium deoxycholate and hydrogen peroxide to facilitate the measurements (Yonetani T, J. Biol. Chem. 236 (1961) 1680)[8]. Basically, the procedure of Mircevova and Simonova (Mircevova L, Simonova A, Coll. Czech. Chem. Coomun. 31 (1966) 4145)[9] was followed for ATPase activity. The composition of the incubation medium was 250 mM Tris-HCl,

75 mM NaCl, 12.5 mM KCl, 3 mM $MgCl_2$, 2.5 mM ATP, and approximately 1-2 mg of membrane protein, all at pH 7.4 and 37° C. Ouabain (5×10^{-5} M) was added to distinguish between Na+ , K+-dependent (Na+, K+-ATPase) and Na+, K+-independent (Mg^{2+}-ATPase) enzyme activities. The release of inorganic phosphate was estimated according to Fiske and SubbaRow (Fiske CH, SubbaRow Y, J. Biol. Chem. 66 (1925) 375)[10] or more readily by the automated method of Van Belle (Van Belle H, Anal. Biochem. 33 (1970) 132)[11].

Hexokinase Activity: Lysates of saline-washed red cells were prepared according to Brewer, et al (Brewer GJ, Powell RD, Swanson SH, Alving AS, J. Lab. Clin. Med. 64 (1964) 601)[12]. Stroma-free supernatants were dialyzed overnight against 0.067 M potassium phosphate, pH 6.3, containing 1 mM disodium EDTA. Hemoglobin was determined after conversion to cyanmethemoglobin. Enzyme assays were performed at pH 7.2 and 25° C in a temperature-regulated Gilford model 240 recording spectrophotometer. The phosphorylation of glucose was followed at 340 nm in a medium containing 33 mM Tris-HCl, 10 mM $MgCl_2$, 6.7 mM ATP, 3.3 mM NADP, 6.7 mM glucose, 0.033 units/ml of glucose-6-phosphate dehydrogenase (Baker's yeast), and 1.67 mg/ml of lysate hemoglobin. As discussed by Brewer, et al (Brewer GJ, Powell RD, Swanson SH, Alving AS, J. Lab. Clin. Med. 64 (1964) 601)[12], there is sufficient endogenous 6-phosphogluconate dehydrogenase present in the lysate so that two moles of NADP will be reduced for each mole of glucose phosphorylated.

RESULTS

Intracellular sodium concentrations were determined in a group of 34 burn patients, whose average age was 23 and mean burn size was 47.8% (24.5% third degree). With three exceptions, all patients were sampled between postburn days 2 and 30. Similar measurements were made in a group of 15 control subjects. As seen in Table 1, the mean sodium concentration was 6.91 ± 0.25 (SEM) mmole/l for burn patients and 6.94 ± 0.28 mmole/l for controls. For both groups the range of observed values was comparable. Similarly, the mean and range of intracellular potassium concentration were nearly identical in the two groups. Thus, in the current

Table 1. Sodium and Potassium Content of Red Cells from Burn Patients and Controls

	Burn Patients	Controls
Subjects	34	15
Sodium	6.91 ± 0.25[1]	6.94 ± 0.28
mmoles/1 of red cells	4.12 - 9.63[2]	4.79 - 8.99
Potassium	82.2 ± 1.7	82.2 ± 2.3
mmoles/1 of red cells	69.4 - 100.7	65.3 - 95.8

[1] mean ± SEM

[2] range of values

population of burn patients and using the method described, there is no evidence of abnormal red cell cation levels.

We nevertheless continued our investigation of sodium transport with a detailed analysis of influx and efflux parameters in 10 burn patients (average age, 22; mean burn size, 51.2%; full-thickness injury, 22.2%; mean day postburn, 20) and nine controls. The results are summarized in Table 2. The total efflux rate constant was identical for the two groups, having a value of 0.376 h^{-1}. However, when this was dissociated into its active (i.e., ouabain-sensitive) and residual components, small differences were noted. Burn patients demonstrated a slightly higher rate constant for ouabain-sensitive sodium efflux, 0.274 h^{-1} as compared to 0.254 h^{-1} for controls; however, this difference was statistically insignificant. On the other hand, the somewhat reduced residual rate constant for the burn patients, 0.102 h^{-1}, was moderately significantly different ($p < 0.05$) from that for the control subjects, 0.122 h^{-1}.

Simultaneous measurements of sodium influx were carried out on these same individuals. What appears to be a marked increase in the mean influx rate constant for burn patients, 0.0224 h^{-1}, when compared with that for controls, 0.0166 h^{-1}, is, in fact, not significant. Among the burn patients were two with unusually high values, namely 0.0388 and 0.0488; the mean rate constant for the remaining eight patients was 0.0170 h^{-1}.

The information regarding influx and efflux rate constants allows a comparison of total sodium influx and efflux. For the control subjects total sodium influx was 2.37 mmoles $l^{-1}h^{-1}$, while total sodium efflux was 2.47 mmoles $l^{-1}h^{-1}$ (Table 2), thereby confirming a steady state in sodium movement across the red cell membrane. For the burn patients, however, there is an apparent deviation from the steady state, but as noted above this is attributable to those two patients whose influx rate constants were 2 and 3 times normal. The other burn patients showed a corresponding balance between sodium influx, 2.43 mmoles $l^{-1}h^{-1}$, and sodium efflux, 2.26 mmoles $l^{-1}h^{-1}$.

Participation of a membrane-bound adenosine triphosphatase activity in the transport of sodium and potassium across the red cell membrane has

Table 2. Rate Constants and Total Flux for Sodium Movements in Red Cells from Burn Patients and Controls

	Burn Patients	Controls	Significance
Subjects	10	9	
Active Efflux Rate Constant[1]	0.274 ± 0.013[2]	0.254 ± 0.029	N.S.
Residual Efflux Rate Constant	0.102 ± 0.003	0.122 ± 0.007	$p < 0.05$
Total Efflux Rate Constant	0.376 ± 0.019	0.376 ± 0.025	N.S.
Total Sodium Efflux[3]	2.26 ± 0.16	2.47 ± 0.24	N.S.
Influx Rate Constant[4]	0.0224 ± 0.0028	0.0166 ± 0.0011	N.S.
Sodium Influx[5]	3.20 ± 0.51	2.37 ± 0.20	N.S.

[1] Rate constant (efflux) expressed as fraction of intracellular sodium extruded per hour (h^{-1})

[2] Mean ± SEM

[3] Intracellular sodium concentrations were 5.97 mM for burn patients and 6.55 mM for controls; efflux expressed as mmoles $l^{-1}h^{-1}$

[4] Rate constant (influx) expressed as fraction of extracellular sodium entering cell per hour (h^{-1})

[5] Extracellular sodium concentration was 143 mM; influx expressed as mmoles $l^{-1}h^{-1}$

been demonstrated by Post, et al (Post RL, Merritt CR, Kinsolving CR, Albright CD, J. Biol. Chem. 235 (1960) 1796)[13]. Both processes, for example, require sodium and potassium in concert, exhibit high specificity toward ATP, have similar kinetic parameters, and are inhibited by ouabain, a cardiac glycoside. Based on our findings of no significant differences between burn patients and controls regarding sodium influx and efflux, we sought confirmation from the various membrane ATPase activities. The results are presented in Table 3. The group of 14 burn patients had a mean Na+, K+-ATPase activity of 0.054 umole $h^{-1}mg^{-1}$, while the control group's value was 0.049 umole $h^{-1}mg^{-1}$. There were also small, but insignificant differences in the Mg^{2+}-ATPase activities. Therefore, there is excellent correlation between the sodium transport data and these membrane enzyme measurements. Interestingly, in both cases the sodium- and potassium-dependent activities of the burn patients were 8-10% higher than the control individuals. The values reported in Table 3 are comparable to those reported by others using intact stroma preparations (Post RL, Merritt CR, Kinsolving CR, Albright CD, J. Biol. Chem. 235 (1960) 1796[13]; Palek J, Brabec V, Vopatova M, Michalec C, Mircevova L, Clin. Chim. Acta 23 (1969) 133[14]).

We examined further the kinetic parameters of the membrane ATPase activities. Upon variation of substrate concentration, in this case ATP, and measurement of subsequent enzyme activity, one may establish the maximum velocity, V_{max}, and Michaelis constant, K_m, for the particular enzyme. The latter term is often equivalent to the binding constant of the enzyme for its substrate. When membrane ATPase of red cells from burn patients and controls is subjected to different concentrations of Mg^{2+}-ATP, linear double reciprocal plots result; that is, there is a direct relationship between $(\text{substrate})^{-1}$ and $(\text{activity})^{-1}$. This finding conforms to a hyperbolic relationship between the two parameters, as well as to the absence of substrate inhibition or enzyme subunit cooperativity. In general, thermal injury led to increases in V_{max} for all ATPase activities, while K_m values for ATP were unaffected (Table 4). The observed normal values of K_m(ATP) and V_{max} for total ATPase are comparable to those reported by Oski, et al (Oski FA, Naiman JL, Blum SF, Zarkowsky HS, Whaun J, Shohet SB, Green A, Nathan DG, New

Table 3. Red Cell Membrane Adenosine Triphosphatase Activities for Burn Patients and Controls

	Burn Patients	Controls
Subjects	14	10
Na^+, K^+-ATPase[1]	0.054 ± 0.006[2]	0.049 ± 0.005
Mg^{2}-ATPase	0.087 ± 0.009	0.099 ± 0.011
Total ATPase	0.141 ± 0.014	0.148 ± 0.013

[1] Activity expressed as micromoles of inorganic phosphate released per hour per milligram of membrane protein

[2] Mean ± SEM

Table 4. Kinetic Parameters of Adenosine Triphosphatase Activities for Burn Patients and Controls

	Activity	Burn Patients	Controls
Subjects		5	3
K_m (ATP) (mM)	Na+,K+-ATPase	ND[1]	ND
	Mg^2-ATPase	0.17[2]	0.16
	Total ATPase	0.28	0.27
V_{max} (μmoles $h^{-1}mg^{-1}$)	Na+,K+-ATPase	0.10	0.07
	Mg^2-ATPase	0.17	0.13
	Total ATPase	0.27	0.20

[1] ND, not determined

[2] Mean value

Eng. J. Med. 280 (1969) 909)[15]. for the Mg^{2+}-dependent activity, our K_m for ATP is identical to that calculated by Godin and Schrier (Godin DV, Schrier SL, Biochemistry 9 (1970) 4068)[16], but our V_{max} is lower by a factor of 3-4. This difference is most likely due to the fact that Godin and Schrier used sheared membranes (Godin DV, Schrier SL. Biochemistry 9 (1970) 4068)[16], while in the present work the membranes are essentially intact (Rosenberg SA, Guidotti G, J. Biol. Chem. 243 (1968) 1985)[7].

An additional area of investigation concerned the activity of red cell hexokinase in burn patients. Rapoport (Rapoport S, in P.N. Campbell, G.D. Greville (eds), Essays in Biochemistry, V.4, Academic Press, N.Y., 1968, p. 69)[17] has discussed the role of hexokinase in red cell glycolysis and concluded that this enzyme is primarily responsible for the overall regulation of glucose metabolism and, therefore, for the net production of such phosphorylated compounds as ATP and 2,3-diphosphoglycerate. As seen in Table 5, a group of 13 burn patients with an average injury of 46% total body surface showed a marked enhancement of hexokinase activity with a mean value of 23.9 umoles of glucose phosphorylated per hour per gram of hemoglobin. The activity of the control group was 13.7, in good agreement with the normal values of 13.5 and 12.9-17.4 reported, respectively, by Brewer, et al (Brewer GJ, Powell RD, Swanson SH, Alving AS, J. Lab. Clin. Med. 64 (1964) 601)[12] and Keitt (Keitt AS, J. Clin. Invest. 48 (1969) 1997)[18]. While not examined, this result suggests that burn patients should demonstrate a significant increase in the conversion of glucose to lactate.

DISCUSSION

The many facets of the current investigation describe a red cell population from burn patients not remarkably different from normal. With the exception of an enhanced hexokinase activity and the capacity, in terms of V_{max}, for increased membrane ATPase turnover, burn patients appear to have normal sodium and potassium transport mechanisms as expressed by sodium influx and efflux rate constants and membrane Na+, K+-ATPase activity. It was not an unexpected finding that the intracellular concentrations of sodium and potassium in these patients were likewise normal.

Table 5. Red Cell Hexokinase Activity for Burn Patients and Controls

	Number of Determinations	Activity[1]	Significance
Burn Patients	13	23.9 ± 3.4[2]	$0.02 < p < 0.05$
Controls	8	13.7 ± 0.6	

[1] Activity expressed as micromoles of glucose phosphorylated per hour per gram of hemoglobin

[2] Mean ± SEM

These results are in contrast to previous investigations of human red cells following thermal trauma. Lantsberg (Lantsberg LA, Fed. Proc. 23 (1964) T515)[1] and Curreri, et al (Curreri PW, Wilmore DW, Mason AD,Jr, Newsome TW, Asch MJ, J. Trauma 11 (1971) 390)[3] reported consistently elevated red cell sodium levels among patients with severe, that is greater than 35% total body surface, burn injury. On the other hand, Welt, et al (Proctor HJ, Smith EKM, Cole C, Welt LG, Surg. Forum 18 (1967) 69[2], Welt LG, Smith EKM, Dunn MJ, Czerwinski A, Proctor H, Cole C, Balfe JW, Gitelamn HJ, Trans. Assoc. Amer. Phys. 80 (1967) 217[19]) studied a group of 21 burn patients, characterized by at least 10% third degree injury, and found only four with red cell hypernatremia; moreover, those same four patients showed a 47% decrease in their ouabain-sensitive sodium efflux rate constant and a 57% decrease in their membrane Na+,K+-ATPase activity. In the current group of 48 burn patients (electrolyte and membrane ATPase measurements were performed on different groups), we have found no examples of abnormal sodium concentration or defective sodium transport. An increase in sodium influx, a passive diffusion phenomenon, was noted in two burn patients, yet their resting sodium concentrations were within the normal range. It should be pointed out that the observed intracellular electrolyte levels agree well with those reported recently by Smith (Smith EKM, Clin. Sci. 42 (1972)447)[20] and Frazer, et al (Frazer A, Secunda SK, Mendels J, Clin. Chim. Acta 36 (1972) 499)[21], although our values tend toward the low end of the normal range.

A trivial explanation may be sufficient to account for our apparently normal burn patients. This derives from the observation by Curreri, et al (Curreri PW, Wilmore DW, Mason AD, Jr, Newsome TW, Asch MJ, J.Trauma 11 (1971) 390)[3] that administration of high caloric diets to burn patients with elevated red cell sodium concentrations led to a rapid return to normal values. These investigators placed burn patients with an average spontaneous daily caloric intake of 1600 kilocalories on a combined oral and parenteral feeding regimen in which minimum intakes of 3000 kilocalories were maintained, and within 3 to 5 days abnormal sodium levels were abolished. This suggests,

as discussed by those authors, a direct relationship between intracellular sodium and nutritional support in the traumatized patient. At present the maintenance of a positive energy balance is a primary consideration to burn patients, especially those with more extensive injury. A careful review of the clinical charts of the current study patients indicated that at least 80% were receiving some form of hyperalimentation. Furthermore, those patients who did not receive additional calories had, in general, smaller (less than 30% total body surface) thermal trauma. It is most likely that, as result of hyperalimentation, the individuals studied in this investigation did not manifest the sodium imbalance and transport deficiency frequently observed in the past.

Our results lend further support to the hypothesis that one of the key factors in regulating red cell sodium concentration is the availability of metabolic energy. In this regard, the burn patients exhibit a nearly two-fold increase in hexokinase activity, the rate-limiting enzyme in red cell glycolysis (Rapoport S, in P.N. Campbell, G.D. Greville (eds), Essays in Biochemistry, V. 4, Academic Press, N.Y., 1968, p. 69)[17]. Thus these patients undoubtedly produce an adequate supply of ATP to drive the membrane sodium-potassium pump, and, in turn, maintain a low intracellular sodium level. Arturson recently observed that burn patients, including those with 40-50% full-thickness injuries, have normal and even supranormal concentrations of ATP in their red cells (Arturson G, Injury 1 (1970) 226)[22]. The increased hexokinase activity is probably not due to increased blood glucose which may result from intravenous infusion during hyperalimentation, since the glycolytic rate is independent of glucose concentration above 7 mM (Garby L, deVerdier CH, Scand. J. Haemat. 1 (1964) 150)[23]. Rather, the increased hexokinase activity is in part, a reflection of the presumably younger mean age of red cells in these burn patients. Brewer and Powell reported approximately 50% greater hexokinase activity in young normal red cells (Brewer GJ, Powell RD, Nature 199 (1963) 704)[24]. In the early postburn period, patients often develop severe hemolysis (Shen SC, Ham TH, Fleming EM, New Eng. J. Med. 229 (1943) 701)[25] and decreased red cell survival

(Davies JWL, Topley E, Clin. Sci. 15 (1956) 135)[26]; these events would necessarily dictate a younger erythrocyte population. Our measurements of hexokinase activity were made, on the average, on the twenty-fourth postburn day.

Abnormalities in cation transport and membrane ATPase, leading ultimately to increased red cell sodium, have been recently discussed by Parker and Welt (Parker JC, Welt LG, Arch. Intern. Med. 129 (1972) 320)[27]. Many disease states elicit these pathologies, among which are hemolytic anemia, hereditary spherocytosis, sickle cell disease, congenital defects of certain glycolytic enzymes, and the "sick cell" syndrome. The last named state, defined by Smith and Welt (Smith EKM, Welt LG, Arch. Intern. Med. 126 (1970) 827)[28], is characterized by a hypoactive sodium pump, decreased Na+, K+-ATPase, and sodium concentrations ranging from 12 to 35 mmoles per liter of red cells. Yet with proper therapeutic treatment all these abnormalities can be corrected. For example, uremic patients following hemodialysis displayed a marked increase in ouabain-sensitive sodium efflux (Welt LG, Smith EKM, Dunn MJ, Czerwinski A, Proctor H, Cole C, Balfe JW, Gitelamn HJ, Trans. Assoc. Amer. Phys. 80 (1967) 217)[19]. Hyperthyroid patients also develop elevated red cell sodium, yet when they become clinically euthyroid, the rate constant for sodium efflux rises to normal and the intracellular sodium concentration decreases (Smith EKM, Samuel PD, Clin Sci 38 (1970) 49)[4]. It is highly significant that hyperthyroidism has classically been characterized by accentuated metabolism (Rawson RW, Sonenberg M, Money WL, in G.G. Duncan (ed), Diseases of Metabolism. 5th ed., Saunders, Phila, 1964, p. 1159)[29]. The reversible nature of the "sick cell" syndrome can now be extended to burn patients. In only an indirect manner may we conclude that supranormal caloric feeding, commonly begun in the early postburn period, was responsible for our inability to demonstrate red cell cation and membrane transport abnormalities among burn patients. The previous observations of others may have arisen from populations of energy-depleted red cells, a not unlikely prospect in light of the hypermetabolic state associated with thermal trauma (Cope O, Nardi

GL, Quijano M, Rovit RL, Stanbury JB, Wight A, Ann. Surg. 137 (1953) 165[30]; Soroff HS, Pearson E, Arney GK, Artz CP, in C.P. Artz (ed), Research in Burns, Amer. Instit. Biol. Sci. Washington, 1962, p. 126[31]; Harrison HN, Moncrief JA, Duckett JW,Jr, Mason AD, Jr, Surgery 56 (1964) 2031[32]).

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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 6973	73 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. MOD'N HIST'N	9A. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
72 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	305
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) Erythrocyte and Plasma Phospholipids in a Military Population with Burn Injuries (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
71 12	73 06	DA	C. In-House

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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 73	.5	19
	CURRENT 74	0	0

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21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede each with Security Classification Code)
(U) Phospholipid; (U) Fatty acid; (U) Erythrocyte membrane; (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 objective of this project is to identify and quantitatively analyze the phospholipids and phospholipid fatty acids present in the erythrocyte membrane and plasma of injured soldiers and to examine the effect of intravenous fat therapy on recognized membrane lipid alterations.

24. (U) The erythrocytes from severely burned patients and appropriate controls were extracted in a manner to yield phospholipids. In turn, these phospholipids were separated by thin-layer chromatography and their component fatty acids analyzed by gas-liquid chromatography. Serum lipids were obtained from the same individuals, quantitated, and likewise analyzed for fatty acid content. Selected patients were monitored serially throughout their clinical course. The effects of intravenous lipid emulsion on these fatty acid patterns are considered.

25. (U) 72 07 - 73 06 Five of the 13 burn patients examined were found to exhibit marked decreases in polyunsaturated fatty acids in the total phospholipid fraction of the erythrocyte membrane. Simultaneously, their plasma lipid fatty acid compositions were near normal. Fat emulsion, Intralipid, was administered to two individuals with return of their fatty acid profile to normal. In the third patient, an oral diet rich in polyunsaturated fat was administered with correction of the polyunsaturated fatty acid deficiency. In the fourth and fifth patients, fat-free intravenous diets were administered, and, although weight gain and some wound healing were achieved, the fatty acid composition of the red cell membrane remained abnormal with marked deficiency of the polyunsaturated fatty acid series.

*Available to contractors upon originator's approval

FINAL REPORT

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

REPORT TITLE: PHOSPHOLIPID AND PHOSPHOLIPID FATTY ACID COMPOSITION OF HUMAN ERYTHROCYTES FOLLOWING SEVERE THERMAL TRAUMA

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

1 July 1972 - 30 June 1973

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ABSTRACT

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Phospholipid and phospholipid fatty acid patterns have been determined by thin-layer and gas-liquid chromatography in red blood cells from 14 adult males with extensive thermal trauma, ranging from 16 to 62% total body surface burn. Five healthy adult males in a comparable age range were included in the survey. The concentrations of phosphatidylethanolamine and phosphatidylserine decreased significantly in the red cell membranes of five burn patients. These same individuals had markedly reduced levels of linoleate (18:2n6, 6.1%) arachidonate (20:4n6, 4.1%), and docosahexaenoate (22:6n3, 0.4%), when compared with the nine other burn patients (18:2 = 9.1%; 20:4 = 14.0%; 22:6 =2.7%) and normals (18:2 = 9.5%; 20:4 = 17.2%; 22:6 = 3.2%). Simultaneously, plasma phospholipid, triglyceride, and cholesteryl ester fatty acid analyses demonstrated only minor deviations from normal values.

Essential fatty acid deficiency in the red cell membrane phospholipids may occur in patients with inadequate preburn nutrition or result from inadequate caloric or extended fat-free parenteral support of these hypermetabolic burn patients in whom turnover and utilization of fatty acids are accelerated following major thermal trauma. The red cell structural and functional abnormalities which accompany thermal injury are discussed in relation to these defects in membrane polyunsaturated fatty acids.

Phospholipid Fatty acid Erythrocyte membrane Humans

PHOSPHOLIPID AND PHOSPHOLIPID FATTY ACID COMPOSITION OF HUMAN ERYTHROCYTES FOLLOWING SEVERE THERMAL TRAUMA

Severe thermal injury alters the fragility, survival, cation concentration, and morphology of red blood cells. Of utmost clinical importance is increased osmotic red cell fragility, a condition which results in hemolysis, hemoglobinuria, and often prolonged anemia (Shen SC, Ham TH, Fleming EM. New Eng. J. Med. 229: 701, 1943[1]; Moore FD, Peacock WC, Blakely E, Cope O. Ann. Surg. 124: 811, 1946[2]). Davis and Alpen (Davis AK, Alpen EL. Am. J. Physiol. 184: 151, 1956)[3] demonstrated decreased survival times for erythrocytes from experimentally burned rats. Elevated intracellular sodium concentrations have been observed in several patient populations with major burns (Lantsberg LA. Fed. Proc. 23: T515, 1964[4]; Curreri PW, Wilmore DW, Mason AD, Jr, Newsome TW, Asch MJ, Pruitt BA, Jr. J.Trauma 11: 390, 1971[5]). Finally, heat damaged erythrocytes, in vivo and in vitro, are characterized by distinct morphological changes, including abnormal discoid and spherical shapes, subnormal cell diameter, and general coarseness of the membrane (Baar S, Arrowsmith DJ. J. Clin. Path. 23: 572, 1970)[6]. Taken together, the above phenomena suggest modifications of the red cell membrane in selected burn patients.

The erythrocyte membrane, whose integrity must be maintained for normal metabolism and survival of the red cell, is composed of approximately equal weight proportions of protein and lipid (Van Deenen LLM, deGier J. In The Red Blood Cell, C. Bishop and D.M. Surgenor (eds). Academic Press, N.Y. 1964, pp. 243-307)[7]. Phospholipids account for three-fifths of the total lipid, with the remainder being distributed between cholesterol (30%) and glycolipid (10%). The present study was undertaken to describe in detail the phospholipid and phospholipid fatty acid composition of red cells of patients with severe thermal injury.

Analyses of serum lipids of burned patients have been reported (Birke G, Carlson LA, Liljedahl SO. Acta Med. Scand. 178: 337, 1965[8]; Dolecek R. Metabolic Response of the Burned Organism, C C. Thomas, Ill., 1969, pp.

107-109[9]), in general, following extensive thermal injury there were initial short-term elevation of free fatty acids, a progressive decrease in the level of total cholesterol, normal or slightly subnormal concentrations of phospholipid, and essentially no change in the quantity of triglyceride. For comparison, we included in our survey the fatty acid compositions of these plasma lipid components.

METHODS

Blood was drawn from healthy male donors and randomly selected male burn patients into heparinized syringes and chilled immediately in ice. After centrifugation at 4° C, the plasma was removed, the buffy coat discarded, and the red cells washed three times with 0.9% NaCl. The cells were resuspended in saline, sampled for a precise hematocrit, and, together with the plasma, stored under nitrogen at -20°C until extraction.

Lipids were extracted from 3 ml of the red cell and plasma fractions by procedure 111 of Ways and Hanahan (Ways P, Hanahan DJ. J. Lipid Res. 5: 318, 1964)[10]. Highest purity, spectroscopic-grade solvents were used without further purification. To retard oxidation of polyolefinic acids, 2,6-di-tert-butyl-p-cresol (BHT)* was used at a concentration of 0.005% (w/v) in the solvents during isolation, storage and chromatography of the lipids.

Phospholipids were quantitatively separated from total red cell lipids by column chromatography on silicic acid (100-200 mesh)** (Ways P, Hanahan DJ. J. Lipid Res. 5: 318, 1964)[10]. After hydrolysis in 2N HCl at 110-120° C for 18-24 hours, fatty acids were extracted with pontane and converted to their methyl esters using boron trifluoridemethanol reagent (Metcalfe LD, Schmitz AA. Anal. Chem. 33: 363, 1963)[11].

Separation of individual phospholipids was achieved by thin-layer chromatography, using commercially prepared plates of silica gel H‡ and a

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** Mallinckrodt Chemical Works, St. Louis, Missouri.

‡ EM Laboratories, Inc., Elmsford, New York.

developing system of chloroform-methanol-acetic acid-water, 25:15:4:1 (v/v). Phosphorus determinations of the phospholipids by direct digestion of silica gel samples were performed as described by Skipski and Barclay (Skipski VP, Barclay M. In Methods in Enzymology. J.M. Lowenstein (ed) Academic Press, Inc., N.Y., vol. 14, pp. 530-598, 1969)[12].

Plasma lipids were chromatographed on 0.25-mm layers of silica gel G* with a development solvent of petroleum ether (b.p. 63-75° C)-diethyl ether-acetic acid, 90:10:1 (v/v).

Commercially available standards allowed identification of triglyceride, cholesteryl ester and various phospholipid regions. These lipids were then eluted from the plates (Skipski VP, Barclay M. In Methods in Enzymology. J.M. Lowenstein (ed) Academic Press, Inc., N.Y. vol. 14, pp. 530-598, 1969)[12] and subjected to acid hydrolysis and methylation. It was necessary to purify further by thin-layer chromatography the methyl esters derived from cholesteryl esters in order to eliminate substances which interfered with subsequent analysis.

Fatty acid methyl esters were finally subjected to gas-liquid chromatography, using a Varian 1800 instrument equipped with dual flame ionization detectors and an electronic integrator. Two paired-column systems were used: (1) 3% EGSS-X** on Gas Chrom Q, 100-120 mesh**, 1/8-inch by 12-foot stainless steel columns, operated between 100-200°C at 3°/min; and (2) 1.25% DEGS‡ on Chromosorb G (H.P.), 100-120 mesh‡‡, 2-mm by 12-foot glass columns, operated between 120-190°C at 4°/min. For both systems helium (18 ml/min) served as the carrier gas. In our experience the DEGS columns performed more satisfactorily. Identification of the various fatty acids was by comparison with commercially available standards, cod liver oil fatty acid methyl esters, and published chromatograms using the EGSS-X liquid phase

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‡ Regis Chemical Company, Chicago, Illinois

‡‡ Varian Aerograph, Walnut Creek, California

(Dodge JT, Phillips GB. J. Lipid Res. 8:667, 1967)[13]. The nomenclature for fatty acids is the following: arachidonic acid, 20:4n6, where 20 is the total number of carbon atoms, 4 is the number of methylene-bridged cis double bonds, and n6 is the position of the double bond nearest the terminal methyl group; in some cases a slightly abbreviated form is used, e.g. 20:4.

RESULTS

Erythrocyte membrane phospholipids. In Table 1 are shown the values for total lipid phosphorus and phospholipid distribution in red cell membrane preparations from normal and burn subjects. It was at once apparent that the burn patients belonged to one of two distinct categories - those with normal phospholipid patterns and those with marked phospholipid alterations. Eight burn patients (Group 1, mean age 33.8; mean burn size 41.1%, mean full-thickness injury 28.4%) had normal levels of membrane phosphorus and a normal distribution of phospholipids among the four major classes. On the other hand, five other burn patients (Group II, mean age 29.2, mean burn siz 45.6%, mean full-thickness injury 23.7%) displayed a marked reduction in lipid phosphorus. The effect of this change on the phospholipid distribution is even more striking. Among the individual phospholipids there were relative increases in phosphatidylcholine and sphingomyelin and a relative decrease in phosphatidylethanolamine. With the overall decrease in membrane phospholipid taken into consideration, however, these variations correspond to absolute reductions in phosphatidylethanolamine (38% of normal) and phosphatidylserine (64% of normal). On a weight basis, phosphatidylcholine and sphingomyelin maintained their normal levels.

Fatty acid composition of the erythrocyte phospholipids. In the analysis of fatty acids derived from the total phospholipid extracts (Table 2), there were only minimal differences between normal red cells and those sampled from the Group 1 burn patients. A slight reduction in arachidonate (20:4n6) was accompanied by similarly small increases in palmitoleate, oleate and nervonate (24:1n9). With these exceptions, all other fatty acids were present in normal concentrations.

The distribution of fatty acids from the erythrocytes of Group II burn

Table 1. Phospholipid Composition of Red Cells from Normal and Burned Patients

	Normal Subjects	Burn Patients Group 1	Burn Patients Group II
Lipid phosphorus	0.119 ± 0.008[a]	0.122 ± 0.004	0.094 ± 00.010
	n = 4[b]	n = 8	n = 5
Phospholipid distribution (weight per cent)	n = 3	n = 8	n = 5
Phosphatidylethanolamine	26.3 ± 1.3[a]	26.4 ± 1.1	13.2 ± 1.0
Phosphatidylserine	13.0 ± 2.5	14.5 ± 0.8	11.8 ± 1.6
Phosphatidylcholine	29.7 ± 2.2	30.2 ± 1.3	37.8 ± 2.2
Sphingomyelin	30.7 ± 1.4	28.4 ± 0.9	37.2 ± 2.6

[a] Mean ± SEM

[b] Number of subjects

Table 2. Fatty Acid Composition of Total Phospholipid from Red Cells of Normal and Burned Subjects

Patient No.	Sex	Age	Burn Size % 3°	Postburn Day of Analysis	Fatty Acid 16:0	16:1n7	18:0	18:1n9	18:2n6	20:4n6	22:6n3	24:0	24:1n9
					g/100 g								
Normals (5)					24.9	0.8	16.6	16.5	9.5	17.2	3.2	5.8	5.7
Mean ± SEM					±1.3	±0.3	±0.6	±0.4	±0.5	±0.8	±0.5	±0.6	±0.2
Group 1 Burn Patients (9)													
1	M	38	40/40	17	23.0	1.3	15.5	17.3	7.9	14.6	3.3	6.4	10.7
2	M	62	41.5/24.5	16	21.7	1.2	15.5	17.6	9.2	14.9	4.2	5.6	10.2
3	M	24	59/28	11	22.6	2.4	17.0	19.2	10.5	13.8	2.2	5.6	6.8
4	M	31	16.5/12.5	58	22.6	1.5	16.3	17.4	10.1	15.1	2.5	5.5	9.0
5	M	25	62/47	2	27.2	3.1	16.9	18.1	10.3	11.5	3.5	4.6	4.8
6	M	27	46/46	13	25.4	1.7	16.6	17.2	9.4	14.6	2.9	5.5	6.7
7	M	19	40/24	25	22.6	2.6	17.4	19.4	8.1	13.1	1.5	6 3	8.5
8	M	58	22.5/21.5	17	24.7	2.5	15.2	18.6	8.7	13.6	1.5	5.2	6.9
9	M	20	42.5/12.5	30	28.3	2.4	16.3	18.5	7.9	14.5	2.9	4.2	8.7
Mean ± SEM					24.2	2.1	16.3	18.1	9.1	14.0	2.7	5.4	8.0
					±0.8	± 0.2	±0.3	±0.3	±0.3	±0.4	±0.3	±0.2	± 0.6
Group 11 Burn Patients (5)													
10	M	40	60/8	17	31.9	2.3	15.6	21.6	6.1	5.5	0.5	7.2	9.3
11	M	19	33.5/33.5	195	29.8	1.8	17.6	21.7	6.1	5.7	0.1	7.6	9.7
12	M	23	55.5/53	96	36.3	0.3	16.7	20.3	5.3	3.7	0.9	8.2	8.3
13	M	41	30/24	2	32.0	2.2	17.8	20.2	6.0	2.2	-	10.3	9.2
14	M	23	49/-	16	24.9	0.8	18.6	23.3	6.8	3.6	0.7	11.4	10.0
Mean ± SEM					31.0	1.5	17.3	21.4	6.1	4.1	0.4	8.9	9.3
					± 1.8	±0.4	±0.5	±0.6	±0.6	±0.6	±0.2	±0.8	±0.3

patients, however, exhibited a pronounced trend toward decreases in all polyunsaturated species. The most dramatic changes included a 76% decrease in 20: 4n6 and an 88% decrease in 22: 6n3. Linoleate was also reduced, but only by 36%. Concurrently, there were substantial increases in 16: 0, 18: 1n9; 24: 0, and 24: 1n9. In these analyses no attempt was made to quantitate fatty aldehyde levels.

When the fatty acid data in Table 2 are converted from a weight distribution to a molar distribution and then grouped according to structural similarities (Table 3), the differences between normal and Group 1 burn subjects, on the one hand, and Group II burn patients, on the other, are more clearly defined. Specifically, the decreases in all members of the n6 and n3 series and the increases in all members of the n9 and saturated series lead to a marked shift in the ratio of saturated to unsaturated fatty acids present within the red cell membrane of phospholipid-deficient burn patients (Group II).

Fatty acid patterns of individual phospholipids. Following isolation of individual phospholipids by thin-layer chromatography, fatty acid distributions were determined within each class for 5 normal and 5 Group II burn patients (Table 4). The composition of sphingomyelin, with its high proportion of saturated and long-chain acids, was unaffected by thermal injury. Phosphatidylethanolamine and phosphatidylserine, however, displayed alterations in nearly all fatty acids quantitated and in the same manner observed in the combined phospholipid analysis. While oleate increased significantly in those two classes, the overall absolute decline in phosphatidylethanolamine and phosphatidylserine resulted in an apparently normal level of oleate in the total phospholipid extract. Of further interest is the finding that changes in linoleate were restricted to phosphatidylcholine, where it is the principal polyunsaturated fatty acid. Finally, of the two species of eicosatrienoate (20: 3) identified and analyzed, there were neither markedly reduced quantities of the n6 acid nor unusually elevated levels of the n9 isomer.

Plasma lipid fatty acids. To ascertain how widespread the fatty acid alterations were, we investigated certain plasma lipid classes. Two normal subjects and four Group II burn patients were selected, and it is evident from

Table 3. Molar Fatty Acid Distribution of Red Cell Phospholipids from Normal and Burned Subjects

	Normal Subjects	Burn Patients	
		Group 1	Group II
		moles/100 moles	
Saturated fatty acids			
Palmitate (16)	28.5	27.8	35.3
Stearate (18)	16.9	16.7	17.4
Lignocerate (24)	4.4	4.0	6.7
Total	49.8	48.5	59.4
Unsaturated fatty acids			
Oleate series (n9)			
Oleate (18)	16.8	18.6	21.6
Nervonate (24)	4.4	6.1	7.1
Total	21.2	24.7	28.7
Linoleate series (n6)			
Linoleate (18)	9.7	9.4	6.2
Arachidonate (20)	15.8	12.9	3.6
Total	25.5	22.3	9.8
Linolenate series (n3)			
Docosahexaenoate (22)	2.7	2.2	0.4
Total unsaturated, including			
Palmitoleate (16:1n7)	50.3	51.6	40.5
Ratio (saturated/unsaturated)	0.99	0.94	1.47

Table 4. Fatty Acid Distribution of the Major Phospholipid Classes of Red Cells from Normal and Burned Patients

Fatty Acid	Phosphatidylethanolamine		Phosphatidylserine		Phosphatidylcholine		Sphingomyelin	
	Normals[a]	Group II Burns[a]	Normals	Group II Burns	Normals	Group II Burns	Normals	Group II Burns
	g/100 g							
16:0	20.2±1.5[b]	25.6±2.0	6.5±1.4	10.4±2.0	31.8±1.1	36.7±1.6	29.4±3.7	27.9±2.9
16:1n7	0.7±0.5	1.5±0.6	0.4±0.2	0.8±0.4	1.3±0.6	2.4±0.5	-	-
18:0	9.7±0.6	15.2±1.2	39.9±3.0	46.4±2.7	13.6±1.4	13.3±1.0	9.7±0.6	10.2±0.5
18:1n9	21.7±0.6	31.4±2.4	11.6±1.1	19.2±1.8	22.1±1.9	28.4±2.0	2.9±0.5	2.8±0.8
18:2n6	6.6±0.2	7.1±0.8	5.5±3.1	4.1±0.9	18.0±0.9	12.3±0.9	0.3±0.2	1.6±1.5
20:0	ND[c]	ND	ND	ND	ND	ND	1.5±0.4	1.8±0.1
20:3n9	0.9±0.4	1.1±0.5	1.1±0.2	2.0±0.7	1.0±0.1	0.9±0.4	-	-
20:3n6	0.9±0.2	0.9±0.4	2.4±0.2	1.8±0.4	1.9±0.4	0.8±0.1	-	-
20:4n6	23.2±1.8	8.4±1.1	21.6±2.9	10.7±2.0	5.0±0.5	1.8±0.3	-	-
22:0	ND	ND	ND	ND	ND	ND	9.1±0.7	9.8±0.9
22:5n3	2.3±0.3	0.2±0.1	1.8±0.3	Trace	Trace	-	-	-
22:6n3	3.8±0.6	0.6±0.4	4.0±0.5	0.6±0.2	0.8±0.1	Trace	-	-
24:0	Trace	0.6±0.2	0.7±0.5	1.8±1.4	1.5±0.4	1.6±0.1	20.1±1.5	21.9±3.9
24:1n9	7.2±0.8	3.3±0.6	3.7±0.3	1.7±1.0	1.4±0.2	1.0±0.2	25.9±2.9	22.8±2.3

[a] Five subjects in each group

[b] Mean ± SEM

[c] ND: Not determined

Table 5 that there are no consistent differences between these groups in the fatty acid compositions of plasma phospholipids, triglycerides, and cholesteryl esters. For example, while two patients had reduced levels of plasma linoleate, the others had normal or somewhat elevated quantities. At the same time, however, minimal fluctuations in arachidonate and n3 unsaturated acids were noted. The appearance of 20: 3n9 was observed in the plasma phospholipid fractions of three of the four burn patients studied.

DISCUSSION

Analysis of the phospholipid and phospholipid fatty acid composition of erythrocytes from individuals with extensive thermal trauma has revealed marked differences between certain burn patients and normal subjects. The red cells of nine patients (Group I) appeared normal in all respects, and agreed closely with phospholipid and phospholipid fatty acid compositions reported by others (Ways P, Hanahan DJ. J. Lipid Res. 5: 318, 1964[10]; Dodge JT, Phillips GB. J. Lipid Res. 8: 667, 1967[13]). Yet, the erythrocyte membranes of five other burn patients with apparently equally severe injuries were characterized by reduced levels of phosphatidylethanolamine and phosphatidylserine, a general decline in membrane lipid phosphorus, and fatty acid patterns entirely consistent with essential fatty acid deficiency. That is to say, significant reductions in the acids of the linoleate series (18: 2n6 and 20: 4n6) and the linolenate series (22: 5n3 and 22: 6n3) were observed. These multiple polyunsaturated fatty acid alterations were reflected in three of the four major phospholipid classes, namely, phosphatidylethanolamine, phosphatidylserine, and phosphatidylcholine. Only sphingomyelin remained unchanged, both in absolute quantity within the erythrocyte membrane and in its fatty acid composition.

Those factors which predispose selected burn patients to these gross red cell lipid abnormalities remain obscure. While age and the extent of injury in this group of 14 individuals appear to have no bearing on these findings, a more meaningful criterion might possibly be the overall metabolic state of the patient. The hypercatabolic response that accompanies burn trauma has been well documented (Soroff HS, Pearson E, Arney GK, Artz CP. In Research in Burns. C.P. Artz (ed). Amer. Institute Biol. Sci. Washington, DC, 1962, pp. 126-136)[14], and the energy requirement of 3000 to 8000 kilo-

Table 5. Fatty Acid Composition of Major Plasma Lipids

Lipid Class	Fatty Acid	Normals	Group II Burns - Patient Nr. 10	11	12	13
			g/100 g			
Phospholipids						
	16:0	30.0	29.6	26.6	26.0	29.7
	16:1n7	0.6	2.5	1.9	0.4	1.4
	18:0	16.0	14.2	16.4	18.1	15.6
	18:1n9	12.3	16.9	16.5	9.7	15.4
	18:2n6	22.8	16.9	15.0	25.9	20.7
	20:3n9	-	1.8	4.2	1.1	-
	20:3n6	2.6	4.7	2.1	3.5	2.8
	20:4n6	9.6	9.5	8.2	9.5	8.2
	22:5n3	0.6	0.4	0.9	0.8	-
	22:6n3	2.0	1.5	2.1	1.7	2.2
	24:0	1.4	0.5	2.1	3.9	1.1
	24:1n9	2.2	2.8	3.9	1.9	2.8
Triglycerides						
	14:0	1.2	1.2	2.1	4.4	0.6
	16:0	25.1	27.5	27.8	17.4	25.1
	16:1n7	5.2	6.3	6.0	4.2	3.8
	18:0	4.9	5.0	5.2	4.0	1.9
	18:1n9	40.6	50.4	47.2	26.4	50.2
	18:2n6	22.8	7.5	8.6	32.8	13.7
	18:3[a]	-	-	-	1.8	-
	20:4n6	1.2	1.2	2.1	1.8	4.6
Cholesteryl Esters						
	16:0	10.4	13.4	15.1	7.1	8.1
	16:1n7	3.6	12.1	8.2	2.3	3.8
	18:0	2.9	2.6	6.6	5.8	0.3
	18:1n9	19.5	30.6	23.9	12.5	22.8
	18:2n6	53.9	34.8	36.6	59.7	56.2
	18:3[a]	-	-	3.2	5.0	0.9
	20:4n6	9.6	6.5	6.3	7.6	8.0

[a] Tentative identification

calories per day can usually be met by combined oral and parenteral feeding (Wilmore DW, Curreri PW, Spitzer KW, Spitzer ME, Pruitt BA, Jr.Surg. Gynec. Obstet. 132:881, 1971)[15]. If, however, for some reason a functional energy deficit persists, the individual will no doubt begin to draw from his own body's store of metabolic energy. Fat deposits are a primary source of such calories, and in the absence of adequate replacement, more rapid mobilization and utilization would necessarily deplete the essential fatty acids. On the other hand, one may argue that the essential fatty acids somehow become unavailable to systems with high cell turnover. It is interesting to speculate that in our patients with major red cell lipid variations, these changes may represent a specific example of widespread essential fat deficiency. Indeed, Farquhar and Ahrens (Farquhar JW, Ahrens EH, Jr. J. Clin. Invest. 42:675, 1963)[16] reported equilibration between dietary fat and erythrocyte fatty acids in normal subjects within 4 to 6 weeks, and Hill, et al (Hill JG, Kuksis A, Beveridge JMR. J. Amer. Oil Chem. Soc. 42:137, 1965)[17] noted significant changes in the fatty acid composition of phosphatidylcholine and phosphatidylserine fractions of red cell lipid after only 16 days of controlled fat feeding. The elevated metabolic rate of burn patients and the absence of fat in the diet would insure an even more dramatic reflection by red cells of total body fatty acid composition. Consistent with this hypothesis is our finding that essential fat deficient burn patients who receive intravenous infusions of a soybean lipid emulsion rich in polyunsaturated fatty acids rapidly exhibit normal red cell lipid patterns.

Plasma lipid fractions fail to undergo as pronounced a change in fatty acid composition as do the red cell phospholipids. This may reflect different turnover rates for these various species, as well as variations in the incorporation of plasma lipids into many diverse lipoprotein classes. Reed (Reed CF. J. Clin. Invest. 47:749, 1968)[18] has argued that the exchange of phospholipids between plasma and red cells is confined primarily to phosphatidylcholine. It is highly significant that the essential fat deficient burn patients have normal levels of this lipid in their red cell membranes; furthermore, the quantity of polyunsaturated fatty acid in phosphatidylcholine is

considerably lower than in both phosphatidylethanolamine and phosphatidylserine. In addition, none of the patients studied in this survey had skin lesions so often characteristic of essential fat deprivation in infants (Hansen AE, Wiese HF, Boelsche AN, Haggard ME, Adam DJD, Davis H. Pediatrics 31: 171, 1963)[19] and some adults (Collins FD, Sinclair AJ, Royle JP, Coats DA, Maynard AT, Leonard RF. Nutr. Metab. 13: 150, 1971)[20]. Nevertheless, the observation of significant levels of 20: 3n9 in certain plasma lipids is consistent with the classic description of essential fatty acid deficiency (Hansen AE, Wiese HF, Boelsche AN, Haggard ME, Adam DJD, Davis H. Pediatrics 31: 171, 1963)[19].

Our observations of altered phospholipid and fatty acid composition of the red cell membrane are similar to those described for a variety of other erythrocyte disorders. Neerhout (Neerhout RC. Clin. Pediat. 7: 451, 1968)[21] in reviewing abnormalities of the red cell, observed that reduced linoleate levels were associated with the following diseases: hereditary spherocytosis, elliptocytosis, certain acquired hemolytic anemias, and acanthocytosis. It has been stressed, however, that both extracellular factors, such as the lipid malabsorption aspect of acanthocytosis, and intracellular factors, such as defective transfer of fatty acids between phosphatidylcholine and phosphatidlyet' anolamine in a particular hemolytic anemia, contribute to these general lipid imbalances (Shohet S.New Eng. J. Med. 286: 577 -583; 638-644, 1972)[22].

Acanthocytosis manifests itself with moderate decreases in red cell membrane lipid phosphorus, slight increases in sphingomyelin and phosphatidylserine, and a marked reduction in phosphatidylcholine (Ways P, Reed CF, Hanahan DJ. J. Clin. Invest. 42: 1248, 1963[23]; Phillips GB, Dodge JT. J. Lab. Clin. Med. 71: 629, 1968[24]). The overall fatty acid composition is described as one of essential fatty acid deficiency, compensated by increases in certain mono- and polyunsaturated fatty acids of the n9 series. In contrast to our findings of decreased levels of linoleate, arachidonate and docosahexaenoate in burn patients, essential fat reduction in acanthocytic red cells is restricted to linoleate; indeed, the quantity of arachidonate is somewhat elevated (Phillips GB, Dodge JT. J. Lab. Clin. Med. 71: 629, 1968)[24] Most

probably, this distribution reflects the altered phospholipid composition described above. On the other hand, hereditary spherocytic cells have a normal phospholipid composition with moderately reduced levels of membrane linoleate but, as in acathocytosis, the level of arachidonate remains undiminished (DeGier J, van Deenen LLM, Verloop MC, van Gastel C.Brit. J. Haemat. 10: 246, 1964)[25]. The fatty acid compositions of hereditary spherocyte phospholipids have recently been confirmed; analysis for fatty acids containing 22 and 24 carbons indicated a virtual absence of those species, both saturated and unsaturated, in all phospholipid classes except phosphatidylethanolamine (Kuiper PJC, Livne A. Biochim. Biophys. Acta 260: 755, 1972)[26]. In comparing the red cell lipid abnormalities of these congenital diseases with those induced by thermal injury, it is apparent that essential fatty acid deficiency of selected burn patients is considerably more widespread, extending to all polyunsaturated fatty acids of the n6 and n3 series.

Other areas of comparison between the above clinical states and that resulting from burn trauma are physiological and biochemical modifications of the red cell membrane. Both acanthocytes and hereditary spherocytes display distinct and unique morphologies (Neerhout RC. Clin. Pediat. 7: 451, 1968)[21] not markedly different from those observed, to a much lesser extent, by Baar and Arrowsmith in thermally damaged red cells (Baar S, Arrowsmith DJ. J. Clin. Path. 23: 572, 1970)[6]. Enhanced osmotic fragility is a well-defined characteristic of hereditary spherocytosis (Neerhout RC. Clin. Pediat. 7: 451, 1968)[21] and early postburn thermal trauma (Shen SC, Ham TH, Fleming EH. New Eng. J. Med. 229: 701, 1943)[1]. The red cells from burn patients are distinguished by supranormal sodium concentrations (Lantsberg LA.Fed. Proc. 23: T515, 1964[4]; Curreri PW, Wilmore DW, Mason AD, Jr, Newsome TW, Asch MJ, Pruitt BA, Jr. J. Trauma 11: 390, 1971[5]); on the other hand, hereditary spherocytes exhibit elevated cation fluxes in order to maintain normal electrolyte gradients (Jacob H, Karnovsky ML. J. Clin. Invest. 46: 173, 1967)[27] and acanthocytes display no alterations in cation permeability (Hoffman JF. Circulation 26: 1201, 1962)[28]. Interestingly, Jacob and Karnovsky (Jacob H, Karnovsky ML. J. Clin. Invest. 46: 173, 1967)[27] correlated their observed

increase in sodium flux in hereditary spherocytes with increased turnover of phosphatidylserine in the red cell membrane. A direct correlation between the specific lipid alterations described in the present communication and functional changes in the red cell membrane is currently under investigation.

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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 OE 6380	73 07 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8b. DISP'N INSTR'N	8c. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
72 07 01	H. TERMINATION	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	310
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) Safety of Parenteral Fat Emulsion as a Caloric Source 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
71 08	Cont	DA	C. In-House

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

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 73	.5	19
	CURRENT 74	0	0

19. RESPONSIBLE DOD ORGANIZATION
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NAME: Basil A Pruitt, Jr, COL, MC DA

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code):
(U) Intravenous Fat; (U) Intralipid; (U) Parenteral Nutrition; (U) Injured Soldiers

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 soybean emulsion Intralipid in the thermally injured soldier in terms of safety, clearance of the fat emulsion from the blood stream, and effect on complete blood count, and liver and pulmonary function.

24. (U) Single 50 ml units of 10% soybean emulsion were administered over a 4-hour period to burn patients and 15 healed controls. Vital signs were monitored prior to infusion and serially taken each hour during the infusion and 8 hours following administration. Fat clearance from the blood was determined by plasma optical densities before the start of the infusion and at 4, 8 and 24 hours post-infusion. CBS and liver function tests were determined before infusion and 24 hours following administration of a single unit of fat. Cardiorespiratory function was determined following a single unit infusion by Xenon 133 scan in eight patients, by standard pulmonary diffusion tests in three patients, and by arterial blood gas analysis in 20 patients.

25. (U) 72 07 - 73 06 No significant thermogenic response to the intravenous fat emulsion occurred in the group of control or burn patients. Complete blood count and liver function studies were unchanged before and after the infusion of one unit of intravenous fat. Clearance curves demonstrated accelerated plasma disappearance of the emulsion in the acutely burned patients when compared with resting controls. No change in pulmonary function occurred by the Xenon 133 technic or by determining pulmonary diffusion capacity following fat infusion. Blood gas measurements were normal following administration of fat given at 1, 2, or 3 gm/kg body weight. This project has been combined with "The Safety and Efficacy of Parenteral Fat Emulsion in Thermally Injured Patients (44)."

*Available to contractors upon originator's approval.

ANNUAL PROGRESS REPORT

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

REPORT TITLE: THE SAFETY AND EFFICACY OF PARENTERAL FAT EMULSION IN THERMALLY INJURED PATIENTS

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

1 July 1972 - 30 June 1973

Investigators:

Douglas W. Wilmore, MD, Major, MC
Joseph A. Moylan, Jr., MD
George M. Helmkamp, Ph.D., Captain, MSC
Basil A. Pruitt, Jr., MD, Colonel, MC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

REPORT TITLE: THE SAFETY AND EFFICACY OF PARENTERAL FAT EMULSION IN THERMALLY INJURED PATIENTS

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

Period covered in this report: 1 July 1972 - 30 June 1973

Investigators: Douglas W. Wilmore, MD, Major, MC
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Basil A. Pruitt, Jr., MD, Colonel, MC

Reports Control Symbol MEDDH-288(R1)

Single unit infusions of a 10% soy bean emulsion (Intralipid)^{R} were evaluated in convalescing man and hypermetabolic thermally-injured patients. No significant thermogenic responses to the emulsions occurred in either group. Vital signs, CBC, and liver function studies remained unchanged. Fat clearance curves demonstrated an accelerated plasma disappearance of the emulsion in the acutely burned patients. ^{133}Xenon perfusion-diffusion studies were normal, and pulmonary diffusion capacity, using the carbon monoxide rebreathing technique, was also normal following the infusion. Blood gas levels did not change following infusion of single or multiple units of Intralipid.^{R}

An essential fatty acid deficiency of the red cell membrane was identified in five patients, with a marked decrease in linoleate, arachidonic, decosahexanoic acids, all members of the polyunsaturated fatty acid series which cannot be synthesized de novo. All patients had extensive burns, and most were on long term, fat free, parenteral diets before this essential fatty acid deficiency occurred. Infusing quantities of soy bean emulsion high in polyunsaturated fatty acids corrected the fatty acid deficiency in the red cell membranes. Administering an isocaloric fat-free diet to an individual for two months resulted in weight gain and wound healing but failed to correct the compositional fatty acid deficiency. Thus, the fatty acid deficiency in red cell membranes appears to be a combination of the stress of thermal injury and nutritional inadequacies, and can be successfully treated by the inclusion of polyunsaturated fatty acids in the diet.

Finally, fat emulsion was administered, along with other caloric support, to 10 critically injured individuals. The fat appeared to be utilized without complication, and the fat emulsion contributed 38% of the total caloric intake in this group of patients. The nitrogen and caloric support of these patients resulted in protein sparing in all, as manifested by varying degrees of nitrogen retention related to both extent of injury and the degree of nutritional support. The availability of this emulsion as an isotonic, high caloric, noncarbohydrate, energy source increases the flexibility of the surgeon's armamentarium for nutritional support in the severely injured patient.

Intravenous fat emulsion
Parenteral nutrition
Intralipid^{R}

THE SAFETY AND EFFICACY OF PARENTERAL FAT EMULSION IN THERMALLY INJURED PATIENTS

Extensive weight loss and nitrogen depletion characterizes the post-traumatic metabolic response following severe thermal injury. Vigorous nutritional support using enteral or combined enteral-parenteral feedings during this catabolic phase of trauma reduces body wasting and often produces caloric equilibrium and weight stabilization (Wilmore DW, Curreri PW, Spitzer KW, Spitzer ME, Pruitt BA Jr, Surg Gynec Obstet 132:881, 1971).[18] The complications of sepsis (Ashcraft KW, Leape LL, JAMA 212:454, 1970),[1] hyperglycemia, nonketotic hyperosmotic coma (Wyrick WJ Jr, Rea WJ, McClelland RN, JAMA 211:1697, 1970),[21] and central venous thrombosis (Warden GD, Wilmore DW, Pruitt BA Jr, In press, J Trauma)[15] which have been related to the use of hypertonic nutritional solutions in severely ill patients limit the use of recently developed techniques of parenteral feedings in patients with major injury. Fat emulsions which are isotonic and contain 1-2 calories/ml may be administered by a peripheral vein, in order to avoid many of the hazards associated with central venous infusion of hypertonic dextrose solutions. The purpose of this study was to determine the safety and efficacy of a 10% soy bean oil emulsion, Intralipid,® as an essential nutrient and caloric source in thermally injured patients.

METHOD AND MATERIALS

Acute Toxicity Studies

Single 500 ml units of 10% soy bean emulsion were administered to 15 healed, convalescing controls and 12 hypermetabolic thermally injured patients. After eight hours of fasting, the emulsion was infused at a constant rate over a four-hour period by way of a forearm vein. Baseline body temperature, pulse rate, blood pressure, and respiratory rate were recorded prior to the infusion and then serially each hour for the next 12 hours. Fat clearance from the blood was determined by measuring plasma spectrophotometric optical density at 700 mμ before infusion and 4, 8, and 24 hours postinfusion. Complete blood count, total serum proteins, albumin, alkaline phosphatase, SGOT, total bilirubin and direct fraction were determined before infusion and 24 hours following administration of the single unit of fat.

Eight patients with normal cardiorespiratory function were given an intravenous bolus of ^{133}Xenon gas dissolved in saline, and serial pulmonary perfusion-diffusion scans made with a scintillation counter.

The overall characteristics of the lung fields were determined by serial anterior-posterior scintigrams and the clearance rate of the gas was studied by measuring disappearance rates of the isotope from the lung fields (Loken MK, Medina JR, Lillehei PJ, L'Heureux P, Kush GS, Ebert RV, Radiology 93:1261, 1969).[9] Following the baseline xenon study, a 500 ml unit of 10% soy bean emulsion was administered, and, at the end of the four-hour infusion, a repeat ^{133}Xenon lung scan was performed.

Pulmonary diffusion capacity was determined in duplicate in five convalescing patients following a 12-hour fast. Before starting with the infusion, duplicate measurements of diffusion capacity were made with the subjects sitting in the upright position, using a standard carbon monoxide rebreathing technique (Lewis BM, Lin T-H, Noe FE, Hayford-Welsing EJ, J Clin Invest 38:2073, 1959).[8] One unit (500 ml) of 10% fat emulsion was administered over a four-hour period and the diffusion capacity was again determined at the end of the infusion and four hours postinfusion, using the same rebreathing technique.

Single or multiple units of intravenous fat were administered to 20 additional severely burned individuals requiring supplemental or total parenteral nutrition. Arterial blood was drawn at the start of the infusion and analyzed for pH, pCO_2, and pO_2.

Red Cell Phospholipid Studies

Heparinized blood was drawn from 13 burn patients, two individuals with chronic enterocutaneous fistulae receiving prolonged fat-free intravenous feedings, and five age-matched normal subjects. Total lipid extraction from red cells and serum was performed with chloroform and methanol, according to the procedures of Ways and Hanihan (Ways P, Hanahan DJ, J Lipid Res 5:318, 1964).[16] Red cell phospholipids were separated by thin-layer chromatography, using silicate gel H; plasma lipids were isolated by thin-layer chromatography using silicate gel G. The method of Dodge and Phillips was followed for fatty acid analysis (Dodge JT, Phillips GD, J Lipid Research 8:667, 1967),[4] and final identification of the various fatty acids was by comparison with commercially available standards, cod-liver oil, fatty acid methylesters, and published chromatograms.

With the identification of fatty acid deficiency, the 10% fat emulsion was administered to two patients in whom enteral dietary support was inadequate or impossible. An oral diet, supplemented with polyunsaturated fatty acids, was used in the third individual, and a parenteral fat-free diet (hypertonic dextrose, protein hydrolysate, minerals, and vitamins) was continued in two patients. Serum and red

cell fatty acid analysis was continued periodically during this supportive dietary therapy.

Long Term Studies

Intravenous fat emulsion was administered 5-46 days to 10 hypermetabolic burn patients who required extensive energy support because of associated injuries or complications following thermal injury (Table 1). In six individuals, the fat emulsion was administered along with other parenteral nutrients to supplement enteral feedings, but in the remaining four patients, fat emulsion was given in combination with dextrose and protein hydrolysate as total parenteral nutrition. The emulsion was administered through a Y-connector, infused simultaneously with a solution containing 5-15% dextrose, 4-5% protein hydrolysate, electrolytes, vitamins and minerals. Central venous cannulae were used for administration of the more hypertonic solutions but peripheral venous routes were frequently used to administer dextrose and amino acid solution containing less than 14% solute concentration. All urine was collected in 24-hour pools and analyzed for sodium, potassium, chloride, creatinine, urinary urea nitrogen, glucose, and total nitrogen. Douglas bag collections of expired gas were performed on selected patients to determine daily metabolic rates and allow calculation of metabolic fuel oxidation. Blood was drawn daily or when clinically indicated to determine blood count, serum electrolytes, glucose, blood urea nitrogen, blood gas pressure, liver and renal function studies.

RESULTS

No significant thermogenic response to the intravenous fat emulsion occurred in the group of control or burn patients. Pulse rate, respiratory rate, blood pressure, and body temperature remained normal in the control group of patients. Complete blood count and liver function studies were unchanged before and after infusion of one unit of intravenous fat (Table 2). Fat clearance curves demonstrated accelerated plasma disappearance rate of the emulsion in the acutely burned patients when compared with the resting controls (Fig. 1). ^{133}Xenon perfusion-diffusion scans remained normal following the administration of the fat emulsion in the eight patients studied. No change in the distribution or rate of clearance of the xenon from the lung fields was noted (Fig. 2).

Pulmonary diffusion capacity measured by carbon monoxide rebreathing technique demonstrated no alterations in the five patients studied (Table 3). Blood gas measurements carried out in 20 patients were unchanged following the infusion of 1 gm/kg, 2 gm/kg, and 3 gm/kg body weight (Table 4). There was no evidence of cyanosis or respiratory insufficiency associated with the fat infusion.

TABLE 1. Characteristics of Patients Studied

Patient	Age	Weight (kg)	Body Surface Area m^2	Per Cent Total Burn	Per Cent Third Degree	Associated Injuries or Complications
1	21	57	1.63	42	7	Stress ulcer bleeding requiring subtotal gastrectomy; postop bowel obstruction requiring lysis of adhesions.
2	41	53	1.65	60	40	Chronic alcoholism, muscular dystrophy.
3	19	77	1.98	75	50	Repeated sepsis; transferred to this unit for final wound coverage.
4	18	81	1.96	56	54	T-E fistula, stress ulcer requiring gastrectomy, respiratory failure, sepsis.
5	54	74	1.86	26.5	18	Hemiplegia, severe leg burns requiring A-K amputations, respiratory failure.
6	9	25	0.94	31	14	Cerebral edema, inhalation injury, stress ulcer requiring subtotal gastrectomy.
7	23	60	1.65	14.5	14.5	Electrical injury resulting in T-1 paralysis, required radical debridement of right neck, right arm & right upper thorax
8	17	65	1.68	32	22	Head injury, associated fractures, respiratory failure.
9	19	72	1.97	28	12.5	Stress ulcer bleeding requiring subtotal gastrectomy.
10	31	52	1.78	75	47	Severe burn required right A-K amputation; gastric fistula, respiratory failure.
	25.2	61.6	1.71	44	27.9	

Table 2. Hematologic Studies and Indices of Liver Function Before and 24 Hours Following Infusion of 500 ml Soy Bean Emulsion in 15 Convalescing Thermally Injured Patients (Mean ± S.D.)

	Before	After
Hematocrit	41 ± 5	42 ± 4
WBC (cu mm)	7,900 ± 2,200	7,700 ± 2,200
Total protein (gm%)	7.3 ± 0.5	7.6 ± 0.4
Albumin (gm%)	3.6 ± 0.6	3.7 ± 0.4
Alkaline phosphatase (K-A units)	13 ± 3	15 ± 4
SGOT (Karmen units)	30 ± 11	34 ± 9
Total bilirubin (mg%)	0.5 ± 0.3	0.5 ± 0.3
Direct bilirubin (mg%)	0.1 ± 0.0	0.0 ± 0.0

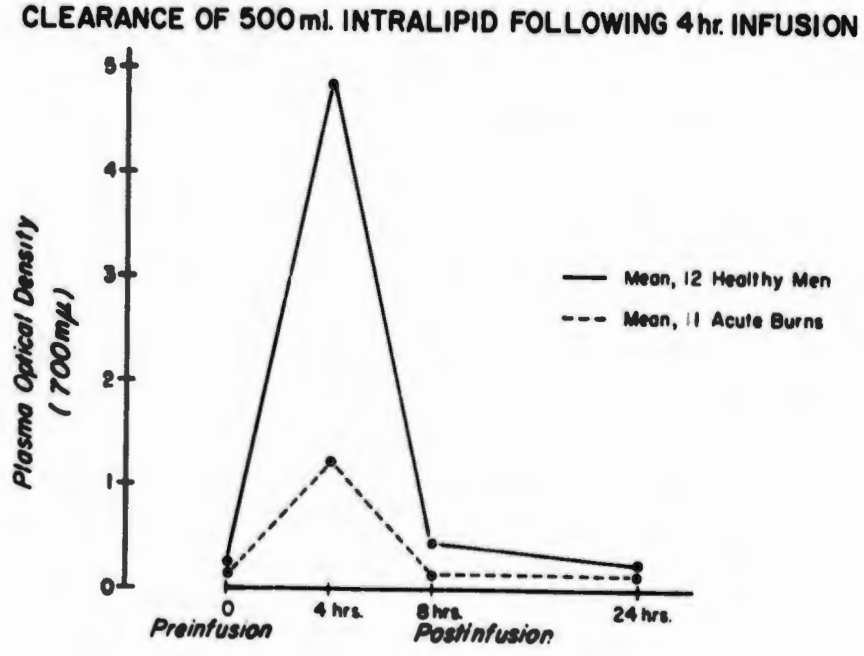

Figure 1. Clearance of 500 ml 10% soy bean emulsion from the blood stream following a four-hour infusion (from 0 to four hours).

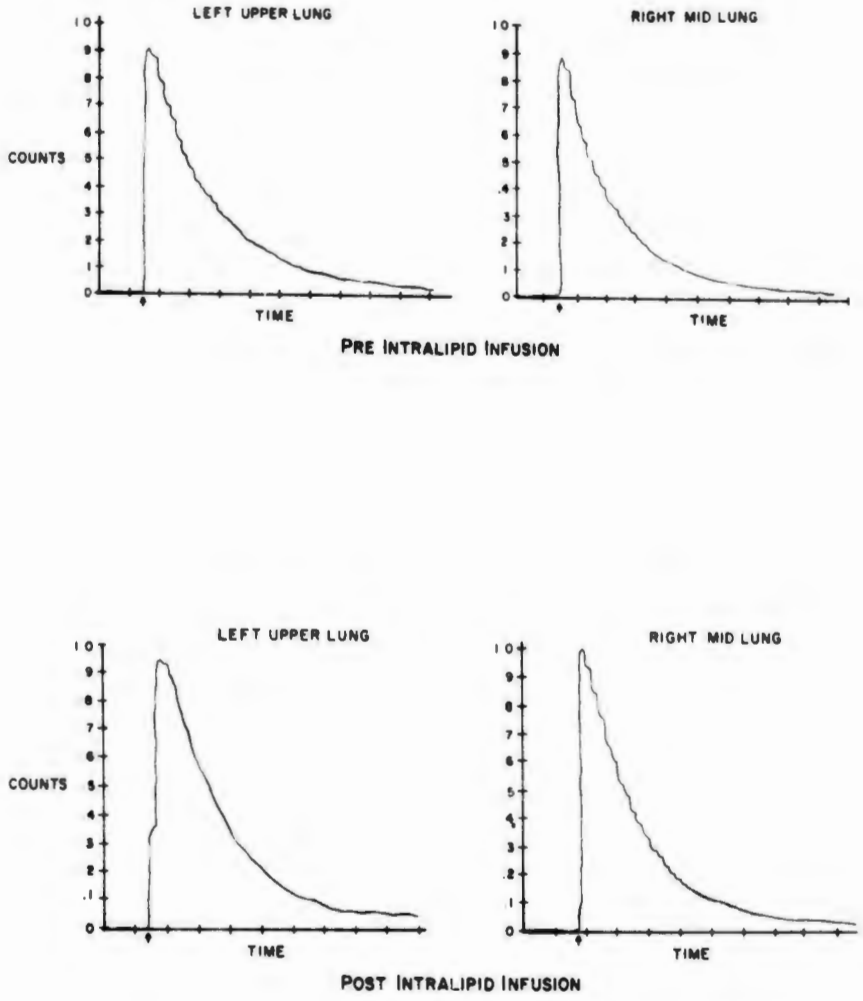

Figure 2. No alteration in clearance of ^{133}Xenon was demonstrated when comparing total lung or regional isotope disappearance curves obtained before (top) and immediately following fat infusion (bottom).

TABLE 3. Diffusion Capacity (DLCO) Before and After 500 ml Soy Bean Emulsion in Convalescing Individuals

Subject	Age	Height (Inches)	Postburn Day Studied	Vital Capacity (Liters BTPS)	Diffusing Capacity in ml CO/min/mmHg (Each Value = Mean of Two Measurements)		
					Pre-Infusion	Immediately Post-Infusion	Four Hours Post-Infusion
1	18	72	97	6.41	36.32	36.32	—
2	20	72	109	6.92	32.92	32.54	33.91
3	21	74	78	5.81	35.04	33.04	35.07
4	21	70	20	5.55	35.99	33.73	35.99
5	20	73	78	4.71	41.0	42.6	38.99

TABLE 4. Effect of Intravenous Fat on Blood Gas Pressures and pH (Mean ± S.D.)

	pO_2 (in mmHg)		pCO_2 (in mmHg)		pH	
	Before	After	Before	After	Before	After
1 gm/kg	91.6 ± 9.5	86.0 ± 9.4	26.9 ± 6.1	27.8 ± 4.4	7.448 ± 0.072	7.466 ± 0.066
2 gm/kg	86.0 ± 22.9	89.7 ± 20.1	28.1 ± 6.5	30.6 ± 7.1	7.454 ± 0.040	7.460 ± 0.060
3 gm/kg	75.2 ± 10.7	78.4 ± 12.5	32.3 ± 3.2	32.1 ± 4.3	7.444 ± 0.046	7.448 ± 0.037

Analysis of the total phospholipid distribution in the red cell revealed that the burn patients fell into two distinct categories, those with normal phospholipid patterns and those with marked phospholipid alterations. Eight burn patients (mean age 33.8, burn size 41.1%, 28.4% full-thickness injury) had normal levels of red cell membrane phosphorus and normal distribution of phospholipids among the four major lipid classes (Table 5). On the other hand, five burn patients (mean age 29.2, burn size 45.6%, 23.7% third degree) revealed a marked reduction of red cell lipid phosphorus. Among the individual phospholipids, there was a relative increase in phosphatidylcholine and sphingomyelin, and a relative decrease in phosphatidylethanolamine. When calculated in terms of absolute changes, with the overall decrease of membrane phosphorus taken into consideration, these alterations of lipid classes corresponded to an absolute reduction of phosphatidylethanolamine (38% of normal), and phosphatidylserine (64% of normal). On this weight basis, phosphatidylcholine and sphingomyelin levels remained normal.

An analysis of the fatty acids derived from the total phospholipid extracts of the red cell revealed only minimal differences between normal red cells and those samples taken from the first group of burn patients (Table 6). However, measurement of fatty acid distribution in the deficient burn patients revealed a pronounced decrease in all polyunsaturated species. The most dramatic changes include a 70% decrease in the acachidonate (20:4n6), an 88% decrease in decosahexanoate (22:6n3), and a 36% decrease in linoleate (18:2n6).* Concurrently, there was a substantial increase in the saturated acids, notably 16:0, 18:1n9, 24:0, 24:1n9. When the relative changes of fatty acids were converted from a weight distribution to molar distribution, then grouped according to structural similarities, the difference between the normal and deficient red cells was more clearly defined (Table 7). Specifically, decrease in all members of the n6 and n3 series, and an increase in all members of the n9 and saturated series, lead to a marked shift in the ratio of saturated to unsaturated fatty acids within the red cell membrane of the phospholipid deficient burn patients.

Isolation of the individual phospholipids by thin-layer chromatography, and interaction and quantification of the fatty acid composition of the individual components, demonstrated that sphingomyelin, with its high proportion of saturated and long chain fatty acids, was

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*The nomenclature for fatty acids is as follows: arachidonic acid, 20:4n6, where 20 is the total number of carbon atoms, 4 is the number of methylene-bridged cis double bonds, and n6 is the position of the double bond nearest the terminal methyl group.

TABLE 5. Phospholipid Composition of Red Cells from Normal and Burned Subjects (Mean ± S.E.M.)

	Normal Subjects (n = 4)	Burn Patients	
		Group I (n = 8)	Group II (n = 5)
Lipid phosphorus*	0.119 ± 0.008	0.122 ± 0.004	0.094 ± 0.010
Phospholipid distribution (Weight per cent)			
Phosphatidylethanolamine	26.3 ± 1.3	26.4 ± 1.1	13.2 ± 1.0
Phosphatidylserine	13.0 ± 2.5	14.5 ± 0.8	11.8 ± 1.6
Phosphatidylcholine	29.7 ± 2.2	30.2 ± 1.3	37.8 ± 2.2
Sphingomyelin	30.7 ± 1.4	28.4 ± 0.9	37.2 ± 2.6

*mg/ml packed cell

TABLE 6. Fatty Acid Composition of Total Phospholipid from Red Cells (Mean ± S.E.M.)

	Number of Patients	Fatty Acid (g/100 g)								
		16:0	16:1n7	18:0	18:1n9	18:2n6	20:4n6	22:6n3	24:0	24:1n9
Normals	5	24.9	0.8	16.6	16.5	9.5	17.2	3.2	5.8	5.7
		±1.3	±0.3	±0.6	±0.4	±0.5	±0.8	±0.5	±0.6	±0.2
Burns	8	24.2	2.1	16.3	18.1	9.1	14.0	2.7	5.4	8.0
Normal fatty acids		±0.8	±0.2	±0.3	±0.3	±0.3	±0.4	±0.3	±0.2	±0.6
Burns	5	31.0	1.5	17.3	21.4	6.1	4.1	0.4	8.9	9.3
Essential fat deficient		±1.8	±0.4	±0.5	±0.6	±0.6	±0.6	±0.2	±0.8	±0.3
Deficient burns following IV fat	2	24.3	0.8	19.5	16.2	10.2	15.9	4.1	6.6	6.4
Deficient burn following fat-free IV diet	1	34.5	0.7	17.3	15.0	8.1	3.8	0.8	10.6	9.1
Deficient burn following high fat oral diet	1	23.8	1.9	15.9	16.8	10.7	16.4	2.4	5.5	6.7
Fistula patients following fat-free IV diet	2	25.0	2.0	15.1	18.9	7.4	13.0	2.6	6.6	9.2

TABLE 7. Molar Fatty Acid Distribution of Red Cell Phospholipids from Normal and Burned Subjects

	Normal Subjects	Burn Patients	
		Group I	Group II
Saturated fatty acids			
Palmitate (16)	28.5	27.8	35.3
Stearate (18)	16.9	16.7	17.4
Lignocerate (24)	4.4	4.0	6.7
Total	49.8	48.5	59.4
Unsaturated fatty acids			
Oleate series (n9)			
Oleate (18)	16.8	18.6	21.6
Nervonate (24)	4.4	6.1	7.1
Total	21.2	24.7	28.7
Linoleate series (n6)			
Linoleate (18)	9.7	9.4	6.2
Arachidonate (20)	15.8	12.9	3.6
Total	25.5	22.3	9.8
Linolenate series (n3)			
Decosahexanoate (22)	2.7	2.2	0.4
Total unsaturated, including			
Palmitoleate (16:1n7)	50.3	51.6	40.5
Ratio (saturated/unsaturated)	0.99	0.94	1.47

unaffected by the nutritional and thermal stress. However, phosphatidylethanolamine and phosphatidylserine were the major subcategories demonstrating the quantitative decrease in polyunsaturated fatty acid similar to the alterations observed with the combined phospholipid analysis. Evaluation of the plasma lipid classes, carried out to determine how widespread the fatty acid alterations occurred following thermal injuries, revealed no consistent difference in the fatty acid composition of the plasma phospholipids, triglycerides, or cholesterol esters. Two patients had a reduced level of plasma linoleate, but the others had normal or somewhat reduced quantities. At the same time, minimal fluctuations of arachidonic and n3 saturated and unsaturated acids were found. The appearance of 5-8-11 eicosatrienoate (20:3n9), which is absent in normal man, was observed in the plasma phospholipid fraction of three of the four deficient patients studied.

Studies of two patients with chronic enterocutaneous fistulas, with normal metabolic rates, who received fat-free parenteral diets for more than a month revealed no significant alterations in the membrane phospholipid fatty acid (Table 6). Evaluation of one of these patients after six months of fat-free feeding demonstrated only a depressed level of linoleate with a normal distribution of arachidonate and decosahexanoate (Fig. 3).

Polyunsaturated fatty acids (the n6 and n3 fatty acid species) cannot be synthesized de novo in man, and therefore must be acquired by dietary intake. The soy bean lipid emulsion was used for replacement therapy in two patients, an oral diet supplemented with high quantities of polyunsaturated fats was used to treat one individual, and a fat-free parenteral diet was continued in two patients.

Following the administration of 10% soy bean emulsion, fatty acid imbalances were gradually corrected to normal levels. For example, patient 1 received 50, 500 ml, units of Intralipid^{R} over a 34-day period and patient 2 received 67 units over a 63 day interval. In the first individual, there was marked improvement of red cell polyunsaturated fatty acids after only two weeks of fat therapy (Fig. 4), and, simultaneously, palmitate and other saturated fatty acids were reduced to a normal level. Patient 2, on the other hand, progressed more gradually to a normal fatty acid distribution when placed on the intravenous fat feedings (Fig. 5). An essentially normal pattern was found on day 260, about 60 days following the onset of lipid infusion. One patient corrected his fatty acid red cell abnormalities following addition of polyunsaturated fats to his diet and the other two patients received hypercaloric fat-free feedings administered by central venous catheter (Table 6). After 68 days, no improvement in erythrocyte fatty acid deficiency was noticed in one individual despite his relative improvement in terms of wound healing and gain in body weight, but

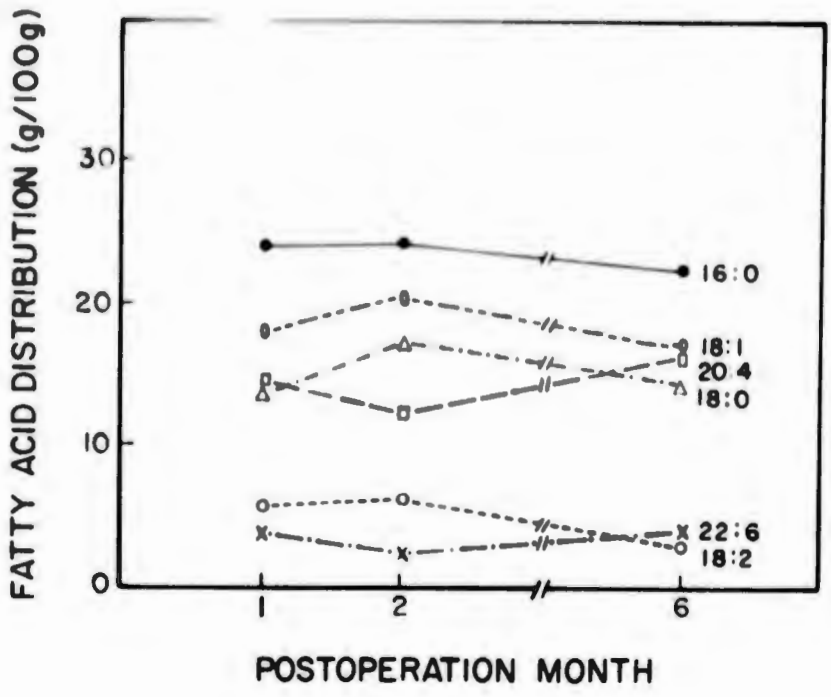

Figure 3. Distribution of selected fatty acids from the erythrocyte phospholipids from a 26 year old female with multiple enterocutaneous fistulae. Her metabolic rate was measured at normal predicted levels and she was maintained by total fat-free intravenous nutrition for six months with minimal alterations in polyunsaturated fatty acids.

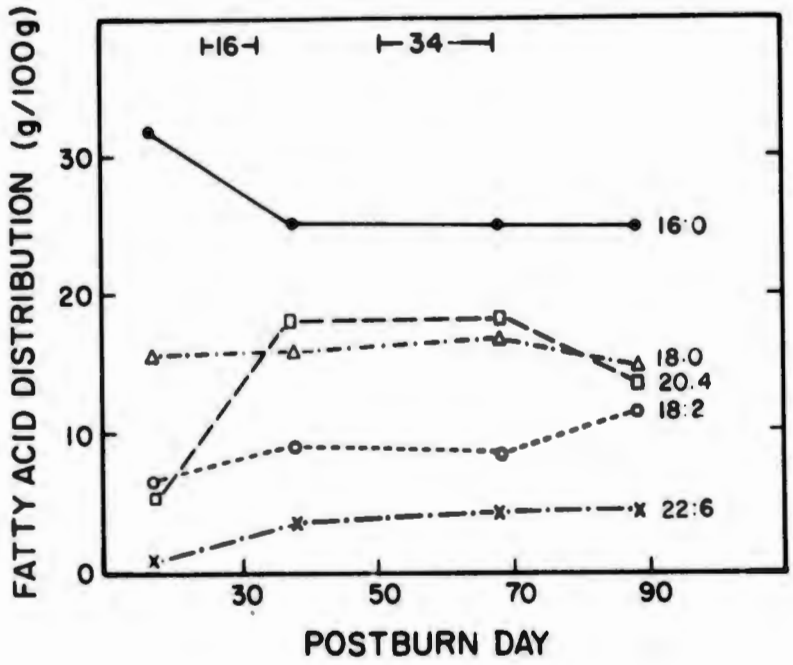

Figure 4. A 40 year old male with 60% total body surface burns corrected his essential fatty acid deficiency (18:2, 22:4, 22:6) with the infusion of 50 units soy bean emulsion. The dose and time relationship of the infused fat is shown at the top of the figure.

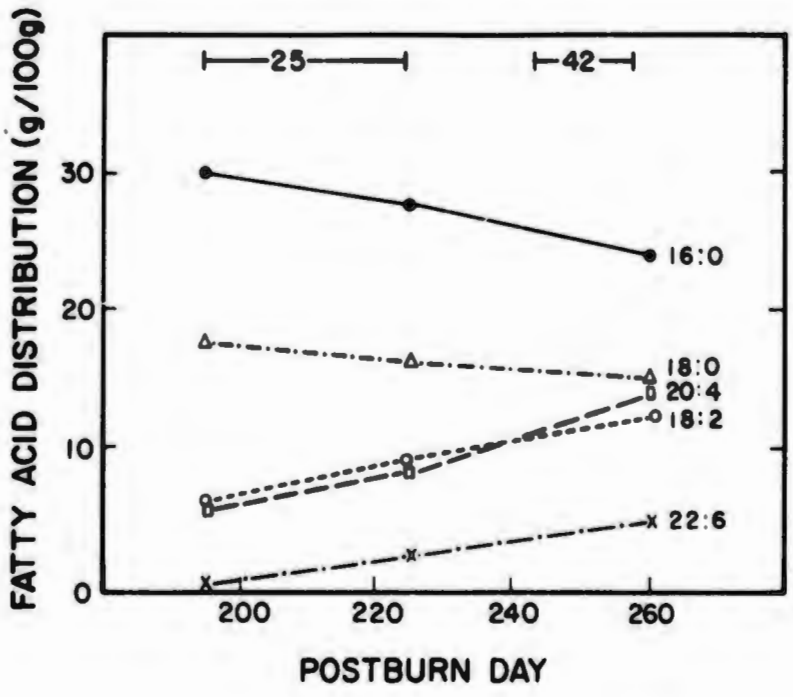

Figure 5. Gradual correction of the polyunsaturated fatty acid deficiency was accompanied by return to normal of the unsaturated fats (16:0, 18:0) with the infusion of intravenous soy bean emulsion in the diet of this 19 year old male with a 75% total body surface burn.

the second patient died from infection and hemolysis before fat could be obtained for administration. Only transient alterations in serum fatty acid levels occurred following the Intralipid[R] therapy.

Two hundred and twenty-two liters of the 10% intravenous fat emulsion were administered to 10 patients with large burns (average burn size 44% total body surface, 27.9 average per cent third degree burn) and associated injuries or complications secondary to their extensive thermal injuries. The emulsion was administered from five to 46 days (mean length of therapy 16.1 days). Although the average dose of the fat emulsion administered did not exceed 3.3 gm/kg/body weight per 24 hours, daily variations occurred and several patients received as much as 2.5 liters of emulsion per day, or a dose of fat equal to 5 gm/kg/body weight per day. No untoward effects could be specifically related to the emulsion in this group of critically ill patients, although a transient plasma lipemia existed while the infusions were in progress. No febrile response was related to the emulsion, and no alterations in liver, renal, or pulmonary function were associated with the doses given to this group of patients. Four of these 10 individuals died, and autopsies obtained in all patients revealed no excess accumulation of fat in liver, lung, or other body tissues.

The average daily caloric intake in the 10 patients studied was 3,770 kcl/day, and 19.2 gm nitrogen/day, with the fat emulsion contributing an average of 38% of the total calories received by these patients (Table 8). Nitrogen retention, determined by comparing nitrogen intake with urinary nitrogen loss, indicated positive nitrogen balance in four individuals (patients 2, 3, 6, and 10), nitrogen equilibrium in three patients (patients 4, 5, and 8), and diminution of the post-traumatic net nitrogen loss in the remaining three individuals. Moreover, vigorous caloric support contributed to stabilization of body weight or actual weight gain, and a good correlation existed between weight change and positive caloric balance.

DISCUSSION

Intralipid,[R] the fat emulsion studied, contains soy bean oil, egg yolk-phosphatide, and glycerol as a 10% emulsion which provides 1,100 calories per liter. The particles are similar to naturally occurring emulsions (such as chyle), and their average diameter of 0.13 μm is similar to that of chylomicrons (diameter of 0.096-0.21 μm) (Wretlind A: Nutr Metab 14 (Supp 1), 1972).[19] The emulsion is isotonic with body fluids and hence may be infused by peripheral vein, and, because of its relatively solute-free nature, provides a source of additional free water to the patient.

TABLE 8. Dietary Support and Metabolic Response

Patient	PBD* Diet Started	No. Days Studied	10% Fat Emulsion		Other Nutrient Support (Exclusive of Fat)				Total Nutritional Intake		Urinary Excretion of Nitrogen	Weight Change
			Total Dose (Liters)	Av. Daily Caloric Contribution	Enteral Feedings		Parenteral Feedings					
				Kcal/Day	Total Kcal/Day	N (Gm/Day)	Kcal/Day	N (Gm/Day)	Kcal/Day	N (Gm/Day)	Gm/Day	Kg/Day
1	41	26	38.0	1650	0	0	1566	17.0**	3216	17.0	21.7	+0.076
2	74	14	19.5	1532	1931	11.9	455	8.3	3818	20.2	10.7	+0.206
3	265	14	11.5	904	3682	22.8	265	8.7	4851	31.4	10.6	+0.129
4	18	10	9.5	1045	1424	11.9	1226	2.2	3695	14.1	10.3	+0.030
5	15	7	10.5	1650	2041	11.6	0	0	3541	11.6	10.6	+0.171
6	30	14	16.8	1320	0	0	1200	16.3	2928	16.3	13.2	+0.280
7	10	12	20.0	1833	0	0	2136	15.9	3969	15.9	29.6	—
8	12	5	7.5	1600	1632	13.0	690	9.6	3922	22.6	20.9	—
9	31	16	18.5	1217	0	0	1295	13.9**	2860	13.9	14.3	0.00
10	89	46	70.0	1711	1957	13.2	1234	15.6**	4902	28.8	21.3	+0.152

*PBD - Postburn day

**Albumin administered but not included in nitrogen intake

Early complications have been reported with other fat emulsions, including fever, dyspnea, cyanosis, flushing, nausea, vomiting, headache, and jaundice. Hyperlipemia, alterations in blood coagulation, liver dysfunction, anemia, and deposition of intravenous fat were late complications associated with the use of other fat emulsions (Wretlind A: The pharmacological basis for the use of fat emulsion in intravenous nutrition. Acta Chir Scand (Supplement) 325:31, 1962).[20] In this study, the emulsion tested did not demonstrate discernible thermogenic reactions, with units taken from 10 different manufacturing batches. Complete blood counts and indices of liver function were unchanged following a one-unit infusion, and critically ill patients given multiple-unit infusions did not demonstrate abnormalities that could be directly related to the emulsion. A report of dyspnea and cyanosis, associated with the administration of fat emulsions (Greene H, Hazlett D, Demarre R, Dremesi J: Effect of intralipid[R] on pulmonary function in normal humans and on electron microscopy of rabbit lung and liver. Presented at the International Congress of Nutrition, Mexico City, 1972),[6] prompted our assessment of the effect of Intralipid[R] on cardiopulmonary function. These studies demonstrated no change in xenon perfusion-diffusion scans or clearance of the isotope from the lung fields after a single unit of fat emulsion. Pulmonary diffusion capacity was unaltered in the five individuals studied, and no changes in blood gas values have been detected after administration of single or multiple units of the fat emulsion.

Prior to wound coverage, the hypermetabolic burn patient demonstrated increased clearance of the intravenous soy bean emulsion from the blood stream. Fat clearance, dependent on concentrations of lipoprotein lipase, is increased following starvation and stress, and may be further accelerated by heparin or insulin. In control adult subjects following an overnight fast, clearance rates have been estimated at 3.8 gm fat/kg/body weight per 24 hours (Wretlind A: Complete intravenous nutrition. Nutr Metab 14 (Supp 1), 1972).[19] While clearances cannot be directly equated with fat utilization, previous studies have demonstrated favorable weight gains and nitrogen retention to the administration of intravenous fat emulsions in animals and man. There is a shift in respiratory quotient toward that of fat oxidation following the administration of the soy bean emulsion, suggesting utilization of the fat (Geyer RP: Parenteral emulsions--formulation, preparation, and use in animals. In Parenteral Nutrition, edited by Meng HC, and Law DH, Springfield, Charles C. Thomas, 1970, p 339).[5] Histologic examination of specimens from our patients showed no lipid accumulation in tissue. Of the series of 10 patients receiving long-term infusion, four died (patients 4, 7, 8, 10), and no abnormalities of fat distribution or lipid accumulation were found. Because the hypermetabolic response to thermal injury provides a neuroendocrine environment favoring triglyercide and fatty acid mobilization and utilization (Carson LA, Liljedahl SO: Lipid metabolism and trauma. Acta Chir Scand 137: 123, 1971),[2] the emulsion apparently equilibrates with available body lipid for utilization or storage as a basic fuel substrate.

Essential fatty acid deficiency occurred in five of the 13 burn patients studied, with a marked decrease in linoleate, arachidonic, and decosahexanoic acids (polyunsaturated fatty acids which cannot be synthesized by the body and hence must be replaced by dietary fat). Although it has been stated that the diet should include approximately 4% of the nonprotein calories as polyunsaturated fat (Collins FD, Sinclain AJ, Royle JP, Coats DA, Maynard AT, Leonard RF, Nutr Metab 13:150, 1971),[3] essential fatty acid deficiencies seldom occur in man, as demonstrated by our two patients with enterocutaneous fistulas, who received fat-free diets for four and seven months with only minor alterations in plasma and red cell polyunsaturated fatty acids. However, the stress of injury in addition to inadequate dietary fat appears to result in a deficiency state, which can also be produced in the stressed young animal or growing infant receiving fat-free feedings (Paulsrud JR, Pensler L, Whitten CF, Stewart S, Holman RT, Amer J Clin Nutr 25:897, 1972).[12] Following major injury, the increased energy demands result in increased mobilization and oxidation of body fat. This study suggests that this increase in fat oxidation is sufficient, when combined with a diet inadequate in polyunsaturated fat, to result in essential fatty acid deficiency. Administering essential fat (Intralipid® 10% contains 43 gm of linoleic acid and 6.5 gm linolenic acid per liter) to our patients who had been maintained on carbohydrate-rich, fat-free, parenteral feedings resulted in return of the red cell membrane fatty acids to normal. That the lipid emulsion played a specific role in correcting the fatty acid deficiency is seen in the patient who received an isocaloric but fat-free intravenous diet and failed to correct the essential fat deficiency, which persisted for two months. Finally, in the patient in which total intravenous support is unnecessary, a diet high in calories containing polyunsaturated fatty acids should correct the characteristics of the essential fatty acid deficiency, as illustrated by the patient who responded to appropriate oral therapy.

Associated with the decrease in the polyunsaturated fatty acid is an increase in saturated acids to abnormally elevated levels. In addition, abnormal fatty acids appear and one component to which significance is attached is 5-8-11 eicosatrienoate (20:3n9), which is absent from most normal tissues (Holman RT, In Progress in the Chemistry of Fats and Other Lipids, New York, Pergamon Press, Limited, 1971, p 275).[7] During deficiency states, this abnormal oleate-derived acid appeared in three out of the four deficient patients examined, and in severe deficiency may account for the major proportion of all polyunsaturated lipid. The contribution of previous dietary history to red cell lipid abnormalities, though difficult to assess, may be an important contribution in the development of this deficiency state.

Although all five patients appeared to be in good health prior to injury, two individuals reported a high consumption of alcohol. Chronic alcoholism may progress to a condition of plasma hyperlipidemia, with an increase of both cholesterol and phospholipid fractions of red cell membrane in chronic alcoholics (Westerman MP, Balcerzak SP, Heinle EW Jr, J Lab Clin Med 72:663, 1968).[17] While our findings are not entirely compatible with this state, it seems unreasonable to assume that the major disproportions of saturated and unsaturated fatty acids observed in patient number 4 on the second postburn day are simply a result of his thermal injury. It therefore appears that the patient susceptible to fatty acid deficiency is the one who has a previous dietary history of low essential fatty acid intake with marginal stores of polyunsaturated fat, is then maintained on a fat-free diet, and has increased essential fatty acid needs as a result of the stress of injury or the need for body repair or growth.

This essential fatty acid deficiency may influence the response to the stress of thermal injury. Fat is an essential component of cell membranes and the proportions of saturated to polyunsaturated fat determine membrane fluidity and, hence, effect transport of water, ions, and other essential nutrients through cell wall and mitochondrial membranes. These observations of altered phospholipid and fatty acid composition of the red cell membrane are similar to those described for a wide variety of red cell disorders (Neerhout RC, Clin Pediat 7:451, 1968).[11] Reduced linoleate levels are associated with hereditary spherocytosis, leptocytosis, certain acquired hemolytic anemias, and acanthocytosis. Investigation into these disease states has resulted in a definition of altered physiology associated with the biochemical modifications of the red cell membrane. Altered osmotic fragility, abnormal sodium and potassium transport, and a decreased cellular half life, have all been associated with similar membrane alterations described in other disease states (Shohet S, New Eng J Med 286:577, 1972).[13] Our preliminary evidence suggests similar correlation between specific lipid abnormalities and functional changes of the red cell membrane in these patients, and further investigations are continuing in this area.

The specific role of fat on protein metabolism must be defined, for fat administered to fasting man may prevent weight loss but apparently has little effect on protein catabolism. Monroe, in an extensive and thorough review of interaction between carbohydrate and fat calories in the diet, points out that an essential amount of dietary carbohydrate is required for nitrogen sparing, but, over and above this quantity, fat and carbohydrate can be interchanged to provide additional calories with similar effects on metabolism (Monroe HN, Phys Rev 31:449, 1951).[10] Fat emulsions provide an isotonic high-caloric source which seems ideal for the burn patient, who requires additional

solute free water because of the marked increase in evaporative water loss from the damaged integument and needs caloric support because of the hypermetabolism associated with thermal injury. Glucose and amino acids can be constituted as 10-14% solutions and delivered simultaneously with the fat emulsion by peripheral vein. Using fat emulsion combined with other feeding techniques, nitrogen equilibrium was achieved in these patients with the administration of approximately 15 gm nitrogen/m^2/24 hours and 2,000-2,200 cal/m^2/day, an estimate comparable to the nitrogen and caloric requirements previously determined at this institute (Soroff HS, Pearson E, Artz CP, Surg Gynec Obstet 112:159, 1961).[14]

Side effects in noninjured patients have frequently been related to the increased plasma lipid that occurs following fat infusion, and accelerated lipid clearance following trauma may account for the relative safety of the fat emulsion following injury. Most of these studies were performed in previously healthy young individuals and guidelines for use of the emulsion must be defined in the noninjured patient during states of resting starvation and in patients with metabolic derangements of fat and carbohydrate metabolism. However, in burn patients, the intravenous fat emulsion allowed a ready caloric source with minimal hazard and could be used to supplement other feeding techniques.

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Wilmore DW, Helmkamp GM, Pruitt BA Jr: Essential fatty acid deficiency in the red cell membrane following thermal injury: Correction with a parenteral fat emulsion. Surg Forum 23:499-500, 1972.

Moylan JA Jr, Wilmore DW, Spitzer KW, Pruitt BA Jr: Pulmonary diffusion characteristics following administration of a parenteral fat emulsion. Surg Forum 23:218-219, 1972.

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Helmkamp GM, Wilmore DW, Johnson A: Essential fatty acid deficiency in red cells following thermal injury: Correction with intravenous fat therapy. In press, J Clin Nutr.

PRESENTATIONS:

Wilmore DW: Influence of Parenteral Diet on Serum and Red Cell Fatty Acid Composition Following Thermal Injury. International Congress of Nutrition, Mexico City, 8 September 1972.

Wilmore DW: Essential Fatty Acid Deficiency in the Red Cell Membrane Following Thermal Injury: Correction with a Parenteral Fat Emulsion. Surgical Forum, San Francisco, California, 3 October 1972.

Moylan JA Jr: Pulmonary Diffusion Characteristics Following Administration of a Parenteral Fat Emulsion. Surgical Forum, San Francisco, California, 3 October 1972.

Wilmore DW: Clinical Evaluation of a 10% Intravenous Fat Emulsion for Parenteral Nutrition in Thermally Injured Patients. American Surgical Association, Los Angeles, California, 27 April 1973.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0C 6978	73 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 INSTR'N	8b. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
72 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	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)
FISCAL YEAR	PRECEDING 73	.4	10
	CURRENT 74	.2	6

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) Gastrointestinal Absorption; (U) High Protein Diet; (U) Trace Elements; (U) Humans

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

23. (U) To evaluate the gastrointestinal absorption and nutritional efficacy of a standard high protein hospital diet in extensively burned patients. To evaluate the effect of alterations in quantities of carbohydrate, fat, and amino acids in the diet of wounded soldiers.

24. (U) Using specially prepared hospital diets with liquid supplements, nitrogen balance has been determined while patients are receiving 40% of their calories from carbohydrate and then 60% of their calories as carbohydrate. Nitrogen intake remains the same during these studies.

25. (U) 72 07 - 73 06 Two patients, to date, have been studied to determine the interaction between fat and carbohydrate calories in nitrogen sparing. Both studies indicate that nitrogen, not calories, is the limiting factor when adequate caloric intake is achieved. Consequently, further evaluation of this dietary program will be carried out early in the postburn period when caloric administration is inadequate and dietary efficiency more appropriate to the wounded soldier.

*Available to contractors upon solicitation's approval

ANNUAL PROGRESS REPORT

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

REPORT TITLE: EVALUATION OF GASTROINTESTINAL ABSORPTION AND NUTRITIONAL EFFICACY OF STANDARD HIGH PROTEIN HOSPITAL DIET IN EXTENSIVELY BURNED PATIENTS

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

1 July 1972 - 30 June 1973

Investigators:

Douglas W. Wilmore, MD, Major, MC
Barbara F. Bristow, 1LT, AMSC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

REPORT TITLE: EVALUATION OF GASTROINTESTINAL ABSORPTION AND NUTRITIONAL EFFICACY OF STANDARD HIGH PROTEIN HOSPITAL DIET IN EXTENSIVELY BURNED PATIENTS

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

Period covered in this report: 1 July 1972 - 30 June 1973

Investigators: Douglas W. Wilmore, MD, Major, MC
Barbara F. Bristow, 1LT, AMSC

Reports Control Symbol MEDDH-288(R1)

Nutritional support following major injury is essential to the care of the wounded soldier. Weight loss is a reflection of the difference between energy expenditure of the patient and calories provided by dietary means and therefore body weight loss is not an obligatory response following injury. Weight loss, per se, has been associated with death following 40 to 50 per cent loss of body mass (loss of approximately one-fourth to one-third of body nitrogen). However, complications associated with weight loss, notably infection and those associated with chronic convalescence, may be related to loss of body proteins, which are utilized as one of the basic substrates for gluconeogenesis during the catabolic state. The purpose of this study is to determine if alterations in the composition of the diet will more effectively preserve lean body mass (optimize nitrogen retention) while a progressive and controlled weight loss occurs following injury.

Intestinal absorption
Hospital diet
Protein sparing
Insulin

EVALUATION OF GASTROINTESTINAL ABSORPTION AND NUTRITIONAL EFFICACY OF STANDARD HIGH PROTEIN HOSPITAL DIET IN EXTENSIVELY BURNED PATIENTS

Two patients have been studied to date while they received high caloric diets. During one study period of at least 10 days, the diet was composed of 40 per cent fat, 40 per cent carbohydrate, and 20 per cent protein and compared with an experimental period, which was placed before or following the control period in a random period, while a diet of 20 per cent fat, 60 per cent carbohydrate, and 20 per cent protein was administered. In the first patient, who received a caloric load in excess of energy demand, nitrogen sparing was maximal with the 40 per cent carbohydrate intake and nitrogen retention was therefore limited by nitrogen intake and not calories or carbohydrate intake. In the subsequent patient, who was maintained slightly below energy requirements, the carbohydrate intake improved nitrogen retention, and this response appeared to be mediated by increased insulin secretion.

Studies with human growth hormone suggest improved nitrogen retention is mediated by a reset in insulin elaboration and alterations in carbohydrate metabolism. Increased gluconeogenesis following injury may require an increased load of carbohydrate during the early catabolic course following injury, and this could be provided by altering dietary composition to provide exogenous glucose and minimize gluconeogenesis and lean tissue breakdown. Moreover, studies in normal and diabetic man indicate that increased insulin elaboration can be achieved through diet alone, and additional studies will evaluate the standard hospital diet and a high carbohydrate diet in the catabolic phase of injury to determine the nitrogen sparing characteristics of each one of these dietary programs.

PUBLICATIONS AND/OR PRESENTATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
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3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
72 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	319
b. CONTRIBUTING				
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11. TITLE (Precede with Security Classification Code)
(U) Human Growth Hormone in Burned Military Personnel (44)

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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 73	.5	16
	CURRENT 74	.5	12

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NAME: James M Long, MAJ, MC
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20. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

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(U) Burns; (U) Growth Hormone; (U) Metabolism; (U) Humans; (U) Human Growth Hormone; (U) Protein Metabolism; (U) Burn Patients

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

23. (U) To administer human growth hormone plus high caloric feedings to burned soldiers during acute thermal injury and evaluate the effects on carbohydrate and protein metabolism.

24. (U) Eight burn patients were studied before wound closure was achieved, and administered a constant intake of nitrogen and calories to meet predicted requirements. Human growth hormone, 10 International Units, was administered daily for one week and compared with a similar week which preceded or succeeded the experimental period. On day 5 of each week of study, a glucose tolerance test was performed. All urine was collected for determination of nitrogen balance and serial blood studies sampled.

25. (U) 72 07 - 73 06 Increased nitrogen retention occurred with HGH therapy in all but one patient, with a mean loss of urinary nitrogen 17.1 grams per day compared with 21.7 grams per day during the control period. BUN decreased from 16 to 13 ($p<0.02$), free fatty acids increased from 1.2 to 1.7, serum protein levels stabilized and liver function tests returned toward normal. Blood glucose increased slightly from 104 to 118 mg%; plasma insulin was reset at almost twice the levels achieved during the control period. The protein sparing effects and other beneficial secondary gains to HGH which enhance recovery of the injured patient appear to be dose related, to require nutrient loading to augment insulin response, and to be mediated by alterations in carbohydrate metabolism in the presence of increased insulin production.

*Available to contractors upon originator's approval

ANNUAL PROGRESS REPORT

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

REPORT TITLE: HUMAN GROWTH HORMONE IN BURN PATIENTS

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

1 July 1972 - 30 June 1973

Investigators:

Douglas W. Wilmore, MD, Major, MC
Joseph A. Moylan, MD
Barbara F. Bristow, 1LT, AMSC
Basil A. Pruitt, Jr., MD, Colonel, MC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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REPORT TITLE: HUMAN GROWTH HORMONE IN BURN PATIENTS

US Army Institute of Surgical Research, Brooke Army Medical Center,
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Period covered in this report: 1 July 1972 - 30 June 1973

Investigators: Douglas W. Wilmore, MD, Major, MC
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Basil A. Pruitt, Jr., MD, Colonel, MC

Human growth hormone was administered to seven severely burned hypermetabolic patients receiving calories and nitrogen predicted to meet or exceed energy demands. A significant increase in nitrogen retention occurred with HGH therapy when compared with control periods of diet alone.

The protein sparing effects and other beneficial secondary gains to HGH which enhance recovery of the injured patients appear to be dose related, to require nutrient loading to augment the insulin response, and to be mediated by alterations in carbohydrate metabolism in the presence of increased insulin production.

Burns
Growth hormone
Metabolism
Humans

HUMAN GROWTH HORMONE IN BURN PATIENTS

Weight loss and negative nitrogen balance characterize the metabolic response following thermal trauma, with the magnitude of the catabolic phase related to the extent and severity of the injury. Modification of the accelerated rate of tissue breakdown and loss of protoplasmic mass is a major priority following extensive injury and this goal may be achieved by vigorous nutritional support (Wilmore DW, Curreri PW, Spitzer KW, Spitzer ME, Pruitt BA Jr, Surg Gynec Obstet 132:881-886, 1971),[1] by prevention of infection in the burn wound and elsewhere, and by timely closure of the surface wound. In spite of optimal care, patients with burns of more than 40 per cent of the body surface sustain a significant loss of body cell mass which may limit wound healing and resistance to infection, prolong hospitalization and convalescence, and delay rehabilitation and satisfactory return to a useful and productive life. Recent studies of hormonal-body fuel interrelationships following starvation (Cahill GF Jr, Herrera MG, Morgan AP, J Clin Invest 45:1751-1769, 1966;[2] Muller WA, Faloona GR, Unger RH, New Eng J Med 285:1450-1454, 1971)[3] and trauma (Lindsey CA, Wilmore DW, Moylan JA, Faloona GR, Unger RH, In press, J Clin Invest)[4] suggest that a disproportionate increase of catabolic hormonal regulators is elaborated following injury to facilitate protein breakdown for conversion to new glucose, which aids wound repair and provides an available supply of readily utilizable energy. Nitrogen sparing could thus be achieved following injury by the administration or stimulation of anabolic hormones to blunt the catabolic response (Hinton P, Littlejohn S, Allison SP, Lloyd J, Lancet 1:767-769, 1971).[5]

Human growth hormone, a molecule with 188 sequential amino acid residues elaborated by the anterior pituitary gland, has a unique physiologic role of promoting growth and improving nitrogen storage. Administered to normal man, this polypeptide promotes retention of nitrogen, phosphorus, and potassium; facilitates intracellular transport of amino acids, and increases ribosomal protein synthesis; stimulates fat mobilization; alters cellular sensitivity to carbohydrates by diminishing insulin responsiveness; and stimulates synthesis of chondrotin sulfate and collagen (Raben MS, Recent Prog Hormone Res 15:71-114, 1959).[6] The anabolic effects of this hormone are dramatically demonstrated when HGH is administered to a child with hypopituitary dwarfism, for it prompts immediate acceleration of linear growth. Because of the specific effects of human growth hormone in maintaining and synthesizing lean body mass, HGH may represent a safe and effective method of reversing the extensive nitrogen loss which occurs following major trauma.

Soroff, Pearson, Green, and Artz (Soroff HS, Pearson E, Green NL, Artz CP, Surg Gynec Obstet 111:259-273, 1960)[7] administered bovine growth hormone to severely burned patients and reported an increased retention of nitrogen and potassium, and concomitant weight gain which occurred in the convalescent state of injury. However, there was accentuation of nitrogen loss when this hormonal preparation was administered during the early catabolic phase of burn trauma. Liljedahl and associates (Liljedahl SO, Gemzell CA, Plantin LO, Birke G, Acta Chir Scand 122:1-14, 1961)[8] gave 10 to 20 mg of human growth hormone daily to burn patients over periods of seven to nine days and noticed an appetite stimulating effect which increased protein and caloric intake, thus increasing nitrogen retention. Roe and Kinney (Roe CF, Kinney JM, Surg Forum 13:369-371, 1962)[9] demonstrated that growth hormone stimulated fat mobilization and increased the proportion of fat utilized as the body's fuel. Following the administration of HGH to convalescing orthopedic patients, there was a rise in monoesterified fatty acids in the serum, an increase in oxygen consumption, and lowering of respiratory quotient.

More recently, Soroff and associates (Soroff HS, Rozin RR, Mooty J, Lister J, Raben MS, Ann Surg 166:739-752, 1967)[10] controlled caloric intake in burn patients at nine grams of nitrogen and 1,500 calories per square meter body surface area, and studied the effects of HGH on both the catabolic and anabolic phase of thermal injury. In general, increased storage of nitrogen, potassium, sodium, and chloride occurred with growth hormone administration, with no apparent predictability in the individual patient's response. Significantly positive gains of intracellular constituents occurred during the anabolic phase of injury but improved nitrogen retention was not found during the catabolic phase. In this study, caloric and nitrogen intake was below predicted levels required to achieve equilibrium during the catabolic phase of injury, and this level of nutrient administration may have limited the anabolic effects of growth hormone in the early postburn period. Studies by Gump and associates (Gump FE, Schwartz MS, Prudden JF, Amer J Med Sci 239:27-32, 1960)[11] in laboratory animals, and Pearson, Soroff, et al (Pearson E, Soroff HS, Prudden JF, Schwartz MS, Amer J Med Sci 239:17-25, 1960)[12] in patients, indicate an intricate interrelationship between the extent of nutrient support and the anabolic effects of growth hormone; that is, a minimal level of nutritional intake must be achieved before HGH can enhance nitrogen storage. The purpose of this study is to evaluate the anabolic effects of human growth hormone when administered with high caloric feedings during the catabolic phase of thermal injury.

MATERIALS AND METHODS

Patients

Seven male patients were studied. The average patient age was 25 years (range 16-41), and mean burn size was 54 per cent total body surface (33.5-75.5). All patients were studied before coverage of the burn wound was achieved, with four of the seven patients entered into the study on the 14th to 17th postburn day (Table 1). Two individuals with excessive burns required staged grafting procedures, and were not healed when studied on the 39th and 84th day postinjury. Finally, the seventh patient, referred to this unit six months following a 75 per cent flame burn, was nutritionally depleted and still required grafting of 34 per cent of the body surface. Because of his severe nutritional disability, he was given human growth hormone eight months following his thermal injury while preparing the wound bed for final skin coverage.

Human Growth Hormone

Human growth hormone is a protein of human origin extracted from pituitary glands obtained at autopsy, following the procedures described by Raben (Raben MS, Science 125:883-884, 1957)[13] as modified by Hartree (Hartree AS, Biochem J 100:754-761, 1966).[14] Following chromatographic extraction, human growth hormone in its pure form appears white, and physical chemical characteristics of the substance confirm its final purity. If satisfactory purification standards are achieved, the potency of the preparation is determined by biologic assay. Potency of HGH is expressed in international units, which is based upon the effect of the extract on skeletal rate of growth in hypothesectomized rats. After biologic assay, the lyophilized hormone is packaged in vials containing 10 international units per receptacle and frozen. Previous studies with the preparation have demonstrated growth stimulation in hypopituitary dwarfs with the administration of 5 to 20 international units per week, with the frequency of administration varying from daily to two or three times weekly. Toxicity with this preparation has not been encountered, although local signs of irritation at the injection site have occurred in dwarf patients. In addition, some individuals have developed resistance to HGH with long-term use, presumably due to the development of antibodies.

Study Design

Following initial resuscitation and the period of ileus and gastrointestinal dysfunction which follows major injuries, all patients entered into the study received predicted nitrogen and caloric intakes necessary to achieve nitrogen and caloric equilibrium. Nitrogen intake

TABLE I. CHARACTERISTICS OF PATIENTS STUDIED

PATIENT	AGE	WEIGHT	PER CENT TOTAL BURN	PER CENT THIRD DEGREE	BODY SURFACE AREA	POSTBURN DAYS STUDIED	
						CONTROL PERIOD	HCH PERIOD
1	41	53.0	60	8	1.65	74–80	81–89
2	19	77.0	33.5*	33.5	1.98	252–258	245–251
3	28	58.0	75.5	57	1.90	46–52	39–45
4	16	62.0	38	14	1.79	21–27	14–20 28–34
5a	18	66.0	64	22	1.88	14–20	21–27
5b						61–67	54–60
6	21	76.5	45	26	1.95	17–23 31–37	24–30
7	34	85.0	60	16.5	2.00	21–27	14–20 28–34
Mean	25	68	54	25	1.88		

*Initial burn 75 per cent; patient partially grafted then transferred to this institute for further care.

was calculated from previously determined guidelines estimated to achieve equilibrium (Soroff HS, Pearson E, Artz CP, Surg Gynec Obstet 112:159-172, 1961),[15] and caloric intake was determined by maintaining a nitrogen to caloric ratio between 1:125 - 1:150. In addition, basal energy requirements were predicted from basal energy production, calculated from oxygen consumption and carbon dioxide production measurements which were obtained using Douglas bag collection of expired gas and gas analysis techniques. Daily caloric and nitrogen intakes were maintained at a constant level for at least two weeks in all patients. In three individuals (Patients 4, 6, and 7), the study was extended an additional week to assess the effect of time on the metabolic changes following thermal injury. Finally, Patient 5 was studied twice, first during the acute phase of injury and later during convalescence.

Each study period was divided into seven-day intervals, one week assigned as the control period (diet alone) and the companion week designated as the experimental period (growth hormone plus same diet). The week of human growth hormone administration was randomized so that half of the patients studied received HGH during the first week of evaluation and the remaining individuals received HGH the week following the seven-day control period. In the three patients studied for a third week, the appropriate switch back was made to continue or discontinue the hormonal therapy in order to match the first study week.

During the experimental period, human growth hormone, 10 international units, was administered intramuscularly daily for seven consecutive days at 8:00 P.M. to augment the normal circadian rhythm of HGH (Glick SM, Goldsmith S, In International Symposium on Growth Hormone, edited by A Pecile and E Muller, Amsterdam, Excerpta Medica, 1968, p. 84)[16] The period of growth hormone administration was compared with the control period when diet alone was given. Because of the need for comparable consecutive weeks for comparison, only patients with a stable course and a prediction of a successful outcome following injury were studied. Individuals who developed complications during the study period, such as pneumonia, systemic sepsis, or gastrointestinal hemorrhage, were dropped from the study.

Dietary Intake

Four of the seven patients received enteral feedings, two patients received combined enteral-parenteral feedings, and one patient studied received all nutrients administered exclusively by the parenteral route (Table 2). All enteral feedings were given as meals, fed three times a day, with interval and nightly dietary supplements, and all food was prepared in the metabolic kitchen under the supervision of the research

TABLE 2. DIETARY INTAKE OF PATIENTS STUDIED

Patient	Route	RESTING METABOLIC RATE		DIET ADMINISTERED				
		Kcal/day	Kcal/m^2/day	Kcal/day	Kcal/m^2/day	Nitrogen (gm/day)	Nitrogen (gm/m^2/day)	Nitrogen-Caloric Ratio
1	Enteral-Parenteral	2269	1375	3818	2314	20.2	12.2	1:189
2	Enteral-Parenteral	2390	1207	4851	2450	31.4	15.8	1:154
3	Parenteral	3192	1716	4320	2274	25.8	13.6	1:167
4	Enteral	2965	1658	4179	2335	30.6	17.1	1:137
5(a)	Enteral	3132	1666	3514	1869	30.8	16.4	1:114
(b)	Enteral	1804	960	3375	1795	25.4	13.5	1:133
6	Enteral	2808	1440	4232	2170	34.2	17.5	1:124
7	Enteral	3788	1894	3810	1905	27.6	13.8	1:138
	Mean	2793	1490	4012	2139	28.2	15.0	1:144
	S.E.	±220	±108	±169	±88	±1.6	±0.6	±9

dietitian. Food was taken from the standard lots with known composition, and nutrient value determined by methods of dietary analysis previously developed at this institute (Spitzer ME, Ritchey C, Glennon JM, Villarreal Y, Mason AD Jr, Amer Diet Ass 62:44-46, 1973)[17] and by composition from food composition tables (Agriculture Department Handbook 8). Parenteral nutrients were administered by the central venous route, using solutions composed of hypertonic dextrose and amino acids, with the nutrient composition considered to be as indicated by the manufacturer. A constant intake was provided daily with only slight alterations in electrolyte and fluid administration, as determined by the clinical condition of the patient and serum electrolyte concentrations.

The predicted dietary intake was calculated to achieve nitrogen and caloric equilibrium, but, as additional patients were studied, it was apparent that positive nitrogen and caloric balance was obtained by the hypercaloric feedings. Thus, as further patients were studied, an ad lib feeding schedule was allowed during the first seven days of study, and the intake of the second week was matched with the first week to obtain a comparable nitrogen and caloric intake. Therefore, the last three patients studied did not receive caloric support comparable to the levels of nutrient intake achieved in the first four individuals.

Collection

All urine was collected in 24-hour pools and analyzed for creatinine, sodium, potassium, glucose, urea nitrogen, and total nitrogen. In the first four patients studied, stool was collected, pooled in weekly aliquots and analyzed for total nitrogen and stool fat content. Because no alterations were demonstrated in quantity of the gastrointestinal losses with growth hormone administration, stool was not collected in the remaining patients.

Body weights were taken daily when possible, and accurate intake and output records were maintained daily. Arterial blood was drawn at 7:00 A.M. on Day 1, 4, and 7 of each study week and analyzed for complete blood count, serum electrolytes, serum protein, urea nitrogen, creatinine, free fatty acids, glucose, calcium, phosphorus, uric acid, cholesterol, triglycerides, total fat, alkaline phosphatase, serum glutamic oxaloacetic transaminase, and bilirubin. On the fifth day of each study week, an intravenous glucose tolerance curve was performed. The patients were fasted after midnight, and at 6:00 A.M. a No. 16 polyvinyl catheter was placed in a large vein, using local anesthesia. Basal blood samples were drawn and 25 gram dextrose in 100 ml sterile water was administered intravenously over four minutes. Samples were

then drawn at 15, 30, 45, 60, 90, and 120 minutes following glucose administration, and blood glucose was measured by the Auto Analyzer, ferricyanide technique, and plasma-insulin was determined by double radioimmunoassay.

General Methods of Management and Patient Evaluation

Patients were treated by the exposed method of burn care until separation of the eschar, with or without biologic dressing, until autografting could be accomplished. All patients were treated with Sulfamylon[R] burn cream, applied every 12 hours to their wounds, and were bathed daily to remove the burn cream and inspect the burn wound. Wound exudate was not collected, for, although it entered into total balance, wound exudate reflects a transudation of body substrate rather than a metabolic end result.

Patients were exercised at frequent intervals and were not operated upon during the periods of study. Blood transfusions were given to two patients during the study in the form of 250 ml packed cells, which was administered because of a low hemoglobin. Patients were evaluated by the nursing staff, who noted their affect, appetite, and activity during the periods of study. The research dietitian recorded interest in food, appetite, and ability to consume all portions of the prepared meals.

RESULTS

Administration of Human Growth Hormone

Human growth hormone, administered as a fixed dose of 10 international units per day for seven continuous days, ranged in dose from 0.1176 I.U. per kilogram body weight per day to 0.1887 I.U. per kilogram per day. The only adverse reaction to the HGH preparation was the occurrence of a rash in one patient, which appeared as a fine macular eruption over the face and trunk and was noted on the fifth day of growth hormone administration. The rash subsided with completion of the seven-day course of human growth hormone concomitant with the withdrawal of several other medications. No local reaction or anaphylaxis was seen with the administration of human growth hormone.

Nitrogen and Mineral Excretion

While on a fixed nitrogen and caloric intake, a reduction of nitrogen excretion occurred in seven of the eight study periods with the administration of human growth hormone, 10 I.U. per day for seven

days (Table 3). This response usually occurred following the first day of therapy and progressed throughout all seven days of growth hormone administration, with some carry over effect noted in the subsequent day or two following the final dose of HGH. Urea was the primary nitrogenous component which decreased following HGH therapy; excretion of creatinine was unaltered by the hormonal administration. No consistent alteration in sodium or water excretion was noted when comparing control weeks with seven-day periods of HGH administration, as demonstrated in three of the patients in which a fixed fluid, sodium, and potassium intake was possible (Table 4). However, increased potassium retention was consistently observed, with the quantity of retained potassium and nitrogen approximating a 3:1 ratio, proportions similar to intracellular concentrations of these substances in muscle mass.

Blood Chemical Values

No alterations were seen in hemoglobin, hematocrit, white blood count, or serum electrolytes with the administration of human growth hormone. The anabolic agent exerted known metabolic effects by significantly decreasing blood urea nitrogen levels and increasing blood glucose, serum calcium, free fatty acids, and total lipids (Table 5). In addition, liver function studies appeared to return toward normal with a decrease in alkaline phosphatase, serum glutamic oxaloacetic transaminase, and total bilirubin.

Carbohydrate Metabolism

Although blood glucose concentration was slightly elevated during the period of HGH administration, this change was not statistically significant. Intravenous glucose tolerance tests, performed on the fifth day of control and HGH periods, demonstrated no differences in the fasting glucose level or peak value achieved with the administration of 25 grams of glucose. In addition, the disappearance rate for glucose (K value) was unchanged during the period of study (Figure 1). However, the asymptotes that the curves approached (the concentration of glucose with the slope [dg/dt] equal to zero) were significantly different in those patients with growth hormone induced nitrogen sparing. There was a higher asymptote or plateau of blood sugar approached by the glucose tolerance curves obtained during the growth hormone therapy when compared with those curves obtained during the control period. In the six patients that demonstrated protein sparing, glucose production appeared to be increased, an observation consistent with enhanced gluconeogenesis induced by HGH.

Insulin values obtained concomitantly during the intravenous glucose tolerance test demonstrated significant differences in insulin

TABLE 3. EFFECT OF HGH ON URINE NITROGEN EXCRETION
(Mean ± S.E.)

PATIENT	TOTAL NITROGEN		UREA NITROGEN		CREATININE	
	CONTROL	HGH	CONTROL	HGH	CONTROL	HGH
1	14.2 ± 1.1	7.3 ± 0.7*	11.9 ± 1.4	7.3 ± 0.5*	0.99 ± 0.08	0.85 ± 0.06
2	11.8 ± 1.5	9.3 ± 1.3	11.4 ± 1.2	8.6 ± 1.3	1.37 ± 0.09	1.42 ± 0.12
3	18.4 ± 1.9	12.4 ± 1.8*	15.6 ± 2.6	9.8 ± 1.3	1.04 ± 0.08	1.03 ± 0.13
4+	23.0 ± 1.8	17.8 ± 1.8	18.2 ± 1.2	15.8 ± 0.9	1.30 ± 0.02	1.23 ± 0.09
5(a)	28.5 ± 1.6	20.1 ± 3.0*	25.5 ± 1.0	17.3 ± 1.9*	1.41 ± 0.03	1.36 ± 0.07
(b)	17.2 ± 1.8	13.7 ± 0.5	14.4 ± 1.3	11.7 ± 0.6	1.18 ± 0.09	1.21 ± 0.07
6+	30.4 ± 1.6	24.3 ± 1.0*	29.1 ± 1.3	22.8 ± 1.3*	2.27 ± 0.12	2.30 ± 0.07
7+	30.0 ± 1.6	31.9 ± 1.8	21.2 ± 1.5	23.7 ± 1.3	2.11 ± 0.09	2.15 ± 0.13
Mean	21.7 ± 2.6	17.1 ± 2.9**	18.4 ± 2.2	14.6 ± 2.2++	1.46 ± 0.17	1.44 ± 0.18

* $p < 0.05$ by comparison of control with HGH period in a single patient

\+ Mean of first and third week compared with mean of second week

** $p < 0.005$ by paired t test

++ $p < 0.02$ by paired t test

TABLE 4. EXCRETION OF WATER, SODIUM, AND POTASSIUM WHILE ON A CONSTANT INTAKE IN THREE PATIENTS STUDIED*

		URINARY EXCRETION	
	INTAKE	CONTROL	HGH
H_2O	4106 ± 241	2571 ± 472	2508 ± 189
Na (mEq)	145 ± 9	73 ± 29	69 ± 34
K (mEq)	143 ± 18	124 ± 24	87 ± 27
Nitrogen (gm)	28.6 ± 2.7	19.3 ± 6.0	13.6 ± 5.4

* Mean ± S.E./24 hours

TABLE 5. BLOOD CHEMICAL VALUES*

	CONTROL	HGH	COMPARISON
Glucose (mg/100 ml)	104 ± 10	118 ± 8	NS+
Blood urea nitrogen (mg/100 ml)	16 ± 1	13 ± 2	$p < 0.01$
Calcium (mg/100 ml)	8.6 ± 0.4	9.1 ± 0.4	NS
Phosphorus (mg/100 ml)	4.3 ± 0.2	4.4 ± 0.4	NS
Total bilirubin (mg/100 ml)	0.7 ± 0.1	0.5 ± 0.1	NS
Alkaline phosphatase (K-A units)	29.2 ± 3.6	25.1 ± 4.8	NS
SGOT (Karoen units)	118 ± 20	89 ± 18	NS
Total proteins (gm/100 ml)	6.1 ± 0.3	6.1 ± 0.3	NS
Albumin (gm/100 ml)	2.6 ± 0.3	2.7 ± 0.3	NS
Uric acid (mg/100 ml)	4.3 ± 0.3	3.8 ± 0.4	NS
Creatinine (mg/100 ml)	0.93 ± 0.03	0.97 ± 0.07	NS
Cholesterol (mg/100 ml)	136 ± 16	127 ± 13	NS
Triglycerides (mg/100 ml)	142 ± 40	172 ± 27	NS
Free fatty acids (mEq/L)	1.2 ± 0.4	1.7 ± 0.6	NS
Total lipids (mg/100 ml)	440 ± 68	463 ± 53	NS

* Mean ± S.E. of values obtained on the fourth and seventh day of each week of study.

+ Comparison by paired t test; NS = Nonsignificant

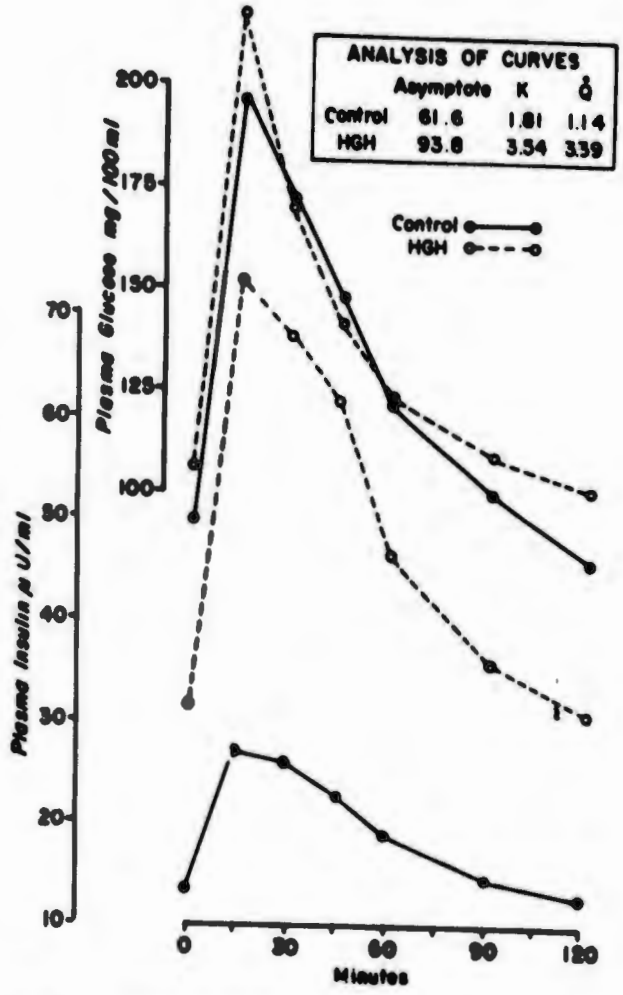

FIGURE 1. Insulin reset (below) occurred in the patients responding to HGH by sparing nitrogen, and the elevation above control levels was significant and related to the dose of HGH (if y = insulin, D = dose in IV/kg body weight, then $y = 17.8 + 120.3\ D$, $r^2 = 0.28$, $F_{1,14} = 5.537$, $p < 0.05$). However, the increased insulin output did not lower blood glucose (above), suggesting increased peripheral resistance exerted by HGH or free fatty acids. Analysis of the glucose tolerance curves demonstrate a significant elevation ($p < 0.05$) in asymptote approached by the curve during HGH therapy, an increased disappearance of glucose (K) into tissues (possibly a reflection of the elevated insulin levels) and increased production of glucose (Q) to support a curve with the characteristics demonstrated.

levels in the patients in whom nitrogen sparing occurred. This reset in basal insulin levels in response to a glucose load seemed unrelated to blood glucose concentration, reflecting the peripheral anti-insulin effect of growth hormone. In contrast to the patients with HGH induced nitrogen retention, one individual demonstrated a slight increase in nitrogen loss with growth hormone administration. His glucose tolerance curve obtained during the period of HGH administration demonstrated a lower calculated asymptote with no significant elevation in the serum insulin (Figure 2).

Subjective Effects

Four of the seven patients demonstrated some mood elevation, which occurred concomitantly with the administration of human growth hormone. In the same four patients, an increase in appetite was noted and there was little difficulty in achieving the desired level of nutrient intake. In the youngest individual, euphoria and an increase in libido was noted during the weeks of hormonal therapy and his mood changed markedly on the alternate control week. Patient families also reported this difference in affect and mood, and these observers could often predict when the patient received HGH.

DISCUSSION

Human growth hormone has the unique physiologic properties of improving nitrogen retention and weight gain in normal man and stimulating linear growth in hypopituitary dwarfs. This anabolic effect is characterized by increased retention of all intracellular constituents, augmenting growth of the lean body mass and the skeleton. Nitrogen retention reaches its maximum level in three to six days of therapy (Henneman PH, Forbes AP, Moldawer M, Dempsey EF, Carroll EL, J Clin Invest 39:1223-1238, 1960)[18] with an increase in nitrogen balance of about 2.4 grams in nitrogen per day in normal healthy man (Forbes AP, Jacobsen JG, Carroll EL, Pechet MM, Metabolism 11:56-75, 1962).[19] This effect is dose related, with little nitrogen storing at 0.1 mg per day, and maximal retention produced with doses of 10 mg per day in normal man (Henneman PH, Forbes AP, Moldawer M, Dempsey EF, Carroll EL, J Clin Invest 39:1223-1238, 1960.[18] In addition, the action of growth hormone may be more prolonged, for normal growth and development have been reported in hypopituitary dwarfs maintained on weekly or twice-weekly administration of the agent. Some sustained effect was noted in this study with improved nitrogen retention seen for up to 48 hours following the last injection of HGH.

The influence exerted by HGH on protein containing mass is reflected in many of the blood chemical parameters studied in these

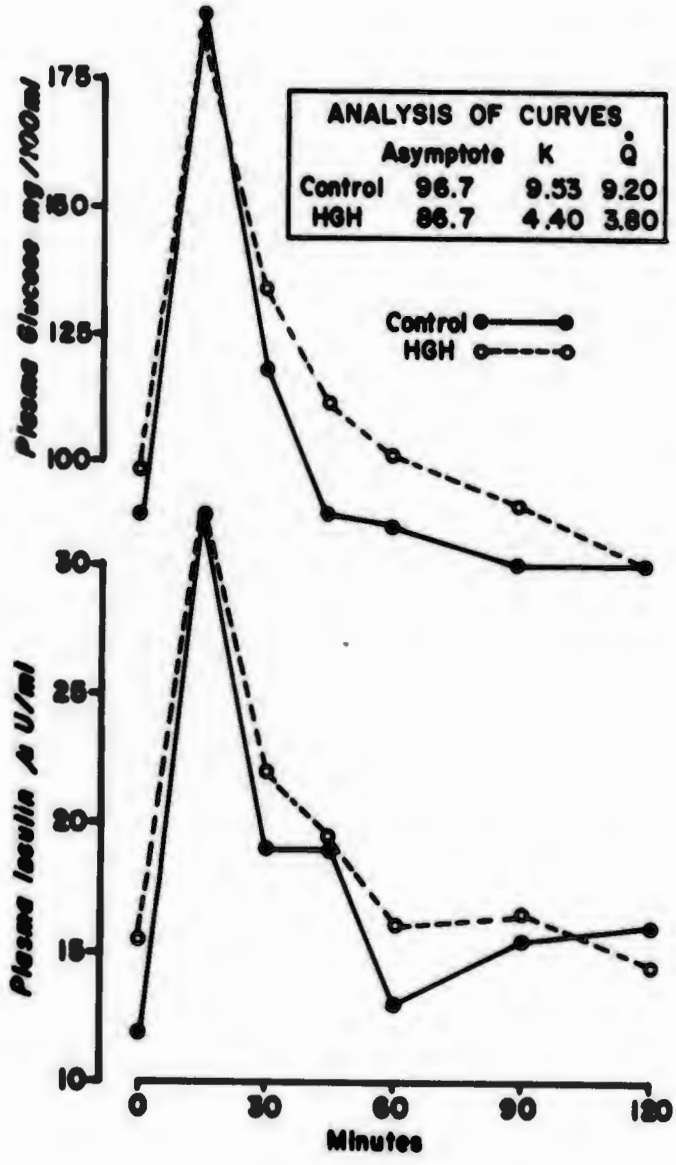

FIGURE 2. The single patient who failed to demonstrate protein sparing had only slight insulin augmentation, blunted glucose flow (K) into tissues, and the most marked increase of serum free fatty acid seen in this group. It appears that the adipokinetic effect of HGH dominated over the insulinogenic effects and no nitrogen sparing occurred, a metabolic response similar to the effects reported following growth hormone administration to fasting man.

patients. The reduction in blood urea nitrogen while on a constant caloric and nitrogen input and while maintaining normal renal function is one indicator of decreased urea production and increased protein sparing. This increased preservation of protoplasmic mass is accomplished by mobilizing free fatty acids from body fat stores, demonstrated by the increased level of free fatty acid that occurred with HGH administration. Increased fat oxidation alters the body's fuel mix, elevating oxygen consumption and lowering the respiratory quotient toward 0.70 (Roe CF, Kinney JM, Surg Forum 13:369-371, 1962;[9] Soroff HS, Rozin RR, Mooty J, Lister J, Raben MS, Ann Surg 166:739-752, 1967).[10] Also associated with the HGH administration was an apparent improvement in liver function as indicated by the decrease in bilirubin, alkaline phosphatase, and enzyme levels. Alterations in hepatic enzymes and bilirubin have been described with fasting and may be related to increased levels of glucagon and epinephrine (Bloomer JR, Barrett PV, Rodkey FL, Berlin NI, Gastroenterology 61:479-487, 1971),[20] or loss of hepatic-cellular protein (i.e., labile nitrogen) which serves as one of the substrates for synthesis of new glucose during starvation or injury (Robinson GA, Butcher RW, Sutherland EW, Cyclic AMP, New York, Academic Press, 1971, Chapter 6).[21] Further evaluations are now in progress to examine the specific interactions between growth hormone and liver function following fasting and trauma. Unger and coworkers (Muller WA, Faloona GR, Unger RH, New Eng J Med 285:1450-1454, 1971)[3] have described the interaction between insulin and glucagon in regulating hepatic gluconeogenesis. That insulin alone can produce marked nitrogen retention and reduction in blood urea nitrogen in burn patients has been demonstrated by Hinton, Littlejohn, Allison, and Lloyd (Hinton P, Littlejohn S, Allison SP, Lloyd J, Lancet 1:767-769, 1971).[5] Administering high doses of insulin (200-600 units per day) with 50 per cent glucose and water infused by vein, normal blood glucose levels were maintained, associated with significant reductions of nitrogen and potassium losses following injury. In some animals, insulin appears necessary for the anabolic effects of growth hormone to be manifested, while in other species (and man) these two hormones seem to act in concert with augmented or additive effects (Scow RO, Wagner EM, Ronov E, Endocrinology 62:593-604, 1958).[22] In hepatic perfusion studies, growth hormone alone enhances transport of amino acid substrates into liver cells and stimulates protein synthesis (Korner A, Ann NY Acad Sci 148:408-418, 1968).[23] It would appear that the anabolic effects of human growth hormone require nutrient intake to first establish a level of hormonal elaboration (insulin output), which is complimented by the administration of HGH. As a result of this hormonal environment, there is preservation of lean body mass and increased utilization of fat as a fuel source, an alteration in metabolism which has previously been described with HGH administration during the hypermetabolic state induced by administration of triiodothyronine (Bray GA, Raben MS, Londono J, Gallagher TF Jr, J Clin Endocr Metab 33:293-300, 1971).[24]

The use of human growth hormone in the controlled manner described by this study may not be the most efficacious method of achieving a drug effect in burn patients. Earlier work by Liljedahl and associates (Liljedahl SO, Gemzell CA, Plantin LO, Birke J, Acta Chir Scand 122:1-14, 1961)[8] indicates that patients improve their intake of calories and nitrogen with HGH administration and hence improve nitrogen and caloric equilibrium. Because this study provided a fixed feeding schedule, the improvement in appetite could only be noted by the acceptance of the diet by the patient. Improved appetite did seem to occur with the hormonal administration in four patients, associated with mood elevation, apparent increased mobility, and willingness to exercise and increase self-care. These gains, if demonstrated in further studies, should have specific effects on overall convalescence and rehabilitation of thermally injured patients.

Although nitrogen retention and maintenance of protoplasmic mass would appear to be a desirable end result in the care of the burn patient, associated gains may be more meaningful clinically. Maintenance of serum protein and improvement in liver function may represent clinical confirmation of laboratory experiments (Liberti JP, Wood DM, DuVall CH, Endocrinology 90:311-315, 1972;[25] Jeejeebhoy KN, Robertson AB, Sodtke U, Foley M, Biochem J 119:243-249, 1970)[26] and be benefits which enhance survival and recovery following injury.

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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 6974	73 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
	A. NEW	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 (Precede with Security Classification Code): (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	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 73	.7	20
	CURRENT 74	.5	15

19. RESPONSIBLE DOD ORGANIZATION
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RESPONSIBLE INDIVIDUAL
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ASSOCIATE INVESTIGATORS
NAME: James M Long, MAJ, MC
NAME: Arthur D Mason, Jr, MD DA

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code): (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 01 - 73 06 Three patients have been studied to date, consisting of a total of five studies. Alpha block was achieved in all five individuals, as determined by orthostatic hypotension and slight nasal stuffiness. No significant decrease in oxygen consumption was seen with alpha blockade. In one patient, adequate beta blockade was achieved with a marked return of oxygen consumption to normal.

*Available to contractors upon originator's approval

ANNUAL PROGRESS REPORT

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

REPORT TITLE: THE EFFECT OF ADRENERGIC BLOCKADE ON THE HYPERMETABOLIC RESPONSE FOLLOWING THERMAL INJURY

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

1 July 1972 - 30 June 1973

Investigators:

Douglas W. Wilmore, MD, Major, MC
James M. Long, MD, Major, MC
Arthur D. Mason, Jr., MD
David W. Johnson, SP4
Robert W. Skreen, PV2
Basil A. Pruitt, Jr., MD, Colonel, MC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

REPORT TITLE: THE EFFECT OF ADRENERGIC BLOCKADE ON THE HYPERMETABOLIC RESPONSE FOLLOWING THERMAL INJURY

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

Period covered in this report: 1 July 1972 - 30 June 1973

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Reports Control Symbol MEDDH-288(R1)

Sympathetic blockade was carried out in four patients with an average burn size of 62%. No consistent effect was seen with alpha blockade alone but a decrease in metabolic activity occurred with combined alpha and beta blockade. This study suggests that increased catecholamines are the neuroendocrine mediator for the post-traumatic hypermetabolic response following burn injury.

Hypermetabolism
Catecholamines
Energy

THE EFFECT OF ADRENERGIC BLOCKADE ON THE HYPERMETABOLIC RESPONSE FOLLOWING THERMAL INJURY

The etiology of the profound hypercatabolic response following burn injury is unknown. Similarities between thermally injured patients and individuals with thyrotoxicosis prompted early endocrine studies by Dr. Cope and his associates, but the increased oxygen consumption could not be related to abnormal thyroid function (Cope O, et al, Ann Surg 137:165, 1953).[1] Increased evaporative water loss from the burn wound results in surface cooling and may stimulate metabolic rate to generate more heat in order to maintain normal body temperature. However, Zawacki and associates found no reduction in metabolic rate when evaporation was blocked with a water impermeable membrane which covered the burn wound (Zawacki BE, et al, Ann Surg 171:236, 1970),[2] Gump and Kinney reported a dissociation between metabolic rate and evaporative water loss (Gump FE, Kinney JM, Surg Clin N Amer 50:1235, 1970),[3] and, more recently, studies in this unit have shown that patients placed in 33° ambient temperature maintain an elevated and sustained metabolic rate.

Hormonal stimulation of heat production occurs with the elaboration of thyroid hormone or catecholamines (Havel RJ, Anesthesiology 29:702, 1968).[4] Thyroid function has been carefully studied, and reports indicate that thyroid activity is normal following burn injury. Catecholamines are calorigenic hormones increasing oxygen consumption when infused or stimulating heat production during cold exposure (Hsieh ACL, et al, Amer J Physiol 170:247, 1957).[5] Catechols are elevated following thermal injury, and their elaboration per unit time has been related to the extent of injury (Hume DM, et al, Ann Surg 143:316, 1956)[6] and to the oxygen consumption of the patient (Harrison TS, et al).[7] Catecholamines stimulate lipolysis resulting in fatty acid mobilization, and epinephrine secretion is related to the peripheral glucose intolerance associated with stress (Porte D, et al, J Clin Invest 45:228, 1966).[8] Sympathetic regulation of insulin and glucagon secretion may be the controlling features of hepatic gluconeogenesis following starvation or trauma (Muller WA, et al, New Eng J Med 285: 1449, 1971).[9] The purpose of this study is to determine the effect of adrenergic blockade on the metabolic response following thermal injury.

MATERIALS AND METHODS

Four adult male patients with an average burn size of 62% (range 51-75%) were studied between the 4th and 30th postburn days. All patients were hypermetabolic with an average metabolic rate of 67.5 Kcal/m^2/hour. Blockade was effected by the intravenous

administration of phentolamine (Regitine [R]), 75 mg infused over a 30 to 45 minute period (alpha blockade), or phentolamine , 75 mg and propanolol (Indural [R]), 75 mg infused over 30-45 minutes (combined alpha and beta blockade). The infusion rate and dose of blocking agents were determined by cardiovascular response during drug administration. Basal studies of pulse rate, blood pressure, respiratory rate, minute volume, metabolic rate, and serum free fatty acids were performed after several hours of fasting and rest, and these studies were repeated after the administration of the blocking agents. Adequacy of beta adrenergic blockade was determined by the absence of response in heart rate or increase in serum free fatty acids following administration of 2-10 mcg Isuprel IV. Orthostatic hypotension and nasal stuffiness were present in patients following alpha blockade.

RESULTS

A varied response occurred with the administration of phentolamine, but a significant decrease in metabolic rate was associated with the combined alpha and beta blockade (Table 1). In one patient, only propanolol was administered with a decrease in metabolic activity comparable to that observed with both alpha and beta blockade. In all patients, there was an associated decrease in minute volume, pulse rate, blood pressure, and free fatty acid level with adequate blockade (Table 1). A large quantity of drug was required for effective competitive blockade of the beta receptor system and the decrease in sympathetic activity appeared to last only two to three hours. In one patient, serial oxygen consumption measurements demonstrated a blocking effect on metabolic rate that appeared to be a dose-related response, with return of metabolic activity to preblockade levels shortly after the end of the drug infusion (Fig.).

DISCUSSION

The response to stress is mediated by way of the adrenergic nervous system, and increased secretion of catecholamines occurs in varying degrees during simple anxiety states, with the stress of starvation, and following major trauma. Adrenergic blockade suppressed tachycardia and increases in free fatty acids and blood glucose in race car drivers under stress conditions (Taggart P, Lancet 2:256, 1972).[11] Similar blockade is reported to suppress the tachycardia and lipid response in public speaking, while taking exams, and during other anxiety states.

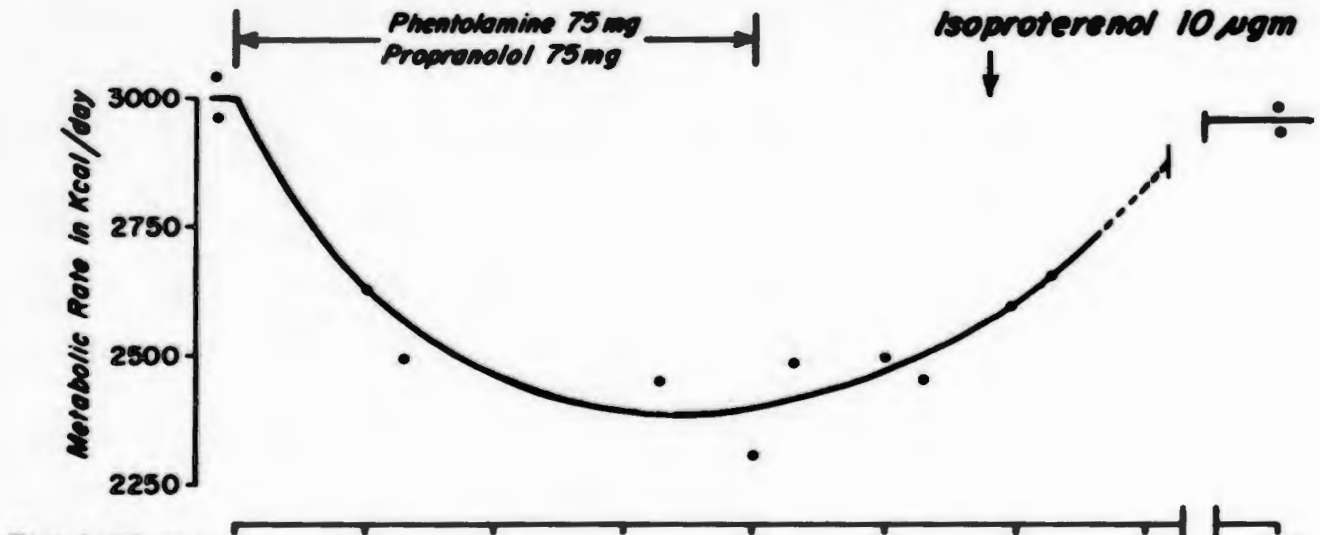
Adrenergic receptor blockade has been used in the treatment of the hypermetabolic state associated with thyrotoxicosis. Stout and associates reported combined alpha and beta blockade over an average 71 days in eight patients (Stout BD, Ann Int Med 70:963, 1969),[12] and

TABLE 1. RESPONSE TO SYMPATHETIC BLOCKADE

	Pulse		Blood Pressure		Respiratory Rate		Minute Volume		Metabolic Rate		Free Fatty Acids	
Patient Study	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Alpha Blockade												
1	100	84	142/70	120/50	12.0	12.5	12.1	16.1	55.0	60.9	–	–
2	120	110	–	–	29.0	32.9	38.2	29.1	80.2	76.8	2.0	8.0
3	126	118	–	–	25.6	24.9	24.3	19.7	76.8	66.7	1.3	3.5
Mean*	115	104			22.2	23.4	24.87	21.63	70.7	68.13	1.65	5.75
SEM	± 7.86	± 10.26			± 5.19	± 5.93	± 7.54	± 3.88	± 7.89	± 4.65	± .25	± 2.25
Alpha and Beta Blockade												
4	118	84	124/72	88/52	40.0	38.0	35.4	32.2	68.0	59.9	4.0	1.7
5	96	88	160/82	124/68	16.0	19.5	18.0	9.4	66.8	44.9	4.6	2.3
6	98	84	128/80	100/70	22.7	24	12.0	10.1	46.9	42.4	5.0	3.5
7	120	96	120/100	90/	17.7	14.0	22.6	19.0	78.7	64.7	–	–
Mean	108	88	131/84	100/64	24.1	23.88	22	17.68	65.1	52.98	4.53	2.5
SEM	± 6.38	± 2.83	± 9.98/± 5.91	± 8.26/± 5.70	± 5.49	± 5.13	± 4.97	± 5.31	± 6.63	± 5.49	± .29	± .53
p	p < .05		p < .01		NS		NS		p < .05		p < .02	

*Effect not statistically different (NS) by paired t test.

RESPONSE TO COMBINED α AND β SYMPATHETIC BLOCKADE



Time in minutes	0	15	30	45	60	75	90	120	430
Blood pressure	140/100	140/100	110/85	80/	80/	90/	97/	100/	135/100
Pulse	120	120	110	110	96	96	100	120	120
Respiratory rate	20	18	16		14	14	14		20
Core temp.	39.4°C							38.9°C	

found oxygen consumption significantly reduced (BMR before therapy +19, after therapy +4). Body weight increased an average of 12 pounds during the same time period. This information may be applicable to the burn patient, for a similar neuroendocrine and metabolic response may occur in all "stress" states.

Finally, if adrenergic output is the effector in the metabolic responses following injury, then the infusion of catecholamines should generate increased heat, raise body temperature and blood pressure, and elevate free fatty acids. Such a response is seen in a hypothermic patient with bacteremia who received an Isuprel infusion and was studied before and 45 minutes following drug administration (Table 2). This data adds supportive evidence to the thesis that stimulation of the adrenergic nervous system may act as the neuroendocrine mediator for the post-traumatic metabolic alterations which are known to occur following injury. Further studies will evaluate the long-term effects of adrenergic blockade in the post-traumatic course following major thermal injury.

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TABLE 2.

METABOLIC RESPONSE TO ISUPREL INFUSION IN SEPTIC MAN

	Preinfusion	Isuprel (5 μgm/min IV)
Temperature (rectal) °C.	95.5	98.2
Pulse (beats/min)	90	124
Blood pressure (mmHg)	115/80	134/70
Urine volume (ml/hr)	155	114
Urine catecholamines (μgm/hr)	930	1,391
Free fatty acids (mEq)	1.85	4.5
Respiratory rate (per minute)	20	24
Minute volume (L/min)	14.2	16.2
$\dot{V}O_2$ (L/min)	0.258	0.314
$\dot{V}CO_2$ (L/min)	0.234	0.282
Metabolic rate (Kcal/m^2/hr)	44.4	53.7
RQ	0.92	0.89

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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 OD 6981	73 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. GRP'G INSTR'G	8A. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
72 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	120
b. CONTRIBUTION				
c. CONTRIBUTING				

11. TITLE (Precede with security classification code): (U) Thrombophlebitis - Etiology and Prevention 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 10	73 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	PROFESSIONAL MAN YRS	FUNDS (In thousands)
FISCAL YEAR PRECEDING 73	.3	10
FISCAL YEAR CURRENT 74	0	0

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: D W McKeel, Jr, MAJ, MC DA

21. GENERAL USE: FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede each with security classification code): (U) Suppurative Thrombophlebitis; (U) Dogs; (U) Thrombophlebitis; (U) Intravenous Catheters; (U) Long-Term Intravenous Therapy

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 role of catheter composition in the etiology of thrombophlebitis. To develop an animal model of suppurative thrombophlebitis. To evaluate the effect of blood flow in the prevention of infusion thrombophlebitis. To prevent infusion phlebitis in injured military personnel.

24. (U) Dog external jugular veins have been catheterized with silastic and polyethylene catheters which have been left in place 10 days. At this time the veins are excised and examined histologically. Dogs will be injected with an intravenous dose of staphylococcus in an attempt to produce suppurative thrombophlebitis. Arteriovenous fistulas will be made in dogs and the venous limb catheterized to evaluate the role of flow in the etiology of thrombophlebitis.

25. (U) 72 07 - 73 03 Polyethylene catheters produce marked thrombophlebitis. There is little reaction about the silastic catheters. Bacteria play an unimportant role in the development of phlebitis secondary to catheterization.

* Applicable to contractors upon notification of approval.

DD FORM 1 MAR 83 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 68 AND 1498-1, 1 MAR 83 (FOR ARMY USE) ARE OBSOLETE.

ANNUAL PROGRESS REPORT

PROJECT NO. 3A161101B71R-01, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: THROMBOPHLEBITIS--ETIOLOGY AND PREVENTION IN BURNED MILITARY PERSONNEL. THE ROLE OF CATHETER COMPOSITION IN THE DEVELOPMENT OF THROMBOPHLEBITIS

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

1 July 1972 - 30 June 1973

Investigators:

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Daniel W. McKeel, Jr., MD, Major, MC
Paul Silverstein, MD, Major, MC
Harrel L. Walker, MS

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A161101B71R-01, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: THROMBOPHLEBITIS--ETIOLOGY AND PREVENTION IN BURNED MILITARY PERSONNEL. THE ROLE OF CATHETER COMPOSITION IN THE DEVELOPMENT OF THROMBOPHLEBITIS

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

Period covered in this report: 1 July 1972 - 30 June 1973

Investigators: Gary W. Welch, MD, Major, MC
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Harrel L. Walker, MS

Reports Control Symbol MEDDH-288(R1)

Cannulation of the external jugular vein with a polyethylene catheter produced marked thrombophlebitis at ten days. Similar catheterization with a silastic catheter produced minimal changes in the vein. Cultures of thrombosed veins indicated an insignificant role of bacteria. A sequential analysis of the development of catheter thrombophlebitis is also described.

Thrombophlebitis
Suppurative thrombophlebitis
Dogs
Longterm intravenous therapy
Intravenous catheters

THROMBOPHLEBITIS--ETIOLOGY AND PREVENTION IN BURNED MILITARY PERSONNEL. THE ROLE OF CATHETER COMPOSITION IN THE DEVELOPMENT OF THROMBOPHLEBITIS

The development of longterm intravenous therapy in medical and surgical patients has created the problem of iatrogenic thrombophlebitis, with its associated morbidity and mortality. In several studies, the incidence of phlebitis varied from 13 to 39 per cent. Indeed, cultures of catheter tips have demonstrated bacterial contamination in a large percentage of the catheters.

Many factors may contribute to the development of thrombophlebitis including (1) pH of infusate, (2) duration of infusion, (3) size of cannula (length and bore), (4) presence of bacteria, (5) infusate, (6) venous flow characteristics, (7) skin preparation and technique of venipuncture, (8) catheter composition.

The purpose of this study was to compare grossly and histologically the phlebitis producing properties of polyethylene catheters and silastic catheters.

MATERIALS AND METHODS

Twenty-one mongrel dogs of various sizes were anesthetized with nembutal. Their necks were shaved and prepped, and bilateral venous cutdowns were made over the external jugular veins. An 18-gauge, four-inch polyethylene catheter was introduced in one vein. The end protruding from the vein was heat sealed and the skin incision closed with Michel clips. The opposite external jugular vein was then cannulated in a similar fashion with a 16-gauge silastic catheter. The skin incisions were closed and the animals returned to the pens and randomly divided into three groups.

The first group of 10 dogs was sacrificed at 10 days and the catheterized veins were excised, leaving the catheter in situ. The tissue specimens, each 8-10 cm long, were fixed in 10 per cent neutral buffered formalin for 48 hours. Transverse sections at 1 cm intervals were embedded in paraffin and sectioned at 5 microns. Staining procedures included hematoxylin-eosin, Verhoeff-Van Gieson for elastic tissue, Masson's trichrome for connective tissue elements, and several types of bacterial stains. This allowed evaluation of the effects of longterm catheter placement upon veins.

A second group of eight dogs were sacrificed in pairs on alternate even days following placement of the catheters. The veins were excised and prepared as above to allow examination of the pathogenesis of catheter thrombophlebitis.

The remaining three dogs were sacrificed at day 10 and their necks prepared with a surgical prep. The catheterized veins, with the catheter in place, were excised aseptically and qualitative and quantitative bacteriologic cultures were done.

RESULTS

The dogs, catheterized and subsequently sacrificed at 10 days, demonstrated striking gross anatomic difference between veins cannulated with the polyethylene and those cannulated with the silastic catheters. The vein containing the silastic catheter showed a small amount of reaction and periadventitial fibrosis about the entry point of the catheter. The vein itself remained patent and a few millimeters proximal to the puncture site it appeared grossly normal. There was no impedance to blood flow.

The veins catheterized with the polyethylene catheter, on the other hand, were consistently thrombosed, fibrotic, and appeared as narrow cords, with very little if any observable blood flow. In addition, there was marked reaction about the length of the vein, necessitating en bloc dissection with surrounding tissue.

Histologic examination confirmed the gross observations. The veins catheterized with silastic showed inflammatory reaction about the puncture site and often demonstrated formation of a small thrombus at the entry site. In contrast, the veins cannulated with polyethylene were extensively thrombosed. By the end of 10 days, there was sufficient reaction within the vein wall to render the thrombus firmly adherent to the intima. Recanalization was well underway and myxomatous degeneration was present within the thrombus.

The veins from the second group of dogs sacrificed at two-day intervals after catheterization permitted examination of the histogenesis of catheter thrombophlebitis. At two days, there was immature thrombus present in both veins. The clot in the vein cannulated with silatic was localized to the site of entry of the catheter in contrast to the vein containing the polyethylene catheter which contained clot extending for several centimeters from the puncture

site. By day 4, the thrombus about the polyethylene catheter was infiltrated by fibroblasts, and during the next four days there was progressive organization of the thrombus. By comparison, there had been little change in the vein containing the silastic catheter.

Approximately eight days after placement of the polyethylene catheter, recanalization had begun within the thrombus and there was sclerosis of the vein wall. At 10 days, the presence of myxomatous cells was noted with concomitant degeneration of the thrombus. Similar changes were noted within the veins containing the silastic catheters. In these veins, however, the process was localized and extended over millimeters rather than centimeters as in the veins containing the polyethylene catheters.

The three veins containing silastic and three containing polyethylene cannulae were cultured. Of these, one containing silastic and two containing polyethylene catheters demonstrated growth of staphylococci. The quantities involved (10^2, 10^3, 10^4 bacteria/ml of tissue), however, lead one to believe this is the result of contamination rather than bacterial or suppurative thrombophlebitis.

DISCUSSION

Intravenous administration of fluids carried with it the risk of infusion thrombophlebitis. Numerous possible causes have been considered as contributing to the development of phlebitis. Fonkalsrud (Fonkalsrud EW: Clin Pediat 8: 135-137, 1969)[1] considered six factors to be involved in the development of infusion phlebitis. There were: (1) pH; (2) duration of infusion, (3) size of cannula, (4) composition of infusion fluid, (5) presence of bacteria, and (6) composition of the catheter. Tse (Tse RL, Lee MW: JAMA 215: 642, 1971)[2] also believed that the pH of the infusion contributed to the development of phlebitis. They found the pH of glucose solutions to vary between 4.40 and 4.70. When Fonkalsrud (Fonkalsrud EW, J Surg Res 8: 539-543, 1968)[3] buffered glucose solutions to a pH of 7.4, there were fewer endothelial changes in the veins of dogs administered the solution as compared to unbuffered solution.

Hastbacka and coworkers (Hastbacka J, Tammisto T, Elving G, and Tiitinen P: Acta Anaesth Scand 10: 9-30, 1966)[4] verified that the duration of infusion contributes to the development of phlebitis. They reported a two-fold increase in infusion phlebitis when the length of infusion was increased from

two to four hours. Size of the catheter, as well as the duration of infusion, was implicated by Swanson (Swanson JT, Rocky Mountain Med J 66: 48-51, 1969)[5] who reported an overall incidence of phlebitis of 28 per cent.

Collins et al (Collins RN, Braun PA, Zinner SH, Kass EH, New Eng J Med 279: 340-343, 1968)[6] report on bacterial contamination of polyethylene catheters used in patients. In their study, 39 per cent of the patients developed phlebitis. Of those catheters removed from patients with phlebitis, 43 per cent grew organisms upon culture and 19 per cent of the cases resulted in bacteremia. Norden (Norden CW: J Infect Dis 120: 611, 1969)[7] attempted to circumvent this complication by applying an antibiotic ointment to the catheter site. Four per cent of the catheter tips were culture positive regardless of treatment. In addition, he reported a higher incidence of Candida with the use of the antibiotic. A comparison of the incidence of thrombophlebitis in the US Army Institute of Surgical Research for the year 1969 with the year 1970 showed no difference although antibiotic ointment was used around catheter sites in 1970.

The role of catheter composition in thrombus formation was examined by Nejad (Nejad MS, Radiology 91: 248-250, 1968).[8] He cannulated the carotid and jugular veins for 30 minutes with 10 centimeter lengths of catheters composed of teflon, polyethylene, and silastic. After removal of the catheters, he measured the amount of clot formed. He found the thrombogenic properties varied according to the manufacturer and catheter composition.

In our study, there is no doubt that the polyethylene catheters produced a marked reaction both within the vein and in the periadventitial structures. All the veins cannulated with the polyethylene catheters were thrombosed by 10 days. Those veins catheterized with silastic, however, remained widely patent and appeared grossly normal. Because there was no infusion fluid, the diameter and length of the catheter composition played a significant role in the development of phlebitis. Culture results likewise do not appear to implicate bacteria in this model. Additional studies are underway to further evaluate the role of bacteria and flow to the development of infusion phlebitis.

SUMMARY

1. Twenty-one mongrel dogs had one external jugular vein catheterized with a polyethylene and the other with a silastic catheter of equal length and diameter.

2. All the veins containing the polyethylene catheter were thrombosed at 10 days.

3. The veins cannulated with the silastic catheters were affected to only a minor degree.

4. Thrombosis occurs by day 2 of catheterization in the veins containing polyethylene and progresses over the next several days.

5. There is no progression of thrombosis in the veins containing the silastic catheter.

6. Culture of the veins reveals only minimal contamination.

REFERENCES

1. Fonkalsrud EW: Postinfusion phlebitis in infants and children. Clin Pediat 8: 135-137, 1969.

2. Tse RL, Lee MW: pH of infusion fluids: A predisposing factor in thrombophlebitis. JAMA 215: 642, 1971.

3. Fonkalsrud EW: The effect of pH in glucose infusions on development of thrombophlebitis. J Surg Res 8: 539-543, 1968.

4. Hastbacka J, Tammisto T, Elving G, and Tiitinen P: Infusion thrombophlebitis. Acta Anaesth Scand 10: 9-30, 1966.

5. Swanson JT: Thrombophlebitis after intravenous infusion. Factors affecting its incidence. Rocky Mountain Med J 66: 48-51, 1969.

6. Collins RN, Brawn PA, Zinner SH, and Kass EH: Risk of local and systemic infection with polyethylene intravenous catheters. New Eng J Med 279: 340-343, 1968.

7. Norden CW: Application of antibiotic ointment to the site of venous catheterization - A controlled trial. J Infect Dis 120-611, 1969.

8. Nejad MS: Clothing on the outer surface of vascular catheters. Radiology 91: 248-250, 1968.

PUBLICATIONS AND/OR PRESENTATIONS

None.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0E 6387	73 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
72 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	316
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) Use of an Intermittent Compression Unit to Decrease Postburn Edema 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	73 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 73	.5	17
	CURRENT 74	0	0

19. RESPONSIBLE DOD ORGANIZATION
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Burn Study Branch
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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
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NAME: Joseph A Moylan, Jr, MAJ, MC DA

21. GENERAL USE: FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code): (U) Military Burn Patients; (U) Intermittent Compression; (U) Postburn Edema; (U) Phalangeal Joints

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 an intermittent compression unit's capability to prevent or reduce edema in the early postburn period in a military population.

24. (U) Eight freshly burned patients with extremity burns will be cleaned and debrided on admission. Measurement of the PIP and DIP joints, and wrists will be made with unmarked sterile aluminum tapes and range of motion of all joints measured. Cultures of the hands will be taken. Extremities will be covered with Sulfamylon burn cream and randomly treated open with evaluation or placed in the compression boot. The machine will cycle 40 seconds on, 20 seconds off at a pressure of 40 mm. At the end of 48 hours the boot will be removed, the extremities measured and recultured.

25. (U) 72 07 - 73 06 Project is completed. Results - Though the intermittent compression unit successfully reduced edema in the first 72 hours following thermal injury, its use did not improve hand function during the first week postburn or result in long term improvement.

*Available to contractors upon originator's approval

FINAL REPORT

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

REPORT TITLE: USE OF AN INTERMITTENT COMPRESSION UNIT TO DECREASE POSTBURN EDEMA IN A MILITARY POPULATION

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

1 July 1972 - 30 June 1973

Investigators:

Roger E. Salisbury, MD, Major, MC
Paul Silverstein, MD, Major, MC
Joseph A. Moylan, Jr, MD, Major, MC
Douglas W. Wilmore, MD, Major, MC
Basil A. Pruitt, Jr, MD, Colonel, MC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

REPORT TITLE: USE OF AN INTERMITTENT COMPRESSION UNIT TO DECREASE POSTBURN EDEMA IN A MILITARY POPULATION

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

Period covered in this report: 1 July 1972 - 30 June 1973

Investigators: Roger E. Salisbury, MD, Major, MC
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Reports Control Symbol MEDDH-288(R1)

Postburn edema of the upper extremity may cause severe functional and rehabilitation problems. The Jobst intermittent compression unit has been found to decrease edema and lymphedema in several pathologic conditions. Therefore, during the first 24 hours postthermal injury in 8 patients with symmetrically burned upper extremities, the Jobst compression unit was applied to one of the arms for 48 hours in an attempt to decrease edema. Limbs treated with the Jobst unit showed a statistically significant decrease in edema when compared with limbs treated in a conventional manner. However, evaluation of hand function at 3 and 21 days postthermal injury revealed no significant differences between the two groups.

Military burn patients Intermittent compression Postburn edema
Phalangeal joints

USE OF AN INTERMITTENT COMPRESSION UNIT TO DECREASE POSTBURN EDEMA IN A MILITARY POPULATION

Normal muscle contraction in an extremity produces pressure that acts on lymphatics and venules, propelling lymph and blood proximally. A patient with a burned extremity has disruption of vascular integrity and also voluntarily immobilizes his arm, two factors that promote disabling edema. An intermittent compression unit has been found useful in reducing primary lymphedema and edema secondary to venous insufficiency and radical mastectomy. Circumferential, even pressure intermittently applied to the extremity simulates the milking action of muscle contraction. The purpose of this study is to evaluate an intermittent compression device's capability to prevent or reduce edema in the early postburn period.

METHODS

During the first 24 hours postthermal injury, 8 patients with bilateral symmetrical upper extremity burns were cleansed and debrided on admission. Measurement of the circumference of the proximal interphalangeal joints (PIP), distal palmar crease (DPC) and wrists were made with sterile unmarked aluminum tapes. Cotton swab cultures were taken of all hands. Each patient's burns were treated with Sulfamylon cream and one extremity, chosen randomly, was placed in the compression device for 48 hours. The opposite limb was treated during this time in the usual manner with elevation, unlimited activity and splints applied at night. At the end of 48 hours, the limb was removed from the device and circumferential measurements were repeated of both extremities. Both limbs were recultured and then treated with elevation and unlimited motion. Measurements of the range of motion of wrist and fingers were made of both hands on the third and twenty-first days postburn and on discharge.

RESULTS

Eight patients with symmetrical burns of the upper extremities were studied and the extremities treated with the intermittent compression unit showed a significantly greater decrease in edema compared with the contralateral arm treated in the standard Institute of Surgical Research fashion

(Table 1). Analysis of the data from these symmetrically burned extremities revealed that limbs treated with the intermittent compression device showed a significant decrease in edema (Table 2), while the opposite limb showed no significant change in edema (Table 3). Significantly, the measurements of the range of motion of the wrist and fingers in the 8 patients revealed no difference in function on the third or twenty-first day postburn.

CONCLUSIONS

Although the arms treated with the intermittent compression device were significantly less edematous on the third postburn day than the arms receiving the standard treatment, there was no difference in function then or on the twenty-first postburn day. Thus, this method of decreasing edema did not improve function in these patients, suggesting that the initial injury itself, with associated pain, is the main reason for decreased function, not the edema. Significantly, by the third week postburn no patient had chronically edematous hands regardless of initial treatment. Since the intermittent compression device does not improve function, is costly, and necessitates additional nursing care, its use is not advocated in burned patients.

PRESENTATIONS

Salisbury RE. "Management of Upper Extremity Edema Following Thermal Injury - Evaluation of Present Treatment" presented at Amer Assoc for Trauma, San Francisco, Calif. October 1972.

PUBLICATIONS

None

Table 1. Treatment of Symmetrically Burned Upper Extremities, 24-72 Hours Postburn

	Compression Device vs Standard Treatment		
	Wrist	DPC	PIP
n	8	8	8
$\bar{d}$*	1.08	0.91	0.44
t	13.50	4.03	3.18
p	<0.001	<0.01	<0.05

$\bar{d}$* = mean difference of decrease in circumference in cm after 48 hours of compression or standard treatment.

Table 2. Treatment of Symmetrically Burned Upper Extremities 24-72 Hours Postburn with an Intermittent Compression Device

	Wrist	DPC	PIP
n	8	8	8
$\bar{d}^*$	1.23	1.23	0.59
t	8.92	6.24	3.7
p	<0.001	<0.001	<0.01

* $\bar{d}^*$ = mean decrease in circumference in cm after 48 hours of treatment.

Table 3. Treatment of Symmetrically Burned Upper Extremities 24-72 Hours Postburn with Standard ISR Protocol

	Wrist	DPC	PIP
n	8	8	8
$\bar{d}^*$	0.15	0.28	0.15
t	N.S.	N.S.	N.S.

* $\bar{d}^*$ = mean decrease in circumference in cm after 48 hours of treatment.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OD 6380	73 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
72 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 Enzmyatic 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)
FISCAL YEAR PRECEDING 73	0.4	14
FISCAL YEAR CURRENT 74	0.3	8

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) Enzmyatic Debridement; (U) Eschar; (U) Thermal Injury; (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) Rapid removal of burn eschar by enzymatic means in rats as a possible future therapy in burned soldiers.

24. (U) Purified proteinases and collagenases will be tested for efficacy in eschar debridement by application to male Sprague-Dawley rats which have been given a 30% total body surface area third degree scald burn.

25. (U) 72 07 - 73 06 Investigation was carried out using Sutilains enzyme in different vehicles on rats. Sutilains enzyme was effective in debriding eschar when used in Plastibase® (Travenol), vanishing cream, and sulfamylon base. Of these, most rapid debridement occurred using Plastibase. A limited clinical trial is planned.

*Available to contractors upon originator's approval.

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

ANNUAL PROGRESS REPORT

PROJECT NO. 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
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1 July 1972 - 30 June 1973

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Arthur D. Mason, Jr., MD

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

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 1972 - 30 June 1973

Investigators: Norman S. Levine, MD, Major, MC
Paul Silverstein, MD, Major, MC
George M. Helmkamp, Jr., Captain, MSC
Arthur D. Mason, Jr., MD

Reports Control Symbol MEDDH-288(R1)

Previous work had indicated that sutilains enzyme was effective in debriding human burn eschar in vitro. The in vivo effect of sutilains in different ointment bases was investigated this year.

Enzymatic debridement
Eschar
Thermal injury
Rats

AN EVALUATION OF THE USE OF ENZYMATIC DEBRIDEMENT OF BURN WOUND ESCHAR TO DECREASE MORBIDITY IN BURNED TROOPS

Enzymatic debridement of the burn wound represents an approach to expeditious eschar removal without the need for surgery or an anesthestic. The goal of this approach is to achieve early closure of the burned wound by prompt removal of the eschar. In theory, this approach could shorten the 3-5 week period necessary for spontaneous eschar separation. Earlier skin grafting and thus a more rapid convalenscence might be expected.

Previous investigation has indicated that sutilains is an effective enzyme in digesting human eschar in vitro. The purpose of this investigation was to test the effect of sutilains in various ointment bases on its ability to debride 20% third degree burns in rats in vivo.

METHODS

Eighteen, 175-185 gm, male, Sprague-Dawley rats were subjected to a 20% third degree scald burn on their backs. These rats were divided into 6 groups of 3 animals each. Each group of animals was subjected to twice-daily application of 5 gm of the following preparations: Group 1, 8200 units/gm of sutilain in Plastibase ointment ^{R}; Group 2, 8200 units/gm sutilain in Sulfamylon base ; Group 4, Plastibase ointment; Group 5, vanishing cream ointment; Group 6, Sulfamylon base ointment. These ointment preparations were left open and not treated with dressings. Animals were graded on days 3 and 5 postburn in terms of per cent debridement achieved. The results are summarized in the following table.

It appears that in rats treated with open applications of equipotent enzyme preparations in different bases, sutilain enzyme was most effective when incorporated in Plastibase $^{(R)}$. This is the preparation which is commercially available. It should be noted, however, that both vanishing cream and the Sulfamylon base showed evidence of drying out in between applications. This was more pronounced when vanishing cream was used than when Sulfamylon ointment base was used. This was surprising to us because sutilains is inactive in a petrolatum base such as Plastibase and, in effect, relies on the ability of a wet eschar to draw dry enzyme from the petrolatum base to an aqueous phase.

Comparison of Various Treatment Groups

Preparation	Average % Eschar Debridement 3 Days	5 Days
Sutilains 8200 U/gm in Plastibase (R)	15	50
Sutilains 8200 U/gm in vanishing cream	5	18
Sutilains 8200U/gm in Sulfamylon base	3	15
Plastibase	0	0
Vanishing cream	0	0
Sulfamylon base	0	0

It could be argued that the Plastibase serves as an occlusive dressing and as such would accelerate debridement by itself. Our ointment base controls showed no evidence of causing debridement within the short time limits of our experiment.

CONCLUSIONS

Twenty per cent third degree burns in live rats showed more rapid debridement when sutilain enzyme was used in Plastibase ointment than when sutilains was used in either vanishing cream or Sulfamylon base at equal unitage per gram. Neither Plastibase, vanishing cream, or Sulfamylon base showed any evidence of causing debridement in our in vivo model when these ointments were used without added enzyme. The reasons for the relative efficacy of the enzyme in Plastibase were not investigated. However, considerable drying of the preparations made up in either vanishing cream or Sulfamylon base was noted. Such findings are compatible with the relatively slow in vivo debridement noted using sutilains enzyme in vanishing cream or Sulfamylon base. Such observations make the possibility of mixing topical chemotherapeutic agents and sutilains in a water-based ointment less attractive unless the frequency of application would have to be increased to prevent such ointments from drying out.

PRESENTATIONS and/or PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0E 6391	73 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
72 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	306
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) Immune Response to Split-Thickness Cutaneous Graft in Allogenic Systems - Develop Wound Coverage Technic 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
72 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 73	.4	14
	CURRENT 74	.6	15

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21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Burn; (U) Skin Homograft; (U) Skin, Split-thickness; (U) Allogenic Systems; (U)Rats

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

23. (U) To investigate the transplantation biology of split-thickness skin grafts in normal and burned rats with known AgB loci, for future application in burned soldiers.

24. (U) Normal and burned Fischer rats received split-thickness and full-thickness grafts from Lewis and Bn donors varying graft size, (2 x 2 cm, 4 x 6 cm), and second set reactions.

25. (U) 72 07 73 06 Unburned Fischer rats receiving allografts from Lewis donors (weak AgB incompatibility) demonstrated rejection of split-thickness allograft at 11.5 days compared to rejection of full-thickness allografts at 15.2 days. Unburned Fischer rats receiving allografts from Bn donors (strong AgB incompatibility) demonstrated rejection of split-thickness allografts at 10.4 days compared to rejection of full-thickness allografts at 13.7 days. These studies suggest that split-thickness grafts are as immunogenic as full-thickness skin grafts and offer no advantages immunologically over full-thickness skin grafts.

*Applicable to contractors upon originator's approval

ANNUAL PROGRESS REPORT

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

REPORT TITLE: THE IMMUNE RESPONSE TO SPLIT THICKNESS CUTANEOUS GRAFTS IN ALLOGENIC SYSTEMS - DEVELOPMENT OF WOUND COVERAGE TECHNIC FOR BURNED SOLDIERS

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

1 July 1972 - 30 June 1973

Investigators:

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William F. McManus, MD, Major, MC
Harrel L. Walker, MS

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

REPORT TITLE: THE IMMUNE RESPONSE TO SPLIT THICKNESS CUTANEOUS GRAFTS IN ALLOGENIC SYSTEMS - DEVELOPMENT OF WOUND COVERAGE TECHNIC 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 1972 - 30 June 1973

Investigators: Glenn D. Warden, MD, Major, MC
William F. McManus, MD, Major, MC
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Reports Control Symbol MEDDH-288(R1)

Permanent transplantation of skin is the ultimate coverage for extensive third degree burns. Although transplantation of kidneys and other organs has been accomplished with varying degrees of success, the skin has been the most difficult to transplant successfully. Medawar (Medawar PB, Bul. War Med. 4:1-4, 1943)[1] demonstrated the transplantation biology of skin using small 0.6 cm^2 "punch" grafts of full thickness skin. During the past 20 years the transplantation biology of full thickness skin grafts has been thoroughly investigated, however, split thickness skin grafts have been examined only histologically. Billingham (Billingham RE, Cell Immunol 2:1-12, 1971)[2] and Steinmuller (Steinmuller D, Transpl. Proc 1:593-6, 1969[3] and Steinmuller D, Hart EA, Transpl. Proc. 3:673-675, 1971[4]) described the main antigenic structures of allograft rejection to be vascular endothelium and passenger leukocytes. In split thickness skin graft both of these determinants are quantitatively reduced, thus the initial sensitization by allograft may be markedly reduced and a split thickness skin graft may respond quite differently from a full thickness skin graft in its ability to induce antigenic response.

Unburned Fisher rats receiving an allograft from Lewis donors, (weak AgB incompatibility) demonstrated rejection of split thickness autografts measuring 4 x 6 cm at 11.5 days compared to rejection of full thickness autograft at 15.2 days. Unburned Fisher rats receiving an allograft from BN donors (stron AgB incompatibility) demonstrated rejection of split thickness autograft at 10.4 days compared to full thickness allograft at 13.7 days. The difference between rejection of split thickness grafts and full thickness grafts is most likely due to

the rejection evaluation procedure as the actual sloughing process was longer with the thicker skin. Although evaluation in burned animals remains to be elucidated, it would appear in intact animals that the antigenic stimuli necessary for graft rejection are present in split thickness autograft and produce standard rejection times. Further studies to elucidate the antigenic stimuli of split thickness autograft to produce second set rejection, enhancement, and effective immunosuppression are presently being evaluated.

Burn
Skin Homograft
Skin, Split-Thickness
Allogenic Systems

THE IMMUNE RESPONSE TO SPLIT THICKNESS CUTANEOUS GRAFTS IN ALLOGENIC SYSTEMS - DEVELOPMENT OF WOUND COVERAGE TECHNIC FOR BURNED SOLDIERS

The overall mortality of patients with thermal injury of more than 60% of the total body surface has remained unchanged even with the advent of effective topical agents. The cause of death is usually related to infection--either burn wound or pulmonary. The usual approach to large severe thermal injuries is expeditious debridement and closure of the burn wound to protect the patient from infection and return metabolic functions to normal.

Allograft and porcine xenograft have been useful as temporary biological dressings to protect the burn wound. However, they are still only temporary dressings and the problems of prolonged or permanent coverage of the larger burn wounds still exist.

Permanent transplantation of skin is the ultimate coverage for large third degree burn wounds. Although transplantation of kidneys and other organs has been accomplished with varying degrees of success, the skin has been the most difficult to transplant successfully. Gardner at Bellevue Hospital in 1881 transplanted cadaveric allograft to a patient with severe electrical injury and had a 75% take. For the next 60 years there was continual debate about permanent cutaneous allotransplantation. Medawar (Medawar PB, Bul. War Med. 4:1-4, 1943)[1] demonstrated the transplantation biology of skin but used small 0.6 cm^2 "punch" grafts of full thickness skin. During the past 20 years the transplantation biology of full thickness skin grafts has been thoroughly investigated, however, split thickness skin grafts have been examined only histologically. Billingham (Billingham RE, Cell Immunol 2:1012, 1971)[2] and Steinmuller (Steinmuller D, Transpl. Proc. 1:593-6, 1969[3] and Steinmuller D, Hart EA, Transpl. Proc. 3:673-675, 1971[4]) described the main antigenic structures of allograft rejection to be vascular endothelium and passenger leukocytes. In split thickness skin both of these determinants are quantitatively reduced, thus initial sensitization by allograft may be markedly reduced, and split thickness skin grafts may respond quite differently from full thickness skin grafts in their ability to induce an antigenic response. The purpose of this study was to investigate the transplantation biology of split thickness skin grafts in inbred rats with known AgB loci.

METHODS

Inbred strains of rats, (Fisher, BN and Lewis) weighing approximately 200 to 250 gms were used for all experimental studies with Fisher rats being the recipient animals in all experiments. The BN to Fisher combination has been shown to demonstrate strong histoincompatibility whereas Lewis to Fisher combinations demonstrate weak histoincompatibility. A full thickness skin defect measuring approximately 4 x 6 cm was

surgically created on the back of 40 Fisher rats. In group I (20 animals) the animals received grafts from Lewis donors while in group II (20 animals) the animals received grafts from BN donors. In each group ten animals received split thickness autograft taken with a Brown electric dermatome at a thickness of approximately 0.12 inches while the other ten animals received defatted full thickness grafts. All grafts were maintained in position with surgical clips and were not covered. The grafts were examined daily and rejection time was evaluated as a 50% slough of the graft.

RESULTS

Lewis to Fisher combinations: Ten animals received split thickness autograft with a mean rejection time of 11.5 days $\pm$ 1.35. Full thickness grafts were rejected at a mean 15.2 $\pm$ 1.61.

BN to Fisher combinations: Ten animals received the split thickness autograft with a mean rejection of 10.4 $\pm$ 0.81 days. The 10 animals who received full thickness autografts underwent rejection at 13.7 $\pm$ 0.82 days (Table I)

DISCUSSION

Although the study is still in progress, initial comparison of full thickness and split thickness autograft in inbred strains of rats with known AgB loci suggests no difference between split thickness autograft and full thickness grafts. The differences between rejection of split thickness skin grafts and full thickness grafts is most likely due to the rejection evaluation procedure as the actual sloughing process is longer in those with the thicker skin. Although the evaluation of antigenic response in burned animals remains to be carried out it would appear in intact animals that the antigenic stimuli necessary for graft rejection are present in split thickness autograft and produce standard rejection time. Further studies to define the antigenic stimuli of split thickness autograft, to produce second set rejection, enhancement, and effective immunosuppression are in progress.

REFERENCES

1. Medawar PB: Notes on problem of skin homografts. Bul War Med 4:1-4, 1943.

2. Billingham RE: The passenger cell concept in transplantation immunology. Cell Immunol 2:1-12, 1971.

3. Steinmuller D: Allograft immunity produced with skin isografts from immunologically tolerant mice. Transpl Proc 1:593-596, 1969.

4. Steinmuller D, Hart EA: Passenger leukocytes and induction of allograft immunity. Transpl Proc 3:673-675, 1971.

TABLE I

Split-thickness Allografts	Day of Rejection
BN -- Fisher	10.4 ± 0.81 days
Lewis -- Fisher	11.5 ± 1.35 days
Full-thickness Allografts	**Day of Rejection**
BN -- Fisher	13.7 ± 0.82 days
Lewis -- Fisher	15.2 ± 1.61 days

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0E 6397	73 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
72 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	320
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) Clinical Evaluation of Etoxadrol (CL1848C) For Use 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 10	72 10	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 73	.3	11
FISCAL YEAR CURRENT 74	0	0

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

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Etoxadrol; (U) CL1848C; (U) Burn Anesthesia; (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) Etoxadrol (CL1848C), a new intravenous, dissociative anesthetic similar in effect to ketamine, is under investigation concerning its efficacy in the anesthetic management of thermally injured troops.

24. (U) Fifteen patients at the USAISR are to be anesthetized with CL1848C without premedication except for atropine.

25. (U) 72 07 - 72-10 Two patients have thus far received CL1848C. Anesthesia appears identical to that produced by ketamine. Blood pressure is elevated. Ventilation and arterial blood gases remain normal. Random movements are common, and muscle relaxation is poor. The patients appear to be awake but dissociated from the environment. In neither case was it possible to produce satisfactory anesthesia. In one, movement in response to pain, and in the other, vocalization and random movements could not be overcome with additional doses of the anesthetic. Postoperatively, dreams occurred in both patients, on occasion pleasant, and at other times, unpleasant. Sensory alterations, frequently reported with ketamine, occurred but were not prominent. Analgesia was profound and of extended duration. The patients, however, did not feel completely recovered from anesthesia for 40 and 96 hours, postoperatively. The study was discontinued after manufacturers withdrew drug from clinical trials on the basis of our and others' similar results.

*Available to contractors upon originator's approval

FINAL REPORT

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

REPORT TITLE: CLINICAL EVALUATION OF ETOXADROL (CL1848C) FOR USE IN BURNED MILITARY PERSONNEL

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

1 July 1972 - 30 June 1973

Investigators:

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Stephen Slogoff, MD, Major, MC
James V. Wessels, MD, Major, MC*
Lois A. Johns, Lieutenant Colonel, ANC

*From the Department of Anesthesiology, Brooke Army Medical Center, Fort Sam Houston, Texas 78234

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

REPORT TITLE: CLINICAL EVALUATION OF ETOXADROL (CL1848C) FOR USE 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 1972 - 30 June 1973

Investigators: Gary W. Allen, MD, Major, MC
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Lois A. Johns, Lieutenant Colonel, ANC

Reports Control Symbol MEDDH-288(R1)

A short clinical evaluation of this new ketamine-like anesthetic was conducted by the Anesthesia Section, US Army Institute of Surgical Research. Two patients who underwent burn wound debridement were anesthetized with CL1848C, using total doses of 1.25 mg/kg and 2.25 mg/kg. Intraoperatively, the anesthetic appeared to produce effects similar to those of ketamine except that random movements of head and extremities, and vocalization occurred, which could not be alleviated with additional doses of CL1848C, and which interfered with completion of the proposed surgery. Postoperative recovery was quite prolonged. The two patients did not feel completely normal until 40 hours and 86 hours postoperatively. Postoperative analgesia was good, but perceptual alterations, dreams, and fear of death made the recovery period unpleasant.

The study of this agent has subsequently been discontinued because of reports of prolonged emergence both here and at other institutions. A complete review of our experience, with case histories, was reported in the Annual Research Progress Report last year (June 1972).

Etoxadrol
CL1848C
Burn anesthesia

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0E 6381	73 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. OTHER INSTR'N	8a. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
72 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	122
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Procede with Security Classification Code): (U) Positive Pressure Ventilation and Surface Tension in Lungs - Animal Model to Evaluate Therapy of Injured Troops (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
71 07	73 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		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 73	.3	10
	CURRENT 74	0	0

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

22. KEYWORDS (Precede Each with Security Classification Code): (U) Surfactant; (U) Surface Tension; (U) Compliance; (U) Hyperventilation; (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) Thermally injured soldiers with total body surface burns greater than 40% invariably have large increases in respiratory minute volumes (up to 70 L/min) and in tidal volumes (up to 30 ml/kg body weight). This study was undertaken to determine whether this marked increase in ventilation is harmful to the lungs.

24. (U) The effect of positive pressure ventilation for 6 hours with room air to ventilator peak pressures of 40 cm H20 was studied using goats as the experimental model. Four controls without treatment, five controls receiving tracheostomy and ultrasonic humidification for 30 hours, and 4 experimental goats receiving positive pressure ventilation then 24 hours of ultrasonic humidification, prior to sacrifice, were studied.

25. (U) 72 07 - 73 03 Excised lung air and saline compliance curves have shown only minor inconsistent differences between these three groups, while surface tension balance studies of bronchial washings have revealed no changes attributable to ventilation. (a) Lung total phospholipids and lecithins were essentially the same for all three groups. (b) Lungs of ventilated goats demonstrated areas of atelectasis grossly, pulmonary edema and atelectasis histologically, and were heavier on a grams per kilogram body basis than were the control lungs. Ventilation with large tidal volumes appears to have no effects on the pulmonary surfactant system of the goat.

*Available to contractors upon originator's approval

FINAL REPORT

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

REPORT TITLE: POSITIVE PRESSURE VENTILATION AND SURFACE TENSION IN LUNGS - ANIMAL MODEL TO EVALUATE THERAPY OF INJURED TROOPS

US ARMY INSTITUTE OF SURGICAL RESEARCH
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1 July 1972 - 30 June 1973

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Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

REPORT TITLE: POSITIVE PRESSURE VENTILATION AND SURFACE TENSION IN LUNGS - ANIMAL MODEL TO EVALUATE THERAPY OF INJURED TROOPS

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

Period covered in this report: 1 July 1972 - 30 June 1973

Investigators: Gary W. Allen, MD, Major, MC
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Thermally injured patients with total body surface burns greater than 40% invariably have large increases in respiratory minute volumes (up to 70 L/min) and in tidal volumes (up to 30 ml/kg body weight). This study was undertaken to determine whether this marked increase in ventilation is harmful to the lungs.

The effect of positive pressure ventilation for six hours with room air to ventilator peak pressures of 40 cm H_2O was studied using goats as the experimental model. Four controls without treatment, five controls receiving tracheostomy and ultrasonic humidification for 30 hours, and four experimental goats receiving positive pressure ventilation then 24 hours of ultrasonic humidification, prior to sacrifice, were studied.

Excised lung air and saline compliance curves have shown only minor inconsistent differences between these three groups, while surface tension balance studies of bronchial washings have revealed no changes attributable to ventilation. Lung total phospholipids and lecithins were essentially the same for all three groups. Lungs of ventilated goats demonstrated areas of atelectasis grossly, pulmonary edema and atelectasis histologically, and were heavier on a grams per kilogram body basis than were the control lungs. Ventilation with large tidal volumes appears to have no effect on the pulmonary surfactant system of the goat.

Surfactant
Surface tension
Compliance
Hyperventilation
Goats

EFFECT OF POSITIVE PRESSURE VENTILATION WITH LARGE TIDAL VOLUMES ON SURFACE TENSION PROPERTIES OF LUNGS

A study by Greenfield, et al, in 1964 (Greenfield LJ, Ebert PA, Benson DW, Anesthesiology 25:312-316, 1964)[1] reported the effect of positive pressure ventilation in dogs using very high tidal volumes. The pressures used during ventilation were those required to produce a palpable paradoxical pulse in the femoral artery. It was found that ventilation at this pressure (usually 26-32 cm H_2O) produced no change in surface activity of lung extracts obtained immediately after the period of ventilation. However, extracts obtained 24 hours post-ventilation were noted to have a significantly elevated minimum surface tension, and the lungs were noted grossly to have patchy atelectasis and edema. Surface tension and gross examination of the lungs became progressively more normal when examined at 48 and 72 hours post-ventilation.

Thermally injured patients with total body surface burns greater than 40% invariably have large increases in respiratory minute volumes (up to 70 L/min) and in tidal volumes (up to 30 ml/kg body weight). This study has been undertaken to determine whether this marked increase in ventilation is detrimental to the lung, and to confirm or disprove the work reported previously.

METHODS

Angora goats were anesthetized with pentobarbital and tracheostomy performed. The animals were allowed to recover from anesthesia and then were allowed to breathe spontaneously for a period of 24 hours utilizing ultrasonic nebulization for humidification of the airway. The experimental group (five goats) was subjected to ventilation using a Morch piston-driven ventilator at peak pressures of 40 cm H_2O with room air humidified to 100% relative humidity at 37° C. for a period of six hours. Ventilator pressures were measured by a Sanborn differential pressure transducer connected to a T-tube extension of the cuffed endotracheal tube. Tidal volumes were measured intermittently with a Stead-Wells 13.5 liter spirometer, and the volumes were corrected for internal compression of the ventilator and to BTPS. Carbon dioxide in quantities sufficient to maintain the arterial pCO_2 in the range of 25 to 35 torr was added at the O_2 port of the ventilator. Following the period of ventilation, the goats were allowed to ventilate spontaneously again with ultrasonic humidification for a period of 24 hours. They were then sacrificed with potassium chloride, and the lungs were excised and examined grossly.

The left lung was weighed, and air and saline compliance determined in the manner described below. The mainstem bronchus of the left lung was catheterized with a large bore plastic cannula. The lung was then placed within an airtight three-liter plastic box connected to a one-liter spirometer. The lung was inflated and deflated with a constant-rate motor-driven syringe, allowing 30 seconds for the total respiratory cycle with air and 30 minutes with saline. Pressure was determined by a Sanborn differential pressure transducer with one limb connected by t-tube between the syringe and lung and the other limb connected to the plastic box containing the lung. Volume changes were determined by means of a potentiometer mounted on the spirometer. Both pressure and volume signals were amplified and recorded on a Sanborn four-channel recorder.

The lungs were inflated two or three times with pressures of 30 cm H_2O and then volume-pressure curves were recorded during the subsequent six respiratory cycles with inflations to 20 cm H_2O and deflations to 0 cm H_2O. Air compliance or total lung compliance (Cl) was determined by the volume change during deflation from 15 to 5 cm H_2O according to the method of Beckman and Weiss (Beckman DL, Weiss HS, J Appl Physiol 26:700-709, 1969).[2] The lungs were then inflated with normal saline to the same volume obtained with air at 20 cm H_2O and deflated. The tissue compliance (C_{tis}) was calculated by dividing the volume change by the pressure change over the flat portion of the saline deflation curve (Beckman DL, Weiss HS, J Appl Physiol 26:700-709, 1969).[2]

The compliance due to surface forces (C_{surf}) was estimated by a formula used by Beckman (Beckman DL, Weiss HS, J Appl Physiol 26:700-709, 1969).[2]

$$1\ C_L = 1/C_{tis} + 1/C_{surf}$$

Because of unequal size among the goats, C_L, C_{tis}, and C_{surf} were divided by body weight or by lung weight to obtain data that would be equivalent from animal to animal.

Sections of the right lung were taken for light and electron microscopy. Surfactant samples were obtained with transbronchial saline flush of the excised right lung and evaluated with a modified Wilhelmy balance and Langmuir trough as described by Comroe (Comroe JH Jr, In Ciba Foundation Symposium on Pulmonary Structure and Function, edited by AVS deReuck and M O'Conner. Boston, Little, Brown & Co, 1962, pp 176-185).[3] The disc-driven barrier speed was 0 - 3.2 mm/sec.

Approximately 10 grams of tissue from the right lung was frozen and later analyzed for lung total phospholipids and lecithin. Total lipids were extracted by the method of Weinhold (Weinhold PA, Villes CA, Biochemica et Biophysica Acta 106:540-550, 1965),[4] and phospholipids were separated by a technique described by Ways (Ways P, Hanahan DJ, J Lipid Research 5:318-328, 1964).[5] The phospholipid extract was separated chromatographically on silica gel H, using a chloroform-methanol-acetic acid-water solvent in volume ratios of 25/15/3/0.75. The spot corresponding to lecithin was removed and its phosphorus content was measured by the method of Bartlett (Bartlett GR, J Biol Chem 234:466, 1959).[6] Lecithin was estimated as 25 times the phosphorus content.

Total phospholipids were calculated from phosphorus content of the phospholipid extract.

Five goats were used as controls and treated in the same manner as the experimental animals except that they were not subjected to the period of positive pressure ventilation.

Four other goats were sacrificed without treatment of any kind and their excised lungs were analyzed in the manner described above.

RESULTS

One goat in the experimental group was noted after excision of the lung to have a severe bronchopneumonia which cultured Staphylococcus aureus, coagulase positive. The data from this goat was subsequently deleted from the study.

The other four experimental goats received positive pressure ventilation for six hours at a mean peak pressure of 40.9 cm H_2O. The mean tidal volume was 642 ml or 51.3 ml/kg body weight. The mean respiratory rate was 16.8 with an average expired minute volume of 10,750 ml.

Gross examination of the excised lungs of the experimental group revealed a considerable amount of patchy atelectasis and occasionally emphysematous blebs appeared on the lung surface indicating that the degree of hyperinflation was great enough to produce alveolar wall rupture.

Compliance data for the three groups of goats are given in Table 1. C_{surf} for the experimental goats when divided by the lung weight was 0.134 ml/cm H_2O/gm as compared to a mean of .0162 ml/cm H_2O/gm for the controls. This represents a decrease of 17%. C_{surf} divided by

body weight was .56 ml/cm H_2O/kg for experimental goats and .62 ml/cm H_2O/kg for experimental goats and .62 ml/cm H_2O/kg (10% decrease) for control animals. These differences were not significant. The hyperinflated lungs were slightly heavier on a gm/kg body weight basis, and the edema which this represents may have produced these small differences in compliance.

Histologically, experimental lungs had areas of atelectasis interspersed with sections of overdistended alveoli. An interstitial edema was present in all of the hyperinflated lungs, but this was of a mild degree and was occasionally found even in control lungs.

The qualitative activity of the surfactant determined by the Silheimy balance and Langmuir trough was well within the normal range for both the experimental and control goats.

Quantitative assay of lung total phospholipids and lung lecithin (Table 1) revealed no significant differences between the control and experimental groups.

SUMMARY

Angora goats mechanically ventilated for a period of six hours with tidal volumes of 51 ml/kg body weight and ventilator peak pressures of 40 cm H_2O had no significant differences in excised lung compliance or surfactant activity 24 hours after ventilation. The ventilator pressures used were extremely high and may have been responsible for the structural changes seen within the lungs, namely, overdistention of alveoli and atelectasis of adjacent areas of lung tissue.

In conclusion, we have been unable to show consistent changes in lung surfactant following mechanical ventilation with large tidal volumes. The reason for the discrepancy between the results of this study and that by Greenfield (Greenfield LJ, Ebert PA, Benson DW, Anesthesiology 25:312-316, 1964)[1] are unknown.

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1. Greenfield LJ, Ebert PA, Benson DW: Effects of positive pressure ventilation on surface tension properties of lung extracts. Anesthesiology 25:312-316, 1964.

2. Beckman DL, Weiss HS: Hyperoxia compared to surfactant washout on pulmonary compliance in rats. J Appl Physiol 26:700-709, 1969.

3. Comroe JH Jr: Physiological and biochemical effects of pulmonary artery occlusion. In Ciba Foundation Symposium on Pulmonary Structure and Function, edited by AVS deReuck and M O'Conner, Boston, Little, Brown, and Co., 1962, pp 176-185.

4. Weinhold PA, Villes CA: Phospholipid metabolism in the liver and lungs of rats during development. Biochemica et Biophysica Acta 106:540-550, 1965.

5. Ways P, Hanahan DJ: Characterization and quantification of red cell lipids in normal man. J Lipid Research 5:318-328, 1964.

6. Bartlett GR: Phosphorus assay in column chromatography. J Biol Chem 234:466, 1959.

PUBLICATIONS AND/OR PRESENTATIONS

None

Table 1. Compliance Data on Three Groups of Goats

	Tracheotomy + Positive Pressure Ventilation	Tracheotomy	Untreated
No. of Goats	4	5	4
Lung Weight (gm)	53.9	84.5	56.8
Body Weight (kg)	12.8	22.0	14.4
Lung Weight/Body Weight (gm/kg) x 1000	4.29	3.82	3.94
Deflation Compliance/Lung Weight (ml/cm H_2O/gm)			
C_L	.097	.129	.112
C_{tis}	.374	.686	.412
C_{surf}	.134	.166	.158
Deflation Compliance/Body Weight (ml/cm H_2O/kg)			
C_L	.41	.48	.43
C_{tis}	1.61	2.50	1.58
C_{surf}	.56	.62	.62
Total Lung Phospholipids (µgm/mg wet lung)	16.65	17.08	21.26
Lung Lecithin (µgm/mg wet lung)	7.81	7.81	9.83

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0D 6978	73 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
72 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	194
b. CONTRIBUTING				
c. CONTRIBUTING				

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

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 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. 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 73	.4	14
	CURRENT 74	.3	7

19. RESPONSIBLE DOD ORGANIZATION
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NAME: John L Hunt, MAJ, MC DA

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

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

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

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

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) 72 07 - 73 06 Modification of the drape material has still further improved its draping characteristics but has necessitated reassessment of the bacterial barrier property of the sheeting material. The material was tested as a laminate and each of its two component parts was tested separately. Four hours of direct exposure of the laminate to broth cultures of Enterobacter, Klebsiella, Providencia, Pseudomonas and Staph. aureus resulted in no passage of these bacteria onto the culture media. Similar exposure of the Spunbonded Olefin material permitted some passage of Klebsiella, Pseudomonas and Staph. aureus, questionable passage of Providencia, and no passage of Enterobacter organisms after four hours of direct exposure. Similar bacteriologic studies of the water repellant cellulosic tissue showed that it permitted passage of all five genera in gross amounts after four hours of direct exposure.

*Available to contractors upon originator's approval

ANNUAL PROGRESS REPORT

PROJECT NO. 3A161102B71R-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

1 July 1972 - 30 June 1973

Investigators:

Basil A. Pruitt, Jr., MD, Colonel, MC
Robert B. Lindberg, Ph D
John L. Hunt, MD, Major, MC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A161101B71R-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 1972 - 30 June 1973

Investigators: Basil A. Pruitt, Jr., MD, Colonel, MC
Robert B. Lindberg, Ph D
John L. Hunt, MD, Major, MC

Reports Control Symbol MEDDH-288(R1)

Loss of the bacterial barrier properties of surgical drapes and gowns contributes to the postoperative wound infection rate of 7% cited by several studies. Standard cotton fabric drapes become freely permeable to bacteria if they become wet, as do cotton gowns. Waterproof paper drapes are superior bacterial barriers but permit not only quantitative fluid "run-off" but instrument "slide-off", and can be torn during an operation, thereby losing their barrier property.

A synthetic drape with greater tensile strength than paper and bacterial barrier properties superior to that of cotton has been evaluated, both clinically and in the laboratory. The initial form of the drape showed excellent bacterial barrier properties but considerable fluid "run-off", and less than ideal draping characteristics. In order to minimize those limitations, a synthetic fabric-cellulosic laminate was constructed. However, it appeared as if the softening process used to improve the draping characteristics of the material destroyed the bacterial barrier with ready passage of test bacteria through the drape material.

An improved drape material has been produced during the past year consisting of a Spunbonded Olefin-cellulosic laminate which has been softened without loss of its bacterial barrier properties. This improved laminate has now been tested in the laboratory and found to be an excellent bacterial barrier. Four hours of direct exposure of the laminate to broth cultures of

Enterobacter, Klebsiella, Providencia, Pseudomonas, and Staphylococcus aureus organisms resulted in no passage of these bacteria onto underlying culture media. Exposure of the Spunbonded Olefin material alone to similar broth cultures showed it to permit some passage of Klebsiella, Pseudomonas, and staphylococcal organisms, questionable passage of Providencia organisms, and no passage of Enterobacter organisms. The water-repellant cellulosic tissue alone exposed in similar fashion to broth cultures permitted passage of gross amounts of all organisms.

Laboratory confirmation of the excellent bacterial barrier properties of the improved laminate indicates that further clinical testing should now be carried out, as will be done over the ensuing year.

Operating room based infections
Surgical gowns
Surgical drapes
Military burn unit

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6973	73 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'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
72 07 01	K. COMPLETION	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	270
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) Effect of Chloride and Extracellular Volume on Correction of Metabolic Alkalosis - A Common Problem in the Injured Troop (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)
FISCAL YEAR PRECEDING 73	.4	14
FISCAL YEAR CURRENT 74	0	0

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) Aldosterone; (U) Chloride; (U) Potassium; (U) Humans; (U) Extracellular Volume; (U) Metabolic Alkalosis; (U) Respiratory Acidosis

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

23. (U) Previous studies reported in the Annual Research Progress Report 1972 suggest that bicarbonate reabsorption in the proximal tubule is not impaired in the aldosterone deficient dog. Further related studies were undertaken to examine the possible site of action of aldosterone in the nephron. Studies were also undertaken to access the role of the renin-angiotensin-aldosterone system on the generation of bicarbonate in the chronic hypercapnic dog as a model of the injured soldier.

24. (U) Study #1 The effect of adrenal insufficiency on sodium reabsorption in the ascending limb was evaluated by the measurement of free water excretion and reabsorption in normal animals who subsequently underwent bilateral adrenalectomy.
Study #2 The role of the renin-angiotensin-aldosterone system on the generation of bicarbonate in the hypercapnic dog was accessed by placing five mongrel dogs on a 50 mEq sodium diet, then after a seven day control period placing these animals in a 10% CO2 atmosphere for nine days and then allowing the dogs to recover for seven days in the normal atmosphere. During this period, serial plasma renin concentrations were measured as well as plasma bicarbonate concentrations. The animals then underwent bilateral adrenalectomy and the same procedure repeated in the aldosterone deficient state.

25. (U) 72 07 - 73 06 Study #1 The evaluation of free water excretion and free water reabsorption in the normal and aldosterone deficient dog suggests that aldosterone has no inhibitory effect on sodium reabsorption in the ascending limb. This study and the studies reported last year thus suggest that aldosterone exerts its major effect on sodium and bicarbonate reabsorption in the distal tubule of the dog nephron.
Study #2 Results from this study suggest that hyperaldosteronism is not required for the generation of bicarbonate in the hypercapnic animal.

*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

FINAL REPORT

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

REPORT TITLE: EFFECT OF CHLORIDE AND EXTRACELLULAR VOLUME ON CORRECTION OF METABOLIC ALKALOSIS - A COMMON PROBLEM IN THE INJURED TROOP

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

1 July 1972 - 30 June 1973

INVESTIGATORS

Philip W. Rogers, MD, Major, MC
Neil A. Kurtzman, MD, Lieutenant Colonel, MC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

REPORT TITLE: EFFECT OF CHLORIDE AND EXTRACELLULAR VOLUME ON CORRECTION OF METABOLIC ALKALOSIS - A COMMON PROBLEM IN THE INJURED TROOP

Period covered in this report: 1 July 1972 - 30 June 1973

Investigators: Philip W. Rogers, MD, Major, MC
Neil A. Kurtzman, MD, Lieutenant Colonel, MC

Reports Control Symbol MEDDH-288(RI)

To examine the effect of mineralocorticoid deficiency on sodium transport by the ascending limb of the loop of Henle, free water clearance and reabsorption were measured in the same six dogs under conditions of aldosterone deficiency and mineralocorticoid sufficiency. Aldosterone deficiency was induced by bilateral adrenalectomy with dexamethasone replacement. C_{H_2O} / 100 ml GFR ranged from 4.0 to 19.5 in the aldosterone deficient dogs and 4.5 to 18.6 in the mineralocorticoid sufficient dogs. C_{H_2O} / 100 ml GFR plotted against V/100 ml GFR showed no significant difference between the two groups. $T_{C_{H_2O}}$/100 ml GFR ranged from 1.2 to 6.5 in the mineralocorticoid sufficient group and 2.4 to 8.5 in the aldosterone deficient group. $T_{C_{H_2O}}$/100 GFR plotted against C_{Osm}/100 ml GFR revealed no significant difference between the two groups. Maximal urine concentration in the mineralocorticoid sufficient group was 1356 mOsm/kg $\pm$ 254 (SD) and 1386 mOsm/kg $\pm$ 331 (SD) for the aldosterone deficient group; the difference is not significant. This study failed

to demonstrate any effect of aldosterone deficiency on renal concentrating and diluting capacity; thus there is no detectable net effect of aldosterone on ascending limb sodium reabsorption.

Aldosterone
Potassium
Metabolic Alkalosis
Chloride
Extracellular Volume
Humans

EFFECT OF CHLORIDE AND EXTRACELLULAR VOLUME ON CORRECTION OF METABOLIC ALKALOSIS - A COMMON PROBLEM IN THE INJURED TROOP

The localization of action of aldosterone in the nephron has been quite controversial and has not been clearly defined. Recent studies (Kurtzman NA, White MG and Rogers PW: J Lab Clin Med 77: 931, 1971[1]; Lynch RE, Schneider EG, Dusser TP, Willis LR and Knox FG: Clin Res 19: 539, 1971[2]; Wright FS, Knox FG, Howard SS and Berliner RW: Am J Physiol 216: 869, 1969[3]) suggest that aldosterone has no effect on sodium reabsorption in the proximal tubule. Most of the experimental evidence accumulated thus far suggests that aldosterone acts on the distal tubule (Hierholzer K and Stolte H: Nephron 6: 188, 1969[4]; Vander AJ, Marvin RL, Wilde WS, Lapides J, Sullivan LP, and McMurray VM: Proc Soc Exp Biol 99: 323, 1958[5]); however, studies evaluating its action on the ascending limb are few and contradictory (Jick H, Snyder JG, Moore EW, and Morrison RS: Clin Sci 29: 25, 1965[6]; Sonnenblick EH, Cannon PJ and Laragh JH: J Clin Invest 40: 903, 1961[7]; Garrod O, Davies SA, and Cahill G, Jr: J Clin Invest 34: 761, 1955[8]).

The present study was designed to evaluate the effect of aldosterone on sodium reabsorption in the ascending limb of Henle's loop in the nephron of the dog by studying the effect of aldosterone deficiency on renal concentrating and diluting capacity. We reasoned that if aldosterone were necessary for sodium transport by the

ascending limb of the loop of Henle it's absence should result in impaired concentrating and/or diluting capacity of the kidney.

METHODS

Studies measuring solute free water excretion (C_{H_2O}) and reabsorption ($T_{C_{H_2O}}$) were performed on each of six mongrel dogs in both the mineralocorticoid sufficient and aldosterone deficient state. These studies were performed on three healthy dogs receiving 1.0 mg desoxycorticosterone acetate (DOCA) daily, then repeated after bilateral adrenalectomy while receiving only dexamethasone 0.75 mg daily. The same studies were also performed on three bilaterally adrenalectomized dogs receiving parenteral DOCA 1.0 mg, and dexamethasone 0.75 mg daily, then repeated on the same animals while receiving only dexamethasone 0.75 mg daily. Thus two groups of mineralocorticoid sufficient and aldosterone deficient dogs were studied, each dog being studied on four separate occasions with a total of 24 experiments being performed. Free water reabsorption was also studied in four normal dogs not given exogenous desoxycorticosterone prior to the study and not given ADH during the study. This group was studied to see if the small dose of exogenous DOCA, given to insure adequate amounts of circulating DOCA caused some degree of volume expansion and resultant inhibition of sodium reabsorption in the ascending limb. ADH was not used in this group of

animals since pharmacologic doses have been shown to cause a natriuresis possibly by inhibiting sodium reabsorption in the ascending limb (Crabbe J: Clin Sci 23: 39, 1962[11]; Kurtzman NA and Rogers PW: Clin Res 20: 600, 1972[12]). Dogs in the aldosterone deficient state were allowed to develop hyperkalemia and mild metabolic acidosis after adrenalectomy to make certain that adrenal insufficiency had been induced. The dogs were treated with DOCA and allowed to recover prior to the study. A minimum of 10 days was allowed between studies on the same dog. All dogs were weighed daily.

All the dogs studied during water diuresis were placed on a "zero" electrolyte diet identical to that described by Cohen (Cohen JJ: J Clin Invest 47: 1181, 1968)[13] to which 50 mEq NaCl were added daily. Water ad libitum was given for three days prior to the study. DOCA was withheld for 96 hours prior to those studies done in the aldosterone deficient state. One hour prior to beginning the study 50 ml of water per kg body weight was administered via a nasogastric tube. Water diuresis was maintained by the continuous intravenous infusion of 0.45% saline at 1.0 ml/kg/min for 20 minutes and then the rate of infusion was varied from 0.25 to 1.0 ml/kg/min. The urine osmolality was less than 75 mOsm/kg prior to initiation of the 10 minute collection period.

All dogs studied during solute diuresis were placed on the same "zero" electrolyte diet to which 50 mEq per day of NaCl were added for three days prior to the study. Water was withheld 48 hours and food

withheld 24 hours prior to the study. On the day of the experiment, five units of vasopressin in oil were injected intramuscularly two hours before the study was started, thereafter a continuous infusion of 50 mU/kg/hour of aqueous vasopressin was given. Solute diuresis was achieved by infusing 5% saline; the rate of infusion was varied from 0.5 to 5.0 ml/minute. Collection periods were 10 minutes in length. Solute diuresis in the normal group of dogs not given DOCA and ADH was otherwise achieved in the same manner as just described.

All studies were performed while the dogs were lightly anesthetized with sodium pentobarbital. An endotracheal tube fitted with inflatable cuff was placed in the trachea and connected to a Bird respirator. The P_{CO_2} was maintained between 35 and 45 mm Hg. Arterial blood samples were obtained anaerobically via an arterial catheter in the femoral artery. Urine was collected from an indwelling bladder catheter.

The serum and urine osmolalities were measured with an Advanced Instruments Osmometer. The partial pressure of CO_2 in blood and glomerular filtration rates were determined by methods previously described (Kurtzman, NA: J. Clin. Invest 49: 586, 1970)[14]. Values of C_{Osm}, C_{H_2O}, and $T_{C_{H_2O}}$ were computed in the usual manner.

RESULTS

Free Water Clearance Representative experiments during water diuresis in the same dog (IG4) under conditions of mineralocorticoid

sufficiency and deficiency are shown in Tables I and II respectively. Only small differences in urine flow(V), COsm, C_{H_2O}, and GFR for both states are present.

Figure 1 plots C_{H_2O}/100 ml GFR against V/100 ml GFR for the entire group of animals in both the mineralocorticoid sufficient and aldosterone deficient state. Aldosterone deficiency has no effect on the relationship of C_{H_2O}/100 ml GFR to V/100 ml GFR. The regression line for the data obtained during the mineralocorticoid sufficient state is $Y = 0.43 + 0.73X$ compared to $Y = 0.54 + 0.71X$ for the aldosterone deficient group of dogs. The r value for both groups is 0.94. Determination of the difference between two linear regressions using the Student t test failed to demonstrate any statistical difference between the two groups (Steel RGD and Torrie JH: New York, McGraw-Hill Book Co. Inc., 1960, p.173.)[15]

Free Water Reabsorption

$T_{C_{H_2O}}$ was examined in the same dogs utilized for evaluation of solute-free water excretion. Again, studies were performed in both the mineralocorticoid sufficient and aldosterone deficient state. Representative experiments during solute diuresis under both conditions of mineralocorticoid sufficinecy and deficiency performed on dog IG4 are presented in Tables III and IV. C_{Osm} and $T_{C_{H_2O}}$/100 ml GFR vary only slightly in both states.

Figure 2 plots $T_{C_{H_2O}}$/100 ml GFR against C_{Osm}/100 ml GFR using the data obtained from all six dogs. Aldosterone deficiency has no effect

TABLE I

Solute free water excretion in a mineralocorticoid sufficient dog.

TIME	GFR	V	U_{Osm}	P_{Osm}	C_{Osm}	C_{H_2O}	$\frac{C_{H_2O}}{GFR} \times 100$	$\frac{V}{GFR} \times 100$
Min	ml/min	ml/min	mOsm/kg	mOsm/kg	ml/min	ml/min	%	%
Dog IG4, wt 12.4 kg								
0-20 Infuse 0.45% NaCl at 1 ml/kg/min								
20-30 Infuse 0.45% NaCl at 0.25 ml/kg/min								
30-40	31.2	2.80	69	299	0.65	2.15	6.89	8.97
40-50	33.8	2.85	63	295	0.61	2.24	6.62	8.43
50-60	39.5	3.30	57	294	0.64	2.66	6.73	8.35
Infuse 0.45% NaCl at 0.50 ml/kg/min								
70-80	38.2	3.10	57	293	0.60	2.50	6.54	8.11
80-90	36.5	3.75	62	298	0.78	2.97	8.13	10.27
90-100	39.7	5.00	67	298	1.12	3.88	9.77	12.59
100-110	37.9	4.50	61	302	0.91	3.59	9.47	11.87
Infuse 0.45% NaCl at 0.75 ml/kg/min								
110-120	39.0	4.85	71	303	1.14	3.71	9.51	12.43
120-130	40.5	5.60	82	295	1.56	4.04	9.97	13.82
130-140	40.9	5.20	83	295	1.46	3.74	9.14	12.71

TABLE II

Solute free water excretion in an aldosterone deficient dog

TIME	GFR	V	U_{Osm}	P_{Osm}	C_{Osm}	C_{H_2O}	$\frac{C_{H_2O}}{GFR} \times 100$	$\frac{V}{GFR} \times 100$
Min	ml/min	ml/min	mOsm/kg	mOsm/kg	ml/min	ml/min	%	%
Dog IG4, wt 11.6 kg								
0-20	Infuse 0.45% Saline at 1.0 cc/kg/min							
20-30	Infuse 0.45% Saline at 0.25 cc/kg/min							
30-40	35.8	2.60	65	281	0.60	2.00	5.58	7.26
40-50	38.0	3.30	60	281	0.70	2.60	6.84	8.68
50-60	43.8	3.75	62	282	0.82	2.93	6.68	8.56
Infuse 0.45 NaCl 0.50 ml/kg/min								
60-70	36.6	3.50	56	277	0.71	2.79	7.62	9.56
70-80	36.6	3.70	59	277	0.79	2.91	7.95	10.10
80-90	35.8	4.05	61	277	0.89	3.16	8.82	11.31
Infuse 0.45% NaCl at 0.75 ml/kg/min								
90-100	32.0	4.10	61	280	0.89	3.21	10.03	12.81
100-110	40.3	4.60	67	277	1.11	3.49	8.66	11.41
110-120	33.7	4.10	73	287	1.04	3.06	9.08	12.16
Infuse 0.45% NaCl at 1.0 ml/kg/min								
120-130	31.3	4.20	72	274	1.10	3.10	9.90	13.41
130-140	33.3	5.00	78	283	1.38	3.62	10.87	15.01
140-150	37.0	5.10	77	275	1.43	3.67	9.91	13.78

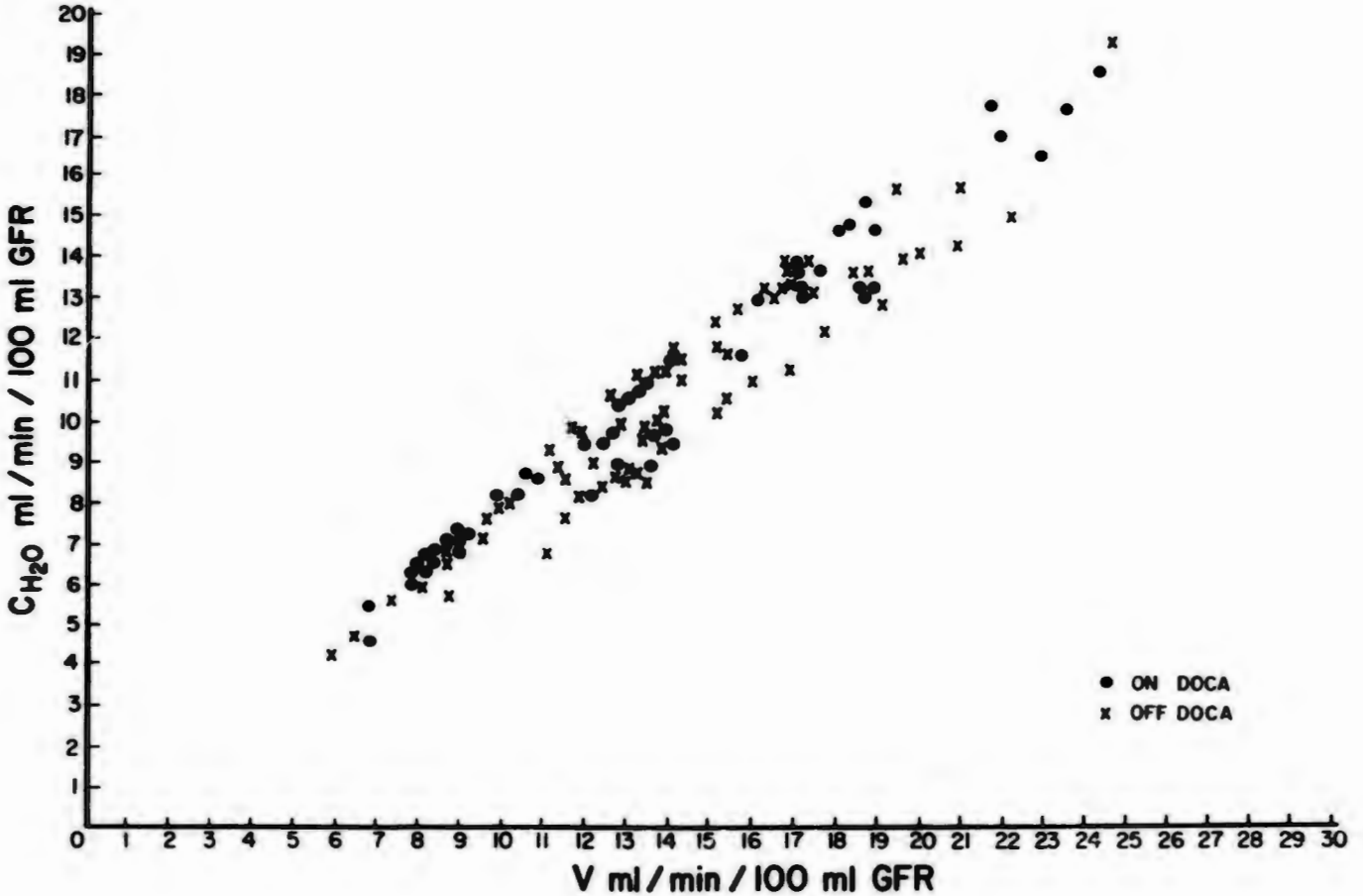

Figure 1: Comparisor of C_{H_2O}/100 ml GFR plotted against V/100 ml GFR for the entire group of animals in both the mineralocorticoid sufficient and aldosterone deficient state.

TABLE III

Solute free water reabsorption in a mineralocorticoid sufficient dog.

TIME	GFR	V	U_{Osm}	P_{Osm}	C_{Osm}	$T_{C_{H_2O}}$	$\frac{C_{Osm}}{GFR} \times 100$	$\frac{T_{C_{H_2O}}}{GFR} \times 100$
Min	ml/min	ml/min	mOsm/kg	mOsm/kg	ml/min	ml/min	%	%
Dog 164, wt 11.8 kg								
0-20 Infuse 5% NaCl at 0.5ml/min + aqueous vasopressin in saline at 50 mU/kg/hr (0.5ml/min)								
20-30	35.5	2.80	506	310	4.57	1.77	12.87	4.98
30-40	32.1	1.60	588	304	3.09	1.49	9.62	4.64
40-50	44.5	1.40	668	307	3.05	1.65	6.85	3.70
Infuse of 5% NaCl at 1.0 ml/min								
50-60	34.3	1.50	567	307	2.77	1.27	8.07	3.70
60-70	34.6	1.60	509	307	2.65	1.05	7.65	3.03
70-80	37.5	1.65	534	307	2.87	1.22	7.65	3.25
Infuse 5% NaCl at 1.5 ml/min								
80-90	32.0	1.35	537	307	2.36	1.01	7.37	3.15
90-100	40.6	1.65	530	303	2.89	1.24	7.11	3.05
100-110	36.6	1.50	526	306	2.58	1.08	7.04	2.95
Infuse 5% NaCl at 2.0 ml/min								
110-120	36.9	1.50	520	306	2.55	1.05	6.91	2.84
120-130	38.8	1.50	534	307	2.61	1.11	6.72	2.86
Infuse 5% NaCl at 3.0 ml/min								
130-140	43.4	1.50	548	304	2.70	1.20	6.22	2.76
140-150	39.4	1.55	539	302	2.77	1.22	7.03	3.09
Infuse 5% NaCl at 4.0 ml/min								
150-160	37.0	1.50	535	300	2.68	1.18	7.24	3.18
160-170	39.5	2.20	522	311	3.69	1.49	9.30	3.80
170-180	40.4	2.50	511	301	4.24	1.74	10.50	4.30

TABLE IV

Solute free water reabsorption in an aldosterone deficient dog

TIME	GFR	V	U_{Osm}	P_{Osm}	C_{Osm}	$T_{C_{H_2O}}$	$\frac{C_{Osm}}{GFR} \times 100$	$\frac{T_{C_{H_2O}}}{GFR} \times 100$
Min	ml/min	ml/min	mOsm/kg	mOsm/kg	ml/min	ml/min	%	%
Dog IG4, wt 11.1 kg								
0-20	Infuse 5% NaCl at 0.5 ml/min+aqueous vasopressin in saline 50mU/kg/hr (0.5 ml/min)							
20-30	30.0	0.25	1406	302	1.15	0.90	3.83	3.00
30-40	33.2	0.35	1171	306	1.37	1.02	4.12	3.07
40-50	26.9	0.35	978	306	1.12	0.77	4.16	2.86
Infuse 5% NaCl at 1.0 ml/min								
50-60	41.7	0.40	1208	304	1.59	1.19	3.81	2.85
60-70	34.0	0.45	1039	308	1.52	1.07	4.47	3.14
70-80	33.5	0.50	1008	307	1.64	1.14	4.89	3.40
Infuse 5% NaCl at 3.0 ml/min								
80-90	41.4	0.95	947	313	2.87	1.92	6.93	4.63
90-100	34.8	1.35	723	331	2.99	1.64	8.59	4.71
100-110	35.9	2.40	631	334	4.53	2.13	12.61	5.93
Infuse 5% NaCl at 5.0 ml/min								
110-120	41.8	4.1	553	350	6.48	2.38	15.50	5.69
120-130	42.0	4.3	525	350	6.45	2.15	15.35	5.11
130-140	42.1	5.1	515	341	7.70	2.60	18.28	6.17

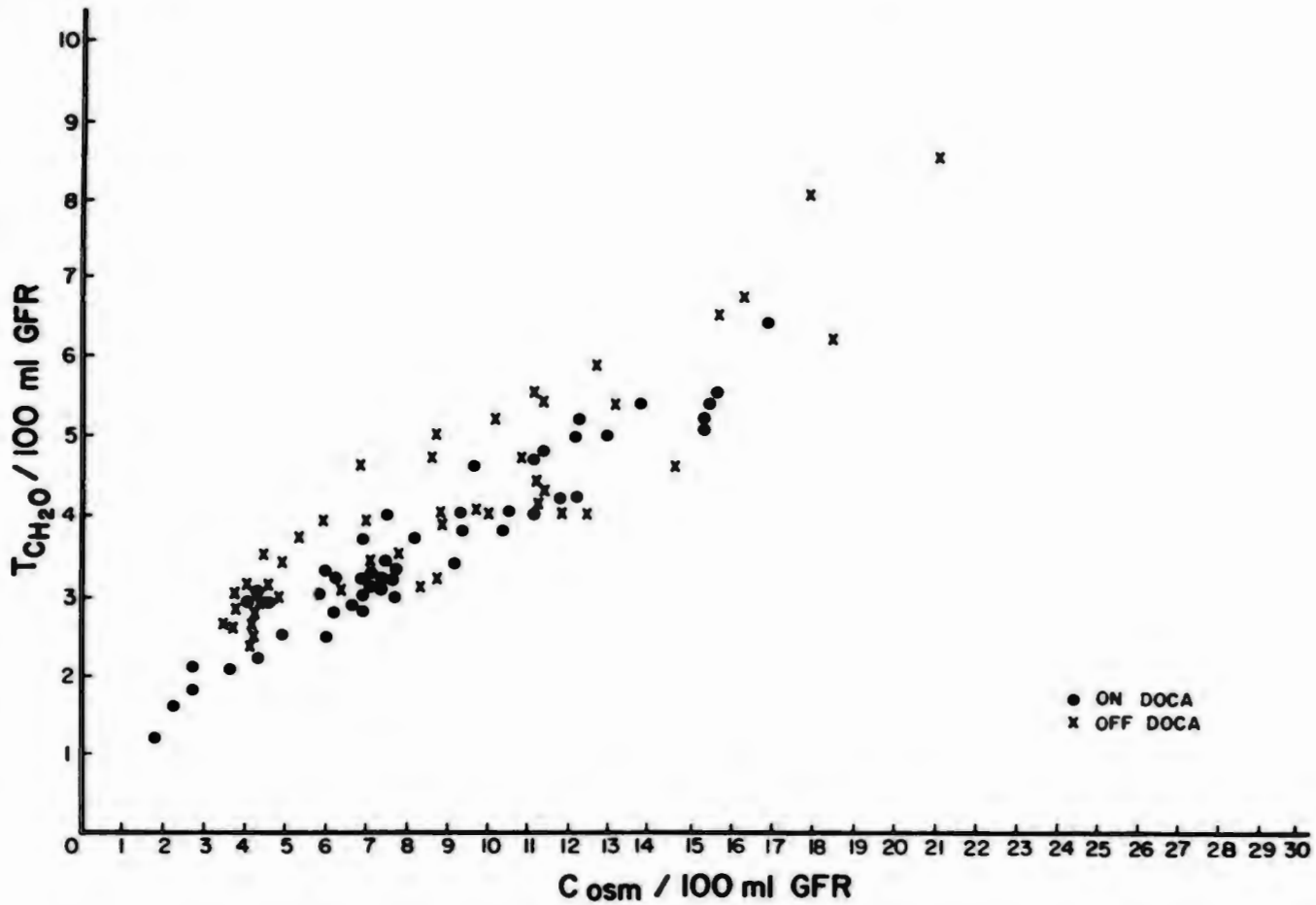

Figure 2: Comparison of T_{CH_2O}/100 ml GFR plotted against COsm/ 100 ml GFR for the entire group of animals in both the mineralocorticoid sufficient and aldosterone deficient state.

the regression line for the data from the normal group is Y = 1.19 + 0.29X as compared to Y = 1.81 + 0.25X in the aldosterone deficient group. The r value is 0.93 in the mineralocorticoid sufficient group and 0.92 in the aldosterone deficient group. Regression analysis by the method described above demonstrated no significant difference between the two groups.

The regression line for the normal dogs not given DOCA or ADH is Y = 2.13 + 0.26X. Determination of the difference between the two linear regressions from the mineralocorticoid sufficient dogs, given DOCA and ADH and the normal group of dogs, not given DOCA and ADH, using the Student t test failed to demonstrate any statistical difference between the two groups. Thus the physiologic doses of DOCA administered to the normal dogs studied in the mineralocorticoid sufficient state resulted in no impairment of sodium reabsorption and and $T_{C_{H_2O}}$ formation as measured with these clearance techniques. The administration of ADH during solute diuresis also apparently had no effect on $T_{C_{H_2O}}$ formation since regression analysis showed no difference between the mineralocorticoid sufficient dogs given 50 mU/kg/min ADH during the study and the normal dogs in which the ADH infusion was not administered.

Maximal urinary concentration in the mineralocorticoid deficient group was 1356 mOsm/kg ± 254 (SD) and 1386 mOsm/kg ± 331(SD) in the aldosterone deficient group. This difference is obviously

not significant. DOCA treatment did not result in weight gain in any of the dogs studied.

DISCUSSION

Although the effects of aldosterone on electrolyte excretion are well known (Yunis, SL, Bercovitch DD, Stein RM, Levitt MF and Goldstein MH: J Clin Invest 43: 1668, 1964[16]; Bartter FC: Metabolism 5: 369, 1956.[17]; Ross EJ, Reddy WJ, Rivers A, and Thorn GW: J. Clin Endocrin 19: 289, 1959[18]; Mills JN, Thomas S, and Williamson KS: J Physiol 151: 312, 1960[19]; Pitts RF: Transactions of the Third Conference on adrenal cortex of the Joshia Macy Jr. Foundation. Progress Assn. Inc., Caldwell, NJ, 1952, p 11.[20]) there has been much controversy with respect to the specific site of action of aldosterone in the nephron. Several investigators have suggested a defect in proximal tubular sodium reabsorption following adrenalectomy (Stolte H, Wiederholt M and Hierholzer K: Berlin/Heidelberg/New York, Springer 1966, p.521.[21]; Cortney MA, Physiologist 9: 158, 1966[22]; Hierholzer K, Wiederholt M and Stolte H: Pfuegers Arch Ges Physiol 291: 43, 1966[23]). Recent studies by Kurtzman, White, and Rogers(Kurtzman, et al, 1971)[1] have provided inferential evidence that aldosterone deficiency results in no significant net depression of proximal sodium reabsorption. Wright, Knox, Howards, and Berliner (Wright, et al, 1969)[3] using direct measurements failed to demonstrate any effect of selective aldosterone deficiency on sodium reabsorption in the proximal tubule.

Since there is suggestive evidence that aldosterone may inhibit sodium reabsorption in the ascending limb of Henle's loop, (Sonnenblick, et al, 1961[7]; Grabbe, et al, 1962[9]; Kessler, et al[10]) further studies were indicated to determine if this segment of the nephron is responsive to the influence of aldosterone.

This study was designed to measure the effect of aldosterone deficiency on the ascending limb of Henle's loop in the nephron of the dog by measurement of solute-free water clearance and reabsorption. Utilizing this model involves several critical assumptions: first that C_{H_2O} is a reasonable index of sodium reabsorption in the diluting segment; and second, that urine flow is approximately equal to the delivery of filtrate to the ascending limb of Henle's loop (Seldin DW, Eknoyan G, Suki WN and Rector FC, Jr.,: Ann NY Acad Sci 139: 328, 1966)[24]. There may be possible sources of error in those assumptions in that back diffusion of water out of the collecting duct would reduce C_{H_2O} not because of decreased sodium reabsorption but because of increased loss of free-water. This back diffusion is minimal, however, with maximal support of ADH as was accomplished in this study with initial urine osmolalities less than 75 mOsm/kg. The use of TC_{H_2O} as an index of sodium reabsorption in the ascending limb also may be somewhat hazardous since increased delivery

of fluid to a normally functioning ascending limb would result in the entrance of large volumes of hypotonic fluid into the collecting duct. With this increased flow rate there may be poor equilibration between the fluid in the collecting duct and medullary interstitium resulting in the excretion of urine which is less concentrated probably due to poor equilibration. Thus the calculated value for $T_{C_{H_2O}}$ may be falsely low and would underestimate the magnitude of sodium reabsorption in the ascending limb during rapid rates of solute diuresis. Experiments utilizing hypertonic saline, however, tend to eliminate this possibility as the cause of impaired $T_{C_{H_2O}}$ (Martinez-Maldonado M, Eknoyan G, and Suki WN: Am J Physiol 218: 1076, 1970)[25]. If aldosterone had a stimulatory effect on sodium reabsorption in the ascending limb of the loop of Henle the clearance of solute free water during water diuresis and the reabsorption of solute free water at any level of urinary flow or osmotic clearance respectively would be depressed in its absence. Although this study was designed to minimize errors in the measurement of C_{H_2O} and $T_{C_{H_2O}}$ in the presence and absence of aldosterone a small effect of aldosterone on sodium reabsorption might be missed using these clearance techniques.

Since our data failed to demonstrate any effect of aldosterone deficiency on free water clearance and reabsorption, or on

maximal urinary concentration, we conclude that aldosterone exerts no gross effect on sodium reabsorption in the ascending limb of the loop of Henle. Another way that a depressive effect of aldosterone deficiency on ascending limb of the loop of Henle. Another way that a depressive effect of aldosterone deficiency on ascending limb sodium reabsorption might be missed would be for DOCA administration and/or saline administration to expand extracellular volume. This volume expansion might result in depressed sodium reabsorption by the ascending limb of the loop of Henle so that the results obtained in our control animals were really depressed values secondary to volume expansion. When these results were compared with those obtained from the aldosterone deficient animals no difference would be discernible and if volume had not been expanded there would have been an apparent difference between the control and aldosterone deficient groups. We think that this series of events played no role in our study for the following reasons. First none of our animals gained weight while receiving DOCA. These animals received only 1 mg/day of DOCA. Our experience indicates that for adrenalectomized animals weighing 7.5 to 15 kg a maintnenance dose of DOCA is 0.5 to 1.0 mg/day. The dose of DOCA required for volume expansion is 1 mg/kg or more. Thus, it is unlikely that volume expansion occurred in any of our animals.

Second, if the curves obtained in this study from plotting C_{H_2O}/GFR VS V/GFR are compared to those of other investigators who did not give DOCA to their animals and who administered 0.45% saline, 0.125% saline, or 2.5% glucose (Seldin, et al, 1966[24]; Martinez, et al, 1970[25]; Eknoyan G, Suki WN, Rector FC, Jr and Seldin DW, J Clin Invest 46: 1178, 1967[26]) it is found that in each instance C_{H_2O} at any level of V is equal or greater in our animals than it is in the others, indicating that ascending limb reabsorption in our animals was not depressed. Similar results are obtained if one compares our $T_{C_{H_2O}}$ data with that of others (Seldin, et al, 1966[24]; Martinez, et al, 1970[25]).

Third, Barton and co workers (Barton LJ, Lackner LH, Rector FC, Jr., and Seldin DW: Kidney Int'nl 1: 19, 1972)[27] have recently demonstrated that in the dog extracellular volume expansion (with DOCA and saline) does not depress sodium reabsorption in the diluting segment of the ascending limb indicating that even if volume had been expanded it would not have influenced our results.

The information presented in this study when added to that which demonstrates no effect of aldosterone on proximal sodium reabsorption in a localized segment of the distal tubule (presumably the sodium for potassium exchange site). Thus under the influence of aldosterone sodium is reabsorbed in exchange for either

with adrenal insufficiency may be the consequence of either inhibition of vasopressin release from the neurohypophysis (Ahmed AB George BC, Gonzalez-Auvert C, and Dingman DF: J Clin Invest 46: 111, 1967)[28] or a direct effect of glucocorticoid on renal tubular permeability (Garrod, et al, 1955)[8].

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PUBLICATIONS AND PRESENTATIONS: None

FINAL REPORT

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

REPORT TITLE: EFFECT OF CHLORIDE AND EXTRACELLULAR VOLUME ON CORRECTION OF METABOLIC ALKALOSIS - A COMMON PROBLEM IN THE INJURED TROOP

THE ROLE OF THE RENIN-ANGIOTENSIN-ALDOSTERONE SYSTEM IN ADAPTATION TO CHRONIC HYPERCAPNIA IN DOGS

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

1 July 1972 - 30 June 1973

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Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

REPORT TITLE: EFFECT OF CHLORIDE AND EXTRACELLULAR VOLUME ON CORRECTION OF METABOLIC ALKALOSIS-A COMMON PROBLEM IN THE INJURED TROOP

THE ROLE OF THE RENIN-ANGIOTENSIN-ALDOSTERONE SYSTEM IN ADAPTATION TO CHRONIC HYPERCAPNIA IN DOGS

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

Period covered in this report: 1 July 1972 - 30 June 1973

Investigators: A Nowakowski, Major, MC
DA Nash, Jr., Major, MC
PW Rogers, Major, MC
NA Kurtzman, Lieutenant Colonel, MC

Reports Control Symbol MEDDH-288(R1)

There is evidence that the renin-angiotensin-aldosterone system plays a role in the generation and maintenance of metabolic alkalosis. To examine the importance of this system for renal compensation of chronic respiratory acidosis, we examined the plasma renin activity (PRA) and serum HCO_3^- response of 5 dogs in the intact and adrenalectomized (ADX) state in a continuous 10% CO_2 environment.

Mean control serum HCO_3 and arterial pH for intact dogs were 22.4 mEq/L and 7.42 respectively. After three days of hypercapnia, mean serum HCO_3^- of 33.0 mEq/L and mean arterial pH of 7.30 were obtained. After ADX, the mean control serum HCO_3^- and arterial pH were 22.7 mEq/L and 7.44 respectively. During chronic hypercapnia the serum HCO_3^- was 32.4 mEq/L and the mean arterial pH was 7.31. The mean PRA for the control period in the intact dogs was 0.67 ng/ml/hr and 2.62 ng/ml/hr for the experimental period (N= 1-3 ng/ml/hr). After ADX mean PRA was 7.94 ng/ml/hr in the control period and 8.48 ng/ml/hr during the experimental period. Increased PRA in the adrenalectomized animals was probably due tc mild intravascular volume contraction.

The changes in the serum HCO_3^- and arterial pH after chronic hypercapnia showed no significant difference between the intact and adrenalectomized dog.

THE ROLE OF THE RENIN-ANGIOTENSIN-ALDOSTERONE SYSTEM IN ADAPTATION TO CHRONIC HYPERCAPNIA IN DOGS

This experiment was undertaken to determine the role of aldosterone in the elevation of serum bicarbonate which occurs in animals and man during the compensation of respiratory acidosis. New bonate is generated in the kidney during aldosterone enhanced hydrogen ion secretion in the distal tubule. This process occurs during metabolic alkalosis associated with primary and secondary aldosteronism. The mechanism of generation of bicarbonate during adaptation to chronic hypercapnia is not known.

METHODS

Five dogs were studied in environmental chambers in a normal, then 10% CO_2 atmosphere. They were studied in the intact and then in the adrenalectomized state. After adrenalectomy, the dogs were placed on maintenance dexamethasone and desoxycorticosterone consisting of 0.75 mg and 0.5 mg IM daily, respectively. Sodium, chloride, and potassium balances were performed and serial plasma renin measurements were obtained during the control and experimental periods.

RESULTS

Shown in Figure 1 are the balance data from a representative dog during the control and experimental periods. Mean serum bicarbonate levels were 22.4 mEq/L in the control period and 33.0 mEq/L with hypercapnia in the intact dog and were 22.7 mEq/L and 32.4 mEq/L respectively in the adrenalectomized state. The mean plasma renin activity in the normal dog was 0.6 ng/ml/hr during the control period and 2.62 ng/ml/hr during the experimental period. After adrenalectomy the mean renin concentration was 7.94 ng/ml/hr during the control period and 8.48 ng/ml/hr during the experimental period. The increase in plasma renin concentration during the aldosterone deficient state is probably related to mild extracellular volume contraction. Statistical analysis of these data by analysis of variance suggests significant difference between the intact and adrenalectomized dog.

CONCLUSION

This study suggests that aldosterone plays no role, in the dog, in elevating serum bicarbonate during compensation for hypercapnia.

ADAPTATION TO HYPERCAPNEA IN THE INTACT VS. ADRENALECTOMIZED DOG

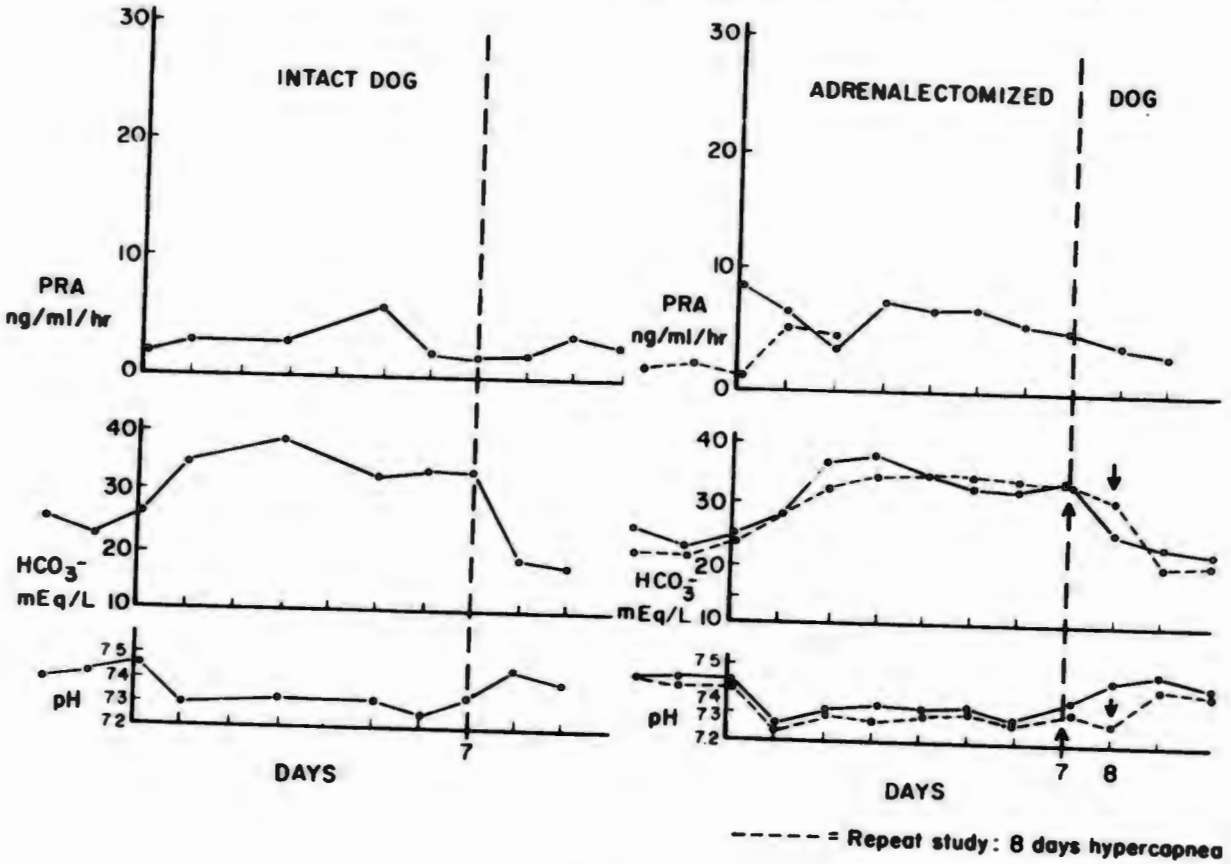

Fig. 1.

PRESENTATIONS AND PUBLICATIONS: None.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6954	73 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
72 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	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 73	.4	19
FISCAL YEAR CURRENT 74	.5	12

19. RESPONSIBLE DOD ORGANIZATION
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RESPONSIBLE INDIVIDUAL
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SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS
NAME: Daniel A. Nash Jr, MAJ, MC
NAME: Andrew Nowakowski, MAJ, MC DA

21. GENERAL USE: FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code): (U) Bicarbonate Reabsorption; (U) Sodium; (U) ADH; (U) Extracellular Volume; (U) Potassium; (U) Burned Soldiers

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 homeostasis 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 anti-diuretic hormone (ADH) and prostaglandin E1 (PGE1) on renal bicarbonate reabsorption in the dog.

24. (U) Study #1 50 mU/kg/min and 50 mU/kg/hr of vasopressin (ADH) were infused into anti-diuretic mongrel dogs. Study #2 PGE1 was infused into the right renal artery at the rate of 2ug/min. Both ureters were cannulated so that the left kidney serves as the control. 0.9M sodium bicarbonate was infused to maintain a plasma bicarbonate concentration of approximately 30 mEq/liter.

25. (U) 72 07 - 73 06 Study #1 The infusion of vasopressin (ADH) was found to be markedly chloruretic with a fractional chloride excretion going from a control value of 0.9% plus or minus 0.31 (SE) to 11.1 plus or minus 2.2(SE). ADH infused into bicarbonate loaded dogs was even more chloruretic with the fractional chloride excretion rising from 1.9 plus or minus 0.3 to 20.2 plus or minus 7.7. Bicarbonate and glucose reabsorption was not inhibited by ADH infusion; however, phosphate reabsorption was markedly depressed with the fraction phosphate excretion rising from a control value of 18.5% plus or minus 10.6 to 55.7% plus or minus 8.9 during the infusion. These data suggest that ADH, in large dosage is a diuretic on the order of potency of ethyacrynic acid. Its main site of action in the nephron is likely the ascending limb of the loop of Henle rather than the proximal tubule. Study #2 The data collected thus far on the infusion of PGE1 into the right renal artery suggest the PGE1 has no effect on bicarbonate reabsorption although there is a mild natiuresis and a mild drop in blood pressure.

* Available to contractors upon originator's approval

ANNUAL PROGRESS REPORT

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 - STUDY # 1 THE EFFECT OF INFUSION OF PHARMACOLOGIC AMOUNTS OF VASOPRESSIN ON RENAL ELECTROLYTE EXCRETION

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ABSTRACT

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REPORT TITLE: THE EFFECT OF INFUSION OF PHARMACOLOGIC AMOUNTS OF VASOPRESSIN ON RENAL ELECTROLYTE EXCRETION

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Aqueous vasopressin was infused to bicarbonate and glucose loaded dogs and to unloaded antidiuretic dogs in doses of 50 mu/kg/min or 50 mu/kg/hr. Both of these doses caused a marked increase in sodium, chloride and water excretion, the larger dose raising the fractional excretion of these ions from 0-2% to more than 20%. Blocking the pressor effects of these doses of vasopressin with sodium nitroprusside did not alter the marked natriuretic and chloriuretic effect of vasopressin. The diuretic effect of vasopressin was not associated with a fall in filtration fraction. The maximal rate of bicarbonate and glucose reabsorption was not depressed by vasopressin infusion; fractional phosphate excretion, however, was markedly increased by vasopressin, reaching values in excess of 50%. Inhibiting distal hydrogen ion secretion by inducing selective aldosterone deficiency failed to uncover a vasopressin induced inhibition of proximal bicarbonate reabsorption which might have been masked by increased distal bicarbonate reabsorption. This study demonstrates an exceptionally potent diuretic action of vasopressin when administered in pharmacologic doses. This diuretic action manifests itself by markedly increasing urine volume as well as electrolyte excretion and is seen in antidiuretic animals as well as those undergoing solute diuresis.

Bicarbonate Reabsorption
Sodium
Extracellular Volume
Potassium ADH
Burned Soldiers

THE EFFECT OF INFUSION OF PHARMACOLOGIC AMOUNTS OF VASOPRESSIN ON RENAL ELECTROLYTE EXCRETION

Vasopressin has been known for some time to cause a natriuresis as well as to increase the excretion of other cations (Sawyer WH, Amer J Physiol 169: 583, 1952[1]; Anslow WP, and Wesson LG, Jr, Amer J Physiol 182: 561, 1955[2]; Ali MN, Brit J Pharmacol 13: 131, 1958[3]; Brooks FP and Pickford M: J Physiol London 142, 486, 1958[4]; Thorn NA andMilewski B: Proc Soc Exptl Biol Med 100: 267, 1959[5]; Sawyer WH: Pharmacol Rev 13: 225, 1961[6]; Chan WY and Sawyer WH: Proc Soc Exptl Biol Med 110:697, 1962[7]; Kramar J and Grinnell EH: Amer J Med Sci 252:53, 1966[8]; Grinnell EH, Kramar J, Duff WM and Lydon TE: Endocrinology 83: 199, 1968[9]; Lindeman RD: Ann N Y Acad Sci 162: 802, 1969[10]; Atherton JC, Hai MA and Thomas S: Arch Ges Physiol 310: 281, 1969[11]; Humphreys MH, Friedler RM, and Earley LE: Amer J. Physiol 219: 658, 1970[12]; Martinez-Maldonado, M, Eknoyan G and Suki WN: Amer J Physiol 220: 2013, 1971[13]). This natriuresis is rapid in onset, of brief duration, and said only to occur during water diuresis (Anslow WP, and Wesson LG Jr, 1955[2]; Ali MN, 1958[3]; Brooks FP and Pickford M, 1958[4]; Chan WY and Sawyer, 1962[7]; Humphreys MH, et al, 1970[12]; Martinez-Maldonado, M, et al, 1971[13]). The magnitude of the increase in sodium excretion resulting from vasopressin administration is not great.

The site in the nephron at which sodium reabsorption is inhibited is proximal to the site at which potassium is secreted. Both the proximal tubule(Martinez-Maldonado, M, et al, 1971)[13] and the ascending limb of the loop of Henle (Humphreys MH, et al, 1970)[12] have been postulated as being the site at which vasopressin exerts its natriuretic effect.

The current studies were initiated to examine the natriuretic effect of vasopressin in further detail by using much larger amounts of the hormone than have previously been administered. We reasoned that by infusing pharmacologic doses of vasopressin we might exaggerate its effects on both electrolyte and water handling by the kidney which would allow us to more precisely separate the two.

METHODS

Eleven groups of dogs were studied as follows. The first group of four animals received 0.9 M $NaHCO_3$ at 1 ml/min and 0.15

M NaCl at 0.4 ml/min. After a 60 minute equilibrium period 2-4 control clearance periods were obtained. The infusion of 0.15 M NaCl was then stopped and an infusion of aqueous vasopressin (Aqueous Pitressin^{R} Parke-Davis) in 0.15 M NaCl substituted for it. Vasopressin was infused at a flow rate of 0.4 ml/min and at a concentration sufficient to deliver 50 mu/kg/min; 4-8 experimental periods were obtained. Vasopressin infusion was then stopped and 0.15M NaCl was infused in its place at a flow rate of 0.4 ml/min; 3-8 post experimental periods were obtained. Fluid losses were replaced with a solution containing three parts distilled water and two parts Ringer's lactate. This fluid replacement was not started until after the conclusion of the second experimental period. All clearance periods were ten minutes in duration.

A second group of seven dogs was studied in a fashion identical to the first except that the blood pressure was kept at control levels by the infusion of sodium nitroprusside. This solution contained 120 mg of nitroprusside dissolved in 250 ml of 5% dextrose in H_2O. The total amount of nitropruisside solution infused to a single dog never exceeded 5 ml.

The third group of five dogs was studied exactly as was the first save that the dose of vasopressin infused was 50 mu/kg/hour.

The fourth group of five dogs was studied exactly as was the second save that the dose of vasopressin infused was 50 mu/kg/hour.

The fifth group of seven dogs was studied exactly as was the first except that Ringer's lactate was infused at 1 ml/min in place of 0.9 M $NaHCO_3$; fluid replacement was with undiluted Ringer's lactate. The control periods were of 20 minutes duration in this group.

The sixth group of four dogs was studied as was the fifth except that the blood pressure was controlled with sodium nitroprusside.

The seventh group of four dogs was studied exactly as was the fifth except that the dose of vasopressin was reduced to 50 mu/kg/hr.

The eight group of four dogs was studied as was the seventh save that the blood pressure was controlled with sodium nitroprusside.

The ninth group of seven dogs was treated identically to the fifth group except that 20% glucose was infused at 2.3 ml/min instead of Ringer's lactate at 1 ml/min.

The tenth group of seven dogs was studied as was the first group except that the animals had previously been subjected to bilateral adrenalectomy. For at least two weeks following surgery they were maintained with daily injections of 0.5 mg of desoxycorticosterone acetate (DOCA) and 0.75 mg of dexamethasone. No DOCA was given 96 hours prior to surgery; we have previously shown that this procedure results in selective aldosterone deficiency (Kurtzman NA, White MG and Rogers PW, J Lab Clin Med 77: 931, 1971)[14].

The eleventh group of four dogs was studied exactly as was the fifth except that the clearance of para-aminohippurate was also measured.

Plasma and urine PO_4 were measured according to the method of Fiske and Subbarow (Fiske CH and Subbarow Y, J Biol Chem 66: 375, 1925)[15] adapted to the autoanalyzer. The methods used to measure GFR, Na, K, Cl, pCO_2, pH and glucose in blood and urine were identical to those previously described (Kurtzman NA: J Clin Invest 49: 586, 1970[16]; Kurtzman NA, White MG, Rogers PW, and Flynn JJ, III, J Clin Invest 51: 127, 1972 [17]) as were the methods of sample collection, measurement of blood pressure, calculation, and statistical analysis.

RESULTS

Group I - IV: The effect of infusing 50 mu/kg/min of vasopressin to a bicarbonate loaded dog is outlined in Table I. Vasopressin infusion increased fractional sodium excretion from 4 to almost 22%, while chioride excretion went from 1.5% to almost 22%. GFR rose, the urine flow rate rose dramatically, from 1 ml/min to almost 16 ml/min, there was also a marked increase in arterial blood pressure. Despite this marked natriuretic and chloriuretic effect of vasopressin infusion, bicarbonate reabsorption changed trivially. The effect of infusing 50 mu/kg/min of aqueous vasopressin to a bicarbonate loaded dog in which the blood pressure was prevented from rising by infusion of sodium nitroprusside is outlined in Table II. The results were similar to those seen in the animals whose blood pressure was not controlled. There was an increased sodium chloride excretion, though not quite as marked, GFR tended to rise, and there was a marked increase in

TABLE I

Effect of Aqueous Vasopressin on Bicarbonate Reabsorption

TIME	BLOOD pH	BLOOD pCO_2	BLOOD HCO_3	GFR	URINE Flow	URINE Na^+	URINE K^+	URINE Cl^-	$\frac{C_{Na}}{GFR} \times 100$	$\frac{C_{Cl}}{GFR} \times 100$	BICARBONATE Filt.	BICARBONATE Excre.	BICARBONATE Reab.	BICARBONATE B.P.
min		mm Hg	mEq/L	ml/min	ml/min		μEq/min		%	%	μEq/min		mEq/L GFR	mm Hg
Dog # 3; wt. 15.5 kg														
0-60	Infuse 0.9 M $NaHCO_3$ at 1 ml/min; infuse 0.15 M NaCl at 0.4 ml/min.													
60-70	7.56	37	32.1	40.4	0.80	185	36	66	3.1	1.5	1297	295	24.8	160
70-80	7.59	35	32.4	45.0	0.85	230	26	68	3.4	1.4	1458	356	24.5	160
80-90	7.54	39	32.2	44.3	0.80	225	19	50	3.4	1.0	1426	270	26.0	158
90-100	7.54	39	32.2	42.5	1.05	270	20	71	4.2	1.5	1369	251	26.3	158
100-105	D/C 0.15 M NaCl; infuse aqueous vasopressin in 0.15 M NaCl at 0.4 ml/min (50 mu/kg/min).													
105-115	7.59	37	34.3	51.6	3.00	645	96	330	8.2	5.8	1770	375	27.0	163
115-125	7.54	43	35.6	52.5	6.50	1203	150	715	14.8	12.3	1869	509	25.9	165
125-135	7.51	44	33.9	71.2	14.5	2306	131	1581	20.8	20.2	2413	601	25.4	177
135-145	7.50	45	33.9	68.5	15.3	2321	127	1622	21.7	21.7	2322	600	25.1	177
	D/C aqueous vasopressin; infuse 0.15 M NaCl at 0.4 ml/min.													
145-155	7.49	45	33.1	68.9	17.0	2346	153	1615	21.7	21.5	2381	573	24.8	177
155-165	7.54	40	33.1	64.8	15.2	2052	152	1398	20.2	19.6	2145	587	24.0	177
165-175	7.52	42	33.4	65.5	14.6	1942	146	1299	18.9	18.0	2188	575	24.6	163
175-185	7.52	43	34.0	64.2	15.6	2059	156	1342	20.6	19.2	2217	568	25.7	150

TABLE II

Effect of Aqueous Vasopressin on Bicarbonate Reabsorption with Controlled Blood Pressure

TIME	BLOOD pH	BLOOD pCO_2	BLOOD HCO_3	GFR	URINE Flow	URINE Na^+	URINE K^+	URINE Cl^-	$\frac{C_{Na}}{GFR} \times 100$	$\frac{C_{Cl}}{GFR} \times 100$	BICARBONATE Filt.	BICARBONATE Excre.	BICARBONATE Reab.	B.P.
Min		mm Hg	mEq/L	ml/min	ml/min		μEq/min		%	%		μEq/min		mEq/L GFR	mm Hg
Dog #7; wt. 13.5 kg															
0-60	Infuse 0.9 M $NaHCO_3$ at 1 ml/min; infuse 0.15 M NaCl at 0.4 ml/min.														
60-70	7.53	43	35.2	43.7	2.30	428	8	117	6.2	2 4	1540	250	29.5	110
70-80	7.57	40	35.5	40.5	2.85	529	7	160	8.1	3.5	1439	270	28.9	110
80-90	7.62	37	36.9	36.9	2.70	564	7	150	7.8	3.0	1361	367	26.9	100
90-100	7.62	35	34.4	36.7	2.40	585	7	148	7.3	2.9	1262	349	24.9	100
D/C 0.15 M NaCl; infuse aqueous vasopressin in 0.15 M NaCl at 0.4 ml/min (50 mu/kg/min); control BP with sodium nitroprusside.															
100-110	7.61	35	34.0	42.4	6.00	1062	10	444	15.0	9.3	1442	360	25.5	100
110-120	7.62	35	34.6	47.9	8.80	1390	11	686	17.1	12.7	1657	520	23.7	100
120-130	7.59	38	35.0	46.3	7.90	1288	11	608	16.3	11.5	1621	495	24.3	100
130-140	7.56	42	36.5	58.6	9.00	1449	9	783	15.9	11.7	2137	474	28.4	100
140-150	7.59	39	36.2	46.2	8.95	1396	8	698	17.0	13.4	1672	506	25.2	100
150-160	D/C aqueous vasopressin and sodium nitroprusside; infuse 0.15 M NaCl at 0.4 ml/min.														
160-170	7.55	40	33.9	50.4	4.80	684	10	346	8.20	6.7	1709	226	29.4	100
170-180	7.56	40	35.0	49.4	4.30	651	12	297	7.90	5.5	1729	341	28.1	100

urine flow rate. Yet again, there was no significant change in bicarbonate reabsorption. The effects of infusing 50 mu/kg/hr of aqueous vasopressin to dogs in which the blood pressure was controlled or uncontrolled is presented in Figure 1. The group III animals (uncontrolled blood pressure) are represented by the closed circles while the group IV (controlled blood pressure) animals are represented by the open circles. The data obtained from the group I and II animals are also presented on this figure and are represented by the closed and open triangles respectively. The points represent the mean values obtained from each group. They were chosen in the following manner. The first point represents the mean data from the final control periods, the second point the mean data associated with the highest excretion of chloride and the final point in each of the four groups represents the mean data from the final post-experimental periods. As is apparent there is a marked increase in chloride excretion in the group I animals was significant at the .001 level. That obtained in the group II animals was significant at .02 level, the data from the group III animals was significant at the .05 level as was the data from the 4th group. Table III presents the mean plasma bicarbonate concentration and bicarbonate reabsorptive rate during the control periods and during vasopressin infusion to each of the four groups. The data was selected by averaging the final control periods and comparing them to the data associated with highest rate of chloride excretion. Vasopressin infusion was associated with no significant change in either the plasma bicarbonate concentration or the bicarbonate reabsorptive rate for each of the four groups.

Group V - VIII: The effect of infusing 50 mu/kg/min of aqueous vasopressin to an antidiuretic dog is detailed in Table IV. Sodium excretion was very minimal during the control periods as was the fractional excretion of chloride following the infusion of vasopressin, fractional sodium excretion rose from 0.3 to 6.3% while fractional chloride excretion rose from 0.2 to a high value of almost 8% and was associated with an increase in GFR and in urine flow rate. Blood pressure rose 20 mm Hg as well. Fractional phosphate excretion is presented on this table and rose dramatically from 7% to a high value of 40%. The phosphaturia, natriuresis and chloriuresis all persisted after discontinuing the vasopressin infusion. The effect of vasopressin infusion on the mean excretion of chloride in the four groups (V-VIII) is plotted in Figure 2. The data were selected as described in the previous figure. Again there was a marked

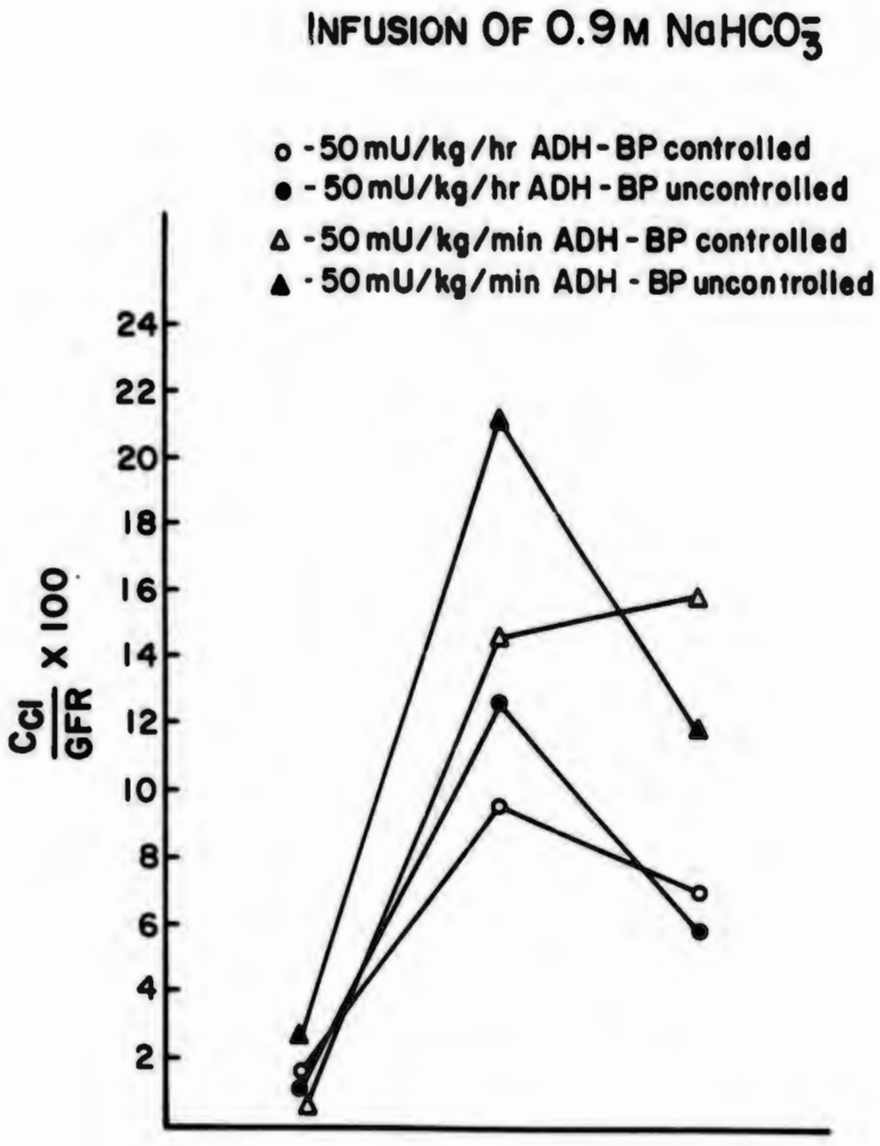

Figure 1. The effect of vasopressin infusion on mean fractional chloride excretion in bicarbonate loaded dogs. The first points are mean control periods, the second mean experimental data, while the final points are mean post-infusion data.

Table III

BICARBONATE REABSORPTION BEFORE AND DURING VASOPRESSIN INFUSION IN GROUPS I - IV

	Plasma HCO_3 mEq/Liter			Reabsorbed HCO_3 mEq/Liter GFR		
	Control		Vasopressin	Control		Vasopressin
Group I (n=4)	31.7 ± 1.1 (SEM)	NS	33 1 ± 1.7	22.7 ± 2.7	NS	21.8 ± 0.8
Group II (n=7)	34.4 ± 1.0	NS	34.6 ± 1.3	24.1 ± 0.4	NS	23.5 ± 1.0
Group III (n=5)	33.7 ± 3.8	NS	34.5°± 3.6	24.2 ± 2.7	NS	24.4 ± 2.6
Group IV (n=5)	38.8 ± 1.9	NS	31.7 ± 1.5	24.9 ± 1.5	NS	23.9 ± 1.7

TABLE IV

Effect of Vasopressin Infusion to an Antidiuretic Dog

TIME	GFR	URINE Flow	URINE Na^+	URINE K^+	URINE Cl^-	$\frac{C_{Na}}{GFR} \times 100$	$\frac{C_{Cl}}{GFR} \times 100$	$\frac{C_{PB_4}}{GFR} \times 100$	B.P.
Min	ml/min	ml/min		μEq/min		%	%	%	mm Hg
Dog # 25; wt. 18.2 kg									
0-60	Infuse Ringer's lactate at 0.9 ml/min; infuse 0.15 M NaCl at 0.4 ml/min.								
60-80	58.0	0.23	29	19		0.3	0.2	6	140
80-100	45.5	0.20	19	13		0.3	0.2	7	140
	D/C 0.15 M NaCl; infuse aqueous vasopressin in 0.15 M NaCl at 0.4 ml/min (50 mu/kg/min).								
100-120	58.0	1.08	147	69	167	1.8	2.5	11	160
120-130	70.5	3.25	475	127	507	4.7	6.1	25	160
130-140	66.0	3.95	608	91	604	6.3	7.9	40	160
140-150	70.7	3.10	542	68	515	5.3	6.3	35	160
150-160	71.2	2.50	483	80	445	4.7	5.4	37	160
	D/C aqueous vasopressin; infuse 0.15 M NaCl at 0.4 ml/min.								
160-170	68.7	4.08	688	112	644	6.9	8.1	45	150
170-180	75.6	3.15	584	107	526	5.4	6.1	43	150
180-190	74.6	2.55	479	110	423	4.5	4.9	42	150
190-200	73.8	2.52	459	93	386	4.3	4.6	36	150
200-210	79.7	2.48	432	91	338	3.8	3.7	32	140

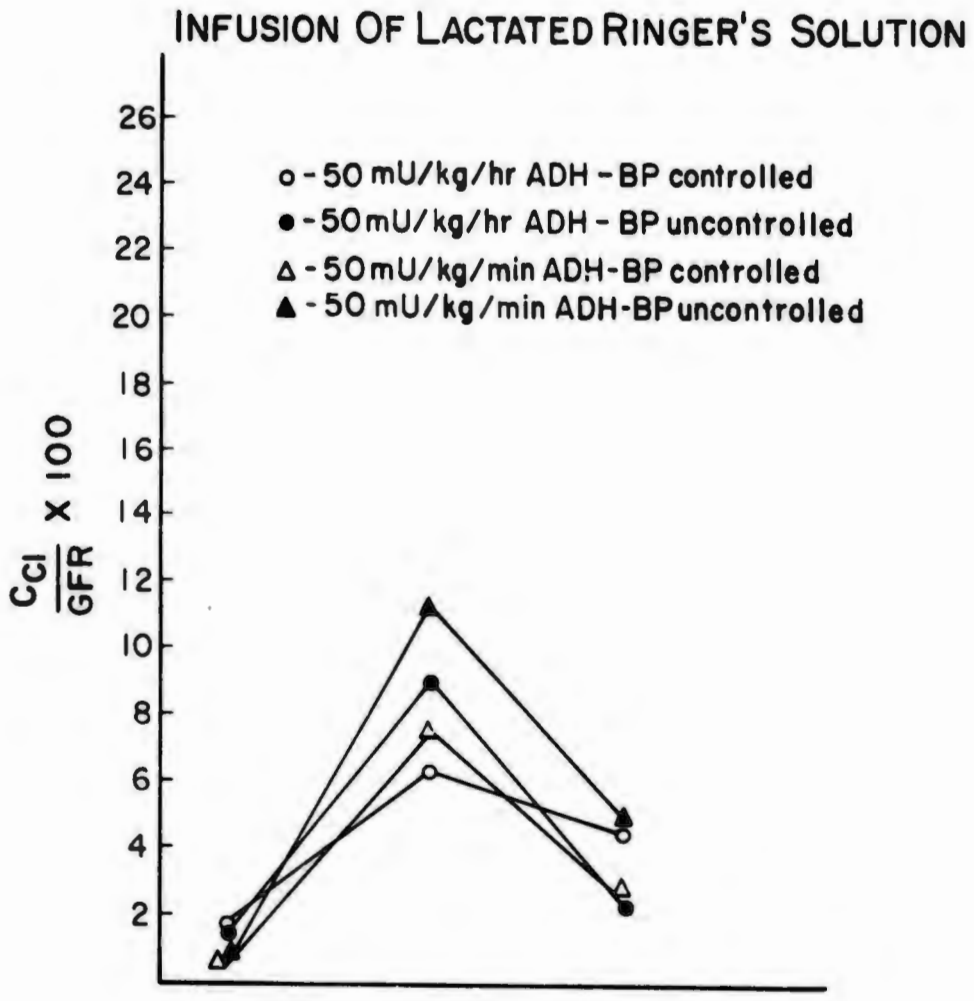

Figure 2. The effect of vasopressin infusion on mean fractional chloride excretion in antidiuretic animals infused with 0.9 ml/min of Ringer's Lactate.

increase in chloride excretion in each of the four groups. The data for the group V animals is plotted with closed triangles, that for the group VI animals with open triangles, that for the group VII animals with closed circles, and that for the group VIII animals with open circles. The increase in chloride excretion for the group V animals was significant at the .01 level, the increase for the group VI animals at the .02 level, the increase for both the group VII and VIII animals at the .05 level. Figure 3 presents the effect on fractional phosphate excretion in each of these four groups. Again, in each of the groups there was a highly significant increase in phosphate excretion; this was significant at the .01 level for group V, at .02 level for group VI and VII, and at the .05 level for group VIII. Also apparent is the fact that the phosphaturia persisted even when the vasopressin was discontinued.

Group IX: The effect of infusion of 50 mu/kg/min of aqueous vasopressin on glucose reabsorption in a glucose loaded dog is presented in Table V. Again there was a marked increase in sodium and chloride excretion, an increase in GFR and blood pressure but no major effect on glucose reabsorption. Table VI presents the mean data obtained from all the glucose loaded animals. The data were selected, again, as described above. There was a highly significant increase in both chloride and sodium excretion but no significant change in glucose reabsorption expressed either as mg/min or reabsorption per unit GFR.

Group X: The effect of the infusion of aqueous vasopressin in a dose of 50 mu/kg/min on bicarbonate reabsorption in aldosterone deficient dogs was also measured. The mean data are presented in Table VII. There was again a marked increase in flow rate and chloride excretion but there was no significant change in bicarbonate reabsorption in these animals. Since aldosterone deficiency markedly impairs distal hydrogen ion secretion these dogs were studied to exclude the possibility that increased distal hydrogen ion secretion might be obscuring the effect of vasopressin infusion on proximal hydrogen ion secretion (Kurtzman, et al, 1971).

Group XI: The effect of aqueous vasopressin infusion of 50 mu/kg/min on flow rate, chloride excretion, GFR, renal plasma flow and filtration fraction is presented in table VIII. There was a highly significant increase in both the flow rate and fractional chloride excretion. While GFR and renal plasma flow rose,

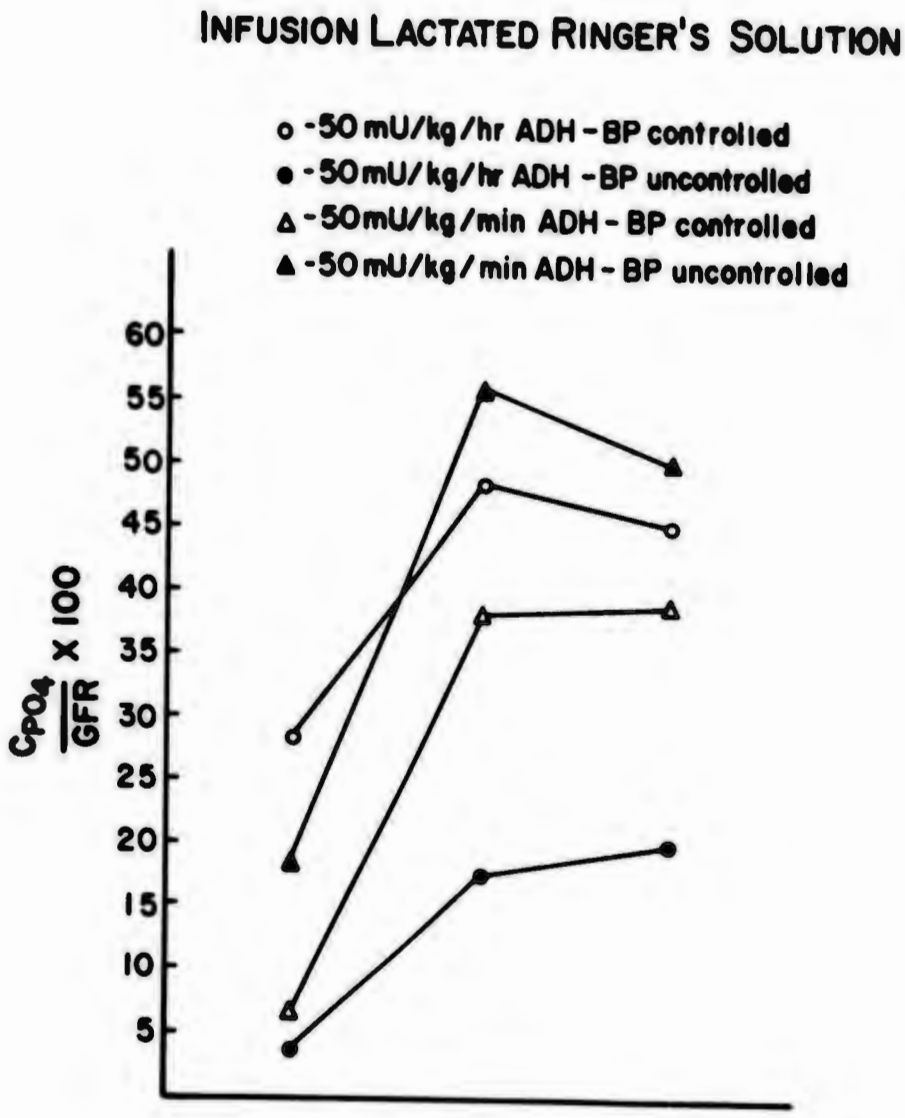

Figure 3. The effect of vasopressin infusion on mean fraction phosphate excretion in antidiuretic dogs.

TABLE V

Effect of Vasopressin Infusion on Glucose Reabsorption

TIME	PLASMA GLUCOSE	GFR	URINE Flow	Na^+	K^+	Cl^-	GLUCOSE Filt.	Excrn.	Reab.	Reab	C_{Na} / GFR x 100	C_{Cl} / GFR x 100	B.P.
Min	mg/100 ml	ml/min	ml/min		μEq/min			mg/min		mg/ml GFR	%	%	mm Hg
Dog # 42, wt 9.2 kg													
0-90	Infuse 20% glucose in H_2O at 2.3 ml/min; infuse 0.15 M NaCl at 0.4 ml/min.												
90-100	503	35.5	1.15	15	9	7	178	92	86	2.43	0.3	0.2	170
100-110	507	32.9	1.15	17	12	9	167	85	82	2.48	0.4	0.3	170
	D/C 0.15M NaCl; infuse aqueous vasopressin at 0.4 ml/min (50 mu/kg/min).												
110-120	587	34.1	1.95	143	33	135	200	109	91	2.68	3.0	3.3	200
120-130	580	43.6	3.80	380	80	265	253	158	95	2.18	6.2	7.7	225
130-140	575	42.5	4.05	417	89	413	244	153	91	2.14	7.0	9.2	225
140-150	600	43.3	4.15	411	91	415	250	169	91	2.10	7.0	9.2	225
	D/C aqueous vasopressin, infuse 0.15 M NaCl at 0.4 ml/min.												
150-160	585	39.2	3.50	324	77	329	229	145	84	2.14	6.1	7.7	220
160-170	565	37.8	2.30	131	58	108	214	134	80	2.12	2.5	2.6	175
170-180	594	37.9	2.15	98	54	73	225	144	81	2.14	1.9	1.9	170
180-190	591	38.7	2.00	64	40	34	229	141	88	2.27	1.2	0.8	170

Table VI

Mean Glucose Data

	$\frac{C_{Cl} \times 100}{GFR}$		$\frac{C_{Na} \times 100}{GFR}$		T_G	$\frac{T_G}{GFR}$
					mg/min	mg/ml
Control	2.1 ± 0.6		1.5 ± 0.5		92.4 ± 16	2.31 ± 0.21
Experimental	15.4 ± 3.6	$p<0.01$	11.8 ± 2.7	$p<0.01$	102.2 ± 22	NS 1.95 ± 0.17 NS
Control	3.2 ± 1.0		2.1 ± 0.8		107.8 ± 26	2.28 ± 0.10

Table VII

Effect of Aqueous Vasopressin on HCO_3 Reabsorption in Aldosterone Deficient Dogs

	Flow Rate	$\frac{C_{Cl} \times 100}{GFR}$	T/HCO_3-
	ml/min	%	mEq/Liter GFR
Control	1.6 ± 0.4	2.1 ± 0.6	22.0 ± 1.0
ADH Infusion	5.9 ± 1.3	14.3 ± 3.0	20.9 ± 1.0
	$p < 0.02$	$p < 0.02$	NS

TABLE VIII

Effect of Aqueous Vasopressin on Filtration Fraction

	Flow Rate	$\frac{C_{Cl}}{GFR} \times 100$	GFR	RPF	F F
	ml/min	%	ml/min	ml/min	
Control	0.5 ± 0.1	0.4 ± 0.2	26.8 ± 6.0	70.3 ± 11.3	0.38 ± 0.05
ADH Infusion	3.0 ± 0.8	13.1 ± 3.7	34.6 ± 4.8	80.8 ± 11.4	0.44 ± 0.03
	p < 0.02	p < 0.02	NS	NS	NS

the increase was not statistically significant. The chloriuresis demonstrated in this table was associated with a rise rather than a fall in filtration fraction though this increase was not statistically significant.

DISCUSSION

This study demonstrates that when aqueous vasopressin is infused at a dose of 50 mu/kg/min it exerts a diuretic action similar in magnitude to that seen following the administration of either ethycrinic acid or furosemide. This marked diuresis is also seen following the infusion of 50 mu/kg/hour of aqueous vasopressin, though the magnitude of this diuresis is less than that seen following the infusion of the higher dose. This vasopressin associated diuresis is characterized by an increase in sodium, potassium, and chloride excretion, as well as a marked increase in urinary volume.

This current study differs from previous studies of the natriuretic action of vasopressin, not only in the size of the dose administered, but in that the natriuresis noted following the administration of this hormone was associated with an increased urinary volume rather than a decreased urinary volume which has characterized most previous studies. Furthermore, the diuretic effect of vasopressin noted in this study was not contingent upon the presence of a pre-existing water diuresis. This diuretic effect was seen in glucose loaded animals, bicarbonate loaded animals, and in anti-diuretic animals. The magnitude of the diuresis is also much greater than previously reported. The site of action of the diuretic effect of vasopressin is difficult to pinpoint with certainty. Previous studies have indicated an effect of vasopressin in both the proximal tubule (Martinez, et al, 1971)[13] and in the ascending limb of the loop of Henle (Hum (Humphreys, et al, 1970)[12]. If the hormone had a generalized inhibitory effect on proximal reabsorption, similar to that seen following volume expansion with saline, one would expect glucose, bicarbonate and phosphate reabsorption to be depressed as well as well as the reabsorption of sodium and chloride (Kurtzman, 1970;[16] Kurtzman, et al, 1972[17]; Massry, et al, 1969,[18]; Suki, et al, 1969[19]). However, in this study there was no effect of vasopressin on the maximal rate of reabsorption of glucose or bicarbonate. These results would seem to indicate that if there is a proximal effect of this agent it is a limited one, and not analogous to the inhibitory effect of volume expansion on proximal reabsorption, in

that vasopressin only depressed phosphate reabsorption. In order to exclude the possibility that a depressive effect on proximal bicarbonate reabsorption was being masked by a coincident stimulatory effect on distal bicarbonate reabsorption the experiments in the aldosterone deficient animals were performed. Aldosterone deficiency results in an inhibition of distal hydrogen ion secretion (Kurtzman, et al, 1971)[14], thus if increased distal bicarbonate reabsorption were masking a proximal effect this maneuver (aldosterone deficiency) should have uncovered it, however, there was no depressive effect of vasopressin administration on bicarbonate reabsorption noted in these aldosterone deficient animals.

The mechanism by which vasopressin exerts such a great diuretic effect is also not completely clear. Since the administration of vasopressin is associated with a marked hypertensive effect it was possible that this hypertensive effect was in some way related to the natriuretic effect of the hormone. However, abolishing this hypertensive effect by administering sodium nitroprusside did not abolish the natriuretic and phosphaturic effects of vasopressin. Thus, our data indicate that the diuretic effect of vasopressin is not related in a major way to changes in systemic blood pressure. Nor is this effect related to a fall in filtration fraction, since filtration fraction was noted to rise, though not significantly, following the administration of vasopressin. Thus it would seem likely that this diuretic effect of vasopressin is a consequence of changes either in intra-renal hemodynamics or is secondary to a direct tubular effect of vasopressin on transport. An alternate possibility is that vasopressin stimulates the release of a natriuretic factor as recently suggested by Diamond and Buckalew(Diamond KA and Buckalew VM, Jr, Clin Res 21: 684, 1973)[20].

There is no reason to believe that vasopressin plays a significant physiologic regulatory role in renal sodium handling. However, the effect that we noted with pharmacologic doses of vasopressin may be mimicing the effect of another, as yet poorly characterized, hypothalamic hormone which may play an important role in controlling renal sodium excretion, much the same way that pharmacologic doses of oxytoxin, a closely related hormone to vasopressin, mimic the anti-diuretic effects of vasopressin. The agent recently described by Gitelman and Blythe (Gitelman HJ and Blythe WB, Clin. Res 20: 594, 1972)[21] may be such a closely related hormone. This study emphasizes the need to exclude vasopressin from crude preparations believed to contain neurohypophyseal hormones thought to play a physiologic regulatory role in renal sodium transport.

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ANNUAL PROGRESS REPORT

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 - STUDY # 2 EFFECT OF PROSTAGLANDIN E_1 ON RENAL BICARBONATE REABSORPTION

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

1 July 1972 - 30 June 1973

Investigators:

Philip W. Rogers, MD, Major, MC
Neil A. Kurtzman, MD, Lieutenant Colonel, MC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

Period covered in this report: 1 July 1972 - 30 June 1973

Investigators: Philip W. Rogers, MD, Major, MC
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Reports Control Symbol MEDDH-288(R1)

Disorders of acid-base homeostasis are very common in the injured or ill soldier. These disorders are maintained, compensated, or corrected by changes in renal bicarbonate reabsorption. Previous studies concerned with this project have evaluated the role of effective extracellular volume, acute respiratory acidosis, potassium deficiency, potassium excess and aldosterone deficiency on renal bicarbonate reabsorption. Studies evaluating the effect of vasopressin (ADH) have just been completed and are reported in this year's Annual Progress Report. Most recently we have undertaken to examine the role of prostaglandin E_1 (PGE_1) on renal bicarbonate reabsorption. These studies are being performed by the infusion of 2 μg/min of PGE_1 into the right renal artery while using the left kidney as a control. Thus far we have found that while PGE_1 in these concentrations causes a mild natriuresis on the order of 5% fractional sodium chloride excretion, there is no change in bicarbonate reabsorption. Also of interest in these studies has been an increase in the glomerular filtration rate during the infusion of PGE_1 as well as an increase in the renal plasma flow. Previous studies by other investigators have suggested that PGE_1 may have its natriuretic effect by inhibiting sodium reabsorption in the proximal tubule; however, our studies thus far suggest that the inhibition of sodium reabsorption takes place at a more distal site. We have not completed enough studies to make a statistical analysis and definite conclusions at this time.

Bicarbonate reabsorption
Prostaglandin E_1
Sodium excretion

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0D 6976	73 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. MOD'N IDENT'N	8a. SPECIFIC DATA CONTRACTOR ACCESS	9. LEVEL OF SUM
72 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:
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e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS ($ Thousands)
FISCAL YEAR PRECEDING 73	1.4	3
FISCAL YEAR CURRENT 74	1.4	5

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21. GENERAL USE: FOREIGN INTELLIGENCE NOT CONSIDERED

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 (Punish individual paragraphs identified by number. Precede text of each with Security Classification Code.)

23. (U) To determine the relationship of the metabolic rate to evaporative water loss in the extensively burned patient, to describe the change of metabolic rate with changes in the ambient temperature and humidity under controlled conditions; to describe the optimal temperature and humidity for treatment areas in terms of energy economy in injured soldiers.

24. (U) The use of a controlled environmental study room to measure metabolic rate at various temperatures and humidity level; concomitantly, measurements of water loss, oxygen consumption, carbon dioxide production, core temperature, and mean skin temperature, with calculation of heat loss component to determine the critical temperature in these conditions to minimize energy demands in the seriously ill patient.

25. (U) 72 07 - 73 06 Four normal men and eight burn patients have been placed and allowed 24 hours stabilization at 25° C. temperature and 33° C. temperature with vapor pressure remaining isobaric. No alterations in metabolic rate were found in normal or burned patients, but there was an alteration in partition of heat loss from all subjects, with a marked increase in evaporative water loss at the warmer temperature. Moreover, heat transfer coefficient demonstrated increased transfer of heat in the burn patient from cooler to ambient, which increased markedly as the patient was placed in the warmer temperature. Metabolic rate and core and skin temperature have been determined in 13 additional patients at 33, 25, 21, and 19 degrees. Alteration in metabolic activity occurred in most patients as the temperature was lowered from 21 to 25 degrees.

*Available to contractors upon submission of approval.

ANNUAL PROGRESS REPORT

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: STUDIES OF THE EFFECT OF VARIATION IN TEMPERATURE AND HUMIDITY ON ENERGY DEMANDS OF THE BURNED SOLDIER IN A CONTROLLED METABOLIC ROOM (Parts I, II, III).

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

1 July 1972 - 30 June 1973

Investigators:

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Robert W. Skreen, PV2
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ABSTRACT

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Over 100 measurements of resting metabolic rate under carefully controlled conditions have been made in 43 patients, studied at one to five ambient temperatures, which ranged between 19 and 33° C. A minimal response in metabolism occurred in normal individuals and patients with less than 50% total body surface burn, when measured between 25 and 33° C. However, in burns greater than 50% total body surface, temperature exhibited an effect on metabolic activity which appeared additive to the hypermetabolism related to the thermal injury. At warmer temperatures, metabolic rate did not return to normal and heat transfer coefficients confirmed the fact that the burn patient is internally warm, attempting to dissipate heat rather than being externally cool and attempting to retain heat. All subjects responded to temperatures below 25° C. by increasing metabolic activity except for four individuals with large burns who appeared unable to elaborate catecholamines in response to this cold stress and demonstrated a marked decrease in metabolism at the lower temperatures. In addition, metabolic rates of septic patients appeared below predicted levels and, in two patients with multiple injuries and moderate sized burns, metabolic rates were elevated above predicted levels, suggesting an additive effect of associated trauma. In an attempt to define sensory input related to ambient comfort temperature in the burn patient, nine burn patients and five normal subjects were allowed to set comfort

temperatures in the metabolic chamber. All burn patients selected temperatures warmer than normal man, and this appeared to be related to burn size. At temperatures of comfort, both core and skin temperatures were greater than those achieved in normal man, suggesting that a possible hypothalamic reset may occur following thermal injury.

Metabolism
Hypermetabolism
Heat loss
Evaporative water loss
Controlled environment
Critical temperature
Burned soldiers

STUDIES OF THE EFFECT OF VARIATION IN TEMPERATURE AND HUMIDITY ON ENERGY DEMANDS OF THE BURNED SOLDIER IN A CONTROLLED METABOLIC ROOM (PART I)

Radiative heat loss is the major route of heat transfer in normal man during conditions of comfort, and may account for up to half of the total heat transfer from the body. Conduction, convection, and vaporization provide mechanisms for transfer of the remainder of body heat and, with intact normal skin, evaporation accounts for only 20 to 25% of the total heat exchange in the resting individual (Hardy JD, Dubois EF, J Nutr 15:477-497, 1938).[1] Thermal injury damages the lipoid complex of the stratum corneum of the skin, altering the vapor pressure barrier, and allowing increased evaporative water loss from burn wound. The quantity of evaporative water loss correlates closely with the extent of body surface injury and patients with major burns therefore have a marked increase in the vaporizational heat loss. The purpose of this study is to evaluate the routes of heat loss in the burn patient and to define alterations in metabolic rate and partition of heat loss at 25 and 33° C. ambient conditions.

MATERIALS AND METHODS

Patients

Eight patients, between 15 and 49 years of age, were 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, and all were male with no other pre-existing disease before thermal injury (Table 1). All patients were studied for 48 hours between the seventh and 18th postburn day, with the mean days of study being the ninth and tenth days following injury. All patients were stable following burn shock resuscitation; had a predictable stable clinical course during the study period; were alert and cooperative during the studies required. They did not have systemic infection, as determined by stable body temperature, had no clinical signs of infection and had negative blood cultures and endotoxin levels before, during, and after the study period.

The control individuals were healthy male subjects working in the Burn Unit who were accustomed to the techniques and methodology of respiratory and metabolic testing. There was no history of previous disease in this group, and all were vigorous, active individuals.

Table 1. Characteristics of Subjects Studied

Subjects	Age	Body Weight (in Kg)	BSA (m^2)	Per Cent Burn (Total/3°)	Per Cent Burn Exposed	PBD* Studied	Comment
Controls							
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		
Burns							
1	43	69.5	1.82	7 / 7	7	8-9	
2	28	63.0	1.79	15 / 2	12	7-8	
3	15	48.0	1.56	26 /18	20	17-18	Associated head injury; tracheostomy
4	49	87.5	2.01	34.5/0	13	8-9	
5	24	87.0	2.09	40 /14	31	8-9	
6	34	85.0	2.00	60 /16.5	27	14-15	
7	18	66.0	1.88	63.5/21.5	37.5	10-11	
8	25	68.0	1.85	84 /76	40	8-9	Tracheostomy

*PBD = Postburn day

Place of Study

The studies took place in an environmental chamber located on the Burn Ward. The chamber, a rectangular room nine feet wide, 17 feet long, eight feet high, contained an air processing system designed to maintain any selected temperature and humidity between +15° C. and +40° C. (range ± 2%), limited by a -1.1° C. and + 35° C. dew point. The makeup air is treated by the air processing system and delivered to the room through a large overhead duct. A delivery fan generates a slight pressure differential transferring the air into the room through fine pores in the ceiling. The air flow is laminar from ceiling to floor and vertical flow is maintained to a level of about two feet above the floor where lateral movement then transports the outgoing air to ducts in the base of the walls and it passes along the inside of the chamber walls back to the air treatment unit. Although the air turnover in the environmental chamber is relatively high, maximum air velocity is less than 50 feet per minute at the level of the bed, three feet above floor level. 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 all patients were housed in the chamber.

Study Design

Patients were studied in the chamber for two consecutive 24-hour periods. During one 24-hour interval, the environmental room was set at 25° C., vapor pressure of 11.88 mmHg (50 per cent relative humidity), and during the other 24-hour study period, the environment was set at 33° C., vapor pressure 11.88 (relative humidity 31%). The order of the ambient conditions was randomized in the patients and normal controls so that half of the subjects were first studied at the cooler temperature followed by the warmer temperature and the other half of the subjects studied at the warmer temperature followed by the cooler temperature.

Study Design

The patients were placed in the room at 9:00 A.M. on the first day of study and allowed to equilibrate for 18 to 20 hours before data collection was undertaken. All burns were treated by the open method with topical Sulfamylon cream applied to the burn wounds. Caloric support was identical during the two 24-hour periods for each individual patient, and standard methods of feeding were employed during the study period. The diet was provided by the metabolic kitchen and the two 24-hour study periods matched to insure identical caloric intakes with

equal quantities of carbohydrates, fat, and protein consumed on the study days. Patients were turned in the bed as required for their burn care but were placed in the supine position several hours before the first test and remained in that position during the final six hours of each study day. All patients were fasted four hours before and during the study period, but received water by the oral or parenteral routes if required to maintain a normal state of body hydration.

The first test was the measurement of insensible weight loss, which was determined by recording serial body weight every 15 minutes for two to four hours, using a Brookline metabolic scale (accuracy ± 2 g) which held the bed containing the patient. No loading or unloading of the bed was permitted during the weighing. At the end of the serial measurements, expiratory gas was collected in two 200 liter Douglas bags to determine oxygen consumption and carbon dioxide production. A mouthpiece and nose plugs or nose clip were used as the interface between the patient and collecting apparatus except in two patients with tracheostomies when a direct connection with the tracheostomy tube could be made. The mouth, lips, gums, and nose of individuals with facial burns were anesthetized with 2% topical viscous Xylocaine to minimize pain to the injured area and allow acceptance of the mouthpiece and nose clip or nose plug with minimal pain. All patients had previously been trained to hold the mouthpiece for collection of expiratory gas, and several trials of Douglas bag collection had been performed before the studies in the environmental chamber. In the patients with tracheostomies, the upper trachea was anesthetized with topical cocaine, the tracheostomy tube and balloon fitted, the balloon inflated, and an air-tight seal insured; 15-20 minutes were allowed after tracheostomy balloon inflation for equilibration before the test was started. The mouthpiece or tracheostomy tube was attached to a low resistance Otis-McKerrow one-way valve which was connected in series to two 200 liter Douglas bags. After an equilibration period of at least 10 minutes with the mouthpiece of tracheostomy, both bags were flushed at least three times with the patient's expiratory gas. After a steady resting state had been achieved, determined by patient comfort, lack of air leak, and steady, stable, respiratory rate, gas collection was started and carefully timed for three to seven minutes of collection for each Douglas bag, with the test terminated when the bag appeared approximately two-thirds full. Number of respirations were counted for each patient during the collection period.

The expiratory gas in the Douglas bag was mixed and samples obtained for gas analysis using a Beckman oxygen analyzer, Model E2, and carbon dioxide using a Godart capnograph. Total volume of each bag was then measured by emptying the contents into a Tissot gasometer. Metabolic rate in kilocalories per square meter of body surface per minute was calculated from the oxygen consumed corrected for respiratory

quotient (Lusk G, J Biol Chem 59:41-42, 1924),[2] without nitrogen excretion, and the results of the two Douglas bag collections averaged.

Thermocouples of copper constantin were used as the thermal sensing devices and attached to a Honeywell recorder for continuous temperature monitoring. A probe was placed six to eight cm in the rectum, with a second probe placed in the external ear in contact with the tympanic membrane. A small hand-held thermocouple measured skin temperature with the surface points used those described by Dubois (Hardy JD, Dubois EF, J Nutr 15:477-497, 1938),[1] for areas not in contact with the mattress, with some modification made for the patients with burns. The extent of burn and unburned skin was determined for each segment of the body and the relative contribution of each area estimated. Multiple temperatures were taken from both burned and unburned surfaces, these measurements weighted mathematically by surface area to determine their overall contribution to mean skin temperature. In the normal individuals, approximately 20 skin temperatures were taken, but in the patients up to 50 surface temperatures were taken for computation of mean skin temperature. Four basic measurements (metabolic rate, insensible weight loss, core temperature, skin temperature) were carried out between the 18th and 24th hour of each study day while the patient was at 25° C. and 33° C.

Calculations

Core temperature stabilized in each ambient situation after six to eight hours. At the time of the study, heat production was equated with heat loss, with total heat loss determined by metabolic rate. A best-fit line was drawn using the method of least squares to predict rate of insensible weight loss. This value was then corrected for the weight of oxygen consumed and carbon dioxide lost, so that the total water loss by evaporation was determined. The body surface area was estimated from the predictions of DuBois (DuBois D, Arch Int Med 17:863, 1916),[3] and metabolic rates were related to body surface area. Following the analysis of Wenger (Wenger CB, J Appl Physiol 32:456-459, 1972),[4] vaporizational heat loss was determined using the heat of evaporation as 0.580 kcal/g. Total dry loss was determined by subtracting heat lost by vaporization from the total heat loss, and radiative heat loss was determined by the Stefan-Boltzmaan equation. Conductive and convection exchange was estimated by subtracting radiational losses from total dry losses and heat transfer coefficients were determined by standard techniques.

RESULTS

Metabolic rate and the partition of heat loss were comparable to previously reported studies in normal man studied at 25° C. (Table 2). In contrast, hypermetabolism was noted in the burned patients and metabolic rate appeared to increase with burn size in a linear manner up to a 50% total body surface burn and then plateaued (Table 3). When combining measurements obtained from both control and burn patients, metabolic rate varied consistently between the 25 or 33° C. ambient temperatures in those patients with burns greater than 50% total body surface (Figure).

Evaporative water loss and therefore vaporizational heat loss increased proportionally with burn size and this relationship was linear at both temperatures studied (Table 3). The route of heat loss was altered by the ambient conditions, and a marked increase in wet heat loss occurred in both normal individuals and the burned patients at the warmer ambient temperature; but the decreased radiative heat loss occasioned by the 33° ambient environment resulted in increased skin temperatures and reduced metabolic rates in the patients with the larger burns (Tables 4, 5).

Calculation of heat transfer coefficients demonstrated a two-fold increase in core to skin transfer of heat in the burned patients when compared with control individuals and this two-fold increase was present at both environmental temperatures. Core to air heat transfer coefficient was relatively constant in all subjects, and skin to air transfer was only changed in the normal subjects studied at 33° ambient temperature, suggesting an increase in body surface area exposed at the warmer temperature due to an unfolding of the arms and legs from the trunk. This increase in skin to air gradient occurred only in the control individuals and was not seen in the burned patients studied (Tables 4, 5).

DISCUSSION

Hypermetabolism characterizes one of the metabolic responses following thermal injury, and this alteration in metabolic activity has frequently been related to the increased evaporative water loss from the burn wound (Harrison HN, Moncrief JA, Duckett JW, Mason AD Jr, Surgery 56:203, 1964;[5] Roe CF, Kinney JM, Surgery 56:212-220, 1964).[6] Covering the wound in experimental animals blocked evaporative water loss and returned metabolic rate to normal (Lieberman ZH, Lensche JM, Surg Forum 7:83, 1956).[7] In addition, burned rats treated at a room temperature of

Table 2. Measured Data

		T = 25 RH = 50			T = 33 RH = 31			
Subjects	Metabolic Rate (Kcal/m^2/hr)	Evap H_2O Loss (gm/hr)	Core Temp	Skin Temp (Mean)	Metabolic Rate (Kcal/m^2/hr)	Evap H_2O Loss (gm/hr)	Core Temp	Skin Temp (mean)
Controls								
1	37.8	19.3	36.7	31.4	39.8	102.4	36.7	33.6
2	31.8	27.0	36.7	31.3	34.5	88.0	36.9	34.8
3	40.1	44.7	37.3	32.0	37.9	100.8	36.9	34.5
4	32.7	23.8	36.5	31.0	33.0	82.3	36.6	33.8
Burns								
1	36.9	43.8	38.5	31.5	37.7	72.1	38.7	35.6
2	49.1	45.8	38.4	32.5	43.6	111.4	37.5	35.1
3	62.0	92.3	39.5	33.9	69.4	123.4	39.0	37.0
4	72.3	84.4	38.8	33.8	71.0	158.5	40.1	37.3
5	58.2	90.6	38.5	33.3	64.9	129.0	38.5	36.5
6	80.0	133.7	38.3	34.8	74.8	201.3	39.7	36.8
7	69.9	114.4	37.5	33.0	69.0	179.0	38.7	36.1
8	79.8	149.6	38.2	32.0	71.6	214.7	36.9	35.0

Table 3. Relationship Between Metabolism, Evaporative Water Loss, and Per Cent Total Body Surface Burn

Ambient Temperature	y	x	Relationship	R^2
25°	Metabolic rate ($Kcal/m^2/hr$)	Per Cent Body Surface Burn	$y = 35.025 + 1.0592x^2 - 0.0065x^2$	0.9184
33°	Metabolic Rate ($Kcal/m^2/hr$)	Per Cent Body Surface Burn	$y = 35.074 + 1.2239x - 0.0096x^2$	0.9162
25°	Evaporative Water Loss ($gm/m^2/hr$)	Per Cent Body Surface Burn	$y = 16.7 + 0.783x$	0.897
33°	Evaporative Water Loss ($gm/m^2/hr$)	Per Cent Body Surface Burn	$y = 45.34 + 0.832x$	0.897

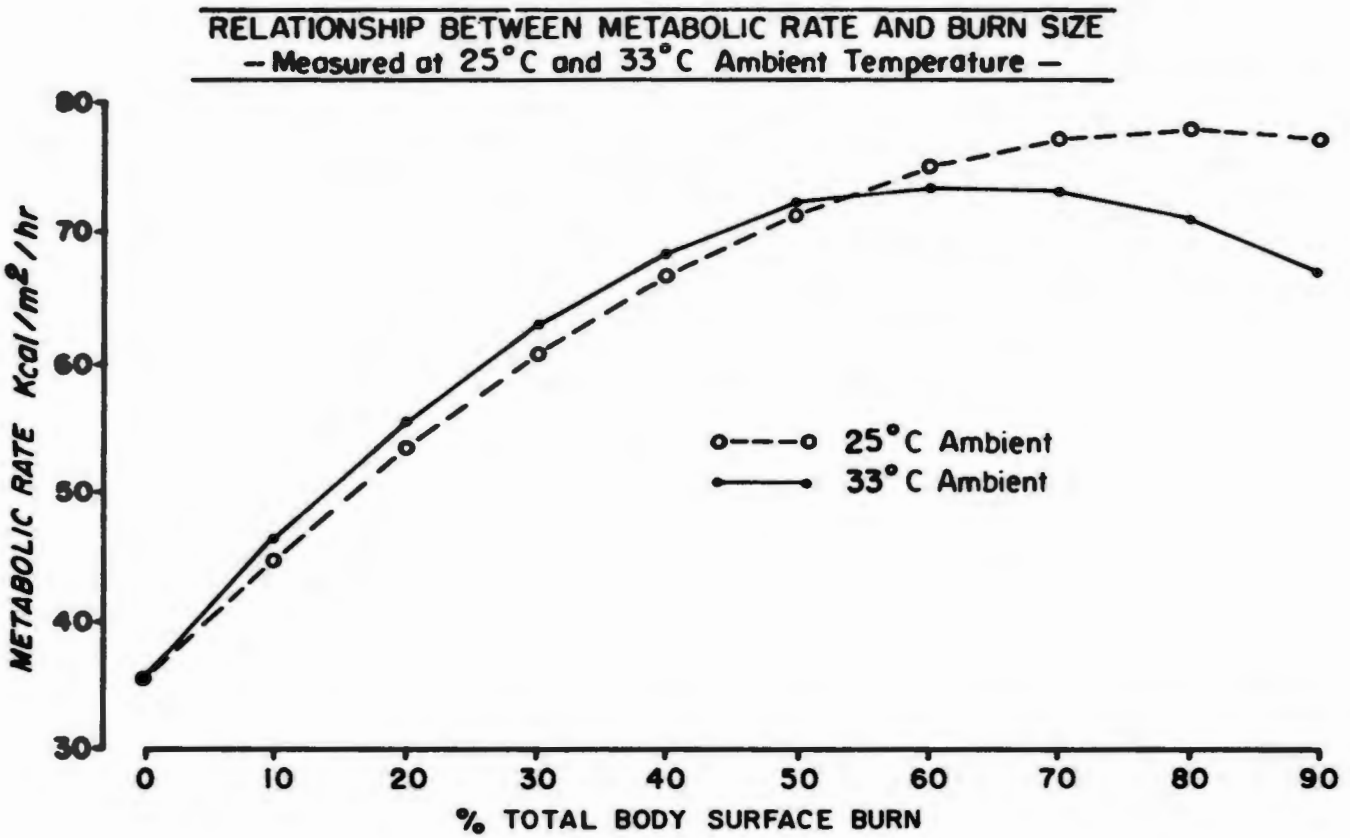
RELATIONSHIP BETWEEN METABOLIC RATE AND BURN SIZE
– Measured at 25°C and 33°C Ambient Temperature –
METABOLIC RATE Kcal/m²/hr
80
70
60
50
40
30
0
10
20
30
40
50
60
70
80
90
% TOTAL BODY SURFACE BURN
25°C Ambient
33°C Ambient

Table 4. Derived Data at 25° C.

	Wet Heat Loss		Dry Heat Loss				Heat Transfer Coefficients		
Controls	Total (Kcal/m^2/hr)	Per Cent of Total	Total (Kcal/m^2/hr)	Per Cent of Total	Radiation (Kcal/m^2/hr)	Cond & Conv (Kcal/m^2/hr)	Core Skin (Kcal/m^2/hr/° C.)	Core Air (Kcal/m^2/hr/° C.)	Skin Air (Kcal/m^2/hr/° C.)
1	5.89	15.59	31.91	84.41	16.39	15.52	7.13	2.73	4.99
2	7.91	24.87	23.89	75.13	16.13	7.77	5.89	2.04	3.79
3	11.95	29.79	28.15	70.21	17.98	10.17	7.57	2.29	4.02
4	6.77	20.69	25.93	79.31	15.33	10.60	5.95	2.26	4.32
Mean	8.13	22.74	27.47	77.26	16.46	10.90	6.64	2.33	4.28
Burns									
1	13.96	37.83	22.94	62.17	16.65	6.29	5.27	1.70	3.53
2	14.84	30.22	34.26	69.78	19.31	14.95	8.32	2.56	4.57
3	34.32	55.35	27.68	44.65	23.08	4.60	11.07	1.91	3.11
4	24.35	33.68	47.95	66.32	22.81	25.14	14.46	3.47	5.45
5	25.14	43.20	33.06	56.80	21.46	11.60	11.19	2.45	3.98
6	38.77	48.47	41.23	51.53	25.53	15.70	22.86	3.10	4.21
7	35.29	50.49	34.61	49.51	20.65	13.95	15.53	2.77	4.33
8	46.90	58.77	32.90	41.23	17.98	14.92	12.87	2.49	4.70
Mean	29.20	44.75	34.33	55.27	20.93	13.39	12.70	2.56	4.24

Table 5. Derived Data at 33° C.

	Wet Heat Loss		Dry Heat Loss				Heat Transfer Coefficients		
Controls	Total (Kcal/m^2/hr)	Per Cent of Total	Total (Kcal/m^2/hr)	Per Cent of Total	Radiation (Kcal/m^2/hr)	Cond & Conv (Kcal/m^2/hr)	Core Skin (Kcal/m^2/hr/° C.)	Core Air (Kcal/m^2/hr/° C.)	Skin Air (Kcal/m^2/hr/° C.)
1	31.26	78.54	8.54	21.46	1.62	6.93	12.84	2.31	14.24
2	25.78	74.72	8.72	25.28	4.88	3.85	16.43	2.24	4.85
3	26.94	71.09	10.96	28.91	4.06	6.90	15.79	2.81	7.31
4	23.40	70.91	9.60	29.09	2.16	7.44	11.75	2.67	12.00
Mean	26.84	73.82	9.46	26.18	3.18	6.28	14.21	2.51	9.60
Burns									
1	22.98	60.95	14.72	39.05	7.07	7.65	12.16	2.58	5.66
2	36.10	82.79	7.50	17.21	5.70	1.81	18.17	1.67	3.57
3	45.88	66.11	23.52	33.89	10.95	12.57	14.70	3.92	5.88
4	45.74	64.42	25.26	35.58	11.79	13.47	25.36	3.56	5.88
5	35.80	55.16	29.10	44.84	9.56	19.54	32.45	5.29	8.31
6	58.38	78.04	16.42	21.96	10.39	6.03	25.79	2.45	4.32
7	55.22	80.03	13.78	19.97	8.45	5.33	26.54	2.42	4.44
8	67.31	94.01	4.29	5.99	5.42	-1.13	37.68	1.10	2.14
Mean	45.92	72.69	16.82	27.31	8.67	8.08	26.61	2.87	5.02

30° C. had lower metabolic rates, lower nitrogen excretion, and a smaller weight loss than rats with similar burns treated at 20° C. (Caldwell FT Jr, Ann Surg 155:119-126, 1962).[8] More recently, burn patients treated in a warm environment (32° C.), demonstrated significant decreases in metabolic rate when compared with treatment at 22° C. (Barr PO, Birke G, Liljedahl SO, Plantin L-O, Lancet 1:164-168, 1968);[9] and this evidence has been used 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, however, Zawacki and associates covered the burn wound with a water-impermeable membrane and thus blocked evaporative water loss but found no alteration in metabolic rate in the burn patients at approximately 25° C. ambient temperature (Zawacki BE, Spitzer KW, Mason AD Jr, Johns LA, Ann Surg 171:236-240, 1970).[10] The present study demonstrates a curvilinear relationship between metabolism and burn size but a direct linear relationship between evaporative water loss and per cent total body surface burn. In the normal subjects studied and burns less than 50%, metabolism was not related to surface cooling by evaporative water loss and hence metabolic rate was unaffected when these individuals were placed in the warmer environments. However, as metabolic rate plateaued in burns greater than 50% total body surface, evaporative water loss increased in a linear manner, contributing to a greater percentage of heat transfer and, at the 25° C. ambient temperature, the result was surface cooling with a mean surface or core temperature below threshold levels for cold receptor firing (Benzinger TH, JAMA 209:1200-1206, 1969).[11] Thus, the patients with larger burns consistently demonstrated a decrease in metabolic rate in the warmer ambient temperature and this effect has been confirmed in the studies of 19 additional patients.

Moreover, this data suggests that evaporative water loss in the burn patient is not the prime stimulator of the hypermetabolic state, but rather the hypermetabolic response is related to an endogenous reset in metabolic activity with additive effects from evaporative water loss only in patients with larger burns. The increased evaporative water loss appeared to provide a convenient route for transfer of this large quantity of heat from the body. In the majority of the burn patients studied, there was no change or an increase in core temperature when the subjects were exposed to the warm environment. In addition, skin temperature increased in all individuals, and there was an increase in core to skin heat transfer coefficient, supporting the thesis that the burn patient is internally warm and attempting to dissipate heat rather than being cold and attempting to vasoconstrict and retain heat. This fact receives further confirmation by the evidence that the burn patients at 33° do not decrease their metabolic rate to normal but rather continue metabolic activity at a previous "set" level. Although

evaporative water loss was markedly altered between 25 and 33° C. ambient environmental conditions, significant alteration in metabolic rate did not occur in burns less than 50%. Only in patients with the larger thermal injuries did evaporation affect metabolism, at a point where vaporizational heat loss increased disproportionately with metabolism.

One of the greatest adaptations of man to his ambient environment is his behavioral response, such as the ability to decrease his exposed surface area or to limit heat transfer by increasing insulation with blankets or clothing. As suggested by our study, normal individuals were able to achieve comfort at 25° C. by decreasing their exposed body surface area (curling up), and then unfolded at warmer temperatures to allow a greater surface area exposure and aid heat transfer as demonstrated by the increased skin-to-air transfer coefficient. This characteristic, however, was not present in the burn patients and extremity and trunk motion appeared limited because of pain and edema associated with their injury.

Treatment of burn patients in warm ambient environments has been proposed as a method for significantly reducing the hypermetabolic response to thermal trauma (Barr PO, Birke G, Liljedahl SO, Plantin L-O, Lancet 1:164-168, 1968).[9] As can be noted in this series of normal weight-nonseptic patients, only burned patients with greater than 50% total body surface injury would have benefited from external heating, with only a 10-12% reduction in metabolic rate. None of those patients returned to their normal preburn metabolic rate while being placed in the warm environment, and the rates of metabolism described in this series of individuals are comparable to those seen by the Swedish investigators in their treatment of patients in warm dry air (Barr PO, Birke G, Liljedahl SO, Plantin L-O, Lancet 1:164-168, 1968).[9] Further studies are now in progress to investigate the clinical benefits of a warm ambient environment in these critically ill patients.

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PUBLICATIONS AND/OR PRESENTATIONS:

None

STUDIES OF THE EFFECT OF VARIATION IN TEMPERATURE AND HUMIDITY ON ENERGY DEMANDS OF THE BURNED SOLDIER IN A CONTROLLED METABOLIC ROOM (PART II)

THE EFFECTS OF AMBIENT TEMPERATURE AND BURN SIZE ON METABOLIC RATE

Metabolic rate increases with burn size, but also increases with exposure to cold ambient temperatures, that is, temperatures below the lower critical temperature. The purpose of this study was to determine the interrelationship between metabolic activity, burn size, and ambient temperature.

MATERIALS AND METHODS

Fourteen burn patients with burns ranging from 7 to 84% of the total body surface were selected for study, along with four normal individuals in the same general age range. The initial group of patients was free of infection as determined by clinical course, vital signs, negative blood cultures, and endotoxin levels obtained before, during, and after study. All patients were studied between the 7th and 25th postburn day.

The patients were placed in the supine position in the metabolic chamber in the early part of the day and allowed three to six hours of equilibration with the 33° ambient temperature. Metabolic rate, core temperature, and skin temperature were then measured as previously described. Hourly urinary collection for determination of catecholamine levels was included in selected individuals. Following the initial determinations at 33°, room temperature was set at 25°, three to six hours equilibration allowed, and the studies repeated. In selected individuals, temperatures were then decreased to 21 and 19° C., with equilibration periods being shortened to one to two hours to minimize cold stress, and the previously described studies were carried out. Vapor pressure was maintained at 11.88 mmHg at all ambient temperature conditions.

After the determinations were performed on the patient group described, 25 additional patients suitable for study were placed in the metabolic room and resting metabolic rates were obtained at temperatures ranging from one to all of the temperatures taken at 2° intervals between 33 and 19°. From this information, a total of 43 patients were studied with 94 observations of metabolic rate obtained at temperatures between 19 and 33.

RESULTS

Basal metabolic rate increased as a function of burn size, and this best fit a curvilinear description (Fig. 1). A slight increase in metabolic rate was found as ambient temperature decreased from 33 to 25° C., but a more dramatic increase in metabolic activity occurred between 25 to 21° C. (Table 1).

The following equation best describes the relationship between metabolism, burn size, and ambient temperature:

$$y = 191.1279 + 1.16399B - 10.7839\ T - 0.008\ B^2 + 0.1807\ T^2 \qquad (r^2 = 0.9323)$$

y = Metabolic rate (kcal/m^2/hr)
B = Burn size in % total body surface
T = Ambient temperature in °C (T > 0)

Although other descriptive equations were evolved (including cube, fourth power, logarithmic, and reciprocal functions), no increase was achieved in the r^2, and the present equation expresses the interaction between temperature and total body surface burn on metabolic activity in a simple usable form.

Entering 67 observations obtained in 31 individuals with burn size ranging from 0-85%, and metabolic rate determined between 33 and 19° C., did not significantly alter the basic equation and only slightly decreased the r^2 value to .90.

Twelve patients studied did not fit this predicted equation for estimating metabolic rates (Table 2, 3). Two of the 12 individuals demonstrated metabolic rates which exceeded predicted values and both patients had associated injuries, which appeared additive to their burn trauma. Six of the 12 patients had positive blood cultures at the time of study, and were consistently below predicted values for metabolic rate obtained from the derived equation. These patients all required thermal support in the form of radiant heat and heating blankets during their care to prevent hypothermia. In the remaining four patients, all of whom died, metabolic rate was normal at 33 and 25° C. However, when these individuals were cold stressed at 21° C., all demonstrated a decrease in metabolic rate in the cool ambient temperature. Urinary catecholamines measured in two of the four

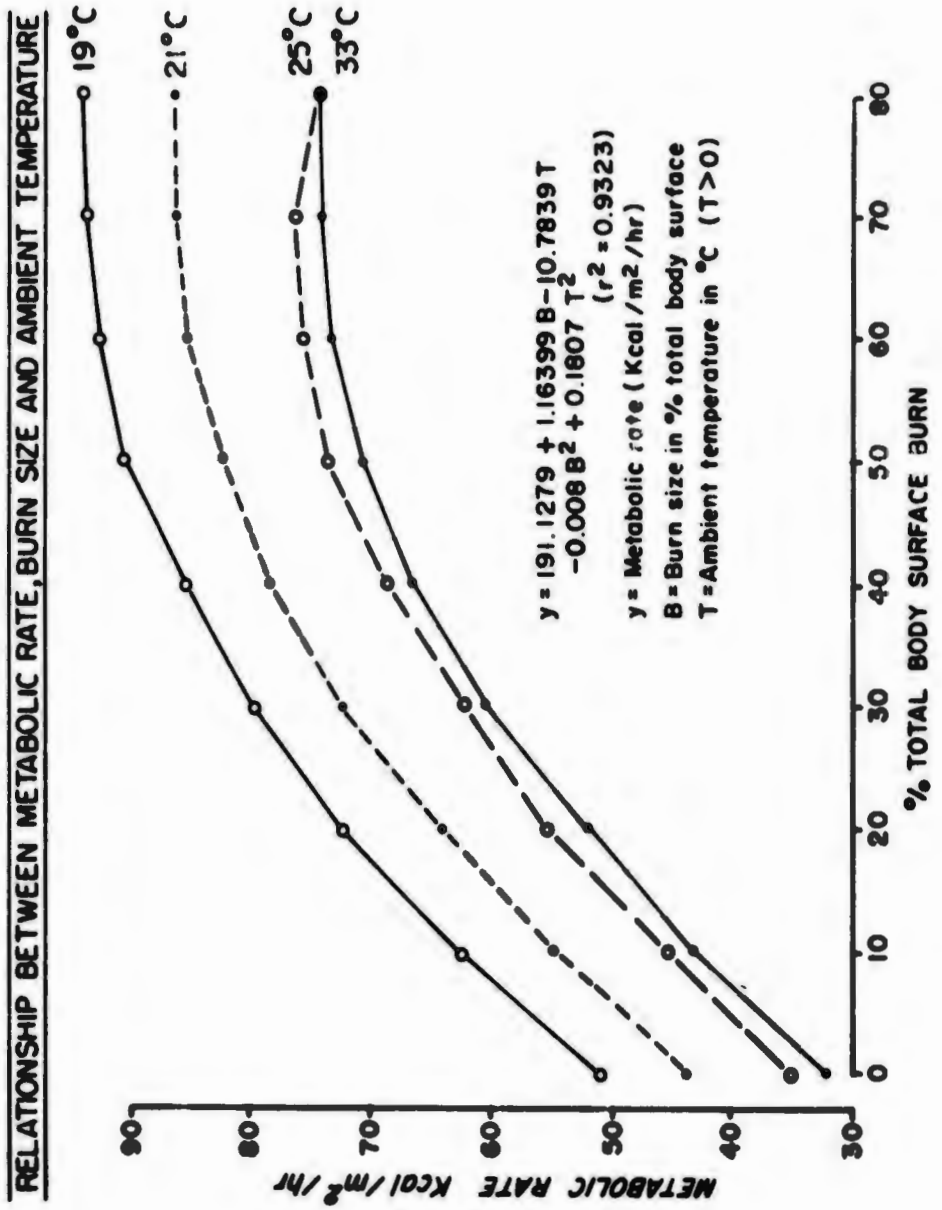
RELATIONSHIP BETWEEN METABOLIC RATE, BURN SIZE AND AMBIENT TEMPERATURE
19°C
21°C
25°C
33°C
y = 191.1279 + 1.16399 B − 10.7839 T
−0.008 B² + 0.1807 T²
(r² = 0.9323)
y = Metabolic rate (Kcal /m² /hr)
B = Burn size in % total body surface
T = Ambient temperature in °C (T>0)
METABOLIC RATE Kcal /m² /hr
90
80
70
60
50
40
30
0
10
20
30
40
50
60
70
80
% TOTAL BODY SURFACE BURN

Table 1. Metabolic Rate at Various Ambient Temperatures ($Kcal/m^2/hr$)

Patient	% Burn	19°	21°	25°	33°
1	64		91.9	69.9	68.9
2	60		94.2	80.4	77.5
3	84			79.7	71.4
4	51			78.8	71.7
5	46		74.1	69.2	69.4
6	39		74.7	67.5	68.8
7	41	82.7	79.2	70.1	57.5
8	53		89.1	76.8	68.0
9	75			78.2	71.7
10	66.5			71.4	65.2
11	7			36.9	37.7
12	15			49.1	43.6
13	34.5			72.4	71.1
14	40			58.3	65.0
15	0	48.9	41.7	37.1	39.8
16	0			31.8	34.5
17	0	49.8	40.3	36.6	37.9
18	0	46.2	41.6	32.6	33.0

Table 2. Patients Differing from Predicted Values of Metabolic Rate

Patient	Burn Size (%)	Ambient Temperature (°C.)	Metabolic Rate ($Kcal/m^2/hr$) Predicted	Observed	Comments
1	26	19	76	89.2	Associated head injury; tracheostomy
		21	69	90.3	
		33	57	69.3	
2	47.5	25	72	88.2	Multiple soft tissue injuries and fractures; tracheostomy
3	55	21	84	66.2	Staphylococcus blood culture
		25	74	51.7	
		33	72	58.8	
4	56.5	25	74	40.6	Klebsiella blood culture; positive endotoxin assay
		29	72	35.6	
5	61	33	73	60.4	Confusion and ileus; later positive Staphylococcal blood cultures
6	63	25	75	54.4	Aeromonas liquefaciens blood culture
7	76	21	86	51.3	Bilateral pneumonia, Providencia stuartii blood culture
		25	75	51.8	
		29	72	48.2	
		33	74	45.5	
8	82	25	74	35.9	E. coli blood culture; positive endotoxin assay
		29	74	35.9	

Table 3. Changes with Cooling in "Normal" Burns, a Septic Patient, and "Nonresponders"

		Metabolic Rate (Kcal/m^2/hr)			Catecholamines (μg/hr)			Mean Skin Temperature (°C.)			Tympanic Temperature (°C.)		
Patients / Burn Size		21	25	33	21	25	33	21	25	33	21	25	33
Predicted Response													
1	41	74.0	69.2	69.4	13.5	12.0	13.5	32.3	33.7	36.0	39.3	39.0	37.8
2	46	79.2	70.0	57.6	91.0	28.0	—	32.2	33.5	36.3	38.8	38.5	37.5
3	76	84	79.3	67.0	96	16.2	6.6	—	—	—	38.1	38.8	38.6
Septic Patient													
	55	62.2	51.6	55.6	668	195	22.8	32.0	34.2	36.2	34.4	35.6	35.4
Nonresponders													
1	51	69.3	78.7	71.7	10.8	17.5	22	29.5	31.4	34.4	37.9	37.5	38.2
2	66.5	68.4	71.4	65.2	7.6	17.6	16.8	30.9	32.5	35.1	36.7	37.1	37.2
3	58	57.6	83.2	84.0	—	—	—	30.4	32.1	34.6	35.9	35.4	36.3
4	84	67.0	79.7	71.4	—	—	—	29.3	32.0	35.0	38.0	38.2	37.0

individuals demonstrated a marked decrease in catecholamine elaboration during this period of cooling (Table 3).

DISCUSSION

Hypermetabolism characterizes the post-traumatic metabolic response to thermal injury related to burn size. In these patients, it appears that a maximum level of metabolic activity occurs with burns greater than 50% total body surface and burn size has minimal effects on metabolism above this level. Burn size interacts with ambient temperature to affect metabolism with small but real changes in metabolic rates between 25 and 33° C., but a marked increase in metabolism between 33° and 21° C. This data is comparable to reports of Liljedahl and associates (Barr PO, Liljedahl SO, Birke G, Plantin LO, Lancet 1:164-168, 1968)[1] who found marked alterations in metabolism between 22 and 32° C, and the proposed formula is likewise in accord with the data of Wilkerson and associates who have studied normal man in the supine position at a variety of temperatures (Wilkerson JE, Raven PB, Horvath SM, J Appl Physiol 33:451-455, 1972).[2]

Two patients with associated injuries had metabolic rates significantly above the predicted levels for burn size and ambient temperature, and it appears that associated injury results in an additive effect, especially in patients with burns of less than 50% of the total body surface. Patients with positive blood cultures demonstrated a decreased oxygen consumption at all temperatures studied, effects comparable to previous reports of infection altering cellular metabolism and oxygen consumption (Duff JH, Groves AC, McLean AP, Surg Gynec Obstet 128:1051-1060, 1969).[3] Finally, four patients did not respond to negative thermal loads, and demonstrated a progressive decrease in oxygen consumption at the lower ambient temperature. Response to cold in man is mediated by the sympathetic nervous system and one explanation of the phenomenon observed would be that some patients with massive injuries (especially older individuals, those patients with extensive burns, and nutritionally depleted patients) are producing catecholamines at their maximum rate and cannot respond to the cold stress by increasing catecholamine production. This hypothesis was supported in two patients who demonstrated decreased production of catecholamines following ambient cooling in contrast to "normal" responding patients who maintained or increased catecholamine output in response to cooling. Goodall and associates have reported a high turnover of catecholamines following burn injury, and a depletion of catecholamines of both sympathetic ganglia and adrenal medulla in burn patients studied at autopsy (Goodall MG, Amer Surg 32:448-452, 1966).[4] If the inability to produce catecholamines to an increased

stress is demonstrated early in the postburn course, hospital care could be modified to minimize additional stress such as environmental cooling, operation, or hydrotherapy in these selected patients. Moreover, possible benefits may be achieved by providing exogenous catecholamines or their bichemical precursors in such "nonresponders."

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PUBLICATIONS AND/OR PRESENTATIONS

None

STUDIES OF THE EFFECT OF VARIATION IN TEMPERATURE AND HUMIDITY ON ENERGY DEMANDS OF THE BURNED SOLDIER IN A CONTROLLED METABOLIC ROOM (PART III)

PATIENT SELECTION OF COMFORT TEMPERATURE

In normal man, metabolic rate is basal at conditions of comfort. The purpose of this study was to establish "comfort" temperature for burn patients in a metabolic chamber and evaluate the various afferent signals which determined optimal comfort.

MATERIALS AND METHODS

Nine burn patients were studied in the second to third week post-injury, along with five normal individuals of the same general age. All individuals were alert, awake, and cooperative. All individuals wore shorts, were placed in bed in the metabolic chamber previously described, and allowed to select optimal temperature for comfort by means of a bedside remote control temperature regulating unit. Relative humidity was maintained at 50% throughout the study. The individuals were maintained in the room from 4 to 24 hours and that ambient temperature obtained at the end of the test period was recorded as the comfort temperature. After satisfactory equilibration with the ambient comfort temperature, mean skin temperature and core temperature (using tympanic thermometry) were determined.

RESULTS

After some trial and error, all individuals studied were able to achieve subjective conditions of comfort. The rapid response of room temperature to alterations in remote bedside control allowed frequent adjustments and minimized wide temperature variations. The mean ambient comfort temperature selected by the normal individuals was 27.8°, which was related to a mean skin temperature of 33.4° C. Core temperature remained normal in these individuals (see Table). In contrast, the burn patients maintained a higher room temperature (mean 30.4), which was associated with an increased core temperature (mean 38.4) and warmer surface temperature (average skin temperature 35.2° C). Comfort temperature was best related to skin temperature (T comfort = -10.64 + 1.15 mean T skin, r^2 = .71), but there was a rough correlation between comfort temperature and burn size (T comfort = 27.54 + 0.069 burn size, r^2 = 0.56). Because sensation of comfort is a combined response arising from skin thermal receptors and central nervous temperature, T comfort was apparently related to both core temperature and skin temperature, and these effects appeared to be additive (T comfort = -38.6 + T core + 0.86 T skin, r^2 = 0.84).

Table Ambient Temperatures of Comfort

Subjects Studied	Burn Size	Room Comfort Temperature (°C.)	T_{Skin} (°C.)	T_{Core} (°C.)
Control				
1	0	29.5	34.2	37.1
2	0	28.5	34.4	36.6
3	0	27.1	33.2	37.0
4	0	26.0	31.2	
5	0	28.0	34.2	37.0
Mean	0	27.8	33.4	36.9
Burns				
1	35	29.0	33.6	39.0
2	65	35.0	37.5	40.0
3	45	29.6	35.5	37.7
4	23	30.0	35.3	38.4
5	43	30.0	35.5	38.3
6	45	29.4	33.6	38.4
7	36	28.5	35.3	37.7
8	35	33.0	34.5	
9	28	29.5	35.8	37.9
Mean	39	30.4	35.2	38.4

DISCUSSION

Thermal comfort depends primarily upon internal temperature (core temperature), but is modified by sensory input from the periphery. Normal man selects an ambient temperature of approximately 28° in order to maintain skin temperature above 33° C., which is considered as the threshold for firing of peripheral cold receptors. Burn patients, however, seem to have a reset in their thermal regulatory system, for all patients felt "comfortable" with increased internal and skin temperatures. Experiments in normal man suggest that the internal set point is shifted or reset in response to peripheral thermal stimulation (Cabanac M, Massonnet D, Belaiche R, J Appl Physiol 33:699, 1972),[1] and initial cooling in the early postburn period or alteration of the integrated peripheral nervous signal could account for this shift in core temperature set. Further studies are in progress to evaluate the role of peripheral thermal regulation in the control of body temperature and hypermetabolism in burn patients.

REFERENCES

1. Cabanac M, Massonnet D, Belaiche R: Preferred skin temperature as a function on internal and mean skin temperature. J Appl Physiol 33:699, 1972.

PUBLICATIONS AND/OR PRESENTATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0E 6953	73 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
72 07 01	A. 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 (Procede 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-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 73	1.1	9
FISCAL YEAR CURRENT 74	1.4	10

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
Burn Study Branch
ADDRESS: Ft Sam Houston, Tx 78234
PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic institution)
NAME: Joseph C McAlhany, Jr, MAJ, MC
TELEPHONE: 512-221-3411
SOCIAL SECURITY ACCOUNT NUMBER:
ASSOCIATE INVESTIGATORS
NAME: Alfred J Czaja, MAJ, MC
NAME: Basil A Pruitt, Jr, COL, MC DA

21. GENERAL USE: FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Procede EACH with Security Classification Code): (U) Evaluation; (U) Gastric Physiologic Disturbances; (U) Thermal Injury; (U) Burn Patients

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 gastric pathophysiology of the thermally injured soldier, so as to better the 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 in 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 post burn. 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 encompose: (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 01 - 73 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 Curling's ulcers would appear to be much higher than previously determined by clinical course operative and autopsy findings. Measurements of mucous, ion flux, coagulation and gastric clearance of radioactive isotope will be evaluated at a later date.

*Available to contractors upon originator's approval

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

ANNUAL PROGRESS REPORT

PROJECT NO. 3A161101A91C-00, IN HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: 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

1 July 1972 - 30 June 1973

Investigators:

Joseph C. McAlhany, Jr., M.D., Major, MC
Albert J. Czaja, M.D., Major, MC
Basil A. Pruitt, Jr., M.D., Colonel, MC
Arthur D. Mason, Jr., M.D.
Robert Lull, M.D., Major, MC
Willard A. Andes, M.D., Major, MC
F. D. Foley, M.D.
*Samuel S. Spicer, M.D.

*Department of Pathology
Medical University
Charleston, S. Carolina 29401

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

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 1972 - 30 June 1973

Investigators: Joseph C. McAlhany, Jr., M.D., Major, MC
Albert J. Czaja, M.D., Major, MC
Basil A. Pruitt, Jr., M.D., Colonel, MC
Arthur D. Mason, Jr., M.D.
Robert Lull, M.D., Major, MC
Willard A. Andes, M.D., Major, MC
F. D. Foley, M.D.
*Samuel S. Spicer, M.D.

Reports Control Symbol MEDDH-288(R1)

The incidence of Curling's ulcer in thermally injured patients is reported to be approximately 12%. The mean time of diagnosis is reported as 12-15 days post burn.

Recent endoscopic observations in patients with multiple organ trauma and severe sepsis reveal gastric mucosal abnormalities early after injury despite the absence of significant bleeding. Progressive mucosal defects and clinical gastric bleeding were commonly associated at later periods post injury.

Endoscopy with a fiberoptic pan-endoscope (Olympus Model GIF-D) has been performed on thermally injured patients with greater than 30% total body surface area injury admitted to the USA Institute of Surgical Research. Initial study is performed at three days post burn and subsequent serial studies are performed between 5 to 7 and 9 to 12 days post burn. Mucosal changes are photographically documented at each study.

The purpose of the endoscopic studies is to define post burn gastric mucosal changes and their relationship to Curling's ulcer and clinically significant gastric bleeding. A true incidence of Curling's ulcer will be obtained as previous studies defined Curling's ulcer only by significant clinical bleeding, operative, and autopsy findings.

Curling's ulcer, Gastric mucosa, Burn, Endoscopy

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*Department of Pathology
Medical University
Charleston, S. Carolina 29401

EVALUATION OF GASTRIC PHYSIOLOGIC DISTURBANCES ASSOCIATED WITH THERMAL INJURY IN A MILITARY POPULATION

The incidence of Curling's ulcer in thermally injured patients is reported to be approximately 12%. The mean time of diagnosis is reported as 12 to 15 days post burn. Serial endoscopy of the upper gastrointestinal tract has been performed on thermally injured patients to define post burn gastric mucosal changes and their relationship to Curling's ulcer and clinical gastric bleeding. A true incidence of Curling's ulcer will be obtained as previous studies defining Curling's ulcer encompassed clinical bleeding, operative and autopsy findings only.

Endoscopy with a fiberoptic pan-endoscopy (Olympus Model GIF-D) has been performed on 21 thermally injured patients of greater than 30% total body surface area injury admitted to the USA Institute of Surgical Research. Initial study is performed at three days post burn and subsequent serial studies are performed between 5 to 7 and 9 to 12 days post burn. Mucosal changes have been photographically documented at each study. Initial study has confirmed the presence of gastric mucosal abnormalities in the majority of patients with greater than 40% total body surface area injury. These abnormalities range in severity from areas of pallor with surrounding mucosal congestion to numerous superficial gastric erosions to discrete gastric and duodenal ulcers. The majority of the changes are limited to the body of the stomach, but antral disease has been present in over 25% of the patients studied. A significant finding has been duodenal mucosal changes of edema, congestion, and friability which we have described as duodenitis. An interesting observation that will need further study is the association of isolated duodenal findings and pancreatitis in the burned patient. The study is continuing at present and the results mentioned above reflect our early observations.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0C 6971	73 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
72 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	075
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Procede with Security Classification Code): (U) Pulmonary Pathophysiologic Changes Following Thermal Injury in Burned Soldiers (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS:
003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
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)
FISCAL YEAR PRECEDING 73	.6	5
FISCAL YEAR CURRENT 74	.4	5

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

RESPONSIBLE INDIVIDUAL
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20. PERFORMING ORGANIZATION
NAME: US Army Institute of Surgical Research
Pulmonary Section
ADDRESS: Ft Sam Houston, Tx 78234

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic institution)
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TELEPHONE: 512-221-4307
SOCIAL SECURITY ACCOUNT NUMBER

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

ASSOCIATE INVESTIGATORS
NAME: Edwin W Hander, ILT, MSC
NAME: DA

22. KEYWORDS (Procede EACH with Security Classification Code)
(U) Shunt; (U) PV Work; (U) Burns; (U) Lung Mechanics; (U) Pulmonary Diffusion; (U) Ventilation/Perfusion Abnormalities; (U) Blood Gases; (U) Wounded Soldiers

23. TECHNICAL OBJECTIVE. 24. APPROACH. 25. PROGRESS (Furnish individual paragraphs identified by number. Procede 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 diffusing capacity. Compare with results of xenon scan and bronchoscopy. In noninhalation injuries, continue prior work with attention to CO2 response curve, better measurement of static lung volumes and use of flow-volume loop, single breath tests, and cardiac output.

25. (U) 72 07 - 73 06 Necessary equipment for the flow volume curves, and single breath tests are on order. Normals are being studied for controls. Apparati for the more accurate measurement of lung volumes are being constructed.

*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

ANNUAL PROGRESS REPORT

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: PULMONARY PATHOPHYSIOLOGIC CHANGES FOLLOWING THERMAL INJURY

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

1 July 1972 - 30 June 1973

Investigators:

Peter A. Petroff, MD, Major, MC
Edwin W. Hander, 1LT, MSC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: PULMONARY PATHOPHYSIOLOGIC CHANGES FOLLOWING THERMAL INJURY

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

Period covered in this report: 1 July 1972 - 30 June 1973

Investigators: Peter A. Petroff, MD, Major, MC
Edwin W. Hander, 1LT, MSC

Reports Control Symbol MEDDH-288(R1)

Burn wounds are a common military problem and pulmonary disease remains the major complication of the burn patient. A group of patients with hyperventilation without apparent dyspnea, beginning about postburn day 3-5, associated only with a decrease in static lung compliance has been identified. Hyperventilation with hypocarbia may result in bronchoconstriction, will affect the oxyhemoglobin dissociation curve unfavorably and decrease cerebral and coronary blood flow. The etiology of the hyperventilation seen in these patients was unexplained. We plan to 1) study the effects of Sulfamylon on ventilation and dead space/tidal volumes ratio; 2) measure CO_2 response curves in patients to see if they respond appropriately to CO_2. In addition, we have constructed a bag-box system for better measurement of lung volumes. We are also initiating the use of single breath tests and flow-volume loops in order to examine the small airway more closely. Lastly, we plan to measure several indices of lung function (static and dynamic compliance, pulmonary resistance, flow-volume loops, VD/VT, and arterial shunt) at the bedside to establish the general course of the patients and principles in the care of the patients.

The equipment necessary for these tests is on order. Where possible, normal values are being obtained.

Burns
Lung mechanics
Pulmonary diffusion
Ventilation/perfusion abnormalities
Blood gases
Shunts

42

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OE 6959	73 07 01	DD DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. GRP'S INSTR'S	8b. SPECIFIC DATA: CONTRACTOR ACCESS	9. LEVEL OF SUM
	A. NEW	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	079
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE: (U) Bacterial and Mycotic Sepsis: Comparative Pathophysiology, Special Reference to Burn Wound Infection 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
72 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	PROFESSIONAL	a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
FISCAL YEAR	73	.3	7
	74	.5	1

19a. 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

19b. PERFORMING ORGANIZATION

NAME: US Army Institute of Surgical Research
Pathology Branch
ADDRESS: Ft Sam Houston, Tx 78234

PRINCIPAL INVESTIGATOR (Punish Rank if U.S. Academic Institution)
NAME: Sanford D Peck, MAJ, MC
TELEPHONE: 512-221-4753
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: Daniel W McKeel, Jr, MAJ, MC
NAME: Harry L Walker, MS DA

20. GENERAL USE: FOREIGN INTELLIGENCE NOT CONSIDERED

21. KEYWORDS: (U) Sepsis; (U) Coagulation; (U) Endotoxin; (U) Injured Soldiers; (U) Rabbits

22. TECHNICAL OBJECTIVE, 23. APPROACH, 24. PROGRESS (Include additional paragraphs identified by number. Precede each with appropriate classification code.)

23. (U) To study parameters of burn wound sepsis as they exist in burned soldiers.

24. (U) Develop a rabbit model of burn wound sepsis upon which coagulation studies and endotoxin levels will be performed.

25. (U) 72 12 - 73 06 A rabbit skin flap model of Pseudomonas burn wound sepsis, resulting in greater than 90% mortality in 36 hours, has been developed. Endotoxin has been detected in the blood and liver, and there is an associated leukopenia and thrombocytopenia. Fibrinogen, thrombin time, partial thromboplastin time and prothrombin time have been performed on all animals using the Fibrometer and indicate a variable pattern of abnormalities as yet not defined. The first three tests have been low, normal or elevated in various patterns.

(Available in computer user's manual)

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

ANNUAL PROGRESS REPORT

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: BACTERIAL AND MYCOTIC SEPSIS: COMPARATIVE PATHOPHYSIOLOGY, SPECIAL REFERENCE TO BURN WOUND INFECTION IN INJURED SOLDIERS

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

1 December 1972 - 30 June 1973

Investigators:

Sanford D Peck, MD, Major, MC
Daniel W McKeel, Jr, MD, Major, MC
Harrel L Walker, MS
Robert B Lindberg, PhD

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: BACTERIAL AND MYCOTIC SEPSIS: COMPARATIVE PATHOPHYSIOLOGY, SPECIAL REFERENCE TO BURN WOUND INFECTION IN INJURED SOLDIERS

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

Period covered in this report: 1 December 1972 - 30 June 1973

Investigators: Sanford D Peck, MD, Major, MC
Daniel W McKeel, Jr, MD, Major, MC
Harrel L Walker, MS
Robert B Lindberg, PhD

Reports Control Symbol MEDDH-288(R1)

Bacterial and mycotic wound infection are commonly found in fatally burned patients, and are sometimes associated with generalized dissemination. The relationship of wound infection to the clinical course is not well understood.

Preliminary results using a rabbit "skin" flap model infected with Pseudomonas aeruginosa indicate bacterial dissemination via the blood stream associated with high visceral counts (10^4 - 10^7 organisms/gram of tissue). There have also been coagulation changes consistent with the diagnosis of disseminated intravascular coagulation, and endotoxin has been detected in the blood of infected animals.

Sepsis
Endotoxin
Coagulation

BACTERIAL AND MYCOTIC SEPSIS: COMPARATIVE PATHOPHYSIOLOGY, DIAGNOSIS AND TREATMENT, WITH SPECIAL REFERENCE TO BURN WOUND INFECTION

The purpose of this study is to describe the mechanism by which experimental wound infection kills the experimental animal. It is striking that although bacterial wound infection disseminates frequently, mycotic infection rarely does. Of particular interest is the role of endotoxin, as reflected by coagulation changes and direct assay, and the anatomic lesions.

Because of the insensitivity of the rat to disseminated intravascular coagulation, and the inability to measure endotoxin in its blood, the previously described burn wound sepsis model could not be used. The rabbit was chosen as an experimental animal because it is sensitive to endotoxin, and endotoxin can be detected in its blood.

RESULTS

Experiment #1 and #3 -

Rabbits were given a 5% scald burn and seeded with Pseudomonas aeruginosa strain VA-134. All five animals survived. A larger burn was not attempted due to previous experience that it, in itself, is fatal.

A 20% dorsal skin flap was then fashioned on the rabbits and then seeded subcutaneously in the traumatized area with either Pseudomonas aeruginosa strain VA-134 or strain 1244. With strain VA-134, 8 of 10 animals died within 24 hours, had positive blood cultures, organ bacterial counts ranging from 10^4 - 10^6/gram of tissue, and histologic lesions including shock lung, glomerular thrombosis, and bacterial abscesses. Using strain 1244, 7 of 8 died within 30 hours, had the same bacteriologic findings as the first group, but no organ lesions.

Coagulation tests on the strain 1244 seeded group revealed a marked decrease in platelets, low fibrinogen (compared to controls), elevated PTT and decreased thrombin time. These animals also had positive blood cultures and positive limulus lysate tests for endotoxin.

Experiment #2 -

Five animals were given 10^7 Pseudomonas aeruginosa strain VA-134 intravenously. All died in 25 hours. The histologic and bacteriologic features were the same as the "flap-seeded" animals with the exception that the bacterial counts were 10^0 - 10^3 per gram of tissue.

Four adult animals were given two doses of E.coli endotoxin, 100 ug/ml, 24 hours apart, and 5 animals were given one dose. Of the first group, all died in from 30 minutes to 48 hours. The lesions found were the same as those in the flap seeded VA-134 animals, but varied from animal to animal so that only the one dying at 48 hours had glomerular thrombosis.

Of the animals given one dose, two were sacrificed at three weeks and had no lesions, three were killed at five hours and had changes resembling those of early shock lung.

SUMMARY AND CONCLUSIONS

A dorsal skin flap on the rabbit beneath which Pseudomonas aeruginosa is seeded constitutes a reproducible model of fatal wound infection. Preliminary data reveals septicemia and high organ counts using two strains of Ps. aeruginosa, however, histologic lesions are present only in animals seeded with strain VA-134. Coagulation data reveals evidence of disseminated intravascular coagulation and endotoxemia in seeded animals. Further studies are needed to statistically confirm the relationship between septicemia, endoxemia, DIC, and visceral lesions. These are in progress using strain VA-134 since this organism has the advantage of producing visable lesions. Later, seeding of the wound with various fungi will be done and the same indices measured.

PRESENTATIONS AND/OR PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0A 6382	73 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
72 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	080
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) Hemodynamics and Pulmonary Vascular Studies in the Early Postburn Period of 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	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 73	.2	7
	CURRENT 74	.3	6

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

RESPONSIBLE INDIVIDUAL
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TELEPHONE: 512-221-2720

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

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic institution)
NAME: Stephen Slogoff, MAJ, MC
TELEPHONE: 512-221-5712
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: Gary W Allen, 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) Burn; (U) Wedge Pressure; (U) Cardiovascular -Hemodynamics; (U) Resuscitation of Fluids; (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 systemic and pulmonary hemodynamics during the resuscitation phase of thermal injury in burned military personnel.

24. (U) Selected patients are studied by means of hemodynamic flow and pressure measurements which are correlated with fluid intake and output.

25. (U) 72 07 - 73 06 One patient has been fully studied, and two pilot evaluations have been performed. Systemic cardiovascular measurements are as predicted by previous studies. Pulmonary hemodynamic changes consist of pulmonary hypertension in the face of systemic vasodilatation without elevation of left atrial pressure. The study is continuing.

*Available to contractors upon originator's approval.

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

ANNUAL PROGRESS REPORT

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: HEMODYNAMICS AND PULMONARY VASCULAR STUDIES IN THE EARLY POSTBURN PERIOD OF BURNED MILITARY PERSONNEL

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

1 July 1972 - 30 June 1973

Investigators:

Stephen Slogoff, MD, Major, MC
Gary W. Allen, MD, Major, MC
Glenn D. Warden, MD, Major, MC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: HEMODYNAMICS AND PULMONARY VASCULAR STUDIES IN THE EARLY POSTBURN PERIOD OF 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 1972 - 30 June 1973

Investigators: Stephen Slogoff, MD, Major, MC
Gary W. Allen, MD, Major, MC
Glenn D. Warden, MD, Major, MC

Reports Control Symbol MEDDH-288(R1)

Little material is available concerning hemodynamic measurements in thermally injured patients. Two studies have been reported in the past which demonstrated classic shock with low cardiac output and increased peripheral vascular resistance immediately post-thermal injury. Pruitt and Mason found a two-fold increase in cardiac output above normal with a commensurate drop in systemic vascular resistance in the second 24 hours postinjury with Brooke Formula resuscitation. No investigations to date have dealt with pulmonary hemodynamics after thermal injury.

In this study, we are attempting to correlate measurement of systemic hemodynamics with pre-existing data and pulmonary vascular measurements. At this time, one patient has been studied and showed the typical response of the systemic circulation to thermal injury and resuscitation, although there was a rising pulmonary vascular resistance associated with minimal pulmonary hypertension in the face of falling systemic vascular resistance. No conclusions can be drawn from this single experience, and this work is continuing.

Burn
Wedge pressure
Cardiovascular hemodynamics
Resuscitation of fluids
Humans

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OE 6393	73 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
72 07 01	D. CHANGE	U	U	NA	NL	[X] YES [ ] NO	A. WORK UNIT

10. (a.) / 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 73	.1	12
FISCAL YEAR CURRENT 74	.1	10

19. RESPONSIBLE DOD ORGANIZATION
NAME: US Army Institute of Surgical Research
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RESPONSIBLE INDIVIDUAL
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20. PERFORMING ORGANIZATION
NAME: US Army Institute of Surgical Research
ADDRESS: Burn Study Branch
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PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
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SOCIAL SECURITY ACCOUNT NUMBER
ASSOCIATE INVESTIGATORS
NAME: Glenn D Warden, MAJ, MC
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 which would be less expensive than currently available forms, guaranteed sterile, indefinitely shelf-storageable and still effective as a temporary biologic wound cover for burned combat casualties.

24. (U) Laboratory evaluation of Lyophilized porcine cutaneous xenograft has been completed. Adequate adherence to the burn wound for longer periods than that achieved with commercially supplied fresh porcine xenograft was achieved. An approach has been developed to study the role of xenograft in altering the mortality caused by a large open surface in the burned rat.

25. (U) 72 07 - 73 06 A model of a 60% third degree scald burn in the rat has been established. Excising 30% of the burn results in a survival of 20%, when no dressing is applied to the excised area. When rat homograft is applied, survival is 100%. The ability of non-viable xenograft to affect survival will be studied.

*Available to contractors upon originator's approval

DD, FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 65 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

ANNUAL PROGRESS REPORT

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: CONTINUED EVALUATION OF SPLIT-THICKNESS CUTANEOUS XENOGRAFT AS A TEMPORARY BIOLOGIC WOUND COVER FOR USE IN BURNED SOLDIERS

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

1 July 1972 - 30 June 1973

Investigators:

Norman S. Levine, MD, Major, MC
Glenn D. Warden, MD, Major, MC
Roger E. Salisbury, MD, Major MC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: CONTINUED EVALUATION OF SPLIT-THICKNESS CUTANEOUS XENOGRAFT AS A TEMPORARY BIOLOGIC WOUND COVER FOR USE 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 1972 - 30 June 1973

Investigators: Norman S. Levine, MD, Major, MC
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Reports Control Symbol MEDDH-288(R1)

During the past decade, the physiologic advantages of cadaver skin allograft in the treatment of second and third degree burn wounds have been well documented. Because of inadequate supply and limited shelf-life, widespread use of cadaver allograft has been restricted. The evaluation of porcine xenograft, canine xenograft, lyophilized xenograft and homograft, and formalinized xenograft and homograft have thus been prompted.

All of these "biological dressings" have, to some extent, been evaluated both in the laboratory and on burned soldiers. All forms appear to show adequate adherence to the wound, whether this is a clean granulating surface on a patient or a freshly excised fascial bed on a rat. Repeated dressing changes on granulating surfaces in patients appeared to "prepare" the wound for the acceptance of autograft. With the exception of homograft, none of these "biological dressings" become vascularized.

The direction of this study has been changed to determine if viability and vascularization of homograft are important in terms of wound coverage. Two models have been developed in which the application of homograft determines survival versus death of an experimentally burned rat. A comparison of formalin-fixed homograft versus fresh homograft will be performed in these two models. The purpose of these experiments will be to determine if the life-saving properties of homograft in an experimental situation can be accomplished with a nonviable biologic dressing.

Cutaneous Xenograft Wound cover Laboratory animals
Humans

CONTINUED EVALUATION OF SPLIT-THICKNESS CUTANEOUS XENOGRAFT AS A TEMPORARY BIOLOGIC WOUND COVER FOR USE IN BURNED SOLDIERS

Previous experience with viable and frozen biologic dressings has indicated that the following properties are common to fresh homograft, viable xenograft (porcine), irradiated-frozen porcine xenograft, and formalin-fixed allograft: (1) they adhere to the wound (2) histologic sections indicate ingrowth of granulation tissue into the lower dermal collagen network of the graft (3) repeated changes of such "biological dressings" help to prepare the bed for autografting.

It has also been established that allograft is the only biologic dressing to undergo vascularization. It has not been established whether or not such vascularization is important in terms of survival of the patient or the animal.

Accordingly, our work this year has been directed toward establishing models in which the life-saving properties of biologic dressings could be investigated.

LABORATORY INVESTIGATION

Three groups of 175-185 gm Sprague Dawley rats were anesthetised and burned to 60% body surface area. Burns were applied by scalding the back of the animal (30% body surface area) in boiling water for 10 seconds and the abdomen of the animal (30% body surface area) in scalding water for 3 seconds. Ten cc of intraperitoneal saline were given to each rat. Following burning, the rats were divided into three groups. In Group 1, (9 animals) no further treatment was administered. In Group 11, 30% of the burn (the entire 3d degree back burn) was excised. No graft was applied. In the third group (10 animals) the 30% back wound was excised and immediately covered with rat allograft. Survival in these three groups of animals is outlined in the Table.

From this experimental data it is clear that fresh rat allograft alters survival favorably when compared to animals in whom half the burn wound

Effect of 30% Excision With and Without Allografting on Survival of Rats Burned to 60% Body Surface Area

Group	No. Animals	Survived 10 Days
60% Burn	9	9/9
60% Burn - 30% Excision-No coverage	9	2/9
60% Burn - 30% Excision-Allograft coverage	10	10/10

is excised and not covered. It will be the purpose of future investigation to determine if survival can be likewise enhanced by using nonviable formalin-fixed allograft instead of fresh rat allograft.

A separate investigation has indicated that immediate surgical excision of a 30% third degree scald burn on the rat together with immediate homografting significantly alters the survival of burned rats challenged 24 hours after burning with an intraperitoneal dose of Pseudomonas aeruginosa, strain 12-4-4. It will be the intention of this study to determine if the application of nonviable, formalin-fixed allograft can affect the mortality of these experimental animals in a fashion similar to fresh viable allograft.

SUMMARY

The emphasis of this investigation has been shifted from the mechanical properties of "biologic dressings" to the "life-saving" properties of such dressings. Two experimental models have been produced as a basis for testing whether or not nonviable allograft is as effective as viable allograft in reducing the mortality in these experimental animals.

PUBLICATIONS AND/OR PRESENTATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0E 6954	73 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
72 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	076
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)

(U) Excision of Eschar 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 73	.1	13
	CURRENT 74	.1	5

19. RESPONSIBLE DOD ORGANIZATION

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

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21. GENERAL USE

FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede 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 toward minimizing blood loss involved in such excisions in burned soldiers.

24. (U) Symmetrical excisions will be performed on patients who are candidates for excision. Laser excisions will be compared to surgical excisions from the standpoint of blood loss, time for excision, graft take and pre and post excision quantitative microbiology.

25. (U) 72 07 - 73 06 The burn wound of three patients has been excised thus far using the laser. Blood loss was less than for a comparable scapel excision. Time for excision was less than with the scalpel. Better than 95% graft takes were achieved on both the laser and electrocautery excised areas in one patient and poor take on both areas was observed in another.

*Available to contractors upon additional 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

FINAL REPORT

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: EXCISION OF ESCHAR IN BURNED SOLDIERS . PART I

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

1 July 1972 - 30 June 1973

Investigators:

Joseph A. Moylan, Jr., MD, Major, MC
John L. Hunt, MD, Major, MC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: EXCISION OF ESCHAR IN BURNED SOLDIERS. PART I.

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

Period covered in this report: 1 July 1972 - 30 June 1973

Investigators: Joseph A. Moylan, Jr, MD, Major, MC
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Reports Control Symbol MEDDH-288(R1)

The goal of burn care is the removal of eschar with autograft closure of the burn wound at the earliest possible moment. Primary excision of extensive burn wounds has been associated with an unacceptable blood loss, and staged excision prior to the development of effective topical chemotherapy was associated with the high occurrence rate of invasive wound sepsis.

The significant improvement in survival of burn patients noted with the use of topical therapy has been confined to patients with burns of less than 60% of the total body surface. This fact has prompted a reevaluation of excision in patients with extensive burns in whom control of bacteria in the unexcised burn is possible, using Sulfamylon burn cream. Janzekovic and Jackson have advocated using tangential excision as a means of excising just the nonviable eschar and minimizing the blood loss and nonspecific trauma associated with a more formal surgical excision.

Laboratory evaluation of a cryogenic device using liquid nitrogen to freeze nonviable thermally injured tissue with subsequent dermatome excision has been performed on 4 Duroc pigs, each of which had been given a 60% full-thickness total body surface burn. These pigs underwent total excision on the second postburn day using a liquid nitrogen cryosurgical instrument to freeze the nonviable tissue and a standard Brown

dermatome. Topical application of liquid nitrogen via a multiperforated delivery head rapidly froze nonviable tissue while the intact circulation of viable tissue presumably dissipated the negative heat loads sufficiently rapidly to keep it from freezing. In this preparation the frozen non-viable tissue was easily differentiated from viable tissue and was readily excised by a dermatome with minimal blood loss. The excised wound accepted homograft skin immediately following excision, and absence of subgraft suppuration confirmed the adequacy of the excision. Autografts applied to the wounds on the animals following a second application of homograft skin showed an excellent take.

This same apparatus was used to perform excisions on 3 patients. A patient with an 80% total body surface area burn underwent excision of the anterior thighs bilaterally with what was judged to be moderate blood loss. The patient was covered with homograft over the excised areas. The patient died shortly thereafter and graft take was not measurable. A second patient underwent excision of abdominal and chest eschar. Bleeding was judged to be excessive and the technic did not appear to work well in these areas. A third patient was treated with cryo-excision of a leg. The blood loss was moderate and the procedure was considered satisfactory.

Cryo-excisions did not proceed as smoothly in patients as in the animal preparation. The use of this technic in treatment of burns of irregular or "yielding" areas such as the anterior chest, buttocks or abdomen has been associated with inadequate viable-nonviable tissue differentiation and excessive blood loss from such areas. Invasive wound sepsis developed in the excised area of one patient despite the use of Sulfamylon burn cream on the adjacent intact eschar. In the clinical situation the cryogenic method appeared best suited for treatment of burns on relatively smooth, relatively unyielding regular surfaces such as the limbs.

In summary, the usefulness of this technic appears limited to certain anatomic surface areas and technicological alterations will be needed to improve the general usefulness of this method of excision, especially in terms of better operative hemostasis. This investigation is being terminated pending such technical improvements and refinements.

Excision Eschar Cryosurgery Liquid nitrogen

ANNUAL PROGRESS REPORT

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: EXCISION OF ESCHAR IN BURNED SOLDIERS. PART II. THE USE OF A CARBON DIOXIDE LASER IN THE DEBRIDEMENT OF THIRD DEGREE BURN ESCHARS

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

1 July 1972 - 30 June 1973

Investigators:

Norman S. Levine, MD, Major, MC
Roger E. Salisbury, MD, Major, MC
John L. Hunt, MD, Major, MC
Daniel W. McKeel, Jr., MD, Major, MC
Basil A. Pruitt, Jr., MD, Colonel, MC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: EXCISION OF ESCHAR IN BURNED SOLDIERS. PART I THE USE OF A CARBON DIOXIDE LASER IN THE DEBRIDEMENT OF THIRD DEGREE BURN ESCHARS

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

Period covered in this report: 1 July 1972 - 30 June 1973

Investigators: Norman S. Levine, MD, Major, MC
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Reports Control Symbol MEDDH-288(R1)

Surgical excision of third degree burns with immediate grafting offers an attractive means of promptly removing the burn eschar and the possibility of a shortened convalescence. Major problems with this technic are twofold: (1) it requires surgical operation under anesthesia; (2) the procedure can involve massive blood loss. Experimental studies with the carbon dioxide laser (10.6 micron wave length) indicate that blood loss can be reduced in such excisions by using this instrument. Bleeding is minimized because of photocoagulation of the small blood vessels. Experiments in pigs have indicated that blood loss with the laser is roughly 30% of that encountered with a cold knife excision. Excellent takes of split-thickness skin grafts immediately applied to the freshly lased surface were uniformily obtained. The purpose of this investigation is to compare the use of the carbon dioxide laser with other instruments used for excision, including the scalpel and electrocautery. A comparison will be made on the basis of blood loss per unit area and on the speed of excision.

In the past 3 months, 3 laser excisions have been performed. In 2 cases the laser was compared to the cold knife. Blood loss in the areas

excised with the laser was less than 30% that obtained using a scalpel in both patients. In one patient a laser excision was compared to an electrocautery excision. Blood loss in this case was reduced to three-quarters of that obtained with the Bovie. In our limited experience, the laser excisions appear faster than cold knife excisions but not quite as fast as the electrocautery excision. A large number of patients will be required before a definite evaluation of laser excision can be made.

Laser Escharotomy Surgical Excision

THE USE OF A CARBON DIOXIDE LASER IN THE DEBRIDEMENT OF THIRD DEGREE BURN ESCHARS

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 technic are twofold: (1) it requires a surgical operation under anesthesia; (2) this procedure involves massive blood loss (up to 40 units of blood per 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 photocoagulation of the small blood vessels. Experiments carried out in 3-month-old Hampshire-Landrase pigs indicate that third degree eschar can be excised safely and effectively with 30% of the blood loss incurred with the cold knife excision. Although minimal injury did occur to the underlying tissue, this did not effect the "take" of split-thickness skin autografts applied immediately to the freshly lased surface. Excisions of up to 15% body surface area burns in pigs have been performed with 90 - 100% graft takes. Prior experience at the Albert Einstein Medical Center with one patient again demonstrated reduced blood loss with graft take comparable to that obtained in a comparable 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 surgical 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 histological evidence. All laser excisions were performed in the operating room under sterile conditions. Plate glass spectacles or ordinary corrective lenses have been worn by the surgical and anesthetic team to protect their eyes from the infrared laser beam. A safety inspection of the laser was made both by 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 "clean fragmentation or smoke" caused by photovaporization of the tissue. Signs designating "LASER IN USE" were posted outside the entrance to the operating room. Nonexplosive anesthetic agents were used.

Preoperative photographs were taken from the areas chosen for both laser and scalpel or both the 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 postexcision from the eschar to be removed (preoperatively) and from the underlying base (postoperatively). Calculation of blood loss in terms of ml/cm^2 and speed of excision in terms of cm^2/minute were made.

RESULTS

Formal laser excision of fresh third degree burn areas have been performed in only 2 patients. A small operative debridement 3 weeks post-burn was performed in a third patient. The results of these excisions are summarized in Tables I and 2. The cases varied considerably in both area and whether or not a tourniquet was used. In all 3 cases, blood loss was less when the laser was used than when either scalpel, scalpel with tourniquet, or electrocautery was used. This difference was dramatic when the scalpel was used for comparision. In both cases 2 and 3, blood loss using the laser was less than one-third of that obtained using the scalpel. Although blood loss with the laser was less than that obtained using the electrocautery, this was not as impressive.

The speed of excision with the laser exceeded the speed of scalpel excision. In the first laser excision, where the laser was compared to the electrocautery, the speed of excision was somewhat less with the laser than with the electrocautery. Part of the problem in the initial excision was getting the operating room team familiar with the use of this new instrument.

Table 1 - Blood Loss

Case No.	Laser	Modality of Excision
1 Both Legs - No tourniquet 20% Excision	0.77 cc/cm^2	1.0 cc/cm^2 (Electrocautery)
2 Both Legs - Tourniquet 35% Excision	0.072 cc/cm^2	0.27 cc/cm^2 (Scalpel)
3 Arm - No tourniquet 2% Excision	0.6 cc/cm^2	2.0 cc/cm^2 (Scalpel)

Table 2 - Speed of Excision

Case No.	Laser	Modality
1 Both Legs - No tourniquet 20% Excision	12.5 cm^2/min	16.6 cm^2/min (Electrocautery)
2 Both Legs - Tourniquet 35% Excision	57 cm^2/min	37 cm^2/min (Scalpel)
3 Arm - No tourniquet 2% Excision	8.3 cm^2/min	6.3 cm^2/min (Scalpel)

The second laser excision, of an even larger area, actually required less time than did the initial laser excision and the laser excision was more rapid than the scalpel excision.

The freshly lased base was tested with immediately applied autograft in only case 1. In this case, graft take was better than 95% on both the laser and electrocautery excised areas.

CONCLUSIONS

From our very limited experience thus far, it appears that using the carbon dioxide laser to excise third degree burns effectively reduces blood loss to one-third of that obtained when the cold knife is used for excision. Although blood loss with the laser was less than that when the electrocautery was used, this difference was less dramatic. The laser excisions appeared to go more rapidly than did the cold knife excisions but not faster than an electrocautery excision. These differences in speed were not dramatic. The small number of patients studied so far precludes our making any significant statistical comparison at this time. The one patient who was autografted immediately following excision showed a better than 95% graft take on the areas excised with the laser and the areas excised by electrocautery. This confirms our experimental findings that the minimal damage caused by the laser to the underlying tissue does not inferfere with graft take.

PRESENTATIONS AND/OR PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0E 6389	73 07 01	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. ORGN INSTR'N	9. SPECIFIC DATA-CONTRACTOR ACCESS	10. LEVEL OF SUM
72 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	3A161102B71P	08	070
b. CONTRIBUTING				
c. CONTRIBUTING				

12. TITLE (Precede with Security Classification Code): (U) Laboratory Evaluation of Artificial Tendons and Homografts for Use in Military Personnel with Severe Flexor Tendon Injury (44)

13. SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 Clinical Medicine

14. START DATE	15. ESTIMATED COMPLETION DATE	16. FUNDING AGENCY	17. PERFORMANCE METHOD
71 09	Cont	DA	C. In-House

18. CONTRACT/GRANT: Not Applicable
a. DATES/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 73	.7	25
	CURRENT 74	.6	15

20. RESPONSIBLE DOD ORGANIZATION
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21. GENERAL USE
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22. PERFORMING ORGANIZATION
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ASSOCIATE INVESTIGATORS
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NAME: F D Foley, MD DA

23. KEYWORDS (Provide each with Security Classification Code):
(U) Artificial Tendons; (U) Flexor Tendon Injuries; (U) Homografts; (U) Chickens

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

23. (U) To study the effects of artificial tendons on undifferentiated connective tissue beds and vascularization of homografts and autografts in tendon sheaths for application in combat wounded soldiers.

24. (U) Ten chickens, 12 weeks old, were anaesthetized and the flexor tendons excised from the right foot, long toe. An artificial tendon was inserted and anastomosed proximally and distally. An artificial tendon was also inserted in the soft tissue of the back. All animals were splinted for 3 weeks and then returned to the operating room. Exploration of back and leg was performed, biopsy of tendon sheath done, and the artificial tendon removed and replaced with autogenous graft or homograft. Animals were sacrificed three weeks later and india ink injections performed. Biopsies of all tendon sheaths were repeated.

25. (U) 72 07 - 73 06 Neosheaths formed about artificial tendons and light microscopy, transmission electron microscopy and scanning electron microscopy revealed them to be similar to normal sheaths. Artificial tendons have been constructed with a sliding sheath that will fit fingers of different sizes. Tensile strength studies of artificial-autogenous tendon anastomosis have been found to be equal to autogenous tendon-tendon anastomosis.

* Available on contributors upon originator's approval

ANNUAL PROGRESS REPORT

PROJECT NO. 3A161102B71P-08, 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
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1 July 1972 - 30 June 1973

Investigators:

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Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A161102B71P-08, 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 of report: 1 July 1972 - 30 June 1973

Investigators: Roger E. Salisbury, MD, Major, MC
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Reports Control Symbol MEDDH-288(R1)

The management of severe flexor tendon injuries of the hand has been dramatically improved by tendon sheath reconstruction with an artificial tendon, followed by conventional tendon grafting. The purpose of this study was to investigate the nature of neosheath formation.

In 25 chickens, the flexor tendons and sheaths in a toe were excised and replaced with a United States Army artificial tendon. An artificial tendon was also placed in the back, and 4 weeks later, tendons and surrounding tissue as well as normal tendon sheaths were excised and submitted for light, transmission electron microscopy and scanning electron microscopy.

In all instances, neosheaths formed around the silastic coated artificial tendon. The neosheaths formed in the foot and back were similar to normal sheath, having the same cellular and fibrous composition. Reconstruction of the normal anatomy with an artifical tendon explains why this surgical technic gives superior results to other less physiologic procedures in patients with severe hand injuries.

Artificial tendons Flexor tendon injuries Homografts Chickens

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* From the US Army Biomechanical Laboratory, Washington, DC

LABORATORY EVALUATION OF ARTIFICIAL TENDONS AND HOMOGRAFTS FOR USE IN MILITARY PERSONNEL WITH SEVERE FLEXOR TENDON SHEATH INJURY

Figures released by the National Safety Council reveal the grim statistics of 115,000 accidental deaths, 11,000,000 temporarily disabled and 400,000 permanently disabled persons, a total cost of 23 billion dollars each year. Upper extremity trauma, among the working population, figures prominently in these statistics. The high cost of time lost from work and the frequent need for new job training, have given impetus to the investigation of new technics to cope with the problems of severe hand injury.

DESCRIPTION AND INTENT

Of all hand injuries one of the most crippling with the poorest surgical results, is the severed flexor tendon. Because of scar deposition following injury and repair, tendon gliding is often compromised in spite of good surgical technic. Historically, (Gonzalez RI. Plast Reconstruc Surg. 23: 535, 1958[1]; Hochstrasser AE, Broadbent TR, Woolf R. Rocky Mountain Med J. 57: 30, 1960[2]; Nichols HM. Ann Surg 129: 223, 1949[3]; Wheeldon T. J Bone Joint Surg 21: 393, 1939[4]) surgeons have utilized many different artificial materials as "blocking agents", to surround the repaired tendon in an effort to restrict scar formation and facilitate gliding. These investigators, however, often used materials such as Ivalon which incited a massive foreign body inflammatory response, resulting in voluminous adhesions and poor function. Other agents, such as silastic, (Pontenza AD. J Bone Joint Surg. 44A: 49, 1962)[5] did not incite inflammation but did block blood supply to the tendon, causing necrosis and anastomotic disruption. Though various artificial tendons (Grau HR. Plast Reconstruc Surg. 22: 562, 1958[6]; Henze CW, Mayer L. Surg Gynec Obstet. 19: 10, 1914[7]; Hunter JM. Am J Surg. 109: 325, 1965[8]; Sakata Y. J Jap Orthop Assn 36: 1021, 1962[9]; Sarkin TL. Brit J Surg. 44: 232, 1956[10]; Arkin AM, Siffert RS. Am J Surg. 85: 795, 1953[11]; Mayer L, Ransohoff N. J Bone Joint Surg. 18: 607, 1936[12]) consisting of silk, wire, nylon or teflon have been periodically tried as replacements for damaged tendons, all relied on sutures for tensile strength, and all eventually disrupted when stress exceeded the mechanical limits of the sutures.

Other investigations with artificial materials have been concerned with the reconstruction of the damaged tendon sheath before tendon grafting. Celloidin tubes, first tried by Mayer and Ransohoff (Mayer L, Ransohoff N. J Bone Joint Surg. 18: 607, 1936)[12], caused a neosheath formation from the soft tissue of the damaged tendon bed, but the tubes were not flexible and stiff finger joints resulted. Laboratory and clinical investigations by Hunter (Hunter JM, Salisbury RE. .J Bone Joint Surg. 53A: 829, 1971[13]; Hunter JM Salisbury RE. Plast Reconstruc Surg. 45: 564, 1970[14]) revealed that the damaged flexor tendon sheath could be consistently reconstructed around a silastic-dacron tendon. Replacing the artificial tendon 3 months later with a tendon graft placed into the newly formed sheath, gave results superior to conventional repair in severely injured hands. Because results of flexor tendon surgery were so dramatically improved by using an artificial prosthesis to promote remodelling of undifferentiated connective tissue into a specific functional organ a unique example of wound healing, it seemed pertinent to further investigate in the laboratory the nature of neosheath formation.

METHOD

The foot of the chicken was chosen for experimentation because the toes are prehensile and the anatomy is analogous to the human finger, each toe having a sublimis and profundus tendon surrounded by a synovial sheath. The United States Army artificial tendon was designed similar to the Hunter artificial prosthesis and consisted of a dacron tape coated with silastic. Major differences, however, included exposure of the knitted dacron tape at both ends, allowing anastomosis of a porous knitted fabric to autogenous tendon. Because the silastic may be peeled back from the tape, only one basic tendon shaft is needed, which can be cut to fit different sized fingers. In 25 chickens the flexor tendons of the second, third, or fourth toes were exposed through midlateral incisions. The sublimis and profundus tendons and the surrounding synovial sheath were excised except for 2 pulleys. An artificial tendon 3 mm wide and 1 mm thick was inserted under the pulleys and anastomosed proximally and distally with 6-0 silk and the leg was cast in flexion for 3 weeks. Four

weeks postoperatively the wounds were re-explored and the tendons and surrounding soft tissue removed as well as the tendon sheath from a normal toe. All specimens were fixed in formalin or glutaraldehyde and submitted for light, scanning electron microscopy and transmission electron microscopy.

RESULTS

In all instances neosheaths formed around the artificial tendon. Thus, the foot is not a privileged location and the connective tissue responds to the silastic in a consistent manner. The sheaths formed in the back were grossly more filmy than normal sheaths, and the neosheaths in the foot, thicker than normal sheaths. H&E stain revealed marked similarities between normal synovial sheaths and the neosheaths in the back and foot. Differences were composed of 2 layers, an inner cellular layer in contact with the tendon and an outer fibrous layer. Normal sheaths displayed areolar and fibrous synovium. The intimal layer of the areolar synovium was 2 to 4 cells thick and composed of cuboidal cells with dark staining ovoid nuclei. Beneath the underlying layer of loose filmy collagen containing many small capillaries was a thick layer of tightly packed collagen. In the fibrous synovium, the intimal layer was less defined and consisted of one cell layer of fibroblasts overlying a dense collagen layer. Neosheaths from the back demonstrated both areolar and fibrous synovium, but the underlying collagen layer was less dense. Neosheaths in the foot were mostly fibrous synovium, with skip areas of bare collagen exposed to the lumen and a sheath wall of collagen markedly thicker than the normal. Specimens from all locations contained reticulum fibers.

Scanning electron microscopy of sheaths from all locations revealed a folded and pleated surface comparable to that seen in ordinary joint synovium. Significantly, pleated synovium (Castor CW. Arth & Rheumat. 3: 140, 1960[15]; Wysocki GP, Brinkhous KM. Arch Path. 93: 172, 1972[16]) is normally seen over areas of movement in joints and in these specimens, the folds ran parallel to the tendon's longitudinal axis of movement. High power views (800-4000X) of the neosheath surface revealed either well ordered collagen bundles with minimal cellularity (fibrous synovium), or a cellular surface, polypoid and poorly organized. Surface cells had numerous cytoplasmic projections which may reflect a secretory role. Pores or cisternae seen between cells may be

openings through which synovial fluid enters from subintimal vessels to lubricate the sheaths (Redler J, Zinny ML. J Bone Joint Surg. 52A: 1395, 1970)[17].

Transmission electron microscopy allowed a further comparison of ultrastructure of the sheaths. The synovial layer of all sheaths was covered by a layer of dense amorphous material. The underlying intimal layer was not a solid wall of cells, nor was there a basement membrane separating cells from subsynovium. The acellular portions of intima consisted of fine intertwining filaments overlying an extremely thick layer of collagen. The surface cells had prominent processes and many cytoplasmic vacuoles. In the absence of a basement membrane, cytoplasmic processes may act as the link between underlying capillaries and sheath surface, selectively transporting proteins and metabolites (Barland P, Novicoff AB, Hamerman D. J Cell Biol. 14: 207, 1962)[18]. In all sheaths, the cell population consisted of fibroblasts, intermediate cells, macrophages, mast cells and occasional Schwann cells. The fibrous component included collagen, elastic, reticulum, and unmyelinated nerve fibers. The fibroblast was the most common cell, having cytoplasm rich in goigi complexes, endoplasmic reticulum, mitochondria and free ribosomes. Cells that were morphologically similar to Type A and B joint synovial cells seen in normal tendon sheaths were also found in the neosheaths from the back and foot. The type A cells had extensive cytoplasmic projections, numerous intracytoplasmic vacuoles, either empty or containing amorphous material, fibrils, filopodia and many vesicles and mitochondria. There were fewer type B cells and these had fewer projections than type A cells, but many dilated cisternae of endoplasmic reticulum filled with amorphous material of medium electron density. Many of the cells contained features of both A and B types, being plumb with abundant cytoplasm, having complex projections and numerous cytoplasmic vacuoles, pinocytic activity and dilated enodplasmic reticulum. Neosheaths from the back and foot also had large cells filled with bundles of fibrils and lysosomes suggesting they were active in collagen production.

DISCUSSION

These studies show that the development of connective tissue sheaths

around artificial silastic-dacron implants results in a structure similar to normal tendon sheaths. It is important to note that some investigators have believed this sheath to be a tube of scar, a very erroneous over-simplification. Transmission electron microscopy has confirmed that a differentiated structure forms with well defined layers and cells other than fibroblasts. Type A and B cells previously described in joint synovium have been identified. It is believed that the A cells are phagocytic, and that B cells with abundant ergastoplasm synthesize and secrete protein. Sheaths from each location contained elements of fibrous and areolar synovium. Though Wysocki associated a wavy, rugated sheath appearance with areolar synovium, it was seen in our specimens with fibrous synovium as well. Both normal and neosheaths had tissue oriented longitudinally along the lines of stress, tendon movement, or shape of the shaft. The successful harvesting of neosheaths from the back comparable to those grown in the foot raises the tantalizing possibility of a tendon sheath free graft. Once the surgeon determines that the tendon injury is severe enough to warrant reconstruction, perhaps an artificial tendon could be implanted in the back and the sheath later transferred to the injured digit with a tendon graft at the time of elective excision of the damaged tendon. This challenge will be the subject of future work.

SUMMARY

Though no technics of end anastomosis have been found to be reliable under unlimited stress, silastic coated artificial tendons placed into the damaged tendon bed for a limited time will facilitate reconstruction of a neosheath. Light, scanning electron microscopy and transmission electron microscopy studies reveal close similarities between neosheaths in the foot and back and normal flexor tendon sheaths, explaining why the reconstruction of the normal anatomy with an artificial implant has produced superior results in tendon repair.

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PRESENTATION

Salisbury RE. "Morphologic observations of neosheath development of undifferentiated connective tissue around artificial tendons" presented at Bioengineering Symposium at Clemson University, Clemson, SC, April 1973.

PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0E 6960	73 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
	A. NEW	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	3A161102B71P	08	068
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) Relationship of Sodium Balance to Plasma Renin Concentration in the Troops With Renovascular Hypertension: A Canine Model (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 73	.8	27
	CURRENT 74	.6	15

19. RESPONSIBLE DOD ORGANIZATION
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NAME: Donald J Johnson, MAJ, MC DA

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Hypertension; (U) Renal Artery Stenosis; (U) Dogs

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 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) Eight mongrel dogs will be given normal dog chow such that each dog will receive 5 mEq/kg sodium daily. Four of the dogs will undergo a 40-50% stenosis of the right renal artery, followed by a 14-day equilabration period and then the creation of a 40-50% left renal artery steonsis again followed by a 14-day eqilabration period and then a right nephrectomy with a third 14-day eqilabration period. The other four dogs will undergo a similar balance and operative procedures except that these animals will have a 90% stenosis of the renal arteries. Sodium balance, measurement of peripheral plasma renin concentrations, weight, glomerular filtration rate, and direct measurement of blood pressure will be performed on each animal prior to and after each of the operative procedures.

25. (U) 73 03 - 73 06 This study has recently begun and the data is insufficient to make any conclusions.

*Available to contractors upon originator's approval

ANNUAL PROGRESS REPORT

PROJECT NO. 3A161102B71P-08, BASIC RESEARCH IN SUPPORT OF MILITARY MEDICINE

REPORT TITLE: RELATIONSHIP OF SODIUM BALANCE TO PLASMA RENIN CONCENTRATION IN THE TROOPS WITH RENOVASCULAR HYPERTENSION: A CANINE MODEL

US ARMY INSTITUTE OF SURGICAL RESEARCH
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1 July 1972 - 30 June 1973

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Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A161102B71P-08, BASIC RESEARCH IN SUPPORT OF MILITARY MEDICINE

REPORT TITLE: RELATIONSHIP OF SODIUM BALANCE TO PLASMA RENIN CONCENTRATION IN THE TROOPS WITH RENOVASCULAR HYPERTENSION: A CANINE MODEL

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

Period covered in this report: 1 July 1972 - 30 June 1973

Investigators: Philip W. Rogers, MD, Major, MC
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Reports Control Symbol MEDDH-288(R1)

Hypertension in the healthy and ill soldier may result from several etiologies which have been previously well investigated and sodium in the hypertension of individuals with renovascular disease is poorly understood. Interference with the blood supply to one kidney in humans and experimental animals results in hypertension which is characterized by increased renin release by the involved kidney. The exact mechanisms responsible for this form of hypertension are not clear but seem to be related to the increased formation of Angiotensin II which has a strong pressor action, secondary aldosteronism, and fluid retention by the ischemic kidney. Renal ischemia also gives rise to increased proximal tubular sodium reabsorption; however when the contralateral kidney has been removed or has decreased blood resulting from a markedly stenotic renal artery lesion, there is no mechanism present to excrete the retained sodium. This results in a positive sodium balance and increased blood volume.

It appears from previous studies that hypertension from unilateral renal artery stenosis is directly related to the elevated plasma renin concentrations (renin hypertension). However, the hypertension associated with bilateral renal artery stenoses may be a "volume hypertension", when the stenoses are

greater than 50%. Hypertension due to a transplanted kidney with renal artery stenosis also appears to be analogus to the hypertension resulting from moderate or severe unilateral renal artery stenosis with contralateral nephrectomy. This would explain the apparent discrepancies of both high and low renin concentrations reported in such patients. This study was undertaken to determine the relationship of sodium balance to plasma renin concentration and resultant hypertension in the conscious dog with chronic unilateral renal artery stenosis and in unilateral renal artery stenosis with surgical removal of the contralateral kidney. This animal model is analogous to soldiers with varied causes of renovascular hypertension.

The studies are conducted on conscious, trained, adult mongrel dogs weighing 15 to 25 Kg. The dogs are divided into two groups with four dogs in each group. The Group I dogs have a 40 to 60% occlusion of the right renal artery produced with a silver clip while the Group II dogs have an 80 to 90% occlusion of the right renal artery. The dogs are fed a weighed portion of dog chow daily containing 6 mEq of sodium per Kg body weight. They are maintained in metabolic cages and have accurate daily measurements of intake and output as well as daily weights. Serum and urinary electrolytes are obtained daily along with serial plasma renin concentrations, and serial determinations of the creatinine clearance. Direct blood pressure measurements by the percutaneous insertion of an intra-arterial cannula attached to a Statham strain gauge manometer are obtained every other day. The study periods are as follows: (1) control periods, 7 days, (2) stenosis of the right renal artery created on day 8 with a fourteen day post operative study period, (3) stenosis of the left renal artery is performed on day twenty-two with another fourteen day post surgical study period. (4) right nephrectomy is performed on day thirty-five with a fourteen day post operative study period.

This study has just been initiated and sufficient data have not been obtained to permit valid conclusions at the present time.

Hypertension
Renal Artery Stenosis
Dog

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0E 6961	73 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
	A. NEW	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	08	067
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) Role of Aldosterone In Alterations of Renin Secretion Associated With K+ Deficiency and K+ Loading In The Injured Soldier (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	PRECEDING/CURRENT FY	a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
FISCAL YEAR	73	.4	22
	74	.6	15

19. RESPONSIBLE DOD ORGANIZATION
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FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Renin; (U) Potassium; (U) Aldosterone Deficiency; (U) Dogs

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

23. (U) Numerous factors affect the secretion of renin in the injured soldier. The control of renin secretion in the adrenal insufficient state is poorly understood. The current study was undertaken to evaluate the role of potassium on renin secretion in bilaterally adrenalectomized dogs.

24. (U) Five mongrel dogs who have previously been adrenalectomized are fed potassium deficient diets, then repleted with potassium and then given a potassium loaded diet containing 150 mEq K phosphate or KCl. Serial plasma renin concentrations will be obtained during the study.

25. (U) 73 03 - 73 06 This study is near completion; however, no definite conclusions can be made at the present time.

*Available to contractors upon originator's approval

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

ANNUAL PROGRESS REPORT

PROJECT NO. 3A161102B71P-08, BASIC RESEARCH IN SUPPORT OF MILITARY MEDICINE

REPORT TITLE: ROLE OF ALDOSTERONE IN ALTERATIONS OF RENIN SECRETION ASSOCIATED WITH K+ DEFICIENCY AND K+ LOADING

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

1 July 1972 - 30 June 1973

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Philip W. Rogers, MD, Major, MC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A161102B71P-08 BASIC RESEARCH IN SUPPORT OF MILITARY MEDICINE

REPORT TITLE: ROLE OF ALDOSTERONE IN ALTERATIONS OF RENIN SECRETION ASSOCIATED WITH K+ DEFICIENCY AND K+ LOADING

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

Period covered in this report: 1 July 1972 - 30 June 1973

Investigators: Andrew Nowakowski, MD, Major, MC
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Reports Control Symbol MEDDH-288(R1)

There are many well defined stimuli to the renin-angiotensin-aldosterone systems in normal and injured soldiers. The control of aldosterone secretion in the anephric state and renin secretion in the aldosterone deficient state have yet to be elucidated. This study was designed to determine the role of potassium deficiency and potassium loading on renin production in the aldosterone deficient animal. Metabolic balance studies were performed on five adrenalectomized dogs placed on a zero potassium diet and then a diet high in potassium (150 mEq/day). No significant difference in plasma renin concentration was noted during potassium deficiency or potassium loading. These studies suggest that aldosterone secretion may indeed be required to induce hyperreninemia in potassium depletion and hyporeninemia in hyperkalemia.

Renin
Potassium
Aldosterone Deficiency

ROLE OF ALDOSTERONE IN ALTERATIONS OF RENIN SECRETION ASSOCIATED WITH K^+ DEFICIENCY AND K^+ LOADING

This study was designed to determine the role of aldosterone during K^+ depletion-induced hyperreninemia and K^+ loading hyporeninemia in the dog. Both phenomena have been recently observed in intact dogs and rats. Potassium deficiency is associated with an early natriuresis which may be the result of suppression of aldosterone secretion secondary to K^+ deficiency. This initial period of sodium depletion rather than K^+ deficiency directly may be the stimulus for the observed increases in plasma renin activity (Figure 1). Potassium loading might cause hypersecretion of aldosterone leading to sodium retention and suppression of renin release by this mechanism rather than by a direct action effect of K^+ loading.

METHODS

Five adrenalectomized dogs maintained on 0.75 mg dexamethasone and 0.5 mg desoxycorticosterone IM daily were studied on a zero K^+ diet over twenty days and then re-studied on a diet containing 150 mEq K^+ (high K^+). Metabolic balance studies were carried out for Na^+. K^+, and Cl^-, plasma renin activities (PRA) were sampled periodically.

RESULTS

Potassium depletion occurred in approximately ten days with a mean cumulative potassium balance of -97 mEq. A mean control serum K^+ was 4.4 mEq/L and fell to 3.1 mEq/L during K^+ depletion. The mean plasma renin concentration rose from 3.2 ng/ml/hr during the control period to 5.8 ng/ml/hr during the experimental period. (Figure 2) There was a mean cumulative sodium balance of +25 mEq.

Potassium loading was associated with marked increases in urinary potassium excretion with a slight or no change in serum potassium. Plasma renin concentrations did not show the expected decreases but increased or remained unchanged (Figure 3).

CONCLUSION

These studies do <u>not</u> show the changes in PRA that have been observed in the <u>intact</u> animal during K^+ deficiency of K^+ loading These studies <u>do</u> suggest that changes in aldosterone secretion may indeed be necessary for K^+ depletion induced hyperreninemia and K^+ loading induced hyporeninemia.

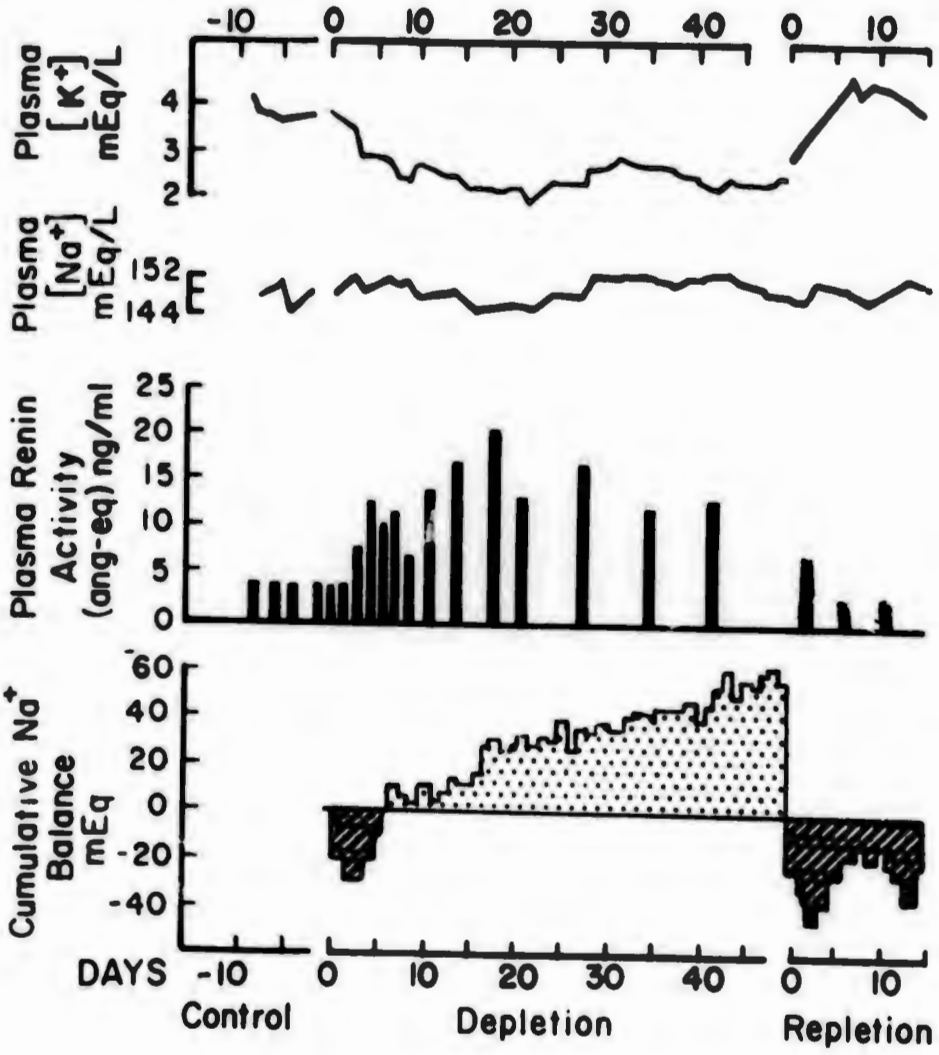

Figure 1: This balance diagram shows the changes in plasma renin concentration with respect with potassium depletion and potassium repletion in the normal dog.

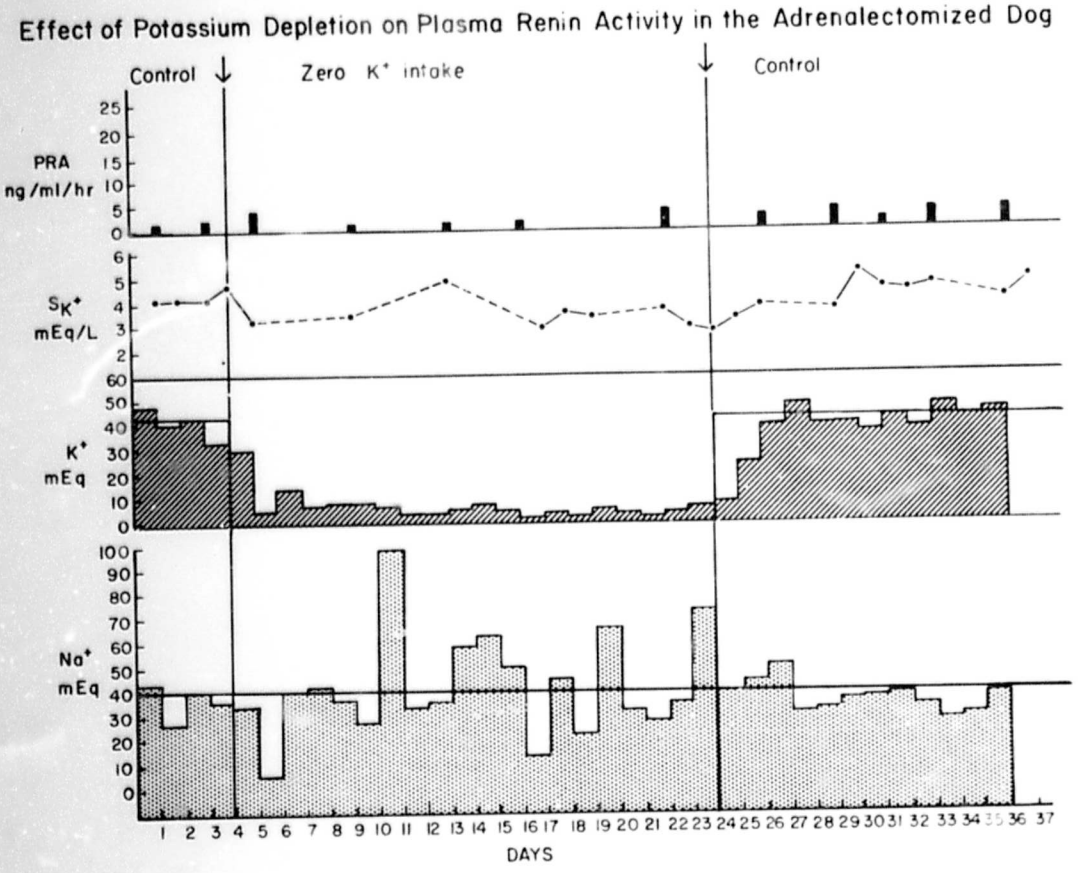

Figure 2: Balance data showing changes in plasma renin activity during the control periods and during the period of potassium depletion in the adrenalectomized dog.

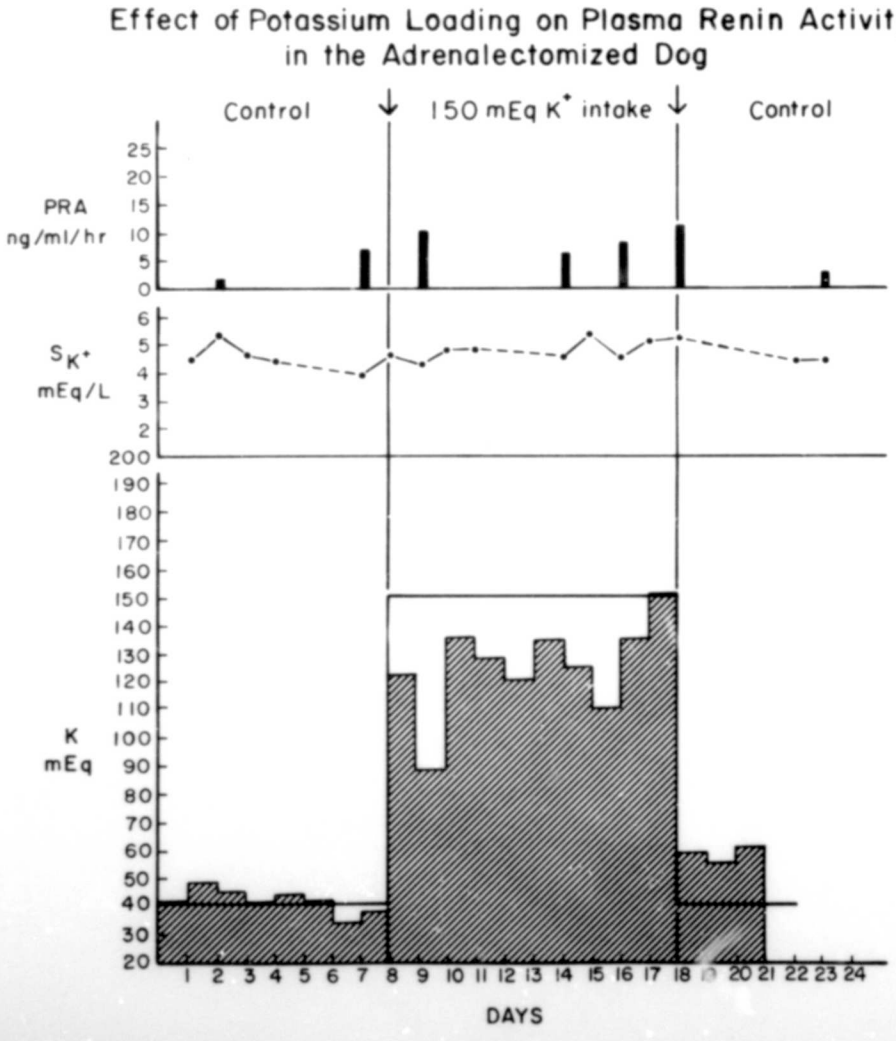

Figure 3: Balance data showing changes in plasma renin activity during control periods and during a period of potassium loading in the adrenalectomized dog.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA 0E 6958	73 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
	A. NEW	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	08	069
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)* (U) A Study of The Renin-Angiotensin-Aldosterone System 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 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 73	.4	15
	CURRENT 74	.4	10

19. RESPONSIBLE DOD ORGANIZATION
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ASSOCIATE INVESTIGATORS
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NAME: DA

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Renin; (U) Azotemia; (U) Hyperaldosteronism; (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) Patients with thermal injury have been noted to have a persistent mild azotemia despite the more vigorous fluid resuscitation regimens such as the Brooke Formula. This study was undertaken to evaluate the renin-angiotensin-aldosterone system in the severely burned soldier.

24. (U) Twenty-seven thermally injured patients ranging from 7-72 years of age with thermal injury ranging from 10 to 82% of body surface were evaluated. Serum BUN, creatinine, electrolytes, total protein and albumin, and urinary electrolytes were measured. The glomerular filtration rate, peripheral plasma renin concentrations, and plasma volumes were measured at various intervals after thermal injury.

25. (U) 72 07 - 73 06 These patients were noted to have uniform depression of the glomerular filtration rate with a mean of 71.3 ml/min plus or minus 6.8 after complete fluid resuscitation and restoration of plasma volume. Mean urinary potassium excretion was 70.4 mEq/liter plus or minus 5.6. These patients were also noted to have avid urinary sodium reabsorption. Plasma renin concentrations were markedly elevated immediately postburn and remained elevated despite restoration of plasma volume. The specific stimulus for the activation of the renin-angiotensin-aldosterone system in these patients at a time when plasma volume was shown to be normal remains unexplained. An attractive explanation for these findings is peripheral A-V shunting at the site of thermal injury. This would account for the great heat loss, the supernormal cardiac output with lowered peripheral resistance previously demonstrated in such patients at a time when plasma volume was normal.

*Available to contractors upon originator's approval.

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

ANNUAL PROGRESS REPORT

PROJECT NO. 3A161102B71P-08, BASIC RESEARCH IN SUPPORT OF MILITARY MEDICINE

REPORT TITLE: A STUDY OF THE RENIN ANGIOTENSIN ALDOSTERONE SYSTEM IN THE THERMALLY INJURED SOLDIER

HYPERRENINEMIA IN THE THERMALLY INJURED PATIENT

US ARMY INSTITUTE OF SURGICAL RESEARCH
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1 July 1972 - 30 June 1973

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Neil A. Kurtzman, MD, Lieutenant Colonel, MC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A161102B71P-08, BASIC RESEARCH IN SUPPORT OF MILITARY MEDICINE

REPORT TITLE: A STUDY OF THE RENIN AGNIOTENSIN ALDOSTERONE SYSTEM IN THE THERMALLY INJURED SOLDIER

HYPERRENINEMIA IN THE THERMALLY INJURED PATIENT

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

Investigators: Philip W. Rogers, MD, Major, MC
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Reports Control Symbol MEDDH-288(R1)

Plasma renin levels were determined in twenty-seven patients with thermal injury of from 10-82% of body surface who received fluid replacement according to the Brooke formula. All patients gained weight and usually developed edema in non-burned areas. Despite a positive salt balance the patients commonly elaborated a salt-free urine. The mean urinary potassium excretion was 70.4 $\pm$ 5.6 (SE) mEq/L and hypokalemia developed unless massive doses of KCl were administered. Serum albumin was depressed, mean 2.6 $\pm$ 0.2 (SE) gm %; GFR was depressed, 72 $\pm$ 7 ml/min; and filtration fraction was high. Renin values were uniformly elevated, 36.5 ng/ml/hr $\pm$ 9, (normal 0.5 $\pm$ 1.5) range 4-129, and are the highest values we have noted utilizing the radioimmunoassay technique. These values remained elevated for as long as 2 months, in the presence of normal measured blood volume. Blood pressure was invariably normal. Cardiac output was high, and pulmonary artery and wedge pressures were normal. The level of plasma renin concentration correlated only with the size of the burn. We postulate that shunting of blood in the area of burn injury results in significant and prolonged renal ischemia resulting in hyperreninemia. Hypoalbuminemia may also contribute slightly to the hyperreninemia.

Renin
Azotemia
Hyperaldosteronism

HYPERRENINEMIA IN THE THERMALLY INJURED PATIENT

The incidence of acute renal failure in the thermally injured patient has greatly diminished as the result of vigorous fluid resuscitation. Such fluid resuscitation prevents vascular collapse, renal ischemia, and the deposition of nephrotoxic substances in the renal tubules. Despite such resuscitation regimens, azotemia remains an unexplained problem in many burn patients.

Previous studies from the US Army Institute of Surgical Research (Pruitt BA, Jr., Mason AD, Jr and Moncrief JA: J. Trauma 11: 36, 1971)[1] have shown a precipitous decrease of cardiac output to 50% of normal during the first 6 hours of injury with a return to normal by 12 hours after injury and an increase to two times normal output after 36 hours with restoration of normal plasma volume. The total peripheral resistance is reciprocal to this curve, - high during the first 6 hours after injury, then continuing to drop over the next 54 hours to approximately 40% of the normal calculated value. Mean arterial blood pressure is slightly diminished or normal throughout most of the postburn course. RISA blood volume determinations reflect complete restoration to normal of the plasma volume at 54 hours. Further recent observations of the thermally injured patient are shown in Table I. These observations suggest marked secondary hyperaldosteronism although the specific stimulus remains unknown. The present study was undertaken to evaluate the role of the renin-angio-tensin system in such patients.

METHODS

Twenty-seven patients, 7 to 72 years of age, with thermal injury of from 10 to 82% of the total body surface were evaluated. These patients did not have heart disease, hypoxia, hyponatremia, or sepsis. They were studied at intervals during their postburn course ranging from the first to the sixty-second postburn day. Fluid resuscitation was administered according to the Brooke formula.

Serum BUN, creatinine, electrolytes, total protein and albumin, and urinary electrolytes were measured on all patients. Glomerular filtration rate was measured by the single injection technique using [^{125}I] Iothalamate. Peripheral plasma renin concentrations were

measured by the radioimmunoassay technique (Haber E, Koerner T, Page LB, Kliman B, and Purudde A: J Clin Endo and Metab 29: 1349, 1969)[2] [normal 1-3 ng/ml/hr]. Blood volume studies were performed by the ^{51} chromium-tagged red blood cell technique.

RESULTS

Shown in Figure 1 is the plasma renin activity on the ordinate and the number of days postburn on the abscissa. Of great interest was the consistent finding of greatly elevated plasma renin activity. The plasma renin activity is greatest during the immediate postburn period and progressively decreases during the convalescence. Figure 2 shows plasma renin concentrations occurring in three patients during their hospitalization. Again, the plasma renin activity progressively decreases as the burn wounds heal or are grafted and approaches the normal value at approximately thirty-days post burn.

Shown in Figure 3 is the relationship of the peripheral plasma renin activity to the percent of burned surface area during the first 14 days postburn. The plasma renin activity plotted on the ordinate ranges from 3 to 128 ng/ml/hr while the percent burn plotted on the abscissa ranges from 10 to 82%. Values obtained during the first seven postburn days are demonstrated by the closed circles, and those obtained from day seven through 14 are demonstrated by the closed triangles. There appears to be a definite relationship between the size of the burn and the magnitude of elevation of plasma renin activity.

The relationship of the plasma renin activity to sodium excretion in the burned patient is shown in Figure 4. Shown on the ordinate is the plasma renin activity, and on the abscissa urinary sodium concentration in mEq/L. The higher values for plasma renin activity are associated with the lower urinary sodium concentrations.

The following formula shows the relationship of these data.

$$\text{Renin (ng/ml/hr)} = -3.85 + .92\ (\%\ B) - .54\ (PBD)$$

certain of these factors are intercorrelated and multiple linear regression analysis shows that the statistically significant values for the best prediction of renin concentration are size of burn and time postburn.

Blood volume studies using ^{51} chromium-tagged red blood cells were done on 7 patients during the first through the twelfth post burn days are shown in Table II. Patients 1, 2 and 4 have measured

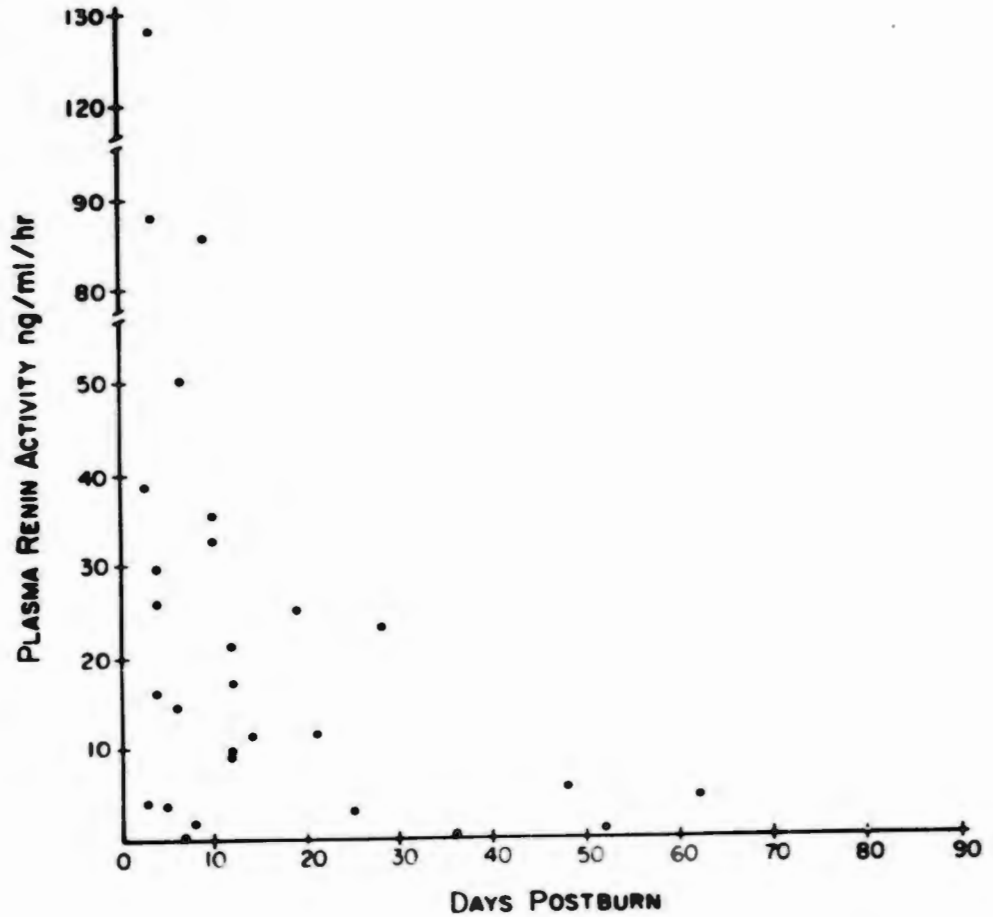

Figure 1: The plasma renin activity plotted on the ordinate is shown for each patient at various intervals after thermal injury as plotted on the abscissa.

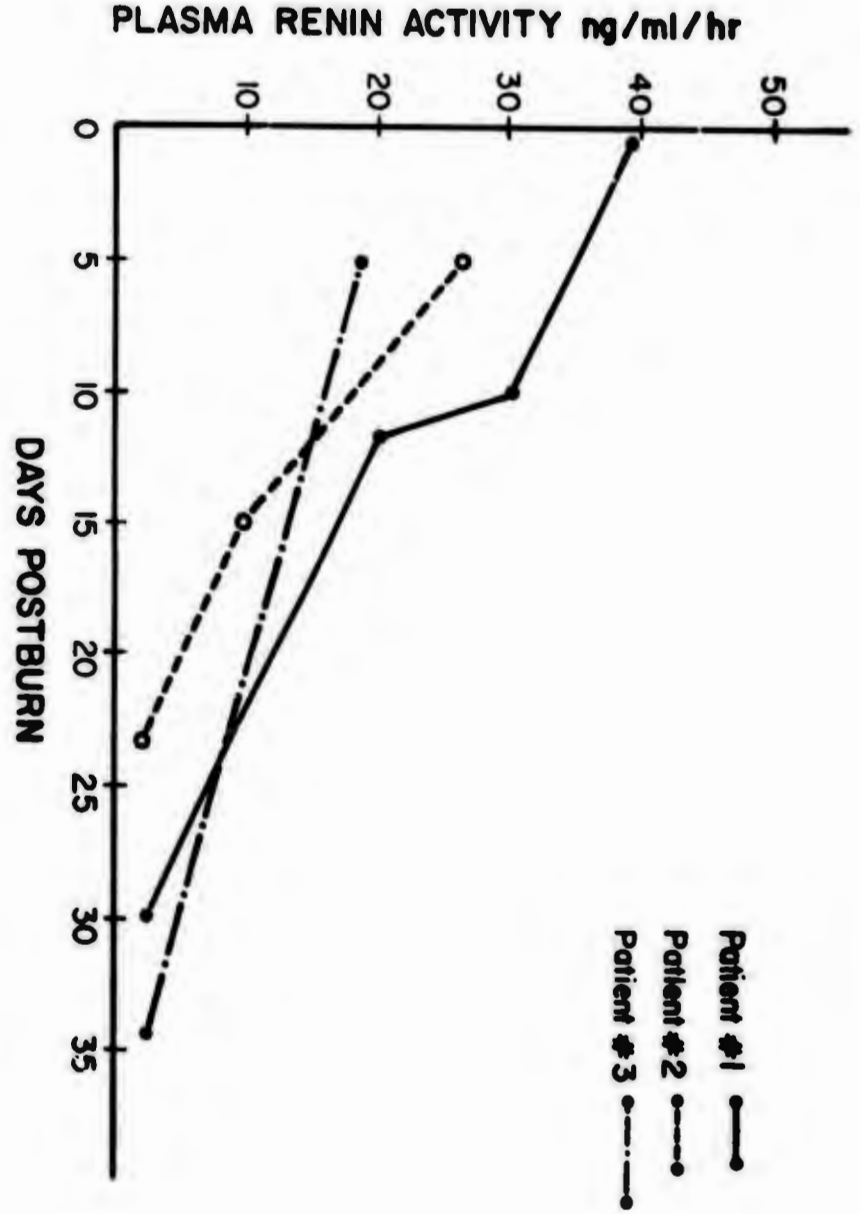

Figure 2: The plasma renin activity on three patients is shown at various intervals during their post-burn course.

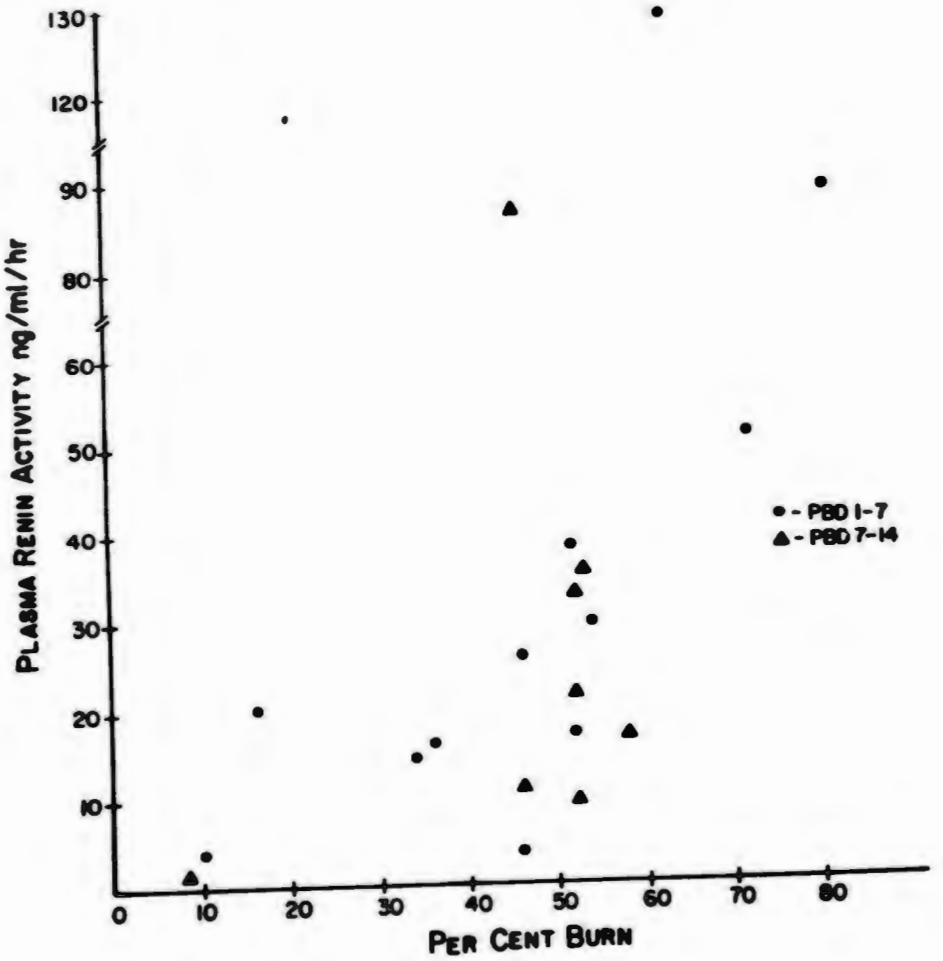

Figure 3: Plasma renin activity is plotted against percent burn for each patient from the first through seventh post burn day as depicted by the closed circles and from the seventh through the fourteenth post burn day as depicted by the closed triangles.

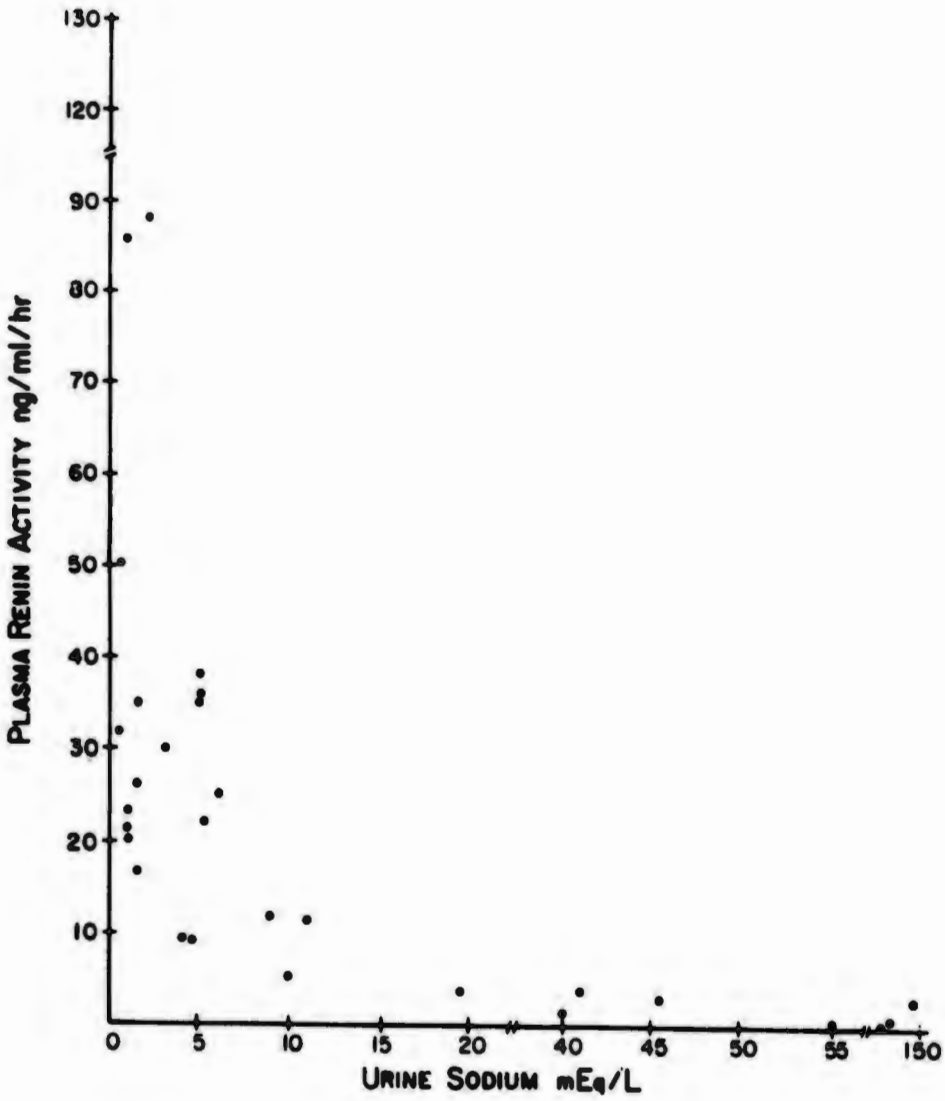

Figure 4: The relationship between the magnitude of plasma renin active is plotted against urinary sodium concentration in the same individuals.

TABLE I

OBSERVATIONS IN THERMALLY INJURED PATIENTS

1. Presence of edema in non-burned areas of the body.

2. Positive sodium balance with the elaboration of urine with a very low sodium content.

3. Uniform depression of glomerular filtration rate with a mean GFR by Glofil(R) technique of 71.3 ml/min ∓ 6.8 SD.

4. Increased loss of potassium in the urine with a mean potassium excretion of 70.4 mEq/L 5.6 SD.

TABLE II

CHROMIUM - 51 BLOOD VOLUME DETERMINATIONS

Patient No.	Days Postburn	% Burn	Pred. Blood Vol. Liters	Calc. Blood Vol. Liters	Plasma Vol. ml/Kg	RBC Vol. ml/Kg	Urine Na+ mEq/L	PRA ng/ml/hr	Serum Albumin Gm %
1	12	58	5.1	6.7	60.1	23.6	38	16.9	2.5
2	6	52	5.1	5.1	43.9	21.8	6	21.3	2.8
3	7	36.5	5.7	5.2	37	18	3	16.3	1.8
4	12	52	5.1	6.9	60.9	24	4	9.38	1.6
5	2	46	4.9	3.1	17.8	26.7	5	24.0	2.4
6	1	40	6.9	5.0	29.2	15.0	2	36.5	2.1
7	4	54	5.2	4.8	39.9	18.8	3	29.7	1.8

blood volumes greater than the predicted value. These values were obtained more than 60 hours postburn. In patient number 1, the urinary sodium concentration is adequate, and yet the plasma renin concentration remains markedly elevated. This disparity was doubtless due to $NaHCO_3$ excretion secondary to the topical application of sulfamylon, a potent carbonic anhydrase inhibitor. The urinary chloride concentration was 4 mEq/L. Patients 2 and 4 have low urinary sodium concentrations and also have elevated plasma renin concentrations. The other patients, who were studied earlier in their postburn course, have blood volumes less than the predicted value and greater plasma renin concentrations.

DISCUSSION

Renal underperfusion may affect sodium excretion in three possible ways (Pruitt, et al, 1971)[1] a fall in glomerular filtration rate, (Haber, et al, 1969)[2] increase in renin production and hence alderstrone secretion, and (Davies JWL: Clin Sci, 1967)[3] peripheral arterial-venous shunting at the site of thermal of injury. Sodium loss from the burn wound is difficult to measure; however, studies by Davies (Davies JWL, 1967) have shown that the maximal loss in large burns is approximately 150 mEq/day. Sodium depletion is unlikely since most patients have a positive sodium balance of 300-500 mEq. Also these patients have edema in nonburned areas. Marked contraction of the extracellular fluid space again is unlikely since normal blood and plasma volumes are associated with elevated renin concentrations.

The most attractive explanation for these findings is peripheral A-V shunting. This would account for the great heat loss, and the supernormal cardiac output with lowered peripheral resistance previously demonstrated in such patients at a time when plasma volume was normal. Peripheral A-V shunting would thus result in contraction of effective arterial blood volume resulting in decreased renal perfusion, increased renin production and secondary hyperaldosteronism. The amount of peripheral A-V shunting depends on the size of the burn wound and is reflected by the magnitude of elevation of plasma renin activity. As was previously mentioned, there is a direct correlation between the percent of surface area burned and the magnitude of elevation of the peripheral arterio-venous shunting in the burn wound might well be that stimulus. It is suggested that the clinical estimation of tissue perfusion and changes in urinary sodium excretion are the most reliable guides to perenteral fluid administration and the adequacy of an "effective" blood volume.

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3. Davies JWL: Some effects of a high sodium intake in burned patients. Clin Sci 32: 101-109, 1967.

PRESENTATIONS:

Rogers PW: A Study of the Renin Angiotensin System in the thermally injured patient. V International Congress of Nephrology, Mexico, City, Oct. 1972.

Rogers PW: Hyperreninemia in the Thermally Injured Patient. Am Burn Assn, Dallas, Texas, April 1973.

PUBLICATION

Nowakowski A, Nash DA, Jr., Rogers PW, Kurtzman NA: The Role of the Renin-Angiotensin-Aldosterone System in Adaption to Chronic Hypercapnia in Dogs. Clin Res 21: 702, Apr 1973.

Rogers PW and Kurtzman NA: A Study of the Renin-Angiotensin System in the Thermally Injured Patient, Fifth International Congress of Nephrology 118: 650, Oct 72.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0E 6970	73 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'S	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
	A. NEW	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 (Precede 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	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 73	.3	10
	CURRENT 74	.4	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) Ketamine; (U) Stress Ulcer; (U) Rats

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

23. (U) To 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) 72 12 - 73 06 In a study of over 200 rats, ketamine has been shown to significantly increase the incidence and severity of ulcers utilizing the restraint technique. This work is continuing.

*Available to contractors upon originator's approval.

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

ANNUAL PROGRESS REPORT

PROJECT NO. 3A162110A821-00, COMBAT SURGERY

REPORT TITLE: THE EFFECT OF KETAMINE ON STRESS INDUCED ULCERATIONS IN THE RAT

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

1 July 1972 - 30 June 1973

Investigators:

David H. Cheney, MD, Major, MC*
Stephen Slogoff, MD, Major, MC
Gary W. Allen, MD, Major, MC

From the Department of Anesthesiology, Brooke Army Medical Center, Fort Sam Houston, Texas 78234

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A162110A821-00, COMBAT SURGERY

REPORT TITLE: THE EFFECT OF KETAMINE ON STRESS INDUCED ULCERATIONS IN THE RAT

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

Period covered in this report: 1 July 1972 - 30 June 1973

Investigators: David H. Cheney, MD, Major, MC*
Stephen Slogoff, MD, Major, MC
Gary W. Allen, MD, Major, MC

Ketamine is an intravenous anesthetic which has, since its introduction in 1967, gradually become the principle agent used in anesthetic management of the thermally injured patient. Although the significant cardiovascular and central nervous system effects of the drug have been intensively investigated, no published data exists on the effects of this drug on the gastrointestinal system, particularly gastric function. Since gastroduodenal ulcers significantly influence the morbidity and mortality of the burn patient, we undertook to evaluate the effect of ketamine on the production of stress ulcers. The rat restraint model was chosen because it had been demonstrated to produce a lesion in the rat histologically compatible with human stress ulcers. The incidence and severity of these ulcers can be influenced by various drugs known to influence ulcer formation in the human.

The model was constructed to produce a 50% incidence of ulcers in the rat when only restraint, after a standard period of starvation, was utilized. Various drugs, in addition to ketamine, were added to the protocol in order to evaluate their influence.

Administration of ketamine has produced virtually 100% incidence of ulcers in the restrained rat, whereas the control restraint model has produced approximately a 40-50% incidence of ulcers. In an effort to delineate whether the effect of ketamine was drug induced or of psychogenic origin, halothane was administered to two groups of rats which were restrained, one of which had ketamine prior to restraint. Neither group formed any stress ulcers. Since halothane has known visceral vasodilating properties, in addition to its anesthetic ability, another group of rats had pretreatment with

a vasodilating dose of dibenzylene in the awake state before restraint with and without ketamine. In these groups, the incidence of ulcers in the control group was unchanged; however, the incidence of ulcers in the ketamine group reverted from the normal 100% to the same incidence as the control groups.

This work is incomplete and requires further evaluation of the mechanism of action of ketamine in producing stress ulcers in the restrained rat. This work is continuing.

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Ketamine
Stress ulcer
Rats

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0E 6967	73 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
	A. NEW	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 (Provide 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 73	.4	14
	CURRENT 74	.6	15

19. RESPONSIBLE DOD ORGANIZATION
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NAME: DA

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Provide 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. Provide 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 133 xenon 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 to treat this 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 double blinded and randomized in the administration of steroids as a means to evaluate them in the treatment of inhalation injury. The 133 xenon lung scan will be used to detect the presence or absence of an inhalation injury.

25. (U) 73 03 - 73 06 As of this report too few patients have been entered into the study and no conclusions can be made as of now.

*Available to contractors upon originator's approval

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ANNUAL PROGRESS REPORT

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
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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 1972 - 30 June 1973.

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Robert J. Lull, MD, Major, MC*

Reports Control Symbol MEDDH-288(R1)

All patients with burns of less than 48 hours duration and between the ages of 15 and 40 years will be included in this study. A double blind randomized study for the evaluation of systemically administered steroids in the treatment of inhalation injuries is the purpose of this study. The criteria for diagnosis of a respiratory burn is solely based on an abnormal ^{133}Xenon lung scan. The admission chest film must be normal. Once the diagnosis of inhalation injury has been made all patients will receive a standard treatment regimen. The steroid to be administered is Solu-Medrol, 30 mgs per kilogram per dose, intravenously in three divided doses for three days. The criteria for effectiveness of steroids in the management of inhalation injury will be comparative analysis of hospital course, chest x-rays and pulmonary function tests of control and treated groups.

Burns
Inhalation Injury
Steroids

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0E 6968	73 07 01	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8A. GRP'S INSTR'S	8B. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
	A. NEW	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	110
B. CONTRIBUTING				
C. CONTRIBUTING				

11. TITLE (Include 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:
b. NUMBER:
c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	PREVIOUS/CURRENT	a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
FISCAL YEAR	73	.5	16
	74	.5	11

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

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: Robert B Lindberg, PhD
NAME: Dwight E McEuen, SP4 DA

22. KEYWORDS (Provide KWOC with Security Classification Code): (U) Fibrinogen; (U) Fibrinogen-Fibrin; (U) Rats; (U) Degradation Products; (U) Thermal Injury

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 marked responses in fibrinogen, fibrin-degradation products, in vitro 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) 72 10 - 73 06 Few differences in the parameters studied have been found between the burned rat and the burned-lethally infected animal. In contrast to previous studies, platelet counts have been virtually identical in the two groups.

*Available to contractors upon originator's approval

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

ANNUAL PROGRESS REPORT

PROJECT NO. 3A162110A821-00, COMBAT SURGERY

REPORT TITLE: FIBRINOGEN-FIBRIN DEGRADATION PRODUCTS IN THE THERMALLY INJURED ANIMAL

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

1 July 1972 - 30 June 1973

Investigators:

Willard A. Andes, MD, Major, MC
Dwight D. McEuen, BS, SP4
Joseph P. Baron, BS, E2

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A162110A821-00, COMBAT SURGERY

REPORT TITLE: FIBRINOGEN-FIBRIN DEGRADATION PRODUCTS IN THE THERMALLY INJURED ANIMAL

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

Period covered in this report: 1 July 1972 - 30 June 1973

Investigators: Willard A. Andes, MD, Major, MC
Dwight D. McEuen, BS, SP4
Joseph P. Baron, BS, E2

The elevations in fibrinogen-fibrin degradation products in the thermally injured animal are well known (Meyers A: Arch. Surg. 105: 404, 1972)[1]. The significance and mechanism of these changes are unknown. Infection may impose an additional lethal insult to such an animal. This study compares such changes and various other indices following burns 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 subsequently seeded with pseudomonas, ISR strain 8283. The latter animals expired in all cases within six days postburn, while the survival of the rats which were only burned was nearly 100%. Methods of measuring the various hematologic indices were those previously used in this lab.

No statistically significant difference in the patterns of response have been noted in fibrinogen-fibrin degradation products, serial thrombin time, or hematocrits. In contrast to previous reports, (Newsome TW, Eurenius K: Surg. Gynecol & Obstet 136: 375, 1973) platelet counts appeared to be insignificantly affected by infection in the thermally injured rat.

Thermal injury
Burn wound
Fibrinogen-fibrin degradation products
Infection

FIBRINOGEN-FIBRIN DEGRADATION PRODUCTS IN THE THERMALLY INJURED ANIMAL

Previous studies in this Institute have shown dramatic changes and close similarities in the concentration of fibrinogen in the thermally injured rat and human. Other investigations have revealed rapid elevations in the titer of fibrin-fibrinogen degradation products following burns and with certain fungal infections. The significance and mechanisms of such changes are unknown.

Infection in the thermally injured animal frequently leads to the death of the animal regardless of the size of the burn. Infection may also cause changes in the fibrinogen concentration or split product titers and these may be helpful in diagnosing the presence of infection or the cause of abnormal bleeding.

Other hematologic indices in the burned animal may also show marked changes but, except in a few instances, have not been correlated with the presence of infection. A need for such studies became apparent when the presence of bone marrow suppression, disseminated intravascular coagulation, or fibrinolysis were seen or suspected in the thermally injured soldier. It seemed possible to compare such changes in a controlled situation by studying the laboratory rat model of burn wound infection(Teplitz C, Davis D, Mason AD, Moncrief JA: J Surg Res 4: 200-216, 1964)[3]. A group of Sprague-Dawley, Holtzman strain, white rats have been given a 30% dorsal scald burn. Immediately after burning approximately one-half of the animals were seeded with a virulent strain of pseudomonas (ISR strain 8283). Sacrifice under methoxyflurane anesthesia was performed by intracardiac puncture and exsanguination. Blood obtained by this technique was used for all subsequent studies. Groups of rats were sacrificed at one and four hours and one, two, three, six, and ten days postburn. Fibrinogen was determined by the turbidimetric technique. Fibrin degradation products were determined by the staphylococcal clumping assay. Platelet counts were performed by phase microscopy. Hematocrits were done by capillary tube methods. Thrombin times were performed on each sample immediately after drawing, at one-half hour, and at one hour intervals post-sacrifice with a standard thrombin solution.

There have been few statistically significant differences in the two groups of rats studied. Maximal fibrinogen values occurred on the second postburn day and averaged 300 mg % in the noninfected group and 600 mg % in the infected group. These values are significant at the 95 % level. The highest values for fibrin-fibrinogen degradation products

were measured by day three in the burned group and by day two in the infected animals. Both levels were about three to four times normal for such animals. Platelet counts have shown no statistically significant differences between the two groups. Peak values were obtained in the burned animals by day 9 and were about one and one-half times normal. Counts in the infected animals had their maximal elevation by day six and were slightly lower than in the burned animals. Thrombin time determinations, done serially as described, showed virtually identical curves in control, burned and infected animals. There was a moderate prolongation of the one hour time as compared with that done at zero time thus indicating measurable in vitro fibrinolytic activity. Hematocrits were not different during the first six days postburn. All animals died within six days-attesting to the virulence of the pseudomonas strain.

At this point in the study, it would appear that the added stress of infection insignificantly changes the pattern of the hematologic indices that have been studied. In particular, neither the elevated fibrinogen nor split product titers were different in the burned or burn-infected groups. This would seem to suggest that in the burned rat, disseminated intravascular coagulation is not an accompaniment of burn wound sepsis as it may occasionally be in its human counterpart. In-vitro fibrinolytic activity was not different in the two groups as evidenced by identical serial thrombin times curves. The similarity in platelet counts in this preliminary report was interesting because previous investigators have shown a marked suppression in infected animals of the usual postburn rise. However, since different investigators have used different strains of pseudomonas, studies are not, perhaps, completely comparable.

Work is continuing in this area to more fully define the pattern of change in the hematologic indices studied and the implications of such changes to the thermally injured or infected animal.

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1. Meyers A: Fibrin split products in the severly burned patient. Arch. Surg 105: 404-407, 1972.

2. Newsome TW, Eurenius K: Suppression of granulocyte and platelet production by pseudomonas burn wound infection. Surg Gynecol & Obstet 136: 375-379, 1973.

3. Teplitz C, Davis D, Mason AD, Moncrief JA: Pseudomonas burn wound sepsis. J Surg Res 4: 200-216, 1964.

PRESENTATIONS AND/OR PUBLICATIONS

NONE

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA 0E 6969	73 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
	A. NEW	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	111
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)* (U) The Effect of Epinephrine and Glucagon on the Rate of Heme Catabolism and Bilirubin Production in 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
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 73	.5	18
FISCAL YEAR CURRENT 74	.5	13

19. RESPONSIBLE DOD ORGANIZATION
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NAME: Douglas W. Wilmore, MAJ, MC DA

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Bilirubin; (U) Heme Catabolism; (U) Epinephrine; (U) Glucagon; (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) In order to better understand the metabolic, hepatic, and hematologic derangements of the burned soldier, the controls of bilirubin production from heme precursors are examined immediately after thermal injury and during convalescence. The rate of bilirubin synthesis is correlated with red blood cell survival, levels of erythroid and nonerythroid heme substrate, glucagon and epinephrine levels.

24. (U) The injured soldier is studied acutely postburn and then during convalescence. Routine hemolytic studies, BSP retention, bilirubin, glucagon and epinephrine levels, and liver function studies are obtained during each study period. The chromium-51 RBC survival time and the rate of endogenous carbon monoxide production are measured simultaneously as a reflection of the rate of hemoglobin destruction and bilirubin production. Convalescent patients and normal controls are studied similarly during infusions of epinephrine, glucagon, or amino acids. The effects of epinephrine, glucagon, hemolysis, ineffective erythropoiesis, and increased non-erythroid heme catabolism on the rate of bilirubin production are determined.

25. (U) 73 03 - 73 06 The rebreathing system to measure endogenous carbon monoxide production has been designed. When the CO chamber for the Van Slyke gas analyzer arrives, controls can be studied, the sensitivity of the system determined, and the study begun.

*Available to contractors upon originator's approval.

ANNUAL PROGRESS REPORT

PROJECT NO. 3A162110A821-00, COMBAT SURGERY

REPORT TITLE: THE EFFECT OF EPINEPHRINE AND GLUCAGON ON THE RATE OF HEME CATABOLISM AND BILIRUBIN PRODUCTION IN THE BURNED PATIENT

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

1 July 1972 - 30 June 1973

Investigators:

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Willard A. Andes, MD, Major, MC
Edwin W. Hander, 1LT, MSC
Robert J. Lull, MD, Major, MC*
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Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A162110A821-00, COMBAT SURGERY

REPORT TITLE: THE EFFECT OF EPINEPHRINE AND GLUCAGON ON THE RATE OF HEME CATABOLISM AND BILIRUBIN PRODUCTION IN THE BURNED PATIENT

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

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Investigators: Albert J. Czaja, MD, Major, MC
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Edwin W. Hander, 1LT, MSC
Robert J. Lull, MD, Major, MC*
Douglas W. Wilmore, MD, Major, MC

Reports Control Symbol MEDDH-288(R1)

Hyperbilirubinemia has been recognized in over 50% of the thermally injured soldiers admitted to this unit, and may be related to hemolysis, ineffective erythropoiesis, intrinsic liver dysfunction, or the increased metabolism of nonerythroid ferroporphyrins, such as hepatic enzymes and circulating myoglobin. Since the early postburn period is characterized by a hypercatabolic state with elevated plasma levels of glucagon and catecholamines, the hyperbilirubinemia may in part represent an over-utilization of heme substrate secondary to caloric deficiencies as well as glucagon and catecholamine stimulation of heme oxygenase activity.

In order to evaluate the regulation of bilirubin production from heme precursors, and to define the contributions of erythroid and nonerythroid heme substrate to the bilirubin pool of the burned soldier, we are comparing the rate of bilirubin production as measured by the rate of endogenous carbon monoxide production with the rate of hemoglobin destruction as measured by chromium-51 red blood cell survival in the immediate postburn and convalescent periods. By correlating changes in the rate of bilirubin synthesis with fluctuations in the levels of heme substrate, glucagon, and epinephrine, we will gain insight into the metabolic controls of bilirubin production and red blood cell survival after thermal injury.

We have accumulated no data as yet, but are in the midst of refining our techniques for the measurement of endogenous carbon monoxide production and serum glucagon levels.

Bilirubin
Heme catabolism
Epinephrine
Glucagon
Burned soldier

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PUBLICATIONS

1 July 1972 - 30 June 1973

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