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

Research Report

ANNUAL RESEARCH PROGRESS REPORT

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3A061102B71P-05,08,BASIC RES.IN SUPPORT OF MIL. MED.

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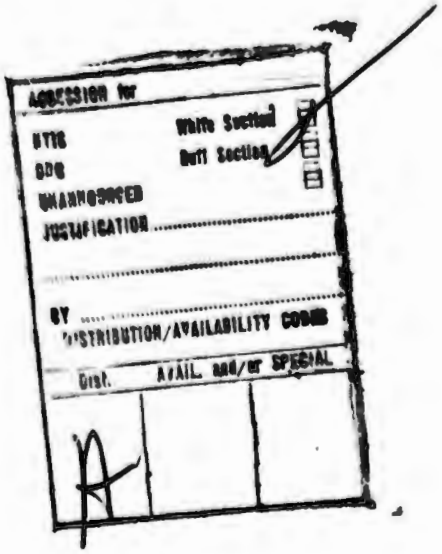

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This report documents the clinical and laboratory activities of the USAISR during fiscal 1972. These activities include patient care, clinical investigation and laboratory research in the areas of (1) burn injury, (2) acute renal failure and (3) general trauma. Special emphasis is placed on the clinical management of burned patients and on studies related to the 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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US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
FORT SAM HOUSTON, TEXAS 78234

31 July 1972

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Basil A. Pruitt Jr.

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

FOREWORD

The importance of the activities of this Institute to the mission of the Army should be apparent to all by virtue of the frequency of combat-generated burn injuries and the number of military burn patients admitted to our wards each year. Military relevance of research is assured by the involvement of all investigators in the clinical activities of the Institute. The multidisciplinary character of both clinical and laboratory studies enables the individual skills and talents of the professional staff members to be applied to problems of clinical significance and avoids the development of "techniques in search of a problem."

The breadth of the studies and activities reported herein reflects the applicability of knowledge of the pathophysiology and complications of thermal injury to the management of all trauma patients and the universality of the burn patient as a model of severe injury. These reports also exemplify the enthusiasm and excellence of our professional staff members, who not only deliver what is popularly called health care but advance such care through research. It is our present and past staff members who have established the national and international reputation of this Institute and who make this the most professionally rewarding assignment in the US Army Medical Corps.

As noted in prior reports, the key to our operation is patient care responsibility, which is widely recognized as critical to any successful medical research program. The importance of such responsibility cannot be overemphasized. The fact that this Institute's focal point is the care of severely burned soldiers and the fact that all research activities have at present an ultimate patient orientation and have in the past resulted in improved survival of the critically injured would seem to question the need for repetitive justification of investigative relevance by phraseology and "key words."

Basil A. Pruitt, Jr.

BASIL A. PRUITT, JR., M.D.
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) 71 01 - 71 12 During 1971, 268 patients were admitted to the institute; 98 patients were evacuated from Viet Nam by way of Japan. Attention to 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 the most 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. 3A061102B71R-01, RESEARCH IN BIOMEDICAL SCIENCES

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

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

1 January - 31 December 1971

Investigators:

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Paul Silverstein, MD, Major, MC
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The US Army Institute of Surgical Research during the calendar year 1971 admitted 268 patients with thermal injury. The Clinical Division places major emphasis on providing optimal clinical care and improving

existing modalities of therapy. New diagnostic and therapeutic regimens are outlined and activities of the Burn Center for 1971 summarized. As in the past, maintenance of an active educational program for the military and civilian medical community in the treatment of thermally injured patients has continued.

Evacuation of burn patients by our burn teams, sent either to Japan for Far East casualties or within the Continental United States, is our prime means of admission. Eighty-eight CONUS flights for 117 patients, a larger number of such flights and evacuated patients than for any previous year, were performed in 1971.

Pulmonary infection continued to be the most common complication of thermal injury and the most common cause of death. Overall mortality rates were essentially unchanged from the prior two years.

Thermal injury
Topical therapy
Autograft
Homograft
Heterograft
Resuscitation
Air Evacuation
Mortality

CLINICAL OPERATION, CENTER FOR TREATMENT OF BURNED SOLDIERS

The Clinical Division of the US Army Institute of Surgical Research continues to maintain as its primary objective the optimal clinical care of the thermally injured. A constant search for new and improved modalities of treatment of the thermally injured plays an important part in the research activities of this Division. With reduction in the total number of admissions for 1971 to 268 as compared to 325 in 1970, attention could more readily be placed upon some of these research endeavors which previously had taken a secondary role. Reduction in admissions was due primarily to a decrease in the number of patients received in transfer from Vietnam.

In 1971,as in the previous year, burn teams from the Institute were sent upon request to the US Army Hospital, Camp Zama, Japan, for preflight evaluation and in-flight care of thermally injured patients evacuated from the Far East. Thirteen such flights from Yokota Air Force Base, Japan, to Kelly Air Force Base, Texas, were made in 1971, and 93 patients were escorted to the Burn Center, compared to 20 flights in 1970 for 172 patients.

In addition, there were 86 emergency air evacuation missions within the Continental United States, as well as one to the Panama Canal Zone and one to Guantanamo Bay, Cuba. Following preflight evaluation and treatment, 117 patients were safely returned to the Institute for definitive care. This is the largest number of such missions, as well as the most patients so evacuated, for any year in the history of the Institute.

CLINICAL MANAGEMENT

For detailed descriptions of the management of the thermally injured as practiced at this Burn Center, the reader is referred to previous annual reports and numerous scientific publications from this Institute. The following paragraphs will deal primarily with new and current methods of clinical therapy.

Continuing evaluation of the Brooke formula has brought about further changes in early resuscitation. In the Annual Report for 1970, mention was made that colloid and electrolyte solutions are now calculated on the basis of the measured total body surface rather than up to a maximum of 50% total body surface burn as the formula was originally written. For the past six months, in the adult 2,000 cc of free water for insensible loss has been eliminated and the patient is given 2 cc/kg/% burn of electrolyte solution in the first 24 hours. The patients are isotonically resuscitated, the sodium of administered fluids being 142 mEq/L and chloride approximately

130 mEq/L. Since the fluid loss into injured tissues is isotonic, it appears appropriate to administer what is lost. Plasma is not given within the first 24 hours, since it is not preferentially retained within the circulation. Colloid does have a greater volume effect than crystalloid in the second 24 hours. With capabilities of blood volume determination, plasma is given in the second 24 hours to make up for the measured blood volume deficit. After 48 hours, fluid administration is primarily 5% dextrose and water to maintain serum isotonicity and supplementary potassium to maintain normal serum values. In children, because of their greater insensible loss per body surface area, insensible water loss is calculated according to the Brooke formula for children. Realizing that these formulas are only guides, adequacy of resuscitation is monitored by following vital signs and urinary output, with rate of administration manipulated accordingly. In most adults, by giving isotonic fluids it has not been necessary to administer the 2,000 cc of insensible water, and there has been no significant increase in the serum sodium levels. Urinary sodium with the classic Brooke formula has been 3-5 mEq/L for the first 5 to 6 days, but with the modified formula it has been 40-60 mEq/L by the second to third day postburn. Weight gain appears to be less with the newer resuscitation procedure.

In an attempt to decrease the extensive weight loss and nitrogen depletion which characterizes the posttraumatic metabolic response following severe thermal injury, vigorous nutritional support using combined enteral-parenteral feedings has been in effect since 1969. However, the use of hypertonic nutritional solutions is not without hazard. Complications of central venous catheterization, associated sepsis and nonketotic hyperglycemic coma have been reported. Intralipid, a soybean fat emulsion which is isotonic and may be administered by peripheral vein, thus eliminating some of the hazards associated with central feedings, has been evaluated in 12 hypermetabolic thermally injured patients and 15 healed, convalescing controls. Single 500 ml units of 10% soybean emulsion which provided one calorie/cc were administered over a 4-hour period to these patients. No significant thermogenic response to the fat emulsion occurred in either group. Vital signs, CBC and liver function studies remained unchanged. Xenon perfusion-diffusion studies, blood gas studies and pulmonary diffusion capacity studies with carbon monoxide rebreathing tests were all normal. Fat clearance curves demonstrated an accelerated plasma disappearance of the emulsion in the acutely burned patients. This soybean emulsion appears to be a promising adjunct to alimentation of the severely burned patient with less hazard than other hypertonic nutritional solutions.

Pulmonary complications with respiratory failure continue to be the leading cause of death in the majority of the patients who expire. Inhalation injury, a complication of thermal injury,

predisposes the respiratory tract to infection and causes a significant increase in the mortality rate associated with thermal injury. Early diagnosis, which is often difficult, may permit early vigorous therapy and thereby minimize disability and fatal septic pulmonary complications. Utilizing ^{133}Xenon perfusion and ventilation tests, 50 consecutive patients with thermal injury due to flame admitted to our Burn Center within 48 hours of injury were studied. There were 15 abnormal scans and 35 normal scans. No false positive or negative diagnoses were made in this series, but both are possible. This incidence of 30% of the patients studied is 10 times higher than previously appreciated following flame burns using the usual diagnostic criteria. This increased incidence represents patients in whom the diagnosis would have been missed for lack of definite clinical signs. The lung scan can be performed in acutely ill burn patients without active patient cooperation. Utilizing the ^{133}Xenon scan as a diagnostic tool, further studies will be carried out to evaluate modes of therapy and the efficacy of drugs such as steroids.

Our pulmonary laboratory has continued to study pulmonary pathophysiologic changes following thermal injury. Multiple indices of lung function have been serially determined in 35 patients during the early postburn period. Often the first clinical suggestion of pulmonary difficulty is the dramatic development of tachypnea and hyperpnea on the third to fifth postburn day. This precedes by several days abnormal findings on chest x-ray, physical examination, and in blood gases. Increases in minute ventilation were seen from the third or fourth postburn day until approximately the 12th postburn day but were not significant before or after this period. Burn size seemed related to the increase in ventilation, since all patients with burns of 40% or greater showed this phenomenon, while only one in the group with less than 40% total body surface burns showed increased minute ventilation. All patients were treated with Sulfamylon topically. No meaningful correlation could be made between the minute ventilatory changes and the serum levels of Sulfamylon and paracarboxybenzene sulfonamide. Static lung compliance and dynamic lung compliance were reduced in both groups of patients, more severely in those with burns exceeding 40% of the total body surface. The abnormal compliance did not correlate with the marked change in minute ventilation. At present, the pulmonary studies suggest that the observed hyperpnea was due to an extrapulmonary cause.

To date, we have had more than 3 years' experience with the use of split-thickness porcine cutaneous xenograft as a substitute for fresh human allograft. Human allograft, if available, continues to be our first choice as a physiologic dressing and in preparation of the burn wound for autografting. Porcine xenograft has proved to be a satisfactory second choice. Since there has been question as to the sterility of the fresh porcine xenograft, lyophilized porcine

xenograft is now being evaluated. This material has an indefinite storage life, is sterile and is easily reconstituted by soaking in normal saline for 40 to 90 minutes. Studies thus far would indicate that it is a satisfactory biologic dressing. While porcine xenograft enhances healing of second-degree burns, if used to promote healing of donor sites, fragments of the xenograft become incorporated into the healing wound and act as foreign bodies, thereby inhibiting healing.

In the past year, isoprene, a synthetic material, has been used to fashion approximately 80% of our splinting devices. This material offers the advantages of being easy to handle, permitting rapid splint manufacture and easy adjustment. It also allows for instant splinting in the operating room and versatility in fabricating devices to suit individual patient needs. Standardized fiberglass splints have remained as "back-up" splints. Although these splints allow repeat sterilization, they are time-consuming and difficult to make, and the material is not readily available.

CLINICAL RESEARCH PROJECTS

In addition to the investigative studies mentioned in the preceding section, additional ongoing research activities include evaluation of the administration of growth hormone on the catabolic phase of thermal injury and the effectiveness of nutritional regimens, etiology and control of hypertrophic scarring, use of an intermittent compression unit to decrease postburn edema, etiology and prevention of thrombophlebitis, metabolic response of hypermetabolic burn patients to cooling, glucose metabolism in the burn patient, coagulation abnormalities in thermally injured patients with special reference to disseminated intravascular coagulation, and erythrocyte and plasma phospholipids in the burn patient.

EDUCATION

The education of the military and civilian community in current treatment of the thermally injured is an important mission of this Institute. It is accomplished through on-the-job training for surgical residents, physicians and paramedical personnel as well as numerous publications and lectures by the clinical staff each year.

During 1971, 4 surgical residents from Brooke General Hospital, 3 from Wilford Hall USAF Hospital, and 2 from civilian institutions participated in the clinical activities of the Institute for periods of from one to 6 months as part of their surgical training. A Public Health Service physician from the Indian Medical Center in Phoenix, Arizona, participated in a 3-month training period, and 2 medical students from the University of Texas Medical School at San Antonio completed 2-month assignments with the Institute. One physician from Israel spent a 4-month training period at the Institute, and one each from Norway and Australia spent a week observing the

the Burn Center's treatment regimen. Two clinical clerks (Army sponsored medical students) received practical experience on the clinical wards for 3-4 months. Finally, approximately 275 civilian and 125 military physicians, students and paramedical personnel visited the Institute in 1971. Twenty-six foreign visitors from Australia, England, Finland, Holland, Honduras, India, Indonesia, Israel, Japan, Laos, Norway, Poland, Saudi Arabia, Sweden and Switzerland received extensive briefings concerning the development and maintenance of burn units in general and the Institute of Surgical Research in particular.

More than 100 scientific presentations dealing with thermal injury were presented by Division representatives at local, state, regional and national meetings, as listed at the end of this section of the Annual Report.

STATISTICAL RESUME

During the calendar year 1971, 268 thermally injured patients were admitted to the US Army Institute of Surgical Research, 98 (36.6%) of whom were evacuated from the Republic of Vietnam. There were 301 dispositions during this period, and all subsequent data will be based upon these dispositions.

The patients ranged in age from 11 months to 76 years and included 259 males and 42 females. The average age of the patients was 25 years, with an average total burn of 30.9% (13.6% third-degree burn). The average burn index was 21.9%. Of the 301 dispositions, 218 had third-degree burn, or 72.4%. Thirty-six patients were under 15 years of age, with an average age of 6.2 years. The average total burn in this pediatric age group was 46%, with 36.4% third-degree (burn index 41). Thirty of the 36 patients had some third-degree burn, or 83.3%.

There were 68 deaths among the 301 dispositions, resulting in an overall mortality of 22.6% as compared to last year's 21.8%. The average age of these patients was 24.7 years, with an average total burn of 60.8% (38% third degree). The average burn index was 48.8%. Of the 68 patients, 3 (4.4%) were evacuated from the Republic of Vietnam, as compared to 65 patients from the Continental United States, which reflects the preselection of patients with thermal injuries from the Far East as a result of the chain of evacuation and the general policy of this Institute to accept only the more severe burn cases within the Continental United States. The average post-burn day of death was 14.9 days. Autopsies were performed on 61 patients, for an 89.7% postmortem examination rate.

Table I depicts the source of admission of patients during 1971. The major area, as in the past 4 years, was the Republic of Vietnam,

Table 1. Source of Admission, 1971

Area	A	AD	AF	AFD	N	ND	VAB	Other	TOTAL
1st Army	4	4	1	0	0	1	0	1	11
3rd Army	7	3	1	3	1	3	2	8	28
4th-5th Army	19	13	2	5	1	0	19	22	81
6th Army	3	1	8	5	2	1	1	4	25
Germany	3	1	0	0	0	0	0	0	4
Viet Nam	111	0	2	0	12	0	0	4	129
Alaska	7	0	4	0	0	0	0	0	11
Thailand	0	0	1	0	0	0	0	0	1
Canal Zone	0	0	0	0	0	0	0	2	2
Japan	0	0	0	0	0	0	1	0	1
Cuba	0	0	0	0	6	0	0	0	6
Korea	1	0	0	0	0	0	0	0	1
Mexico	0	0	0	1	0	0	0	0	1
	155	22	19	14	22	5	23	41	301

A - Army
AF - Air Force
N - Navy & Marine Corps
D - Dependent
VAB - Veterans Administration Beneficiary

Other: Civilian Emergency (15)
Designee of Secretary of Army (21)
US Public Health Service Beneficiary (5)

with a total of 129 (42.8%) patients admitted; active duty or retired military accounted for 196 admissions; active duty or retired military dependents 41; Beneficiaries of the Veterans Administration 23; civilian emergencies 15; Designees of the Secretary of the Army 21; and US Public Health Service Beneficiaries 5.

The mode of injury in patients evacuated from the Far East for the years 1965-1971 is summarized in Table 2. Striking is the marked rise in injuries due to gasoline explosions for 1971 (33%) as compared to previous years. The number of injuries due to white phosphorus increased from 8 to 1970 to 16 in 1971, a larger number than in preceding years. Aircraft accidents, primarily helicopter, continued to be one of the major modes of injury but were reduced to 23% as compared to a high of 30% in 1970.

Table 3 illustrates the effect of age and total body surface burn on mortality. Increased mortality in the very young and very old is readily seen, with 3 survivors out of 13 patients less than 4 years of age and 2 survivors in patients greater than 60 years of age. Mortality of burns greater than 60% remains high despite any treatment modality, being 87.5% or more.

The mortality rates in increments of 10% total body surface burn for 1968-1971 are tabulated in Table 4. Comparison with 1968 reveals a marked increase in the mortality for 30-60% total body burn group for 1969, 1970 and 1971. Even more striking is the increase in mortality at 40-50% total body surface burn in 1971 to 41.2% as compared to 27.7% in 1970 and 12.8% in 1968.

The survival and mortality data for patients with greater than 30% burns (1955-1971) are presented in Table 5. No striking change is noted in 1971.

Table 6 compares the mortality of the years 1962-1963, the pre-Sulfamylon era, to 1964-1971, when virtually all patients admitted to the Institute were treated with topical Sulfamylon cream. Improvement in mortality is seen in burns with 60% or less of the total body surface involved, and essentially no change for those greater than 60%.

The average required total hospitalization for all patients was 50 days. The average postburn day of admission to the Institute was 9 days.

During the year, 2,998 operations were performed on 266 patients, an average of 10 per patient. There were 521 procedures for 197 patients which required general anesthesia, an average of 1.7 per patient. A total of 2,485 procedures were done on the ward which required no general anesthesia. One hundred seventy patients required 414 autografting procedures, with an average of 1.4 per patient.

Table 2. Mode of injury - Patients from Viet Nam, 1965-1971

Cause of injury	1965	1966	1967	1968	1969	1970	1971
Gasoline explosion	4 (21%)	20 (14%)	38 (15%)	48 (17%)	36 (23%)	44 (24%)	42 (33%)
Burning brush	4 (21%)	19 (13%)	7	11	4	4	1
White phosphorus	3 (16%)	7	13	13	9	8	16 (12%)
Aircraft accident	2	24 (16%)	34 (14%)	48 (17%)	33 (21%)	55 (30%)	30 (23%)
Vehicle over land mine	2	11	47 (19%)	22	21 (14%)	16	10
Napalm	1	11	1	15	2	1	1
Burning trash	1	9	29 (12%)	19	0	9	3
Booby trap	1	5	18	7	1	6	1
Mortar round	0	24 (16%)	24 (10%)	19	10	7	5
Powder charge	0	5	13	17	13	15	6
Rocket/recoilless rifle-tank	0	1	19	38 (13%)	21 (14%)	12	9
Electrical	-	-	-	4	1	1	0
Miscellaneous	1	11	8	23	5	6	5
TOTAL	19	147	251	284	156	184	129

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	0	0	1	0	0	0	0	0	0	0	1	0	0
1-2	1	0	0	0	0	3 (3)	0	0	0	0	4	3	75.0
2-3	1	0	0	2 (2)	0	0	1 (1)	0	1 (1)	1 (1)	6	5	83.3
3-4	0	0	0	0	0	0	1 (1)	0	0	1 (1)	2	2	100.0
4-5	1	0	1 (1)	1	0	0	0	0	1 (1)	0	4	2	50.0
5-10	0	2	0	0	3 (2)	1 (1)	2 (2)	2 (2)	0	0	10	7	70.0
10-15	1	2	2	0	1 (1)	1 (1)	1	0	1 (1)	0	9	3	33.3
15-20	9	8	5	2	5 (2)	1 (1)	1 (1)	1 (1)	2 (2)	1 (1)	35	8	22.9
20-30	27	40	39 (1)	25	10 (2)	8 (2)	3 (3)	4 (3)	1 (1)	1 (1)	158	13	8.2
30-40	3	7	2	1	8 (4)	4 (2)	2 (2)	0	3 (2)	1 (1)	31	11	35.5
40-50	5	1	3	4 (2)	4 (1)	1 (1)	1 (1)	0	1 (1)	0	20	6	30.0
50-60	0	5	4	1	2 (1)	1 (1)	0	1 (1)	0	1 (1)	15	4	26.7
60-70	0	0	0	2 (2)	1 (1)	0	0	0	0	0	3	3	100.0
70-80	2	0	0	1 (1)	0	0	0	0	0	0	3	1	33.3
Total	58	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	60.0	91.7	87.5	90.0	100.0			22.6

Note: Deaths shown in parentheses.

Table 4. Per Cent Body Surface Involvement and Mortality, 1968 - 1971

% Burn	0-10	10-20	20-30	30-40	40-50	50-60	60-70	70-80	80-90	90-100	Total
					(1968)						
No. Burned	71	68	84	66	47	35	19	9	2	0	401
Deaths	0	0	3	6	6	6	8	8	1	0	38
% Mortality	0	0	3.6	9.1	12.8	17.1	42.1	88.9	50	0	9.5
					(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

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Table 5. Per Cent Burn Versus Survival, 1955-1971

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

Table 6. Comparison of Burn Mortality Rates, 1962-1963 and 1964-1971

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
1964-71	1259	24	1.9	363	48	13.2	285	85	29.8	164	71	43.3	260	211	81.2

In addition, 1,418 homograft and 961 porcine xenograft procedures were performed on 190 and 159 patients respectively. Cadaver homograft was harvested aseptically from 59 donor bodies.

Escharotomies were required in 66 patients, an incidence of 22%. Some type of amputation involving an extremity was necessary in 31 patients. Disarticulation of the phalanges of the hand was the most commonly performed amputation (19). Because of extensive soft tissue injury, thermal injury, electrical injury or invasive fungal disease, 16 major amputations were required.

Tracheostomies were performed in 36 patients (12%) with respiratory failure or upper airway obstruction. This is approximately half the number performed in 1970, reflecting more prompt attention to vigorous pulmonary therapy at the first indication of a pulmonary problem and the use of endotracheal tubes for short periods (24-72 hours) to tide the patient over.

Pulmonary infection continues to be the most frequent complication following thermal injury. Pneumonia was diagnosed in 79 patients, and 29 patients sustained an inhalation injury along with their thermal injury. Pneumothorax developed in 17 patients, usually as a complication of suppurative pneumonitis with abscess formation.

Of 1,451 blood cultures drawn in 192 patients, 237 yielded microorganisms in 92 patients. Intravenous catheters were placed through cutdowns in 195 patients, while percutaneously placed venous catheters were utilized in the majority of instances for administration of intravenous fluids. Forty-eight patients had exploration of previous cutdown sites or veins excised for suspected thrombophlebitis which was subsequently proved by clinical, bacteriological and histological criteria in 22 patients, an overall incidence of 7.3% (approximately the same as in 1970).

Topical Sulfamylon acetate was applied to the burn wound of 299 patients, with 25 patients showing various degrees of hypersensitivity, an incidence of 8.3%. Eighteen patients received silver nitrate to the burn wound, most of these because of hypersensitivity to Sulfamylon which did not respond to antihistamines.

Associated injuries were noted in 106 patients (35.2%). Thirty-four ears in 25 patients developed chondritis which necessitated operative excision of infected cartilage. Gastrointestinal bleeding occurred in 37 patients (12.3%), and Curling's ulcer was diagnosed in 20. The bleeding in only one of these ulcer patients required operative intervention. Associated fractures were noted in 28 patients. Six patients developed myositis ossificans of the elbow, five requiring surgical removal of the abnormal bone for restoration of function. Pancreatitis developed in 18 patients, an incidence of 6%.

In order to eliminate any bias resulting from preselection of patients evacuated from the Republic of Vietnam, as a result of delay in admission to the Burn Center, during which time the more severely injured patients with significant complications have expired, mortality rates of patients admitted from the Continental United States are considered apart from the patients from the Far East in Table 7. Estimated mortality is derived from probit analysis of survival data at this Institute between 1964-1967. Predicted mortality from 1968 was 17.4% as compared to 21.4%. Actual mortality for CONUS patients is improved over 1970, being 29.9% as compared to 40.2%. Mortality for 1971 appears to be reverting back to that of 1969 when actual mortality was 42% and predicted 32%. Mortality of Vietnam patients remains unchanged.

Pneumonia played a significant role in the demise of 50% of the 68 deaths in 1971 (Table 8). Herpes simplex was cultured from the tracheobronchial secretions in 3 of these and Providencia stuartii in 12. Pulmonary emboli contributed to a fatal outcome in 8 patients as did inhalation injury in 9. Bacterial burn wound invasion in 7 patients, mycotic burn wound invasion in 7 patients, mixed bacteria and mycotic burn wound invasion in 7, and Pseudomonas burn wound sepsis in 3 patients were contributing causes of death. It is interesting that 5 patients had disseminated intravascular coagulation which lead to a fatal outcome and 3 patients expired with pancreatitis as one of the primary causes of death.

SUMMARY

From 1 January to 31 December 1971, 268 patients were admitted to the US Army Institute of Surgical Research, and there were dispositions on 301 patients. Reassessment of fluid resuscitation in the first 48 hours postburn, with modification of the Brooke formula, increased caloric support through peripheral veins with the soybean fat emulsion, Intralipid, increased diagnostic accuracy of inhalation injury with ^{133}Xenon scanning, and study of pulmonary pathophysiologic changes following thermal injury have been some of the clinical studies which have allowed for improved management of the thermally injured patient.

While the number of patients evacuated from the Republic of Vietnam by our flight teams has decreased, emergency air evacuation missions within the Continental United States increased to 88 this year. One hundred seventeen patients were admitted by this means to the Institute, more than in any previous year.

The total care of the burn patient must be a team effort. In no other place has this been more exemplified than at the US Army Institute of Surgical Research by the extraordinary dedication, cooperation, and enthusiasm of the physicians, nurses, medical specialists, paramedical and secretarial staff assigned here.

Table 7. Body Surface Involvement, Mortality, and Predicted Mortality of Patients (Age 15-45 Yrs.) from CONUS and Viet Nam

Per Cent Burn	1969						1970						1971					
	CONUS Patients			Viet Nam Patients			CONUS Patients			Viet Nam Patients			CONUS Patients			Viet Nam Patients		
	No.	Deaths	Predicted Deaths	No.	Deaths	Predicted Deaths	No.	Deaths	Predicted Deaths	No.	Deaths	Predicted Deaths	No.	Deaths	Predicted Deaths	No.	Deaths	Predicted Deaths
0-10	6	0	0	11	0	0	11	0	0	26	0	0	15	0	0	27	0	0
10-20	13	0	0	24	1	0	9	0	0	37	0	0	22	0	0	34	0	0
20-30	9	0	0	37	0	0	10	2	0	47	1	0	15	1	0	32	0	0
30-40	10	3	0.3	37	2	1.9	14	3	0.4	38	2	1.0	10	1	0.3	20	0	0.5
40-50	9	4	1.4	40	4	6.2	14	5	2.2	26	4	4.1	15	7	2.3	12	2	1.9
50-60	8	5	3.2	13	4	5.2	8	7	3.2	6	1	2.4	9	5	3.6	4	1	1.6
60-70	8	6	5.1	7	1	4.5	4	4	2.6	3	3	1.9	7	6	4.5	0	0	-
70-80	10	8	8.1	1	1	0.8	8	8	6.5	0	0	-	5	4	4.1	0	0	-
80-90	4	4	3.7	0	0	-	3	3	2.7	0	0	-	6	5	5.5	0	0	-
90-100	4	4	3.8	0	0	-	1	1	1.0	0	0	-	3	3	2.9	0	0	-
TOTAL	81	34	25.6	170	13	17.7	82	33	18.6	183	11	9.4	107	32	23	129	3	4
Mortality		42%			7.6%			40.2%			6.0%			29.9%			2.3%	
Predicted		32%			10.4%			22.7%			5.1%			21.4%			3.1%	

Table 8. Causes of Death, 1971

Patient	Age	Sex	% Burn Total	% Burn 3°	PBB Death	Cause of Death
1	55	M	100	100	0	*Inhalation injury
2	2	F	99	93	1	Inhalation injury
3	3	M	94½	87½	20	Septicemia (Klebsiella); mycotic (Candida, Cephalosporium) and bacterial (Pseudomonas) invasion of burn wound; bronchopneumonia (Klebsiella)
4	30	M	93½	93½	12	Bilateral interstitial pneumonia (Proteus, Klebsiella, Staph.), with extensive broncho-alveolar exfoliation and hyaline membrane formation; bacterial (mixed) and fungal (mixed) invasion of burn wound
5	21	M	91	91	3	Septicemia (Aerobacter, Proteus); pulmonary hemorrhage; burn shock
6	19	M	90	68	1	Inhalation injury; shock, irreversible
7	4	F	89½	89½	2	Inhalation injury; shock, irreversible
8	30	M	87	0	7	Septicemia (Providence, E. coli); pulmonary hemorrhage, massive, secondary to aspiration; mycotic (Fusarium) and bacterial (Staph, Providence) burn wound infection
9	2	F	86	86	1	Inhalation injury
10	17	M	86	79	6	Septicemia shock (E. coli, Providence); mixed mycotic invasive burn wound infection
11	31	M	86	42	5	Diffuse intravascular coagulation; pulmonary edema and congestion
12	16	F	86	40	9	Aspiration pneumonia; interstitial pneumonitis with hyaline membranes and edema; massive, bilateral pleural effusions
13	40	M	84	14	4	Pulmonary edema
14	20	M	82	42	3	Shock, irreversible

* Autopsy not performed

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Table 8. Causes of Death, 1971

Patient	Age	Sex	% Burn Total	% Burn 3°	POD Death	Cause of Death
15	11	F	80½	80½	11	Inhalation injury; interstitial pneumonitis with hyaline membranes and edema; bronchopneumonia (Providence, Klebsiella, E. coli); mycotic (Absidia sp.) invasion of burn wound
16	28	M	78	41	5	Pulmonary edema, hypoxic encephalopathy
17	6	F	77	55	5	Cerebral edema and infarction; bronchopneumonia (Providence, Pseudomonas, Klebsiella) and interstitial pneumonitis, pulmonary thromboemboli and micro-infarcts; septicemia (Klebsiella, Providence, E. coli); bacterial invasion of burn wound (mixed)
18	7	F	76½	72½	9	Bronchopneumonia (Providence, Enterobacter, Staph., Streptococcus); septicemia (Providence, Enterobacter, Staph.); cerebral edema; anoxic-ischemic encephalopathy
19	52	M	73	62	12	Pancreatitis
20	23	M	70½	27	3	Inhalation injury; bronchopneumonia with abscess formation (E. coli, Serratia)
21	25	M	70½	0	7	Interstitial pneumonitis (Providence) with hyaline membranes; pulmonary congestion and edema; pulmonary thromboemboli, bilateral; bronchopneumonia (Providence); mycotic invasion (Candida) of burn wound
22	15	F	70	2	6	Tracheal obstruction due to impacted mucus; hypoxic encephalopathy with cerebral edema; burn wound sepsis (Pseudomonas)
23	18	F	68	42	57	Mitral valvulitis, staphylococcal, with septicemia
24	34	F	65½	43	27	*Bronchopneumonia, bilateral (Pseudomonas, Providence); fungal invasion of burn wound (Phycomycetes); cerebral edema
25	9	M	65½	42½	4	Hypoxic encephalopathy; pancreatitis
26	2-6/12	F	65	47½	5	Duodenal ulcer, perforated

* Autopsy not performed

Table 8. Causes of Death, 1971

Patient	Age	Sex	% Burn Total	% Burn 3°	POD Death	Cause of Death
27	9	M	65	23	10	Cardiac arrest of undetermined etiology; bronchopneumonia (mixed bacterial) with abscess formation
28	45	M	63½	58½	14	Infarction of left cerebral hemisphere; diffuse intravascular coagulation
29	24	M	63	34	16	Herpes pneumonia; pulmonary emboli; subacute cor pulmonale
30	27	M	63	0	12	*Burn wound sepsis (Pseudomonas); bronchopneumonia, bilateral (organism unknown)
31	3-7/12	F	61	39	3	Pulmonary insufficiency secondary to congestion and edema, interstitial pneumonitis, and thromboemboli; septicemia, staphylococcal
32	29	F	60½	26½	37	Bronchopneumonia, bilateral, with abscess formation (Providence, Pseudomonas, Staph.); septicemia (same organisms); hypoxic encephalopathy
33	33	F	60	20	14	Diffuse intravascular coagulation; pulmonary edema (with hyaline membranes) and congestion; bronchopneumonia (Providence, Candida)
34	39	M	59	54	1	Inhalation injury, hypoxic encephalopathy
35	42	M	58½	39	14	Septicemia (Providence) bronchopneumonia, viral (Herpes) and bacterial (Pseudomonas, Providence); bacterial invasion of burn wound (Pseudomonas, Providence)
36	11	M	57	57	15	Bronchopneumonia, bilateral (Pseudomonas, Providence), confluent
37	55	M	56	0	14	*Bronchopneumonia (Klebsiella, Pseudomonas, Staph.)
38	1-8/12	F	54	54	8	Fungal invasion of burn wound (organism unknown); bronchopneumonia (Klebsiella, Staph.)
39	19	M	54	12	15	Massive pulmonary embolization
40	27	M	54	0	22	Bronchopneumonia (Providence, Pseudomonas) with abscess formation; septicemia (Pseudomonas, Providence)

* Autopsy not performed

Table 8. Causes of Death, 1971

Patient	Age	Sex	% Burn Total	% Burn 3°	PBD Death	Cause of Death
41	1-8/12	M	53½	53½	17	Invasive disseminated Pseudomonas infection; septicemic shock
42	1-8/12	M	53	49½	5	Bronchopneumonia (Klebsiella)
43	21	M	53	28	18	Septicemia (Providence); bronchopneumonia (Providence, Pseudomonas) with abscess formation; pancreatitis
44	34	M	53	0	16	Respiratory insufficiency secondary to interstitial pneumonitis and bronchopneumonia (Providence, Pseudomonas, Staph.), bronchiolitis, pulmonary thromboemboli; necrosis, laryngotracheobronchial mucosa
45	7	M	51	51	2	Inhalation injury
46	22	M	49½	35½	24	Diffuse intravascular coagulation; pulmonary edema and congestion; bacterial suppurative thrombophlebitis, left internal iliac vein
47	31	M	49½	31½	16	*Bronchopneumonia (E. coli, Pseudomonas, Providence); septicemia (Staph.)
48	15	F	49½	23½	54	Hypoxic encephalopathy; pneumonia (Providence, herpesvirus, cytomegalovirus)
49	52	M	49	24½	19	Massive adrenal and retroperitoneal hemorrhage; bronchopneumonia (Providence, E. coli); mycotic (Aspergillus) and bacterial (Providence) invasion of burn wound
50	39	M	48½	0	15	Septicemia (Pseudomonas); mycotic (Fusarium) invasion of burn wound with renal dissemination; bacterial (Pseudomonas, Providence) invasion of excised vein bed; mycotic (Candida) suppurative thrombophlebitis
51	18	F	48	21	34	*Septicemia (Staph.); mycocarditis and pericarditis
52	68	M	47	0	8	Bacterial invasion of burn wound (Staph., Candida); necrotizing bronchopneumonia with abscess formation (E. coli, Klebsiella, Candida); septicemia (Staph., E. coli, Klebsiella)
53	9	F	45½	43½	15	Bronchopneumonia, bilateral (Staph., Klebsiella); necrosing tracheobronchitis (herpetic and bacterial); mixed mycotic invasion of burn wound

* Autopsy not performed

Table 8. Causes of Death, 1971

Patient	Age	Sex	% Burn Total	% Burn 3°	PBD Death	Cause of Death
54	6	F	45	44	10	Bacterial invasion of burn wound (Pseudomonas, Streptococcus, Providence)
55	41	M	45	36	33	Bronchopneumonia with abscess formation (Enterobacter, Pseudomonas, Candida)
56	35	M	45	0	7	Pulmonary embolization; bronchopneumonia (Providence, Pseudomonas)
57	31	M	43	43	41	Pneumonia (Providence), with abscess formation; septicemia (Providence); bacterial invasion of burn wound (Providence
58	22	M	41	30	22	Septicemia (Pseudomonas, Providence); bacterial (Providence, Pseudomonas) and mycotic (Fusarium) invasion of burn wound
59	10	M	40	30½	19	Septicemia (Pseudomonas, Providence); bacterial (Pseudomonas) invasion of burn wound; bronchopneumonia with abscess formation (Pseudomonas, Providence)
60	49	F	38	37	45	*Cause uncertain
61	61	F	35½	25	29	Pulmonary embolization, bilateral, diffuse; gram-negative sepsis (Providence); aspiration of gastric contents.
62	48	M	34½	34½	26	Rhinocerebral mycormycosis; pneumonia
63	2	F	33	26½	26	Cardiac arrhythmia, probably secondary to hyperkalemia
64	74	F	33	0	5	Septicemia (Staph., Klebsiella); gastrointestinal hemorrhagic necrosis, diffuse; bacterial invasion of burn wound (Staph.)
65	68	M	32	17	44	Bronchopneumonia (Klebsiella, E. coli)
66	2-6/12	F	31	31	17	Bacterial (Pseudomonas, Providence) invasion of burn wound; septicemia (Pseudomonas, Providence, Staph.)
67	23	M	22½	2	13	Bilateral pneumonitis with diffuse intra-alveolar hemorrhages (Pseudomonas, Staph., Streptococcus); diffuse intravascular coagulation; thrombosis, left innominate vein, superior vena cava, and right auricular appendage.
68	4	M	22	0	17	Systemic herpes simplex

* Autopsy not performed

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Moncrief JA, Pruitt BA Jr: The massive burn with sepsis and Curling's ulcer, in, (Ed) Hardy JD, Critical Surgical Illness, WB Saunders Co, 1971, pp 207-226.

Pruitt BA Jr, Curreri PW: The burn wound and its care. Arch Surg 103:461-468, 1971.

Asch MJ, Mason AD Jr, Pruitt BA Jr: Regional blood flow in the burned, unanesthetized dog. Surg Forum 22:55-56, 1971.

Pruitt BA Jr, Curreri PW: The use of homograft and heterograft skin in the treatment of burns, in, (Eds) Polk HC Jr, Stone HH, Contemporary Burn Management. Little, Brown & Co, Boston, 1971, pp 397-417.

Asch MJ, Moylan JM Jr, Bruck,HM, Pruitt BA Jr: Ocular complications associated with burns. A review of a five-year experience including 104 patients. J Trauma 11:857, 1971.

Pruitt BA Jr: Management of infections in the seriously burned patient. Symposium, Changing Patterns of Bacterial Infections and Antibiotic Therapy, 1971, pp 92-96.

Pruitt BA Jr, Mason AD Jr: Hemodynamic studies of burned patients during resuscitation, in, Research in Burns, 1971, pp 93-98.

Curreri PW, Eurenius K, Pruitt BA Jr: A study of coagulation factors in the thermally injured patient, in, Research in Burns, 1971, p 594.

PRESENTATIONS

Curreri PW: Hyperalimentation in Burn Patients. ENT Clinical Staff, BGH, BAMC, Fort Sam Houston, TX, 5 Jan 71.

Pruitt BA Jr: Current Perspectives in Management of the Burn Patient. Surgical Staff, Cedars of Lebanon Hosp, Los Angeles, CA, 9 Jan 71.

Pruitt BA Jr: Member, NIH Study Sect B Mtg, San Diego, CA, 11-12 Jan 71.

Pruitt BA Jr: Attendee, NIH Conf on Effects of Artificial Boundaries of Moving Blood, San Diego, CA, 13-15 Jan 71.

The following presentations were made to Physical Therapy Students, MFSS, BAMC, Fort Sam Houston, TX, 15 Jan 71:
Galloway KF: Nursing Care of the Burn Patient.
McManus WF: Medical Treatment of the Burn Patient.
Kirkman EM: Physical Therapy for the Burn Patient.
Palm L: Occupational Therapy for the Burn Patient.

Curreri PW: Platelet Kinetics and Coagulopathies Following Thermal Injury. Shriners Burns Institute, Cincinnati, Ohio, 19 Jan 71.

Curreri PW: Carbenicillin--Clinical and Laboratory Experience in Thermal Injury. Univ. of Cincinnati Med Sch, Cincinnati, Ohio, 19 Jan 71.

The following presentations were made to the Global Med Course, USAF Sch of Aerospace Med, Brooks AFB, TX, 22 Jan 71:
Pruitt BA Jr: Use of Physiologic Dressings and Skin Graft in the Treatment of Thermal Injury.
Moylan JA Jr: Resuscitation of the Extensively Burned Patient.
Inge WW Jr: Topical Chemotherapy of the Burn Wound.
McManus WF: Complications of Thermal Injury.

Galloway KF: Nursing Care of the Burn Patient. Incarnate Word Coll Sch of Nursing, San Antonio, TX, 26 Jan 71.

Inge WW Jr: Treatment of Burns. Officer Basic Course, MFSS, BAMC, FSHT, 29 Jan 71.

Galloway KF: Nursing Care of the Burn Patient. Flight Nurses and Med Technicians, Sch of Aerospace Med, Brooks AFB, TX, 3 Feb 71.

The following presentations were made to the Medical Staff Program, Center Pavilion Hosp, Houston, TX, 4 Feb 71:
Pruitt BA Jr: Current Method of Treatment of the Extensively Burned Patient.
Curreri PW: Treatment of Burns.

Curreri PW: Management of the Acute Burn Patient with Particular Reference to Common Complications. Symp on Acute Surg Problems and Their Management. Bowman Gray Sch of Med, Wake Forest Univ, Winston-Salem, NC., 12 Feb 71.

Pruitt BA Jr: Discussed paper on, "Topical Therapy of Burns." Soc of Univ Surgeons Mtg, New Haven, Conn, 11-13 Feb 71

McGranahan BG: Nursing Care of the Burn Patient. Univ of Texas Med Sch at San Antonio Sch of Nursing, San Antonio, TX, 26 Feb 71.

Pruitt BA Jr: (1) Summary of Topical Therapy of Burns; (2) Management of Complications in the Burn Patient; (3) Workshop, "Management of Burns". Trauma Seminar, Las Vegas, Nev, 1-3 Mar 71.

Pruitt BA Jr: Viral and Nonbacterial Surgical Infections. ACS Comm on Control of Surgical Infections. Pre- and Postop Care Committee, Wash DC, 8,9 Mar 71.

Johns LA: Nursing Care of the Burn Patient. Licensed Vocational Nurses Assn of Texas, Div 81, Jourdanton, TX, 11 Mar 71.

Pruitt BA Jr: Management of the Burn Patient--Complications in Burn Patients. ACS Sectional Mtg, New Orleans, LA, 15-17 Mar 71.

Pruitt BA Jr: Moderator, Conf on Mesenteric Circuit in Shock, Gastroenterology Sect, Walter Reed Army Inst of Research, WRAMC, Wash DC, 17 Mar 71.

Johns LA: Nursing Care of Burn Patients with Orthopedic Problems. 9th Annl Symp, Air Force Nurse Corps, Wilford Hall Med Ctr, Lackland AFB, TX, 19 Mar 71.

Pruitt BA Jr: (1) The management of Pulmonary and Urinary Complications; (2) The Pulmonary Burn; (3) Moderator, Panel on, "Supportive Care". Symp. on Thermal Injury, Shriners Burns Inst, Cincinnati, O, 18,19 Mar 71.

Pruitt BA Jr: Discussed papers on: (1) Superior Mesenteric Artery Syndrome; (2) Frozen, Viable Homografts in the Treatment of Burns. Amer Surg Assn Mtg, Boca Raton, Fla, 25-27 Mar 71.

McGranahan BG: Nursing Care of Burn Patients. Incarnate Word Coll Sch of Nursing, San Antonio, TX, 31 Mar 71.

The following presentations were made to the Symposium on, Surgical & Orthopaedic Aspects of Trauma", Brooke Gen Hosp, BAMC, FSHT, 5-9 Apr 71.

Pruitt BA Jr: Hemodynamic Changes Following Injury and Resuscitation.

Moylan JA Jr: Considerations in the Early Care of Extensively Burned Patients.

Inge WW Jr: Common Complications of Thermal Injury.

Bruck HM: Fungal and Viral Infections in Burn Patients.

Curreri PW: Energy Requirements and Hyperalimentation in Burn Patients.

Moylan JA Jr: Treatment of Burns. ANC Off Adv Course, MFSS, BAMC, Fort Sam Houston, TX, 13 Apr 71.

The following personnel attended and/or made presentations to the American Burn Assn Third Anl Mtg, San Antonio, TX, 16,17 Apr 71:

Pruitt BA Jr: Attendee.

Munster AM: Myositis Ossificans in Burns: A Prospective Study.

Inge WW Jr: Systemic Candidiasis in the Burn Patient--An Emerging Opportunistic Disease.

Asch MJ: Liver Disease in Burn Patients--Review of 40 Cases.

Bruck HM: Heterotopic Calcification of the Elbow Complicating Thermal Injury.

Wolfe JE: Nursing Care of the Patient with Burns During Air Transport.

Johns LA: Use of Air-Fluidized Bed in Care of Burned Patients.

Pavlakovic D: Operating Room Nursing in a Burn Unit.

Munster AM: Acalculous Cholecystitis in Burned Patients: Southwestern Surg Congr, Las Vegas, Nev, 19 Apr 71.

Moylan JA Jr: Tracheostomy in Burn Patients--Analysis of a 5-year Experience. Southeastern Surg Congr, Miami Beach, Fla, 20 Apr 71.

The following presentations were made to the Clinical Pastoral Education for Chaplains Course, BGH, BAMC, Fort Sam Houston, TX, 26 Apr 71:

Johns LA: Nursing Care of the Burn Patient.

Munster AM: Procedures Used in the Treatment of Burn Patients.

Johns LA: Nursing Care of Burn Patients. Laredo AFB Hosp, Laredo AFB, TX, 17 May 71.

Munster AM: Transportation and Management of Burn Patients. Med Air Evac Command, Scott AFB, Ill, 18 May 71.

McGranahan BG: Nursing Care of the Burn Patient. Flight Nurses, Sch of Aerospace Med, Brooks AFB, TX, 20 May 71.

The following presentations were made to the Sympon the Air Fluidized Bed, Med Univ of So Carolina, Charleston, SC, 5 Jun 71.

Pruitt BA Jr: Experiences with the Air Fluidized Bed at the Institute of Surgical Research.

Newsome TW: Use of the Air Fluidized Bed for the Burn Patient.

McGranahan BG: Current Treatment of the Burn Patient. Dist #17, TX Nurses Assn, Corpus Christi, TX, 16 Jun 71.

Newsome TW: Treatment of Burns. Off Adv Course, MFSS, BAMC, Fort Sam Houston TX, 17 Jun 71.

Curreri PW: Current Therapy of the Complications of Thermal Injury. Woodbury Med Soc, Sioux City, Iowa, 17 Jun 71.

Pruitt BA Jr: Committee on Control of Surgical Infections, ACS, Atlantic City, 22,23 Jun 71.

McGranahan, BG: Nursing Care of the Burn Patient. Flight Nurses & Med Technicians, Sch of Aerospace Med, Brooks AFB TX, 30 Jun 71.

Curreri PW: Treatment of Burn Injuries and Their Complications. Kuakini Hosp and St Francis Hosp, Honolulu, Hawaii, 2 July 71.

Curreri PW: Treatment of Burn Injuries and Their Complications. Tripler Army Hosp, Honolulu, Hawaii, 6 Jul 71.

Curreri PW: Treatment of Burn Injuries and Their Complications. Queens Hosp and Kaiser Hosp, Honolulu, Hawaii, 7 Jul 71.

Newsome TW: Treatment of Burns. Off Basic Course, MFSS, BAMC, Fort Sam Houston, TX, 29 Jul 71.

Silverstein P: Management of Burn Patients. Fort Sam Houston Optimists Club, Fort Sam Houston, TX, 29 Jul 71.

McManus WF: Care of the Thermally Injured Patient. Univ of Nebraska Coll of Med Postgrad Seminar, Omaha, Neb, 1 Aug 71.

Moylan JA Jr: Treatment of Burns. Off Basic Course, MFSS, BAMC, Fort Sam Houston, TX, 12 Aug 71.

McGranahan BG: Nursing Care of the Burn Patient. Baptist Hosp Sch of Nursing, San Antonio, TX, 18 Aug 71.

Pruitt BA Jr: (1) Curling's Ulcer in Burn Patients, (2) Fungal and Viral Infections of Surgical Importance. Staff, Gen Surg Svc, Wm Beaumont GH, El Paso, TX, 24-26 Aug 71.

Silverstein P: Treatment of Burns. Off Basic Course, MFSS, BAMC, Fort Sam Houston, TX, 1 Sep 71.

Pruitt BA Jr: Methods of Resurfacing Denuded Skin Areas. Transplantation Soc Symp on Current Status of Artificial Organs for Clinical Transplantation, New York, NY, 9,10 Sep 71.

McManus WF: Treatment of Burns. Off Basic Course, MFSS, BAMC, Fort Sam Houston, TX, BAMC, Fort Sam Houston, TX, 16 Sep 71.

Pruitt BA Jr: The Use of Sulfamylon in the Treatment of Burns. Mtg of Soc of Plastic & Recon Surgeons, Galveston, TX, 16-18 Sep 71.

Pruitt BA Jr: Metabolic Changes in Thermal Injury. Parenteral Nutrition Conf, Bermuda, 21-25 Sep 71.

Pruitt BA Jr: Current Status of Topical Therapy of Burn Injury--Results in over 2100 Burn Patients. Texas Surg Soc Mtg, Waco, TX, 3-5 Oct 71.

Warden GD: Treatment of Burns. ANC Off Adv Course, MFSS, BAMC, Fort Sam Houston, TX, 4 Oct 71.

McGranahan BG: Nursing Care of the Burn Patient. Sch of Nursing, Univ of Texas Med Sch at San Antonio, San Antonio TX,5 Oct 71.

Salisbury RE: Treatment of Burns. Off Basic Course, MFSS, BAMC, Fort Sam Houston, TX, 6 Oct 71.

Johns LA: Nursing Care of the Burn Patient, Disaster Planning--Regional. Northweast Texas Hosp Admin Gp, Continuing Edu for Nurses, Tejas Village, Lake of the Pines, TX, 8 Oct 71.

McManus WF: Treatment of Burn Patients. Assn of Operating Room Nurses, Grand Island,Neb, 9 Oct 71.

Johns LA: Nursing Care of the Burn Patient. Laughlin AFB Hosp, Laughlin AFB, TX, 14 Oct 71.

The following personnel attended and/or made presentations to the Amer Assn for Surgery of Trauma Mtg, New York, NY 14-16 Oct 71.
Pruitt BA Jr: Attendee.
Bruck HM: Curling's Ulcer in Children: A 12-Year Review of 65 Cases. Amer Assn for Surg of Trauma, New York, NY
Reckler JM: Superior Mesenteric Artery Syndrome as a Consequence of Burn Injury.

Pruitt JR: Hemodynamic Changes Following Thermal Injury. Surg Biol III Club, Atlantic City, NJ, 17 Oct 71.

Hunt JL: Management of Mass Burn Casualties. Medical Aspects of Advanced Warfare Course, Sch of Aerospace Med, Brooks AFB Tex, 18 Oct 71.

Pruitt BA Jr: Attendee, Amer Coll of Surgeons Meeting, Atlantic City, NJ, 18-22 Oct 71.

The following presentations were made to the Clinical Pastoral Edu for Chaplains Course, BGH, BAMC, FSHT, 26-27 Oct 71:
McGranahan BG: Nursing Care of the Burn Patient.
Silverstein P: Current Techniques of Burn Care.

Silverstein P: Current Problems in Burn Therapy. Plastic Surg Dept, Univ of Tex Med Sch at San Antonio, San Antonio, TX, 27 Oct 71.

McManus WF: Treatment of Burn Patients. St Joseph's Hosp, Huron, SD, 28 Oct 71.

Johns LA: Pharmacy Support of the Burn Patient. Pharmacy Svc, BGH, BAMC, FSHT, 28 Oct 71.

McGranahan BG: Nursing Care of the Burn Patient. Flight Nurses, Sch of Aerospace Med, Brooks AFB, TX, 3 Nov 71.

Silverstein P: Management of Burns. Orange Co Med Ctr, Orange, CA, 3 Nov 71.

Wilmore DW: Treatment of Burns.Off Basic Course, MFSS, BAMC, Fort Sam Houston, TX, 4 Nov 71.

Pruitt BA Jr: Amer Assn for Academic Surg, Philadelphia, PA, 18-20 Nov 71.

Johns LA: Problems of the Burned Individual. Nursing Svc Inservice Program, BGH, BAMC, Fort Sam Houston, TX, 30 Nov 71.

Pruitt BA Jr: Recent Advances in Burn Treatment. Surg Staff Conf, Presbyterian Hosp, New York, NY, 2 Dec 71.

Pruitt BA Jr: Pulmonary Changes in the Early Postburn Period. Surg Metabolic Unit Conf, Presbyterian Hosp, New York, NY, 3 Dec 71.

Pruitt BA Jr: Panelist, Panel on Closed Blunt Injuries of Chest. Postgrad Course: Chest Trauma. Recent Advances in Emergency Management, Amer Coll of Chest Surgeons, NY Univ Med Ctr, New York, NY, 3-4 Dec 71.

Pruitt BA Jr: Respiratory Burns: Diagnosis and Treatment. New York Univ Med Ctr Postgrad Course, New York, NY, 5 Dec 71.

Moylan JA Jr: Current Research in Burn Therapy. Dept of Surg, Univ of New Mex Sch of Med, Albuquerque, NM, 10 Dec 71

Welch GW: Treatment of Burns. Off Adv Course, MFSS, BAMC, Fort Sam Houston, TX, 10 Dec 71.

Pruitt BA Jr: Panelist, Panel on Trauma and Resuscitation, New York State Soc of Anesthesiologists, Inc, New York, NY, 12-15 Dec 71.

Moylan JA Jr: Burn Care. Dept of Surg, Harbor GH (UCLA) Torrance, CA, 13 Dec 71.

EXHIBITS

The following exhibits were displayed at the 120th Anl Conv of the Amer Med Assn, Atlantic City, NJ, 20-24 Jun 71:

"Burns in Children."

"Emergence of Opportunistic Infection in the Burn Wound."

"Management of Orthopedic Complications in the Thermally Injured", displayed at the Ani Conv of the Amer Coll of Surgeons, Atlantic City, NJ, 18-22 Oct 71.

"Occupational Therapy for Thermally Injured Patients", displayed at the Ani Conv of the Amer Occupational Therapy Assn, Cleveland, Ohio, 1-5 Nov 71.

MOTION PICTURES

"Dressing the Burn Wound", shown at Third Ani Meeting of the Amer Burn Assn, San Antonio, TX, 16 Apr 71.

"Management of a Massive Thermal Injury by Skin Transplantation Between Monozygotic Twins", shown at the Ani Conv of the Southeastern Surg Congr, Miami Beach, FL, 20 Apr 71.

The following motion pictures were shown at the 120th Ani Conv of the Amer Med Assn, Atlantic City, NJ, 20-24 Jun 71:

"The Use of Mesh Autografts in the Treatment of Burns."

"The Management of Burns in Children."

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. Agency Accession	DA OA 6983
2. Date of Summary	72 07 01
Report Control Symbol	DD-DR&E(AR)636
3. Date Prev Sum'ry	71 07 01
4. Kind of Summary	D. CHANGE
5. Summary Scty	U
6. Work Security	U
7. Regrading	NA
8a. Dissem Instr'n	NL
8b. Specific Data-Contractor Access	☒ Yes ☐ No
9. Level of Sum	A. WORK UNIT

10. No./Codes	Program Element	Project Number	Task Area Number	Work Unit Number
a. Primary	61102A	3A061102B71R	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: 62 02

14. ESTIMATED COMPLETION DATE: Cont

15. FUNDING AGENCY: DA

16. PERFORMANCE METHOD: 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

Fiscal Year		a. Professional Man Yrs	b. Funds (In thousands)
Preceding	72	1.4	41.2
Current	73	1.4	43.3

19. RESPONSIBLE DOD ORGANIZATION

NAME: US Army Institute of Surgical Research

ADDRESS: Ft Sam Houston, Tx 78234

RESPONSIBLE INDIVIDUAL

NAME: Basil A Pruitt, Jr, LTC, MC

TELEPHONE: 512-221-2720

20. PERFORMING ORGANIZATION

NAME: US Army Institute of Surgical Research Surgical Study Branch

ADDRESS: Ft Sam Houston, Tx 78234

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

NAME: Paul Silverstein, MAJ, MC

TELEPHONE: 512-221-5712

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Basil A Pruitt, Jr, LTC, MC

NAME: DA

21. GENERAL USE

FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code): (U) Laboratory animals; (U) Combat casualties (U) Trauma; (U) Immunity; (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 the Branch have included both purely clinical studies, involving patients on the ward, laboratory studies involving animal models, and a combination of the two.

25. (U) 71 07 - 72 06 Achievements of the Surgical Study Branch have involved both purely clinical responsibilities and research endeavors. Clinical duties included aeromedical evacuation of burn patients and their care until discharge from the unit. Ward officer coverage was also provided for ward 13B.

Research projects dealt with biologic wound covers of human and xenograft origin, development of a synthetic skin substitute, enzymatic debridement of burn eschar, creation of an animal model for hypertrophic scar study and a clinical study on therapy of hypertrophic scar and postburn edema.

*Available to contractors upon originator's approval.

ANNUAL PROGRESS REPORT

PROJECT NO. 3A061102B71R-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 - 31 December 1971

Investigators:

Paul Silverstein, MD, Major, MC
Andrew M. Munster, MD, Lieutenant Colonel, MC
Lois A. Johns, Lieutenant Colonel, ANC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A061102B71R-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 - 31 December 1971

Investigators: Paul Silverstein, MD, Major, MC
Andrew M. Munster, MD, Lieutenant Colonel, MC
Lois A. Johns, Lieutenant Colonel, ANC

Reports Control Symbol MEDDH-288(R1)

The Surgical Study Branch has continued to render clinical care to burn patients admitted to the Institute from all three branches of the Armed Forces in addition to veterans and civilian emergencies. Branch members also participate in air evacuation of burned individuals from referring physicians within and without CONUS to the Institute of Surgical Research.

Responsibilities secondary to delivery of medical care involve research concerned with problems related to burn and traumatic injuries, and participation in various teaching programs.

Research projects under way include broad topic categories such as biologic wound dressings, synthetic skin substitutes, bacteriology of the wound treated with biologic dressings, enzymatic debridement of the burn wound, immunologic responses of wounds to xenograft, and the problems of etiology and therapy of hypertrophic burn scars.

Trauma
Immunity
Biologic dressings
Enzymes
Hypertrophic scar

CLINICAL OPERATION, SURGICAL STUDY BRANCH FOR TREATMENT OF INJURED SOLDIERS

The Surgical Study Branch has continued its activities in: (1) primary delivery of medical and surgical care of acutely burned individuals admitted to this Institute, (2) clinical and laboratory research in problems related to care and rehabilitation of the burn patient, and (3) various teaching duties.

Delivery of medical care is the prime purpose of the branch and responsibility starts at the time of air evacuation from the referral center in Japan or CONUS. A surgeon accompanies each patient during his evacuation and is responsible for his care after admission to the Institute. Initial resuscitation, convalescence, and surgical intervention, when required in the process of wound healing, are supervised during the patient's initial hospitalization. At the time of discharge, all wounds are healed and the patient is referred to specialty centers for attention to specific reconstructive and rehabilitation problems or returned to duty. Some patients residing in this geographical area return to the Institute for reconstructive surgery, space and personnel permitting. Special attention has been paid to functional reconstruction of hand burns and cosmetic reconstruction of facial deformities due to hypertrophic scar, graft contractures, ectropion, etc.

Clinical research has been continued in the use of biologic dressings as a temporary burn wound cover. Following the evaluation of fresh and frozen irradiated porcine xenograft, a new dosage form, lyophilized porcine cutaneous xenograft, is being evaluated after successful laboratory study. The lyophilized skin appears to be acceptable in assisting wound debridement and preparing the granulating wound for autografting. It appears to be effective in supporting re-epithelialization of second degree burn wounds. The ease of shelf storage and guaranteed sterility are attractive properties of the lyophilized product and it is currently being assessed clinically.

Other biologic dressings evaluated included formalin-fixed allograft and xenograft and lyophilized allograft (supplied by the Naval Medical Research Laboratories, Bethesda, Maryland). Formalinized skin was applied to 12 patients with generally favorable results. However, because of its stiffness, it was considered to be less desirable than porcine skin. Lyophilized allograft is currently being evaluated.

In the laboratory, synthetic skin substitutes of various con-

struction are being tested on excised rat wounds. Basic design of the skin substitutes conforms to the bilaminar physical structure of natural skin. While none of these products is ready for trial in the clinic, progress has been made towards designing thinner, more elastic, and more bio-adherent dressings.

Enzymatic debridement of burn wounds has been studied in both test tube and animal models. Sutilains was found to be superior to Bromelain as a nonspecific protease, primarily because of less toxicity at therapeutic dosages. Sequential therapy with sutilains and clostridial collagenase was found to be superior to the use of either enzyme alone or combined. Ongoing experiments are directed to development of an enzyme dosage form compatible with existent topical antibacterial burn wound medications.

Clinical experience in the treatment and prevention of hypertrophic scars confirms work published from other burn centers supporting the use of compression devices designed to apply 40 mm Hg pressure to wounds from the time of complete healing until scar maturation is complete. Pressure is applied by custom made elastic stockings, sleeves, gloves, vests, and face masks. Scars treated within 3 months of appearance show a better response than those treated later. Average duration of therapy ranges between 6-12 months.

Clinical experience with intralesional and topical application of triamcinolone to hypertrophic scars has been disappointing.

Laboratory investigation to develop an animal model in which to study hypertrophic scar formation and the biochemistry of collagen synthesis and turnover has been successful in producing a scar model in the young, female, Duroc pig. Histologic studies confirm similarity of the porcine scar tissue to human scar in both maturation cycle and cellular morphology. Future investigations will determine whether or not biochemical similarities also exist in the specific activity of collagen.

PUBLICATIONS

Munster AM, Pruitt BA, Jr: Recent advances in the management of burns. Med J Australia 1: 484-489, 1971.

Munster AM, DiVincenti FC, Foley FD, Pruitt BA, Jr: Cardiac infection in burns. Amer J Surg 122:524-527, 1971.

Silverstein P, Raulston GL, Walker HL, et al: Evaluation of formalin-fixed skin as a temporary dressing for granulating wounds. Surg Forum 22: 60-62, 1971.

Pruitt BA, Jr, Silverstein P: Methods of resurfacing denuded skin areas. Transplantation Proc 3: 1537-1545, 1971.

MOTION PICTURES

Silverstein P, McManus WF: Laboratory and clinical evaluation of porcine cutaneous xenograft in the treatment of burns (10 minutes; color and sound). Submitted to the ACS Clinical Congress.

PRESENTATIONS

Johns LA: Nursing Research in the Army Nurse Corps. Adv Nursing Course, MFSS, BAMC, FSHT, April 1971.

Silverstein P: Wound Coverage. BGH Surg & Ortho Aspects of Trauma Symposium, BAMC, FSHT, April 1971.

Johns LA: Nursing Research and the Army Nurse Corps. Sch Nursing, Univ Tx Med Sch, San Antonio, Tx, April 1971.

Johns LA: Findings and Recommendations on Nursing Roles and Functions. Tx Nurses Assoc (Region No. 5), Dallas, Tx, May 1971.

Johns LA: Problems and Concepts, Panel participation, Symp on the Air-Fluidized Bed. Med Univ So Carolina, Charleston, SC, June 1971.

Silverstein P: Burn Wound Covers: Dead or Alive. Alcon Labs Sci & Technol Div, Ft Worth Tx, July 1971.

Silverstein P: Enzymatic Debridement of Burn Eschar. Baxter Labs Med Res Div, Chicago, Ill, August 1971.

Johns LA: A Planning System for Nursing Care. San Antonio OR Nurses, San Antonio, Tx, September 1971.

Silverstein P: Use of Cadaver Allograft in the Treatment of Burns. Amer Soc Plast Reconstr Surg Symp on Burns, Galveston, Tx, September 1971.

Johns LA: Nursing Research in the Army. ANC Adv Off Course, MFSS, BAMC, FSHT, September 1971.

Silverstein P: Evaluation of Formalin-Fixed Skin as a Temporary Dressing for Granulating Wounds. Amer Coll Surg, Surg Forum, Atlantic City, NJ, October 1971.

Johns LA: Patient Psychology. Clin Pastoral Ed Tng Course, BGH,

BAMC, FSHT, October 1971.

Johns LA: Ethics in Nursing Research. Sch Nursing, Incarnate Word Coll, San Antonio, Tx, November 1971.

Johns LA: Patient Psychology. AMEDD Adv Course (ANC, MSC), MFSS, BAMC, FSHT, November 1971.

Silverstein P: Synthetic Skin. Lederle Labs Res Symp, Pearl River, NY, November 1971.

Silverstein P: Biologic Wound Dressings: Dead or Alive. Assoc Acad Surg, Philadelphia, Pa, November 1971.

Johns LA: Nursing Research. AMEDD Adv Nursing Course, MFSS, BAMC, FSHT, December 1971.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0A 6956	72 07 01	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. GROUP NUMBER	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
71 07 01	DI, 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	3A061102B71R	01	141
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE: (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

	PRIOR YEARS	a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
FISCAL YEAR	72	4.3	60.5
	CURRENT 73	1.0	15.0

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

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

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

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

ASSOCIATE INVESTIGATORS
NAME: Neil A Kurtzman, LTC, MC
NAME: DA

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede each with Security Classification Code)
(U) Renal Failure; (U) Hemodialysis; (U) Peritoneal Dialysis; (U) 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 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, and peritoneal dialysis, glomerular filtration rates, metabolic balance studies.

25. (U) 71 01 71 12 Twelve patients were treated for renal insufficiency during the reporting period. Two of these patients presented with acute renal failure, and 10 with chronic renal insufficiency. Three patients underwent renal homotransplantation. There were 940 patient days in the period covered by this report. In addition, the Renal Section performed 85 determinations of glomerular filtration rate, and two glucose titration tests. Studies of the renin-angiotensin system were completed in 28 thermally injured patients.

* Available to contractors upon requestor's approval.

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

ANNUAL PROGRESS REPORT

PROJECT NO. 3A061102B71R-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 - 31 December 1971

Investigators:

Philip W. Rogers, MD, Major, MC
Neil A. Kurtzman, MD, Lieutenant Colonel, MC
Martin G. White, MD, Major, MC

Reports Control Symbol MEDDH-288(R1)

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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 January - 31 December 1971

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Fourteen patients were treated in the Renal Section during the reporting period. This represents a total of 940 patient days, and 331 hemodialysis. Four patients presented with acute renal failure with 2 survivors. Ten patients were treated with chronic renal insufficiency, 4 of these have received renal homotransplantation. In addition, glomerular filtration rates were performed on 86 patients.

Renal failure
Hemodialysis
Peritoneal dialysis

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

All patients treated in this Section of the Institute were dialyzed using the Travenol Twin Coil No. 145 Artificial Kidney or the extracorporal coil EX 01 or EX 03 Artificial Kidney. Dialyses on acutely ill patients were performed using percutaneous insertion of catheters into appropriate arteries and veins. Anticoagulation of the dialyzing coil was obtained with either regional or total body heparinization, depending on the individual circumstances. Of the 4 patients with acute renal failure, 2 patients survived while the other 2 patients expired from complications unrelated to their renal disease. Six patients with chronic renal failure continued to do well during the year. Four patients with chronic renal failure were referred for renal homotransplantation.

Presented in the Table are the characteristics of patients treated for acute and chronic renal failure in the renal section during 1971.

Patient Nr. 1 was a 38-year-old man who had been treated on chronic hemodialysis since 1964. His course during the past year included one episode of septicemia, secondary to Staphylococcus aureus, coagulase positive related to an arterial shunt infection. The patient tolerated this complication well and in February 1971 was referred to the Bexar County Hospital for renal homotransplantation.

Patient Nr. 2 was an 8-year-old girl admitted with acute salicylate intoxication. She required only one hemodialysis with marked improvement in her condition. She was later discharged from Brooke General Hospital without further complication.

Patient Nr. 3 was a 46-year-old man admitted to the Medical Service at Brooke General Hospital with a 6-year history of poorly controlled hypertension with resultant renal failure and hypertensive cardiovascular disease. He initially was dialyzed several times by peritoneal dialysis, then referred to the Veterans Administration Hospital in Houston, Texas where he was trained for chronic home hemodialysis. He has been followed in the nephrology clinic, Brooke General Hospital and has received hemodialysis on 3 occasions due to difficulty with hemodialysis at home as a result of an inadequate internal arterio-venous fistula. A new internal arterio-venous fistula was created and the patient is now resuming his home hemodialyses.

3-2

1 January 71 thru 31 December 71

No.	Age	Sex	Diagnosis	No. of Hemodialysis	No. of Hospital Days	OUTCOME	OTHER ACTIONS
1	38	M	Glomerulonephritis, Chronic	14	49	Survived	1971 Transplanted
2	8	F	Salicylate intoxication, Acute	1	1	Survived	
3	46	M	Glomerulonephritis, Chronic	3	12	Survived	
4	37	M	Post-Operative Acute Tubular Necrosis	6	15	Survived	
5	57	M	Nephrosclerosis, Chronic	103	289	Survived	1971 Transplanted
6	28	F	Burn; Acute Cortical Necrosis	3	3	Expired	
7	47	F	Nephrosclerosis	58	152	Survived	1972 Transplanted
8	33	M	Nephrosclerosis	52	109	Survived	
9	13	F	Glomerulonephritis, Chronic	18	56	Survived	1971 Transplanted
10	33	M	Nephrosclerosis	28	71	Survived	
11	94	F	Glomerulonephritis, Chronic	3	7	Expired	
12	43	M	Glomerulonephritis, Chronic	35	120	Survived	
13	32	F	Barbiturate intoxication, Acute	1	1	Expired	
14	66	F	Pyelonephritis, Chronic	6	15	Survived	
			TOTAL	331	TOTAL 940		

1 January 71 thru 31 December 71

No.	Age	Sex	Diagnosis	No. of Hemodialysis	No. of Hospital Days	OUTCOME	OTHER ACTIONS
1	38	M	Glomerulonephritis, Chronic	14	49	Survived	1971 Transplanted
2	8	F	Salicylate intoxication, Acute	1	1	Survived	
3	46	M	Glomerulonephritis, Chronic	3	12	Survived	
4	37	M	Post-Operative Acute Tubular Necrosis	6	15	Survived	
5	57	M	Nephrosclerosis, Chronic	103	289	Survived	1971 Transplanted
6	28	F	Burn: Acute Cortical Necrosis	3	3	Expired	
7	47	F	Nephrosclerosis	58	198	Survived	1972 Transplanted
8	33	M	Nephrosclerosis	52	189	Survived	
9	13	F	Glomerulonephritis, Chronic	18	56	Survived	1971 Transplanted
10	33	M	Nephrosclerosis	28	71	Survived	
11	34	F	Glomerulonephritis, Chronic	3	7	Expired	
12	43	M	Glomerulonephritis, Chronic	35	128	Survived	
13	32	F	Barbiturate intoxication, Acute	1	1	Expired	
14	66	F	Pyelonephritis, Chronic	6	15	Survived	
			TOTAL	331	TOTAL 988		

3-2

Patient Nr. 4 is a 37-year-old man admitted to the Cardiovascular Surgical Service for aortic valve replacement. He had a formefrust of Marfan's syndrome with marked aortic insufficiency. This patient had previously undergone a right nephrectomy for pyelonephritis involving the same kidney. He developed acute renal failure postoperatively due to prolonged hypotension and massive hemoglobinemia and hemoglobinuria due to prolonged perfusion on the heart-lung machine as well as poor oxygenation due to a defective oxygenator during the insertion of the aortic valve prothesis. He was dialyzed every other day on the Cardiovascular Surgery Service without complication. He had gradual return of complete renal function. He was later medically retired from the Army because of his cardiovascular problem.

Patient Nr. 5 is a 57-year-old man with a history of hypertension since 1967 which was inadequately treated resulting in arteriolar nephrosclerosis with resultant chronic failure. An external A-V shunt was inserted in one arm and an internal A-V fistula was created in the other arm and hemodialyses performed utilizing the external shunt while the internal A-V fistula matured. His course was initially complicated by an uncontrollable thirst. Peripheral renin determinations were obtained and were found to be markedly elevated. Since there is information suggesting that angiotensin stimulates the thirst center in the hypothalamus, it was felt that this patient's excessive thirst was causally related to the high plasma renin concentrations. In October 1971, the patient underwent bilateral nephrectomy and had an uncomplicated postoperative course. This operative procedure resulted in marked clinical improvement of his peripheral neuropathy and also resulted in a dramatic cessation of his craving for water. His average weight gain between dialyses prior to nephrectomy was 4 to 6 kg, and after nephrectomy ranged from 1 to 1.5 kg. His course throughout the remainder of the year remained uncomplicated. He has subsequently had a cadaveric renal transplant.

Patient Nr. 6 was a 28-year-old woman who sustained 48% total body surface burns in an automobile accident. She developed disseminated intravascular coagulation during her burn course with an associated acute cortical necrosis and acute renal failure. She had three uneventful hemodialyses during her hospitalization and expired as a consequence of her thermal injury.

Patient Nr. 7 was a 47-year-old Puerto Rican lady with a history of systemic arterial hypertension since 1965 which had been poorly controlled resulting in progressive azotemia and uremia. This was hastened by an accelerated phase of her hypertension prior to her admission to Brooke General Hospital. Despite adequate antihyper-

tensive therapy, her renal function never improved. She was evaluated for renal transplantation at Wilford Hall U.S. Air Force Medical Center. This patient underwent bilateral nephrectomy as part of the preparation for transplantation. In November, a D-matched cadaver kidney became available and the patient was transferred to Wilford Hall U.S. Air Force Medical Center for renal homotransplantation. The patient's immediate postoperative course was uncomplicated; however, early in 1972 the patient experienced a chronic rejection which could not be reversed, so nephrectomy was performed. The patient was then returned to Brooke General Hospital for chronic maintenance hemodialysis. This patient continues to do well at the present time.

Patient Nr.8 was a 33-year-old black man who was found to have severe hypertension approximately 2 years prior to his admission to Brooke General Hospital. Although this patient remained on active duty, his hypertension was never adequately followed or treated. He was admitted to Brooke General Hospital with accelerated hypertension and renal failure. Complete evaluation revealed no known cause for his hypertension. Despite adequate treatment this patient's renal function never improved. He was continued on chronic hemodialysis 3 times weekly via an internal A-V fistula. Dialysis has been uncomplicated.

Patient Nr. 9 was a 13-year-old black girl with sickle cell trait, situs inversus, and a 2-year history of hypertension, proteinuria, microscopic hematuria, and azotemia. Renal biopsy was performed in 1970 and was compatible with a chronic proliferative glomerulonephritis. Her renal function continued to deteriorate and in December 1970 an internal arterio-venous fistula was created so that chronic hemodialysis could be accomplished. The patient as well as her family members had HLA typing and it was found that there was only a 1 antigen mismatch between her and her mother. After complete medical, urologic and psychiatric evaluation both daughter and recipient were admitted to Wilford Hall U.S. Air Force Medical Center in March 1971 where the living related kidney transplant was performed. The operative procedure was uncomplicated and the patient did well for approximately 2 months and then had an acute rejection episode caused by the discontinuation of immnuosuppressive medication by the patient. She was immediately hospitalized and the rejection episode treated with pulse therapy with Prednisone resulting in adequate suppression with slight reduction in renal function. The patient continues to do well at the present time.

Patient Nr. 10 was a 33-year-old black man with a long history of hypertension and nephrosclorosis and slowly progressive renal insufficiency and finally renal failure. He had been evaluated and

followed at Brooke General Hospital for 2 years and in September 1970 was referred to the Veterans Administration Hospital in Houston, Texas, for evaluation for renal homotransplantation and/or chronic maintenance home hemodialysis. Because of extenuating circumstances with the family, the patient was returned to Brooke General Hospital for training for the home hemodialysis. The patient was markedly uremic, had a severe metabolic encephalopathy and a severe peripheral neuropathy which only improved after months of hemodialysis. The patient also later sustained a mild cerebrovascular accident resulting in paresis of the left lower extremity. The patient has shown marked improvement with 3 times weekly hemodialysis. He participates in an active physical therapy program and is presently being considered for cadaveric renal transplantation.

Patient Nr. 11 was a 34-year-old white lady with undifferentiated schizophrenia and a 15-year history of chronic glomerulonephritis with progressive azotemia and renal failure. She had been maintained for several months on peritoneal dialysis and was finally placed on chronic hemodialysis after an episode of peritonitis. There was much difficulty in the placement of an external arterio-venous shunt due to the marked calcification of her vessels from secondary hyperparathyroidism. Because of multiple operative procedures required, she refused further hemodialysis. This desire was shared by her husband and parents. Chronic hemodialysis was then terminated and the patient later expired in the hospital as a result of her uremia.

Patient Nr. 12 was a 43-year-old black man with a 7-year history of chronic recurrent ureteral calculi necessitating right nephrectomy in 1967. He was noted to have moderate hypertension at the time of his operative procedure; however, therapy and followup remained inadequate. The patient was finally referred to Brooke General Hospital for evaluation of his hypertension and was found to have severe azotemia. Treatment and close observation resulted in no improvement with continual decline of his renal function. Finally, he was placed on chronic hemodialysis and has been evaluated for cadaveric renal homotransplantation. He and his wife have undergone hemodialysis training so that hemodialysis in the future can be accomplished at home by them.

Patient Nr. 13 was a 32-year-old white lady admitted to Brooke General Hospital with acute barbituate intoxication, Stage IV coma, and severe aspiration pneumonitis. The patient was admitted some 4 to 6 hours after ingestion. She was dialyzed for 4 hours without improvement in her level of consciousness or neurologic response. Despite orotracheal intubation, positive pressure breathing, and frequent tracheal suctioning, there was much difficulty in keeping the

patient well oxygenated. As a result of hypoxia the patient had a cardiac arrest and was unable to be resuscitated.

Patient Nr. 14 was a 66-year-old white lady with a history, since the age of 7, of recurrent urinary tract infections and resultant chronic pyelonephritis. A left ureteroplasty was performed in 1947 which was unsuccessful and resulted in the loss of the left kidney. The patient has over the years had progressive azotemia culminating in renal failure. Since she had no other systemic illness, she was felt to be a candidate for center hemodialysis. An internal arterio-venous fistula was created in the left arm which has provided access for hemodialysis. The only complication has been the development of a pseudoaneurysm on the venous limb of the A-V fistula. The patient is currently being maintained on twice weekly hemodialysis and is doing well.

In addition to the hemodialyses performed by the Renal Section, glomerular filtration rates were assessed in 84 patients during the reported period, using glofil (^{125}Iothalamate I) excretion as an index of renal function. These studies were performed on patients within the Institute of Surgical Research as well as patients in Brooke General Hospital. The staff and corpsmen of the Renal Section also staff the Nephrology Clinic of Brooke General Hospital. This clinic meets weekly and has approximately 12 to 16 outpatient visits per clinic week. The staff of the Renal Section of Metabolic Branch serve as consultants to Brooke General Hospital on matters of renal disease, acid-base balance, hypertension, and other nephrologic and metabolic problems.

PRESENTATIONS

White MG: Acid Base Disorders. BGH Surg Staff, BGH, FSHT, 16 Mar 1971

White MG: Acute Renal Failure. BGH Surg Staff, BGH, FSHT, 22 Mar 1971

Spitzer ME: Nutrition in Burns. 4th Nutrition Symposium, WRAIR, Wash, DC, 5 Aug 1971

Rogers PW: Treatment of Accelerated Hypertension. Dept Med Grand Rounds, BGH, FSHT, 3 Sep 1971

Spitzer ME: Role of the Dietician. The Kidney Fnd., Univ Tex Med Sch, San Antonio, Tx, 9 Sep 1971

Rogers PW: The Role of Hemodialysis in the Management of Acute

Poisoning. Dept Med, BGH, FSHT, 14 Sep 1971

Spitzer ME: Rationale of the Renal Failure Diet. BGH Food Svcs Div, BGH, FSHT, 15 Oct 1971

Spitzer ME: Nutritonal Support to the Burn Patient. BGH Food Svcs Div,BGH, FSHT, 18 Oct 1971

Rogers PW: Renovascular Hypertension and Renal Physiology. Dept Pediatrics, BGH, FSHT, 13 Nov 1971

Spitzer ME: Normal Nutrition in Burn Patients. Social Workers Svcs, BGH, FSHT, 18 Nov 1971

Rogers PW: Effect of Aldosterone Deficiency on Solute Free Excretion and Reabsorption in the Dog Nephron. Amer Soc Nephrology, Wash, DC, 22 Nov 1971

Spitzer ME: Metabolic Methods of Nutritional Studies. BGH Dietetic Interns, BGH, FSHT, 1 Dec 1971

PUBLICATIONS

None

FINAL REPORT

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

REPORT TITLE: BILATERAL RENAL ARTERY STENOSIS AND SURGICALLY CORRECTABLE HYPERTENSION WITH LOW PLASMA RENIN CONCENTRATION

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

1 July 1971 - 30 June 1972

Investigators:

Neil A. Kurtzman, MD, Lieutenant Colonel, MC
Philip W. Rogers, MD, Major, MC

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A patient with bilateral renal artery stenosis was found to have hyporeninemia and hypertension. Surgical repair of the stenosed vessels resulted in amelioration of the hypertension with elevation of the plasma renin concentration to normal. The patient re-stenosed both renal arteries and once again developed hyporeninemia and hypertension. Bilateral renal artery stenosis results initially in an increase in renin release, salt retention and expansion of extracellular volume. Volume expansion then suppresses renin release. The resultant new steady state is characterized by hypertension on a volume rather than a pressor basis. Surgical correction of bilateral renal artery stenosis results in diuresis and restoration of extracellular volume to normal thus allowing the previously suppressed renin levels to return to normal. These findings suggest that the decision to correct bilateral renal artery stenosis should be based on clinical grounds rather than the concentration of renal vein or peripheral vein renin.

Hyporeninemia
Hypertension
Renal artery stenosis

BILATERAL RENAL ARTERY STENOSIS AND SURGICALLY CORRECTABLE HYPERTENSION WITH LOW PLASMA RENIN CONCENTRATION

Patients with renal artery stenosis make up a majority of those patients afflicted with surgically correctable hypertension. Such patients' hypertension is deemed likely to respond to surgical correction of the lesion if there is a ratio of 1.6:1 or greater of the concentration of renin in renal vein blood of the involved kidney as compared to that from the normal kidney (Bourgoignie, Kurz, Catanzaro, et al).[1] The likelihood of surgical cure of hypertension is even greater if the peripheral plasma renin concentration is elevated (Meyer, Ecoiffier, Alexandre, et al)[2] While information on patients with unilateral renal artery stenosis is abundantly available, virtually nothing has been published concerning the status of the renin-angiotensin system in patients suffering from bilateral renal artery stenosis and hypertension.

We have recently had the opportunity to make detailed observations of a patient with such a bilateral lesion. The findings we have made, while unexpected, seem quite logical when compared to related animal studies.

CASE REPORT

The patient is a 44-year-old white male, who was first told that he had hypertension in March 1967, at the time of his retirement physical examination from the U. S. Army. His subsequent course is summarized in the Table. Blood pressure determination at that time was 180/114 mm Hg supine and 152/110 mm Hg standing. An IVP showed poor excretion of the contrast material by the left kidney. A Hippuran ^{131}I renogram showed decreased delivery of the isotope to the left kidney as well as impaired excretion. Technecium-99M renal scan showed poor concentration of the isotope by the left kidney. Urinary vanil-mandelic acid excretion was 6.3 and 2.4 mg/24 hours (normal 0 - 10 mg/24 hours). A percutaneous transfemoral aortogram performed in January 1968 revealed bilateral renal artery stenosis secondary to atherosclerotic plaques at the origin of both renal arteries and involving the entire length of a smaller artery supplying the upper pole of the right kidney. Serum creatinine at that time was 1.2 mg/100 ml. He was treated with alpha methyldopa 500 mg four times daily, reserpine, 0.25 mg three times daily, guanethidine 100 mg daily, and hydrochlorithiazide, 50 mg daily. He was seen in December 1970, and was again evaluated for his hypertension, which was poorly controlled on the above medications. Blood pressures at this time were in the range of 180-200/120-130 mm Hg. He complained

Bilateral Renal Artery Stenosis

	FEB 1967	NOV 1967	JAN 1968	OCT 1970	JAN 1971	FEB 1971	MAR 1971		MAR 1971	APR 1971	OCT 1971		NOV 1971	MAR 1972
BLOOD PRESSURE	150/114	170/110	170/110	180/120	200/110	170/100	170/130		145/92	150/94	200/130		140/80	140/80
SERUM CREATININE (mg/100ml)	1.0	1.2	1.3				1.8		1.1		1.5		1.2	1.1
GLOMERULAR FILTRATION RATE (ml/min)		130		95			58		100	105	60		100	99
RENIN CONCENTRATIONS ng/ml/hr														
RIGHT RENAL VEIN				0.15			6.05			2.61	0.61			
LEFT RENAL VEIN				0.14			3.65			2.43	0.88			
INFERIOR VENA CAVA				0.10		0.73	3.67			2.89	0.80			
BRACHIAL VEIN				0.10							0.88			
PERIPHERAL RENIN														
STANDING													1.65	
SUPINE													1.54	
SPLIT RENAL FUNCTIONS														
GFR (ml/min) R/L				40.7/55.9										
Na(mEq/L) R/L				65/55										
Osmolality(mOsm/kg) R/L				480/520										
Flow (ml/min) R/L				2.5/4.1										
RPF (ml/min) R/L				163/246										
URINARY ALDOSTERONE EXCRETION (ugm/24hrs)					16.4									

FIRST OPERATIVE PROCEDURE

SECOND OPERATIVE PROCEDURE

LOW Na+ DIET AND IV FUROSEMIDE

THERAPY

ALPHA-METHYLDOPA	500 mg qid
ALPHA-METHYLDOPA	250 mg bid
HYDROCHLOROTHIAZIDE	50 mg daily
PROPRANOLOL	80 mg tid
CIMETIDINE	300 mg daily
CLONIDINE	200 mg daily
SPIRONOLACTONE	400 mg daily
DIAZIDE	1 cap. bid

Bilateral Renal Artery Stenosis

	MAR 1967	NOV 1967	JAN 1968	DEC 1970	JAN 1971	FEB 1971	MAR 1971	MAY 1971	APR 1971	OCT 1971	NOV 1971	MAR 1972
BLOOD PRESSURE	160/116	170/118	170/110	180/120	200/110	170/100	170/120	145/96	150/94	200/130	110/80	130/86
SERUM CREATININE (mg/100ml)	10	20/1.2	21/1.3				40/1.8	25/1.1		20/1.9	25/1.2	25/1.1
GLOMERULAR FILTRATION RATE (ml/min)		130		95			50	100	105	40	100	90
RENIN CONCENTRATION ng/ml/hr												
RIGHT RENAL VEIN				0.15			6.86		2.41	0.61		
LEFT RENAL VEIN				0.14			5.65		2.43	0.58		
INFERIOR VENA CAVA				0.10		0.73	3.67		2.03	0.89		
BRACHIAL VEIN				0.10						0.68		
PERIPHERAL RENIN STANDING											2.05	
SUPINE											1.54	
SPLIT RENAL FUNCTIONS GFR (ml/min) R/L				40.7/55.9								
Na(mEq/L) R/L				65/95								
Osmolality(mOsm/kg) R/L				486/536								
Flow (ml/min) R/L				2.5/4.1								
RPF (ml/min) R/L				163/246								
URINARY ALDOSTERONE EXCRETION (ug/24hrs)					10.4							

FIRST OPERATIVE PROCEDURE

SECOND OPERATIVE PROCEDURE

LOW Na+ DIET AND IV FUROSEMIDE

THERAPY

ALPHA-METHYLDOPA	500 mg qid
ALPHA-METHYLDOPA	250 mg bid
HYDROCHLOROTHIAZIDE	50 mg daily
[illegible]	25 mg tid
[illegible]	100 mg daily
GUANETHIDINE	200 mg daily
SPIRONOLACTONE	400 mg daily
DIAZIDE	1 cap. bid

4-2

of recurrent morning headaches and visual scotomata. Physical examination at that time revealed retinal arteriolar narrowing, AV nicking, and mild cardiomegaly. There was no evidence of peripheral edema on this examination or on any of the subsequent physical examinations. Chest x-ray showed mild cardiomegaly with left ventricular prominence.

Laboratory data revealed a glomerular filtration rate (iothalamate ^{125}I) of 95 ml/min. A repeat percutaneous transfemoral aortogram again showed marked stenosis of both the left and right renal arteries, and a barely visible stenotic renal artery supplying the upper pole of the right kidney (Fig. 1). Selective renal vein renin determinations [measured by the radio-immunoassay technic (Haber, Koerner, Page, et al)[3]] with the patient on an unrestricted salt intake and off all drugs for 3 days were obtained as follows: right renal vein 0.15, left renal vein 0.14, inferior vena cava 0.10, brachial vein 0.10 ng/ml/hr (normal 1-3 ng/ml/hr).

Renal split-function studies were also obtained and were as follows: GFR, right 40.7, left 55.9 ml/min; sodium concentration, right 65, left 95 mEq/liter; urine osmolality, right 480; left 520 mOsm/kg, flow rate, right 2.5, left 4.1 ml/min; renal plasma flow, right 163, left 246 ml/min.

An angiotensin infusion test was performed; a 20 mm rise in diastolic blood pressure resulted from the infusion of 2.02 ng/kg/min of angiotensin II. A technecium-99M renal scan revealed a slight decrease in concentration of the isotope bilaterally; a hippuran ^{131}I renogram demonstrated diminished arterial blood flow to both kidneys.

Subsequent to these studies, the patient was treated with 400 mg of spironolactone daily, which resulted in no significant decrease in his blood pressure over a 2 week period. The patient was then placed on alpha methyldopa, 500 mg four times daily, guanethidine, 200 mg daily and Diazide^{R} (25 mg hydrochlorothiazide, and 50 mg triamterene) one capsule twice daily. He was followed as an outpatient, and was noted to have diastolic blood pressures of 120-130 mm Hg while receiving these medications. Urinary aldosterone excretion on no drugs and on an unrestricted salt intake was 14.4 µg/24 hours (normal 5-19 µg/24 hours).

He was admitted to the hospital on 25 Feb 71, with uncontrollable hypertension. His GFR had decreased from 95 to 58 ml/min; his serum creatinine had increased from 1.2 to 1.8 mg/100 ml. Renogram and renal scan showed marked deterioration of renal function on the right, as compared to the previous study.

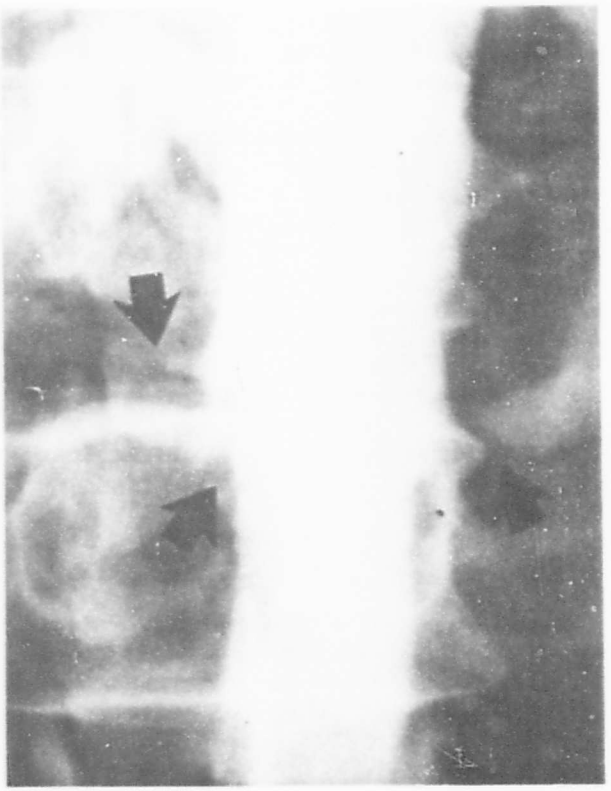

Fig. 1. Percutaneous femoral aortogram (December 1970) demonstrating the marked stenoses (arrows) of both renal arteries and the nearly obliterated artery to the superior pole of the right kidney.

Physical examination was unchanged except for the presence of a Grade II/ VI diastolic murmur heard at Erb's point and over the aortic area. ECG showed left ventricular hypertrophy with ST-T wave changes. Chest x-ray again showed mild cardiomegaly. A brachial vein renin obtained on an unrestricted salt diet was 0.73 ng/ml/hr. The patient was then given 80 mg of furosemide intravenously and placed on a 10 mEq/day sodium diet for 3 days which resulted in a 2.5 kg weight loss. Selective renal vein renin determinations following this regimen were as follows: right vein 6.06, left renal vein 3.65, and brachial vein 3.67 ng/ml/hr. Despite this 2.5 kg weight loss, the patient's blood pressure remained elevated at 180/120 mm Hg. Repeat aortogram showed worsening of the stenosis of the superior right renal artery, with no change in the stenosis of the lower right and left renal arteries. The patient was transferred to the Surgical Service, where, on 2 March, bilateral aorto-renal artery grafts, using autogenous saphenous vein, were performed. At operation, the surgeon was unable to pass a probe through either renal artery orifice. Postoperatively, the patient's creatinine dropped to 1.0 mg per 100 ml, and his GFR rose to 108 cc/min, with selective renal vein renins (on an unrestricted salt intake) as follows: right renal vein 2.41, left renal vein 2.43, inferior vena cava 2.83 ng/ml/hr.

The patient's blood pressure was 120/80 mm Hg at the time of discharge from the hospital on no medication. He was followed in the outpatient clinic for 3 months with no change in GFR. Blood pressure remained in the range of 140-150/90-94 mm Hg on no medication.

On 19 Oct 1971, the patient was seen with complaints of severe headache, dizziness, and visual scotomata. Physical examination revealed a blood pressure of 200/130 mm Hg in the right arm, with no change in blood pressure from sitting to standing positions. He had gained 2.5 kg. There was bilateral Grade II KW retinopathy. Cardiopulmonary examination was unremarkable except for the presence of an audible S4 and mild cardiomegaly. A bruit was audible over the left flank. Laboratory data revealed a serum creatinine of 1.9 mg/100 ml, BUN 28 mg/100 ml and an iothalamate ^{125}I GFR determination of 40 ml/min. Renal vein renin determinations, on an unrestricted salt intake and off all drugs for 3 days, were as follows: right renal vein 0.61, left renal vein 0.80, inferior vena cava 0.80, brachial vein 0.68 ng/ml/hr.

A percutaneous transfemoral aortogram was performed, and revealed marked bilateral renal artery stenosis at the site of the previous saphenous vein grafts (Fig. 2). Renogram and renal scan again showed moderate decrease in blood flow to both kidneys with decreased concentration of the isotope bilaterally. A ^{51}Chromium blood volume determination revealed a blood volume of 500 cc greater

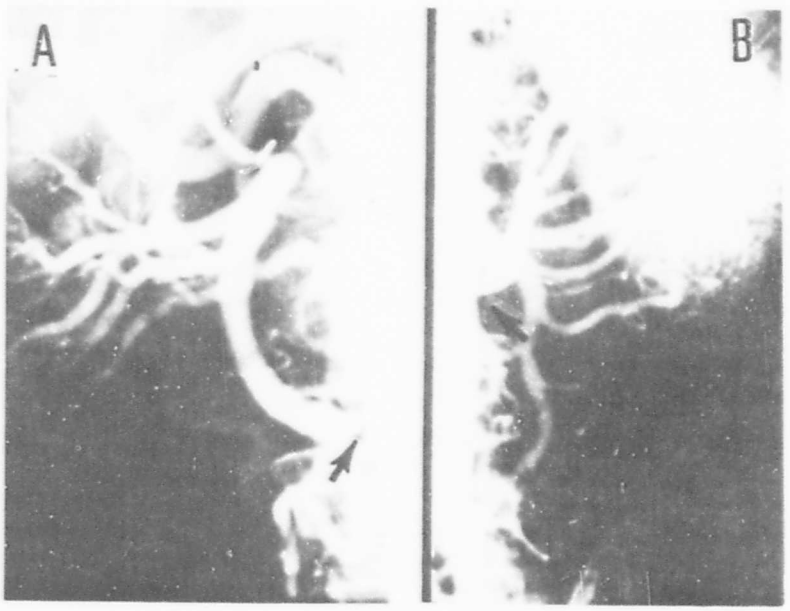

Fig. 2. Percutaneous femoral aortogram (October 1971) demonstrating the bilaterally stenosed (arrows) renal artery autogenous saphenous vein grafts-A, right renal artery; B, left renal artery.

than the predicted blood volume.

On 29 Oct, the patient was taken to the operating room, where both saphenous vein grafts were opened longitudinally, close to the aorta, saphenous vein patches were applied bilaterally resulting in widely patent renal arteries with good flow. The patient tolerated the procedure well and postoperatively his blood pressure was well controlled with alpha methyldopa 250 mg twice daily and hydrochlorthiazide 50 mg daily. Over the ensuing 2 weeks, his serum creatinine gradually dropped to 1.2 mg/100 ml and his GFR increased to 100 ml/min. Also, postoperatively, his weight decreased by 2.5 kg to his usual weight of 70.5 kg. Postoperative renin determinations on an unrestricted salt intake and on no drugs were 2.05 ng/ml/hr in the standing position and 1.34 ng/ml/min in the supine position. ^{51}Chromium blood volume equaled the predicted value. The patient continues to do well at the present time.

DISCUSSION

This patient presented with the unexpected findings of hypertension, bilateral renal artery stenosis, and low plasma renin concentration both in renal vein and peripheral venous blood. The patient was quite appropriately sensitive to infused angiotensin. That our patient was able to elaborate renin was demonstrated when he was placed on a low salt diet and given furosemide. These maneuvers resulted in a greater than thirty-fold increase in both renal and peripheral plasma renin concentrations, but did not decrease his blood pressure. This also resulted in a right renal venous renin concentration greater than 1.6 times that of the renin concentration of the left renal vein. This lateralization of renin production only became apparent with contraction of the extracellular fluid space with the low sodium diet and furosemide. This maneuver, however, did not result in a markedly elevated peripheral plasma renin concentration. Because of these findings our patient was not felt to be a candidate for the surgical correction of the renal vascular lesions. Repair of these lesions was undertaken only when renal function had deteriorated markedly. It was a surprise therefore to find that renal artery surgery not only restored the glomerular filtration rate to normal, but also resulted in marked relief of the patient's hypertension, a type of hypertension that had previously been refractory to multiple drug therapy. Of great interest was the seemingly paradoxical rise of the plasma renin concentration to the normal range.

When the patient re-developed hypertension in October 1971 subsequent to re-stenosis of the saphenous vein-renal artery grafts, he was again noted to have hyporeninemia. His blood volume was 500 cc

greater than predicted. Repair of the stenosis resulted in amelioration of his hypertension, return of his blood volume to the predicted value, a 2.5 kg weight loss and a rise in plasma renin concentration to the normal range.

Interference with the blood supply to one kidney in humans and experimental animals results in hypertension (Goldblatt, Lynch, Hanzel;[4] Gross, Brunner, Ziegler;[5] Regoli, Brunner, Peters, et al[6]). This form of hypertension is characterized by increased renin release from the involved kidney. The exact mechanisms responsible for this form of hypertension are not clear, but they are related to increased formation of angiotension II which has a strong pressor action, secondary aldosteronism, and fluid retention by the ischemic kidney. Marked fluid retention does not occur because of increased salt excretion by the non-ischemic kidney. Another cause of such hypertension may be the release of an additional pressor agent as postulated by Grollman and Krishnamurty.[7]

In the animal model of unilateral renal artery stenosis, removal of the non-ischemic kidney results in a fall of the plasma renin concentration to normal, nevertheless hypertension persists or worsens (Regoli, Brunner, Peters, et al;[6] Gross[8]). Treatment of animals with unilateral renal artery stenosis with antirenin antibodies results in relief of the hypertension; this treatment has no effect on hypertension however, when the uninvolved kidney has been removed (Brunner, Kirshman, Sealey, et al.)[9].

The animal model of a solitary ischemic kidney appears analogous to bilateral renal artery stenosis observed in this patient in that the ischemic kidney or kidneys are responsible for maintaining sodium balance. It seems likely to us that the following events transpire when the entire renal mass is ischemic. Initially there is an increase in renin release and salt retention, both secondary to renal ischemia, which results in hypertension and extracellular volume expansion. Volume expansion eventually results in suppression of sodium reabsorption and renin release analogous to that seen in "DOCA escape" (Wright, Knox, Howards, et al)[10]. Thus a new steady state is reached which is characterized by hypertension, on a volume rather than a pressor basis, suppressed renin release (resulting in depressed or normal plasma renin concentration) and expanded extracellular volume; these are the features which characterized our patient. Normally, in such patients, hypertension in the steady state is associated with an expanded blood volume but not associated with hyperreninemia; blood volume contraction via salt restriction and diuretic therapy should not relieve the hypertension since the beneficial effect of volume contraction would be counter-balanced by increased release of renin as was the case in our patient. It is

also possible that the hypertension seen in patients with unilateral renal artery stenosis, not associated with significant differences in renal vein renin concentration is maintained by a volume rather than pressor mechanism. Volume contraction by means of salt restriction, diuretic therapy, and the upright posture allows difference in renal perfusion to be expressed thus converting volume hypertension to pressor hypertension. Further studies are necessary to determine if this hypothesis has a solid basis of fact.

The implications of our findings are several. First one would not expect bilateral renal artery stenosis severe enough to result in bilateral renal artery ischemia to be characterized by hyperreninemia. The decision as to whether or not to operate on such patients should be made on grounds other than the plasma renin concentration such as hypertension refractory to medical therapy, or hypertension associated with deteriorating renal function. Second, one would not expect ischemia to the total renal mass to result in increased renin release until the ischemia had progressed to the point where the kidney is no longer able to maintain salt balance no matter what the level of extracellular volume. This point has relevance to the hyperreninemia seen in patients with endstage renal disease (Weidman, Maxwell, Luper, et al)[11] and to the increased renal renin release seen in patients with a transplanted kidney undergoing an acute rejection episode (Gunnells, Stickel, Robinson)[12]. Volume expansion occurs in both types of disorders but the degree of renal disease present is so marked that salt balance can no longer be maintained and renin release cannot be suppressed. The mechanisms by which volume expansion and sodium excretion inhibit renin release are still unclear, as is the reason explaining how the kidney that has lost its excretory function still preserves the ability to form supernormal amounts of renin.

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PRESENTATIONS AND/OR PUBLICATIONS

None

ANNUAL PROGRESS REPORT

PROJECT NO. 3A061102B71R-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 - 31 December 1971

Investigators:

Gary W. Allen, MD, Major, MC
Stephen Slogoff, MD, Major, MC
Joseph M. Garfield, MD, Major, MC

Reports Control Symbol MEDDH-288(R1)

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ABSTRACT

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

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In 1971, 179 of 301 patients whose disposition was completed at the US Army Institute of Surgical Research were given 476 anesthetics at this institute. Of the anesthetics given, 47.3% were halothane with or without nitrous oxide in oxygen; 27.3% were ketamine with the remainder consisting of nitrous oxide, methyoxyflurane, barbiturate anesthesia, local anesthesia, and regional blocks. Of those patients receiving anesthesia at the Institute of Surgical Research, the mean number of anesthetics per patient was 2.65. Two major intra-operative complications occurred during the year and will be discussed in detail in the text. No intra-operative deaths occurred.

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 4 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[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 anorectic 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.[1]

TYPES OF ANESTHESIA USED

A. GENERAL ANESTHESIA

1. Halothane with or without nitrous oxide in oxygen: about half (47.3%) of the anesthetics at our institution are performed with this combination of agents due to ease of administration, tranquil induction and emergence, relative lack of longlasting 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.[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.[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.[4]

3. Ketamine: Ketamine is an intravenous "dissociative" general anesthetic which has been available for clinical use for approximately two years. Over one-fourth (27.3%) 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. In 1970, one such complication occurred and was discussed in that annual report. No such complications, however, occurred this year. One intra-operative complication occurred with ketamine in 1971, and will be discussed in the Case Report Section.

B. REGIONAL ANESTHESIA

Again, in 1971, regional anesthesias, in particular, axillary brachial plexus block, supraclavicular brachial plexus block, spinal, ulnar nerve block, and superior laryngeal nerve block, were used to a significant degree (3.8% of all anesthetics given). 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. Serial 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. 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. Hypoxic Arrest in a Two-year Old

This 25-month-old Caucasian female was admitted with a 33% total body surface scald burn of which 26-1/2% was third degree. Her resuscitation was uneventful. On the third postoperative day, the patient had an upper gastrointestinal bleeding which responded to a milk and Maalox regimen. On the 10th postburn day, she was noted to have an increased respiratory rate (50-60/minute) and a fall in blood pressure with decreased urine output, which was corrected by the intravenous administration of fluids.

On the 11th day postburn, and again on the 13th day postburn, areas of fungal burn wound invasion were excised under anesthesia. On the 14th postburn day, the patient was hypotensive with a high CVP (15 cm H_2O). Arterial pH was 7.33 with Pco_2 of 41. Fluids and bicarbonate were administered and the patient was rapidly digitalized.

Because of rapid spread of the fungal wound invasion, the patient was scheduled for radical burn wound excision of both lower extremities on the 15th postburn day. The patient was brought to the operating room with a blood pressure of 150/80, pulse 140. Oxygen was administered by mask for five minutes and N_2O 30% with O_2 70% inhalation begun. Curare, 6 mg (0.7 mg/kg), was given intravenously, and ventilation was controlled beginning one minute after administration. Two minutes later, intubation was attempted but the patient developed laryngospasm and could not be intubated. The laryngospasm persisted, and two minutes later the patient was noted to be cyanotic; a sinus bradycardia (10/minute) was noted on ECG and pulses were absent. External cardiac massage was begun, the patient's trachea was intubated and 100% O_2 given; 0.1 mg epinephrine was given intravenously and good pulses were obtained. The operation was completed with 50% N_2O and oxygen.

Postoperatively, the oral endotracheal tube was left in place and the patient was ventilated with a Bird respirator.

Collapse of the right upper lung lobe was noted on the 17th postburn day, but this resolved and the patient was extubated on the 19th postburn day.

However, the patient died on the 26th postburn day with invasive burn wound sepsis.

Comment: Intubation was attempted too quickly after the curare administration, at a time when she was inadequately anesthetized.

Lack of palpable pulses during the bradycardia, indicating inadequate circulation, necessitated external massage which effectively prevented hypoxic arrest and CNS damage or death.

Case No. 2. Grand-Mal Convulsions Following Ketamine

The second patient was a 22-year-old white male admitted to the Institute of Surgical Research 5 days following a 50% total body surface (35% third degree) burn sustained in an automobile collision.

Initial care had been uncomplicated. On the fifth postburn day, the patient became disoriented and febrile, and the burn wound margins contained some cellulitis for which Lincomycin was begun. Bilirubin was 2.9 mg%, and the elevation was attributed to intravascular hemolysis. Despite normal chest X-rays, the arterial P_{O_2} was 50-60 torr. IPPB and O_2 were administered with clinical improvement.

On the 21st postburn day, the patient began having blood cultures positive for Providencia and a left groin catheter tip grew Providencia stuartii resistant to all antibiotics tested.

Despite a depressed sensorium on the 23rd postburn day, the patient was scheduled for debridement of burns and homografting. Arterial blood gases were as follows: P_{O_2} 74 torr (F_{IO_2} 0.21), P_{CO_2} 36 torr, pH 7.306. Sodium was 133.5 mEq/L and potassium 3.1.

Lungs were clear to auscultation and by X-ray, and vital signs were stable.

The patient was taken to the operating room, and atropine, 0.6 mg, was given intravenously. Anesthesia was induced with ketamine, 2 mg/kg (150) and supplemented thereafter with incremental doses of one mg/kg ketamine.

Twenty-five minutes after induction, the patient became completely apneic and required oxygen and positive pressure ventilation by mask. The apnea lasted three minutes, and normal ventilation was then resumed. No cyanosis, bradycardia, or hypotension

was noted. Oxygen was given throughout the remainder of the anesthetic (total anesthesia time--2 hours), and 2 more similar periods of apnea were noted.

The anesthetic was otherwise uncomplicated except for mild hypotension (90/60), occurring toward the end, which responded to the administration of fluids including whole blood. Rectal temperature at the end of the procedure was 90.5°F despite warming of blood and of the patient by a K-thermia blanket.

Postoperatively, the patient required nasotracheal intubation for continuing apneic episodes. Despite IPPB with a Bird Mark XIV ventilator, pulmonary function deteriorated. Arterial blood gases drawn 3 hours postoperatively revealed a Po_2 of 59 torr ($FIo_2$0.5), Pco_2 38 torr, pH 7.281. Recurrent grand-mal seizures required large amounts of intravenous phenobarbital.

The patient became hypotensive, and large amounts of fluid, blood, and vasopressors failed to maintain the circulation. The patient died 7-1/2 hours postanesthesia.

At autopsy, evidence of burn wound colonization with bacteria and fungi and diffuse intravascular coagulation was found. No gross CNS lesions were noted.

Comment. Recent studies have revealed that ketamine may induce seizure activity and indeed this may be the mechanism of ketamine anesthesia. Whether ketamine was responsible for the seizures noted postoperatively in this patient is unknown. CNS hypoxia from inadequate circulation or from microthrombi associated with diffuse intravascular coagulation may also produce seizures.

However, ketamine anesthesia cannot be discounted as a factor contributing to this patient's demise.

SUMMARY

When surgery is performed early in the hospital course in the burn patient (i.e., during the first 6 weeks, general anesthesia is usually necessary due to the nature of the procedure and to potential contamination at regional injection sites. Although halothane, with or without nitrous oxide in oxygen, is generally used, other agents are satisfactory. Ketamine is being used more and more as a general anesthetic in our Institute for debridement, chondrectomies, orthopedic procedures, and other ward procedures.

For surgery during the reconstructive period of the burn (6 weeks and beyond), regional anesthesia can often be used, as long

as the patient is not septicemic and does not have infected skin areas at or immediately adjacent to the siteof nerve blocking. Ketamine is also indicated for certain procedures done at this time.

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PRESENTATION

Garfield JM: The Influence of Preoperative Information of Psychologic Responses to Ketamine and Halothane Anesthesia in Burn Patients. Clinical Session, Amer Burn Assn, San Antonio, Tex, 16-17 Apr 1971.

TABLE I. OVERALL PATIENT DATA, USAISR (1968-1971)

	YEAR	1968	1969	1970	1971
A	TOTAL NO. OF PATIENTS	389	294	321	301
B	NO. OF PTS. RECEIVING ANES. (ISR + ELSEWHERE)	259	189	208	190
C	% OF PTS. RECEIVING ANES. (ISR + ELSEWHERE)	66.6%	64.3%	64.8%	63.1%
D	TOTAL NO. OF ANESTHETICS ADMIN. (ISR + ELSEWHERE)	921	662	585	532
E	TOTAL NO. OF ANESTHETICS ADMIN. (ISR ONLY)	794	601	497	476
F	MEAN NO. OF ANESTHETICS PER PATIENT (D/A)	2.37	2.25	1.82	1.77
G	MEAN NO. OF ANESTHETICS PER PATIENT WHO RECEIVED ANESTHESIA (D/B)	3.56	3.50	2.81	2.80
H	TOTAL BODY SURFACE - %	29.5%	36.2%	30.3%	30.9%
	THIRD DEGREE - %	8.8%	11.5%	11.9%	13.6%

TABLE 2

NATURE OF SURGERY (PRIMARY) 1971

PROCEDURE	ISR NO	ISR %	ELSEWHERE NO	ELSEWHERE %
DEBRIDEMENT AND/OR HOMOGRAFT	74	15.5	41	73.2
AUTOGRAFT	252	52.9	2	3.6
ORTHOPEDICS	62	13.0	2	3.6
EAR (CHONDRECTOMY)	19	4.0	0	0
EYE AND LID	18	3.8	2	3.6
INTRA-ABDOMINAL	8	1.7	5	8.9
TRACHEOSTOMY AND BRONCHOSCOPY	22	4.6	4	7.1
OTHER	21	4.4	0	0
TOTAL	476		56	

5-9

TABLE 3

TECHNIQUES OF ANESTHESIA - 1971

	ANESTHESIA ISR	PER CENT OF TOTAL	ANESTHESIA ELSEWHERE	PER CENT OF TOTAL	TOTAL ANESTHESIA	PER CENT OF TOTAL
TOTAL	476	100.0	56	100.0	532	100.0
GENERAL ANESTHESIA	449	94.3	54	96.4	503	94.5
HALOTHANE	225	47.3	24	42.9	249	46.8
N_2O	89	18.7	12	21.4	101	19.0
METHOXYFLURANE	1	0.2	3	5.4	4	0.8
KETAMINE	130	27.3	14	25.0	144	27.1
BARBITURATE	1	0.2	0	0.0	1	0.2
OTHER OR UNKNOWN	3	0.6	1	1.8	4	0.8
LOCAL ANESTHESIA	27	5.7	2	3.6	29	5.5
REGIONAL BLOCKS	18*	3.8	0	0.0	18	3.4
LOCAL	9	1.9	2	3.6	11	2.1

AXILLARY BRACHIAL PLEXUS BLOCK - 8
SUPRACLAVICULAR BRACHIAL PLEXUS BLOCK - 2
SPINAL - 4
ULNAR NERVE BLOCK - 1
SUPERIOR LARYNGEAL NERVE BLOCK - 3

TABLE 4

EMPLOYMENT OF ANESTHETIC AGENTS AT ISR, 1964-1971 (IN PER CENT)

AGENT	1964	1965	1966	1967	1968	1969	1970	1971
HALOTHANE	87.0	68.3	92.9	97.0	99.4	86.9	66.8	47.3
N_2O, O_2	0.6	3.5	1.3	0	0.3	4.7	8.4	18.7
METHYOXYFLURANE	0	20.0	0	0	0.1	0.8	0.4	0.2
CYCLOPROPANE	4.8	0.6	0.7	0	0	0	0	0
NEUROLEPTANALGESIA	0	0	2.0	3.0	0	1.0	0.4	0
KETAMINE	0	0	0	0	0	4.8	18.7	27.3
REGIONAL BLOCK AND LOCAL	6.0	8.0	1.2	0	0.3	1.8	5.2	5.7
OTHER OR UNKNOWN	1.6	0.0	1.9	0	0	0	0	0.6
TOTAL NO. OF ANESTHETICS	332	495	713	670	794	601	497	476

TABLE 5

MULTIPLE HALOTHANE ADMINISTRATION*

YEAR	NO. PATIENTS RECEIVING ANESTHESIA	NO. PATIENTS RECEIVING MULTIPLE HALOTHANE	PER CENT
1969	189	132	69.8
1970	208	94	45.2
1971	190	52	27.4

*INCLUDES ANESTHETICS RECEIVED PRIOR TO ARRIVAL AT ISR

TABLE 6

GENERAL ANESTHETIC INDUCTION AGENTS, ISR-1971

AGENT	NO. OF INDUCTIONS	PER CENT OF TOTAL
IV BARBITURATE	194	43.2
IV KETAMINE	120	26.7
IM KETAMINE	17	3.8
IV OTHER	11	2.4
INHALATION	107	23.8
TOTAL	449	99.9

TABLE 7

USE OF MUSCLE RELAXANTS, ISR - 1971

TOTAL GENERAL ANESTHETICS	NO. OF ANESTHETICS WHERE MUSCLE RELAXANTS USED	dT-CURARINE	GALLAMINE	SUCCINYLCHOLINE
449	81	19	50	14
% OF TOTAL GEN. ANESTH.	18.0%	4.2%	11.1%	3.1%

	NO. OF ANESTHETICS	% OF TOTAL GENERAL ANESTHETICS
MUSCLE RELAXANT USED	81	18.0%
USED FOR INTUBATION	62	13.8%
USED FOR RELAXATION	38	8.5%

AGENT	NO. OF ANESTHETICS	NO. OF ANESTHETICS WHERE MUSCLE RELAXANT USED	%
N_2O, O_2	89	66	74.2%
HALOTHANE, N_2O, O_2	225	14	6.2%
LOCAL FOR TRACH.	9	1	

5-13

TABLE 8

TYPE OF AIRWAY DURING GENERAL ANESTHESIA, ISR-1971

AIRWAY	NO. OF ANESTHETICS	% OF TOTAL NO. OF GENERAL ANESTHETICS
MASK	167	37.2
ENDOTRACHEAL TUBE		
ORAL	147	32.7
NASAL	12	2.7
TRACHEOTOMY	13	2.9
NATURAL AIRWAY	110	24.5
TOTAL	449	100.0

TABLE 9

MORTALITY OF THOSE RECEIVING ANESTHESIA, ISR-1971

TOTAL GIVEN ANESTHESIA	179	INTRAOPERATIVE DEATHS	0
DEATHS	28	DIED WITHIN 24 HOURS	3
PER CENT	15.6%	24 HOURS TO 1 WEEK	16
OVERALL MORTALITY	25.4%	GREATER THAN 1 WEEK	9

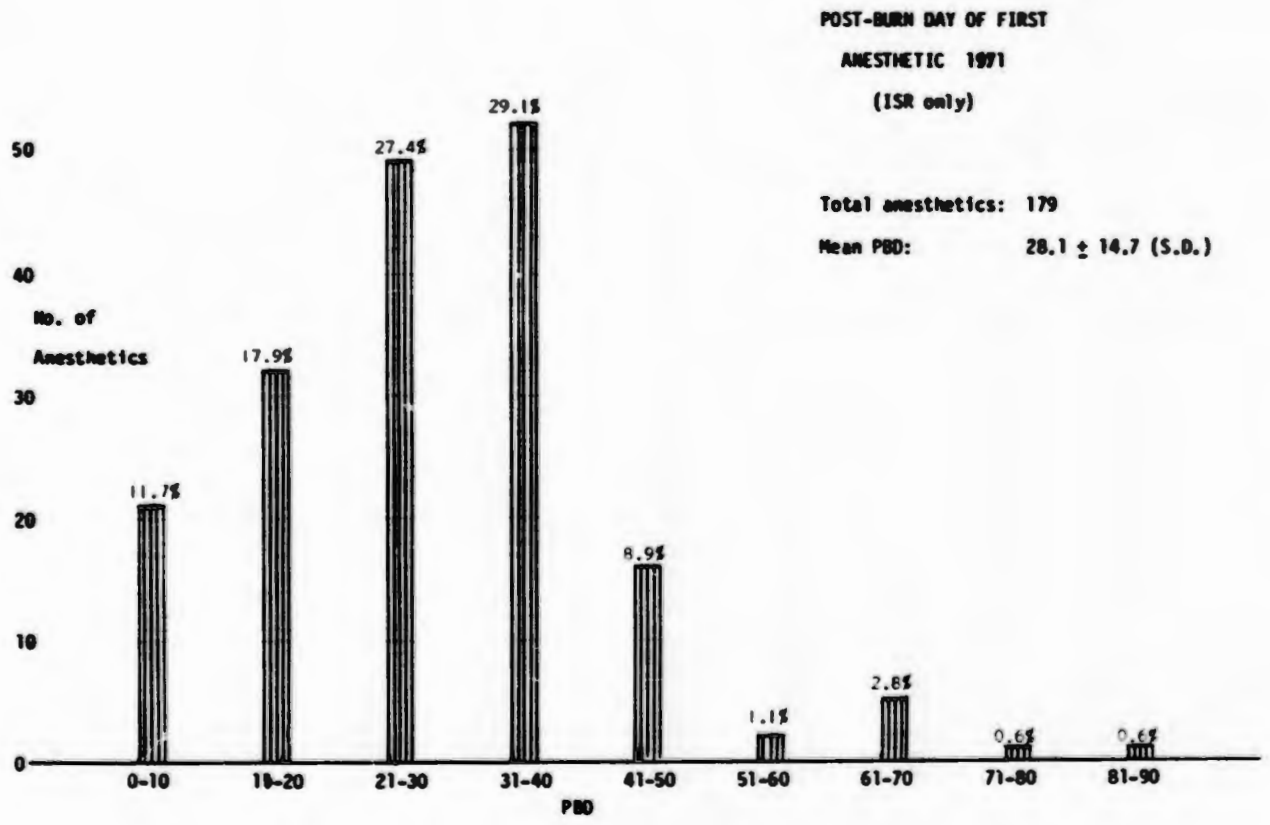
POST-BURN DAY OF FIRST
ANESTHETIC 1971
(ISR only)
Total anesthetics: 179
Mean PBD: 28.1 ± 14.7 (S.D.)
No. of
Anesthetics
50
40
30
20
10
0
11.7%
17.9%
27.4%
29.1%
8.9%
1.1%
2.8%
0.6%
0.6%
0-10
10-20
21-30
31-40
41-50
51-60
61-70
71-80
81-90
PBD

Figure 1

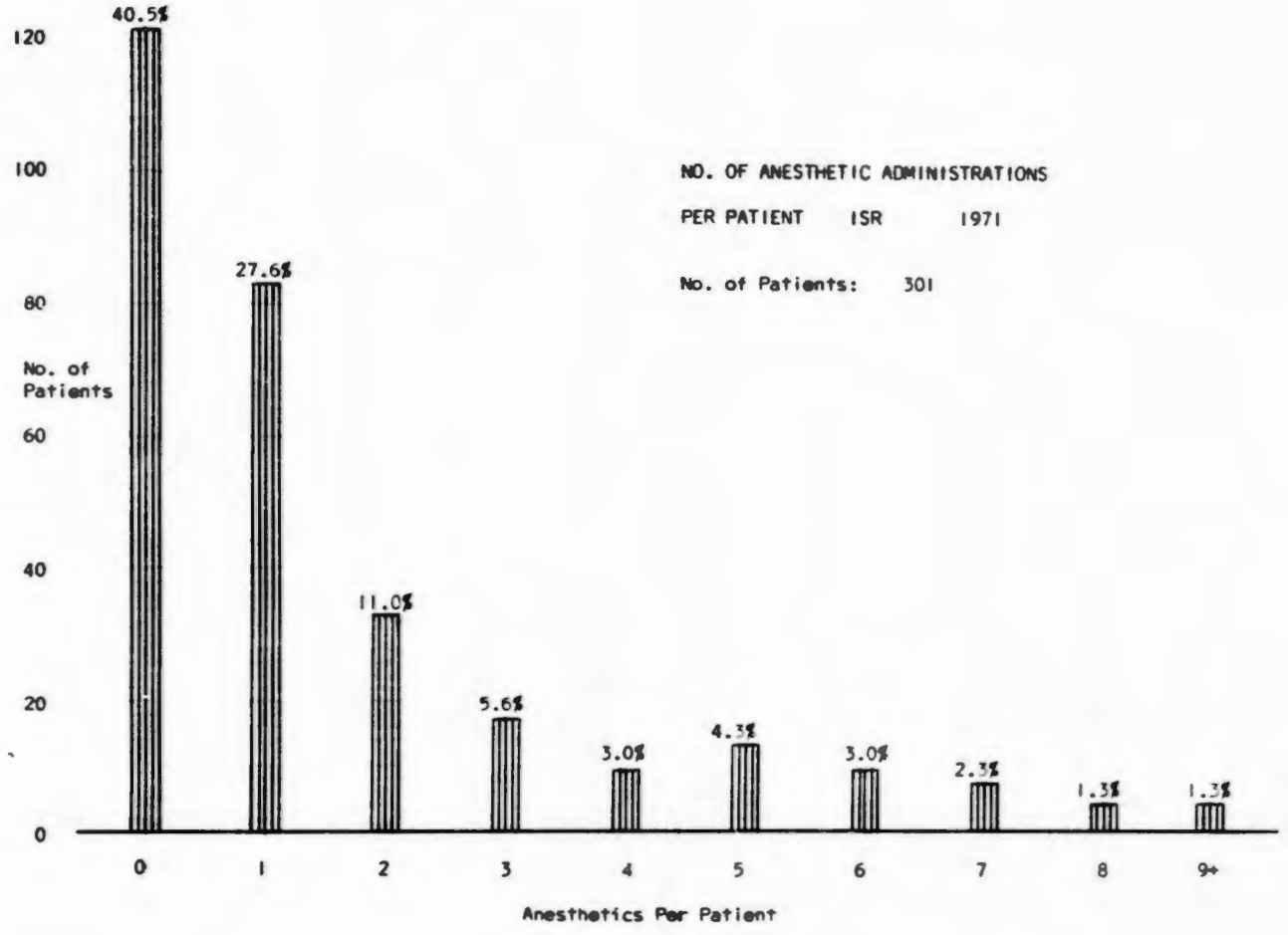
NO. OF ANESTHETIC ADMINISTRATIONS
PER PATIENT ISR 1971
No. of Patients: 301
No. of Patients
120
100
80
60
40
20
0
40.5%
27.6%
11.0%
5.6%
3.0%
4.3%
3.0%
2.3%
1.3%
1.3%
0
1
2
3
4
5
6
7
8
9+
Anesthetics Per Patient

Figure 2

6

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OD 6980	72 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	3A061102B71R	01	309
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Procede with Security Classification Code): (U) Use of 133 Xenon in Early Diagnosis of Inhalation Injury in Burned Military Personnel (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS:
003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
71 06	Cont	DA	C. In-House

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

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
FISCAL YEAR	PRECEDING 72	0.3	15.9
	CURRENT 73	0.1	4.0

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 A Moylan, Jr, MAJ, MC
TELEPHONE: 512-221-3301
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: Douglas Wilmore, 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) Inhalation Injury; (U) 133 Xenon Lung Scan; (U) Blast Injury; (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 develop an objective test for the early diagnosis of inhalation injury in combat wounded personnel.

24. (U) All patients with flame or blast injuries admitted to the USAISR receive a 133 Xenon lung scan on admission. The results of the scans are correlated with the clinical course and autopsy findings.

25. (U) 71 06 - 72 06 Initial evaluation of the results demonstrate the abnormal 133 Xenon lung scans provide an early diagnosis of inhalation injury prior to onset of clinical signs. Further studies will be carried out to assess the value of this test.

*Applies 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. 3A061102B71R-01, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: USE OF ^{133}XENON IN EARLY DIAGNOSIS OF INHALATION INJURY IN BURNED MILITARY PERSONNEL

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

1 July 1971 - 30 June 1972

Investigators:

Joseph A. Moylan, Jr., MD, Major, MC
Douglas W. Wilmore, MD, Major, MC
David E. Mouton, MD, Major, MC *
Basil A. Pruitt, Jr., MD, Colonel, MC

* Nuclear Med Off, Department of Medicine, Brooke General Hospital, Brooke Army Medical Center, Fort Sam Houston, Texas 78234

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

REPORT TITLE: USE OF ^{133}XENON IN EARLY DIAGNOSIS OF INHALATION INJURY IN BURNED MILITARY PERSONNEL

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

Period covered in this report: 1 July 1971 - 30 June 1972.

Investigators: Joseph A. Moylan, Jr., MD, Major, MC
Douglas W. Wilmore, MD, Major, MC
David E. Mouton, MD, Major, MC *
Basil A. Pruitt, Jr., MD, Colonel, MC

Reports Control Symbol MEDDH-288(R1)

Prompt diagnosis and treatment of inhalation injuries following thermal trauma are often hampered by lack of early objective findings of respiratory tract injury. Patients who subsequently develop wheezing and produce thick carbonaceous sputum followed by severe bronchorrhea and progressive pulmonary insufficiency may initially have no facial, oral, or pharyngeal burns and have normal chest roentgenograms, spirograms, and blood gases.

Using ^{133}Xenon, the pulmonary alveolar diffusion and ventilatory characteristics were prospectively studied in 50 patients following extensive thermal trauma (20 to 95% total body surface burn injury). One-third of the patients demonstrated inhalation injury documented by characteristic clinical and pathologic criteria. Ventilation clearance of ^{133}Xenon gas was significantly delayed by the tracheobronchial injury. This change in scanographic findings occurred in patients before the onset of clinical signs or symptoms. Moreover, the course of improvement in inhalation injury was accompanied by serial improvement of ^{133}Xenon clearance as documented with scanograms. The perfusion-diffusion scans of all patients were correlated with clinical findings, chest roentgenograms, pulmonary function tests, blood gas analysis, tracheal cultures, bronchoscopic examination and autopsy data. The use of the ^{133}Xenon perfusion-diffusion scan allows an objective evaluation of various therapeutic modalities in the treatment of inhalation injuries.

Inhalation injury
^{133}Xenon lung scan

* Nuclear Med Off, Dept of Medicine, Brooke General Hosp., Brooke Army Medical Center, Fort Sam Houston, Texas 78234

USE OF ^{133}XENON IN EARLY DIAGNOSIS OF INHALATION INJURY IN BURNED MILITARY PERSONNEL

Chemical tracheobronchitis follows inhalation of smoke and other incomplete combustion products. The severity of mucosal injury is probably proportional to the concentration and duration of contact of the noxious agents. Inhalation injury predisposes the respiratory tract to infection and causes a significant increase in the mortality rate associated with thermal injury.[1] Signs of inhalation injury often appear later in the postburn period and in some cases are evident only by autopsy. Early diagnosis, previously difficult, permits early therapy and may minimize both acute disability and fatal septic pulmonary complications.

^{133}Xenon perfusion and ventilation tests are precise measurements of regional function in patients with pulmonary disease.[2,4] This study using ^{133}Xenon lung scans evaluates the early postburn period disturbances in ventilation in the absence of gross roentgenologic changes in burn patients and correlates these findings with the presence of inhalation injury.

METHODS

Fifty consecutive patients with thermal injury due to flame, admitted to the US Army Institute of Surgical Research within 48 hours of injury, have been studied. All patients with scald and chemical burns were excluded from this report. Upon admission, a complete history was obtained, with care being taken to define pre-injury respiratory disease and smoking history. Following a thorough physical examination, a chest x-ray was performed and arterial blood was drawn for gas analysis.

In the radioisotope laboratory, a ^{133}Xenon scan was performed, using a Nuclear/Chicago scintillation camera with an 11.5-inch sodium iodide crystal and a diverging collimator by injecting 6-10 microcuries of ^{133}Xenon dissolved in saline into either the antecubital or femoral vein. Serial scintiphotograms were obtained every 4 seconds during the initial 28-second period, then every 30 seconds until ventilatory clearance of the radioactive gas was completed.

A normal scan demonstrated complete and equal clearance of the ^{133}Xenon within a 90-second period while deviations from this pattern were considered abnormal.

The physical findings, clinical course and laboratory results of all patients and the autopsy results for those patients who died were carefully recorded and related to the observed scintiphotographic changes.

RESULTS

Fifteen of the 50 patients studied had abnormal ^{133}Xenon scans on admission to this Institute. The average burn size was 42% total body surface for the total group and 50% for those patients with abnormal xenon scans. Forty-one patients were injured in an enclosed space, although only 29 accidents were considered "closed space injuries." Eighty-seven per cent of the patients with abnormal ^{133}Xenon scans and 22% of patients with normal scintiphotograms were injured in a "closed space."

Facial burns were present in 86% of patients with normal xenon scans, while burns of the head and neck occurred in two-thirds of those with delayed isotope clearance (see table). Two patients with abnormal scintiphotograms had intra-oral burns. Carbonaceous sputum was produced in one-half of the patients with ^{133}Xenon ventilation disturbances and was not produced by patients with normal scans. In this series, carbonaceous sputum was seen early in some patients but was usually observed first on the third postburn day. Hoarseness was noted in 4 of the 15 patients with abnormal scans, beginning the second postburn day, and did not occur in any patients with a negative scan. Wheezing was subsequently recorded in 7 patients who had delayed isotope clearance, usually starting the third day postinjury. None of the patients with normal scans had wheezing during their hospitalization.

Pulmonary atelectasis or infiltrates demonstrated by roentgenogram were observed during the initial week postinjury in two-thirds of the patients with delayed isotope clearance, while patients with normal scans had a 6% incidence of abnormal chest X-ray findings.

Clinical Findings Versus Scans

	Abnormal Scans	Normal Scans
Face burns	10/15 (66%)	30/35 (86%)
Intra-oral burns	2/15 (13%)	0/35 (0%)
Carbonaceous sputum	8/15 (53%)	0/35 (0%)
Hoarseness	4/15 (27%)	0/35 (0%)
Wheezing	7/15 (47%)	0/35 (0%)
Mortality rate	9/15 (60%)	10/35 (28%)

Bronchoscopy, carried out in 5 patients with abnormal scintiphotograms, revealed severe inflammation of the mucosa, areas of mucosal sloughing, tissue casts and carbonaceous material. Admission Pa_{O2} on room air was 82 mmHg for the normal scan group and 77 mmHg for the abnormal ^{133}Xenon scan group.

The mortality rate was significantly higher for the patients with abnormal scintiphotograms (60%) compared with the other group (28%). Autopsies were performed in all 19 patients in this study who expired. Six of the 9 patients dying in the group with abnormal scans had pathological pulmonary findings of carbonaceous material, mucosal inflammation or sloughing without infection, and bronchial casts, and all were diagnosed as having inhalation injuries. The remaining 3 cases had mucosal damage with superimposed bacterial infection. The autopsies of the 10 patients with normal scans who expired revealed normal lung tissue in 8 and hematogenous pneumonia in 2.

DISCUSSION

^{133}Xenon and scintiphotograms are widely employed to measure both the perfusion and the diffusion-ventilation phases of pulmonary function. The accuracy of this technic in documenting abnormalities of the components of respiratory function has been reported by several investigators. Since inhalation injury primarily affects the airways, ^{133}Xenon scans were employed to evaluate ventilatory changes in the lungs of burn patients.

There was uniform agreement between the ^{133}Xenon scan diagnosis of inhalation injury and clinical pathological findings. In patients with abnormal scans, the diagnosis of inhalation injury was confirmed clinically by the production of carbonaceous sputum, hoarseness, wheezing, visual changes at bronchoscopy and/or autopsy findings. Two or more clinical diagnostic criteria for inhalation injury were present in each case except for 2 patients who expired within 72 hours of injury and demonstrated conclusive autopsy findings. Although most patients ultimately exhibited multiple clinical signs of inhalation injuries, these findings were often not discernible until roentgenologic changes were obvious or the individual developed a complication secondary to the inhalation injury.

X-ray abnormalities were common in the group with abnormal scans but were often nonspecific and frequently developed after the third postburn day. Over 90% of the patients with normal xenon clearance at admission had no pulmonary complication requiring treatment during their hospital course and only 2 patients in this "normal" group had radiological findings consistent with pneumonia. In each of these patients, septicemia was documented prior to the onset of the x-ray abnormalities, and the pneumonia was considered hematogenous in origin.

Although no false positive or negative diagnoses were made in this series, both are possible. A complete history of preinjury pulmonary disease and cigarette smoking should be obtained. Diseases such as bronchitis, bronchiectasis, asthma, and viral pneumonia can produce ventilatory abnormalities on xenon scintiphotograms similar to those we have observed following inhalation injury. The admission chest film, usually normal immediately following inhalation injury,[1] may be helpful in diagnosing pre-existent pulmonary disease. Patients with a prior history of respiratory tract disease or heavy smoking whose xenon scan is abnormal on admission should have the test repeated after a brief but vigorous period of intermittent positive pressure breathing with bronchodilators. Such therapy may change the xenon clearance pattern of patients with medical pulmonary disease, reducing the incidence of false positive diagnoses.

Conversely, hyperventilation could theoretically result in such rapid clearance of the radioactive gas from the lung that inequalities in the ventilation pattern might be missed, giving a false negative result. All patients in this series had a respiratory rate of 20 or less at the time of initial testing.

Postponement of the scan examination to the fourth postburn day or later may be another source of false negative tests. The maldistribution of ventilation and delay of gas clearance are presumed to be secondary to airway inflammation and occlusion which, if minimal, may resolve promptly. Xenon clearance patterns reverted to normal by the fourth postburn day in 80% of the patients, although gas "trapping" persisted for up to 10 days in severe cases.

The perfusion patterns observed with xenon scan technic employed in this study were normal in every patient. Although both the intravenous or inhaled routes using xenon gas can be used to demonstrate ventilation abnormalities, intravenous administration technic is more adaptable to the burn patient, for no active patient cooperation is required. The inhalation technic with ^{133}Xenon requires active patient participation, including lengthy periods of breath holding, often not possible in severely ill patients. In addition, the ^{133}Xenon scan is easy and safe to perform. Calculations show that an intravenous injection of 40 microcuries of ^{133}Xenon, or 4 times the amount used in this study, results in a 10-millirad gonadal dose, a small percentage of the gonadal dosage delivered by conventional pelvic x-ray.[3]

In this series, evidence of inhalation injury occurred in 30% of the patients, an incidence 10 times higher than previously appreciated following flame burn using usual diagnostic criteria. This increased incidence of inhalation injury represents cases which may not have been diagnosed without the ^{133}Xenon scan because of lack of definite clinical signs.

SUMMARY

A 50-patient prospective study was completed to evaluate the use of ^{133}Xenon lung scans in the diagnosis of inhalation injury. Results demonstrated that ^{133}Xenon scintiphotography prior to the fourth postburn day is an easily performed, accurate diagnostic test for inhalation injury prior to the onset of symptoms, the validity of which has been confirmed by clinical and autopsy correlations. The lung scan can be performed in acutely burned patients without active patient cooperation. With careful attention to the individual's prior medical history, the admission chest x-ray and early scanning, the incidence of false interpretations of the xenon is low. Diagnosis by ^{133}Xenon scan allows institution of early therapy of this injury which may reduce secondary bacterial complications and lower the high mortality (60%) associated with inhalation injury.

REFERENCES

1. DiVincenti FC, Pruitt BA Jr, and Reckler JM: Inhalation injuries. J Trauma 11:109, 1971.

2. Jones RH, Coulam, CM, Goodrich JK, Sabiston DC: Radionuclide quantitation of lung function in patients with pulmonary disorders. Surgery 70:891, 1971.

3. Lassen NA: Assessment of tissue radiation dose in clinical use of radioactive inert gases, with examples of absorbed doses from 3-H2, 85-Kr and 133-Xe. Minerva Nucl 8:211, 1964.

4. Loken MK, Medina JR, Lillehei JP, L'Heureux P, Kush GS, Ebert RV: Regional pulmonary function evaluation using Xenon 133, a scintillation camera, and computer. Radiology 93:1261, 1969.

5. Loken MK, Westgate HD: Using Xenon-133 and a scintillation camera to evaluate pulmonary function. J Nucl Med, 9:45, 1968.

PUBLICATIONS AND/OR PRESENTATIONS

None

7

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OD 6970	72 07 01	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. SMSN INSTR	9. SPECIFIC DATA-CONTRACTOR ACCESS	10. LEVEL OF SUM
71 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	3A061102B71R	01	189
b. CONTRIBUTING				
c. CONTRIBUTING				

12. TITLE (Precede with Security Classification Code)

(U) A Critical Evaluation of Resuscitation Used in Burned Soldiers (44)

13. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 Clinical Medicine

14. START DATE	15. ESTIMATED COMPLETION DATE	16. FUNDING AGENCY	17. PERFORMANCE METHOD
70 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

FISCAL YEAR	a. PROFESSIONAL MAN YRS	b. FUNDS ($ in thousands)
PRECEDING 72	0.4	12.7
CURRENT 73	0.8	20.8

20. RESPONSIBLE DOD ORGANIZATION

NAME: US Army Institute of Surgical Research

ADDRESS: Ft Sam Houston, Tx 78234

RESPONSIBLE INDIVIDUAL

NAME: Basil A Pruitt, Jr, LTC, MC

TELEPHONE: 512-221-2720

21. GENERAL USE

FOREIGN INTELLIGENCE NOT CONSIDERED

22. PERFORMING ORGANIZATION

NAME: US Army Institute of Surgical Research
Laboratory Division

ADDRESS: Ft Sam Houston, Tx 78234

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

NAME: Joseph A Moylan, Jr, MAJ, MC

TELEPHONE: 512-221-4652

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: A D Mason, Jr, MD

NAME: DA

23. KEYWORDS (Precede each with Security Classification Code)

(U) Thermal Injury; (U) Resuscitation; (U) Cardiac Output; (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 evaluate the resuscitory effects of volume, sodium and colloid on thermal injury shock in order to improve therapy of the thermally injured soldier.

24. (U) 36 dogs received 30, 60 or 120 ml/kg; 3, 6, or 12 mEq Na/kg and 0 or 1 gram albumin/kg intravenously in all combinations. Two additional dogs served as untreated controls. Blood gases, electrolytes, renal indices and cardiac output, glomerular filtration rate, sodium clearance and balance were studied serially.

25. (U) 71 07 - 72 06 Volume and sodium administration are statistically significant independent factors in resuscitation. Colloid exerted no greater effect on cardiac output. Multiple regression analysis of the relative effects of sodium and volume ($y = a + b\,(vol) + c\,(Na)$) indicates that one milliequivalent of salt exerted a resuscitative effect on cardiac output equaled by 13 ml of volume and that these effects were additive.

Plasma sodium and renal sodium clearance were higher in animals receiving hypertonic solutions. Arterial pH was normal in all resuscitated animals. GFR paralleled cardiac output. Further work is being carried on to validate the accuracy of the formula in predicting return of cardiac output.

*Available to contractors upon additional request

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

ANNUAL PROGRESS REPORT

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

REPORT TITLE: A CRITICAL EVALUATION OF RESUSCITATION USED IN BURNED SOLDIERS

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

1 July 1971 - 30 June 1972

Investigators:

Joseph A. Moylan, Jr., MD, Major, MC
Arthur D. Mason, Jr., MD

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

REPORT TITLE: A CRITICAL EVALUATION OF RESUSCITATION USED 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 1971 - 30 June 1972

Investigators: Joseph A. Moylan, Jr., MD, Major, MC
Arthur D. Mason, Jr., MD

Reports Control Symbol MEDDH-288(R1)

The controversy concerning the primacy of volume or sodium in reversing postburn shock has been rekindled by studies of hypertonic saline resuscitation. Further, the position of colloid in such resuscitation is not certain. This study evaluates the individual effects of volume, sodium, and colloid on early postburn hemodynamics in awake, burned dogs.

Thirty-six dogs were randomly assigned to treatment groups in a factorial design, receiving 30, 60, or 120 ml/kg, 3, 6, or 12 mEq Na/kg, and 0 or 1 gm albumin/kg intravenously in all combinations. Two additional dogs served as untreated controls. Blood gases, electrolytes, renal indices, and cardiac output were measured pre-injury and serially during the study. Glomerular filtration rate, sodium clearance, and balances were calculated.

Volume and sodium administration are statistically significant independent factors in resuscitation. Colloid exerted no greater effect on cardiac output. Multiple regression analysis of the relative effects of sodium and volume ($y = a + b\,[vol] + c\,[Na]$), indicates that one milliequivalent of salt exerted a resuscitative effect on cardiac output equaled by 13 ml of volume and that these effects were additive.

Plasma sodium and renal sodium clearance were higher in animals receiving hypertonic solutions. Arterial pH was normal in all resuscitated animals. GFR paralleled cardiac output. Further work is being carried on to validate the accuracy of the formula in predicting return of cardiac output.

Thermal injury
Resuscitation
Cardiac output

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0E 6387	72 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. SMRY 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	3A061102B71R	01	316
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE: (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	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 PREVIOUS 72	0.3	6.3
FISCAL YEAR CURRENT 73	0.2	6.0

19. RESPONSIBLE DOD ORGANIZATION
NAME: US Army Institute of Surgical Research
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20. PERFORMING ORGANIZATION
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Burn Study Branch
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SOCIAL SECURITY ACCOUNT NUMBER:

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NAME: Joseph A Moylan, Jr, MAJ, MC DA

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS: (U) Military burn patients
(U) Intermittent Compression; (U) Postburn Edema; 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) Thirty 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 elevation 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 01 - 72 06 Preliminary evaluation reveals that extremities treated with the compression boot became less edematous than with conventional treatment. Removal of the boot is not followed by increased edema.

*(Available in continuation pages with preparer's approval)

DD, FORM 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. 3A061102B71R-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 1971 - 30 June 1972

Investigators:

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Paul Silverstein, MD, Major, MC
Joseph A. Moylan, Jr, MD, Major, MC
Douglas W. Wilmore, MD, Major, MC
Robert B. Lindberg, PhD
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Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A061102B71R-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

Period covered in this report: 1 July 1971 - 30 June 1972

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Reports Control Symbol MEDDH-288(R1)

Normal muscle contraction in an extremity produces pressure that acts on lymphatics and venules, propelling lymph and blood proximally. The 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 varicose veins and post 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.

Thirty early postburn patients with bilaterally symmetrical extremity burns will be cleansed and debrided on admission. Measurement of the PIP, DIP joints and wrists will be made with sterile unmarked aluminum tapes. Cotton swab cultures will be taken of all hands. Each patient's burns will be treated with Sulfamylon burn cream and one extremity, chosen randomly, will be placed in the compression boot for 48 hours. The opposite limb will be treated during this time in the usual manner with elevation, unlimited activity and night splints. At the end of 48 hours the limb will be removed from the boot and circumferential measurements will be repeated of both extremities. Both limbs will be recultured and then treated with elevation and unlimited motion. Range of motion of wrists and fingers will be made of both hands

at 21 days postburn and on discharge.

To date 6 patients with symmetrical burns of the upper extremities have been studied and in all 6 cases the arm treated with the intermittent compression machine showed a greater reduction in edema after 48 hours than the standard treatment. However, at 21 days there was no difference in function between the two hands receiving the two forms of therapy. At 21 days there was no difference in size between the two hands.

It is planned to study 24 more patients. However, if there is no difference in functional result or in demonstrable edema between the two methods of treatment, then the standard ISR therapy of immediate elevation, unlimited activity and no dressings would seem preferable to the intermittent compression machine, the use of which requires nursing time and special care.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0E 6392	72 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
	K. COMPLETION	U	U	NA	NL	☑ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62110A	3A062110A821	00	110
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Provide with security Classification Code): (U) Postburn Edema of the Upper Extremity: Evaluation of Present Treatments 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 08	72 01	DA	C. In-House

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

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PREVIOUS 72	0.3	7.4
FISCAL YEAR CURRENT 73	0	0

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21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Provide each with security Classification Code)
(U) Edema; (U) Upper Extremity

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 validity of present types of therapy thought to decrease upper extremity edema and to discover which if any is best.

24. (U) (a) One half of all patients with burned extremities will be treated with elevation, immobilization and a compression dressing for the first 72 hours. The other half will be treated open. Measurements of the limb will be made at 24 and 72 hours to see which group has more swelling.

(b) All patients will have extremity elevation at night for 8 hours. Measurements at night and the morning will determine if elevation decreases edema.

(c) All joints of the hand will be taken through a passive range of motion for 15 minutes. Measurements before and after will confirm if this decreases edema.

(d) Patient will be measured before and after 15 minutes of hydrotherapy with active mobilization to see if this decreases edema.

25. (U) 71 08 - 72 06 (a) Though edema decreased in both groups, extremities placed in a compression dressing were less edematous after 48 hours than controls.

(b) Eight hours of elevation successfully decreased edema.

(c) Passive manipulation did not decrease edema.

(d) Hydrotherapy and active exercises for 15 minutes did not decrease edema.

* (applicable to contractors upon originator's approval)

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

FINAL REPORT

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

REPORT TITLE: POSTBURN EDEMA OF THE UPPER EXTREMITY: EVALUATION OF PRESENT TREATMENTS IN MILITARY PERSONNEL

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

1 July 1971 - 30 June 1972

Investigators:

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Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

REPORT TITLE: POSTBURN EDEMA OF THE UPPER EXTREMITY: EVALUATION OF PRESENT TREATMENTS 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 1971 - 30 June 1972

Investigators: Roger E. Salisbury, MD, Major, MC
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Reports Control Symbol MEDDH-288(R1)

Edema, a universal sequelae of thermal injury to the upper extremity, impedes early function and may prolong eventual rehabilitation of the burned hand and forearm. Because postburn edema impairs restoration and eventual rehabilitation of the upper extremity, elevation, active and passive exercises and compression dressings are often employed to minimize postburn swelling. The purpose of this study was to evaluate some present methods of management of upper extremity edema.

Eighty-two extremities were studied. Unmarked aluminum tapes were used to measure the effects of open treatment versus compression dressings, nightly elevation, passive motion, hydrotherapy and an intermittent compression device on edema. One thousand four hundred seventy-six measurements over 492 joints were performed. Compression dressings with elevation were more effective in reducing edema than active exercise and elevation in the first 48 hours postburn. Nightly elevation for 8 hours consistently reduced edema. The intermittent compression devise significantly reduced edema without active patient participation. Neither passive nor active exercise of short duration were effective in reducing edema.

Edema
Upper extremity

POSTBURN EDEMA OF THE UPPER EXTREMITY: EVALUATION OF PRESENT TREATMENTS IN MILITARY PERSONNEL

Postburn edema of the upper extremity often presents a severe functional rehabilitation problem. The limb becomes swollen with a proteinaceous transudate that makes movement difficult and painful. The patient voluntarily immobilizes his hand in a comfortable, non-functional position which is thought to promote edema formation. The end result may be periarticular fibrosis, ankylosed joints and tendon adhesions. Thus, the surviving burn patient often experiences limitation of function that necessitates future reconstructive surgery. A review of the literature reveals a significant lack of agreement on early burn management to retard such edema with many impressions, but few controlled studies. For the first 48 hours, some authors advocate elevation, immobilization and a compression dressing (putting the hand at rest). Others believe in no elevation, complete mobilization and only a night splint. Some recommend passive mobilization of joints, other condemn it as dangerous because if injudiciously performed more trauma and edema may result. Hydrotherapy is popular with some authors, while others insist that the open granulating bed absorbs water, becomes boggy and less mobile. The purpose of this study was to evaluate the present types of therapy thought to decrease upper extremity edema and to discover which, if any, is effective.

METHOD

All burned extremities were cleansed and debrided on admission. Provisional diagnosis was made of second and third degree burn (correct depth of burn was determined later by the patient's need for grafting). Twenty-four hours after burn, one-half of the extremities (picked in random fashion) had Sulfamylon burn cream applied, were wrapped in a soft compression dressing in the position of function and elevated in a Thomas splint, arm at sternum level and forearm flexed to 90° and immobilized for the next 48 hours. The rest of the burned extremities had Sulfamylon burn cream applied and were allowed unlimited activity for the next 48 hours. Sterilized unmarked aluminum tapes were used to measure the circumference at the PIP, distal palmar crease and wrist 24 hours postburn and 48 hours after the patient was begun on the study.

Following the 48 hour measurements all patients were treated alike according to the usual ISR protocol. However, each night from 2200 hours to 0600 hours all burned extremities were elevated with the arm at the level of the sternum, forearm and hand at 90° to the upper arm in a Thomas splint or from an IV pole. Measurements of the proximal interphalangeal joints, distal palmar crease and wrist were done before and after elevation.

The burned wrist, MP, PIP and DIP joints were gently taken through a full range of flexion and extension 25 times per minute for 5 minutes each day by the physical therapist. Measurements of these joints were made immediately before and after exercise.

All patients received 15 minutes of hydrotherapy each day. Debridement was performed when necessary and active motion of the fingers was encouraged. Measurements were made immediately before and after hydrotherapy.

RESULTS

One thousand four hundred seventy-six measurements over 492 joints were performed. Table 1 reveals that the standard therapy for the first 48 hours of elevation and unlimited motion did not significantly decrease upper extremity edema. In fact, the wrist actually increased in size, though this was not significant. Table 2 reveals that those patients placed in a compression dressing for 48 hours experienced a statistically significant decrease in wrist swelling. Eight hours of nightly elevation significantly decreased wrist edema (Table 3), but not edema of the fingers or distal palmar crease. Neither passive exercise (Table 4) nor active exercise in the Hubbard tank (Table 5) were effective in reducing edema at any time in the postburn course.

CONCLUSIONS

None of the techniques studied dramatically decreased edema in the early postburn period. All patients, however, were followed for a minimum of 30 days post injury and none developed a chronically edematous hand. In all patients most gross edema had subsided by 5 days postburn, as indicated by subsequent measurements of the involved parts. The techniques of therapy tested had little effect on early postburn edema, but it is very possible that aggressive mobilization of the burned extremity, both active and passive, favorably affects edema resolution and function as indicated by absence of chronic edema in these patients.

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the burned hand. Br J Plast Surg 12:129, 1959.

4. Drummond JA: The management of burned hands. Surg Clin NA 44:977, 1964.

5. Gronley JK, Yeakel, MH, Grant AE: Rehabilitation of the burned hand. Arch Phys Med Rehabil 43:508, 1962.

PUBLICATIONS AND/OR PRESENTATIONS

None

Table 1. Effect of Standard Therapy

	Pretreatment			Post-Treatment		
	PIP	DPC	Wrist	PIP	DPC	Wrist
Mean	7.4	22.2	19.9	7.2	21.7	20.4
Nr. of Ob.	7	7	7	7	7	7
S.D.	0.6	1.5	2.4	0.5	1.3	2.5
S.E.	0.2	0.6	0.9	0.2	0.5	0.9
T	0.6275	0.6172	- 0.3535			
	NS	NS	NS			

Table 2. Effect of Compression Dressing

	Pretreatment			Post-Treatment		
	PIP	DPC	Wrist	PIP	DPC	Wrist
Mean	7.8	24.0	19.6	7.6	22.7	18.6
Nr.of Ob.	5	5	5	5	5	5
S.D.	0.2	1.5	0.4	0.2	0.9	0.6
S.E.	0.1	0.7	0.2	0.1	0.4	0.3
T	1.4144	1.4865	2.7746			
	NS	NS	$p < 0.05$			
			$p > 0.02$			

Table 3. Effect of Passive Exercise

	Pretreatment			Post-Treatment		
	PIP	DPC	Wrist	PIP	DPC	Wrist
Mean	8.0	22.9	19.4	8.0	22.9	19.2
Nr.of Ob.	28	28	28	28	28	28
S.D.	0.8	0.9	1.3	0.8	0.8	1.2
S.E.	0.1	0.2	0.2	0.1	0.2	0.2
	NS	NS	T = 0.58			
			NS			

Table 4. Effect of Hydrotherapy and Short Term Active Exercise

	Pretreatment			Post-Treatment		
	PIP	DPC	Wrist	PIP	DPC	Wrist
Mean	7.4	21.8	18.0	7.4	21.6	18.0
Nr.of Ob.	26	26	26	26	26	26
S.D.	0.5	1.2	1.0	0.5	1.4	1.1
S.E.	0.1	0.2	0.2	0.1	0.3	0.2
	NS	T = 0.5428	NS			
		NS				

Table 5. Effect of 8 Hrs of Elevation

	Pretreatment			Post-Treatment		
	PIP	DPC	Wrist	PIP	DPC	Wrist
Mean	7.6	22.2	18.8	7.3	22.0	17.9
Nr.of Ob.	16	16	16	16	16	16
S.D.	0.5	1.6	1.2	0.6	1.2	1.0
S.E.	0.1	0.4	0.3	0.2	0.3	0.3
T	1.4880	0.3874	2.2321			
	NS	NS	$p < 0.05$			
			$p > 0.02$			

10

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0E 6381	72 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
	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	3A061102871R	01	122
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Include 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	Cont	DA	C. In-House

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

18. RESOURCES ESTIMATE	PROFESSIONAL MAN YRS	FUNDS (In thousands)
FISCAL YEAR PRECEDING 72	0.3	8.5
FISCAL YEAR CURRENT 73	0.3	10.0

19. RESPONSIBLE DOD ORGANIZATION
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NAME: DA

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Provide KEY 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 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 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 water is being studied using goats as the experimental model. A pilot study with 4 normal goats, 5 goats subjected to tracheotomy, ultrasonic mist, and ventilated for 6 hours at high pressures, then sacrificed 24 hours post-ventilation, is currently being completed.

25. (U) 71 07 - 72 06 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. Lungs of ventilated goats have consistently had areas of atelectasis grossly, are slightly edematous, and are heavier on a grams per kilogram body weight basis than are the lungs of control goats. Currently, it is planned to attempt lecithin analysis of lung tissue using isotope-labelled lecithin.

* available in exceptions 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.3A061102B71R-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
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1 July 1971 - 30 June 1972

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Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A061102B71R-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 1971 - 30 June 1972

Investigators: Gary W. Allen, MD, Major, MC
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Reports Control Symbol MEDDH-288(R1)

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 harmful to the lung.

The effect of positive pressure ventilation for six hours with room air to ventilator peak pressures of 40 cm H_2O is being studied using goats as the experimental model. A pilot study with 4 normal goats, 5 goats subjected to tracheotomy, ultrasonic mist, and ventilated for six hours at high pressures, then sacrificed 24 hours post-ventilation, is currently being completed.

Excised lung air and saline compliance curves have shown only minor inconsistent differences between these 3 groups, while surface tension balance studies of bronchial washings have revealed no changes attributable to ventilation. Lungs of ventilated goats have consistently had areas of atelectasis grossly, are slightly edematous, and are heavier on a grams per kilogram body weight basis than are the lungs of control goats. Currently, it is planned to attempt lecithin analysis of lung tissue using isotope-labelled lecithin.

Surfactant
Surface tension
Compliance
Hyperventilation

POSITIVE PRESSURE VENTILATION AND SURFACE TENSION IN LUNGS-- ANIMAL MODEL TO EVALUATE THERAPY OF INJURED TROOPS

A study by Greenfield, et al., in 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% invariable 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 (5 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 P_{CO_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 a 3-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 4-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 6 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.[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.[2]

The compliance due to surface forces (C_{surf}) was estimated by a formula used by Beckman:[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.[3] The disc-driven barrier speed was 0 - 3.2 mm/sec.

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 4 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 3 groups of goats are given in the table. C_{surf} for the experimental goats when divided by the lung weight was .0134 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 for experimental goats and .62 ml/cm H_2O (10% decrease). 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 Wilhelmy balance and Langmuir trough was well within the normal range for both the experimental and control goats.

Quantitative assay of lung lecithin is currently in progress but is not expected to reveal any significant differences.

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[1] are unknown.

Table 1. Compliance Data on Three Groups of Cats

	Tracheotomy + Positive Pressure Ventilation	Tracheotomy	Untreated
No. of Cats	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

It is currently planned to add 3 more goats to each group and to complete the analysis of lung lecithin.

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3. Comroe JH Jr: Physiological and biochemical effects of pulmonary artery occlusion. In Ciba Foundation Symposium on Pulmonary Structure and Function. Ed by AVS deReuck and M O'Conner. Boston, Little, Brown, and Co, 1962, pp 176-185.

PRESENTATIONS AND/OR PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
				DA OD 6966	72 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. DISB'N INSTR'N	9a. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
71 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	3A061102B71R	01	162
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code) (U) Bacteriologic Survey of Inhalation Therapy Equipment in Burn Unit - Potential Source of Airborne Pneumonia 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 07	72 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 72	0.5	9.0
	CURRENT 73	0	0

19. RESPONSIBLE DOD ORGANIZATION
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TELEPHONE: 512-221-4307
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ASSOCIATE INVESTIGATORS
NAME:
NAME: DA

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Nosocomial Pneumonia; (U) Nebulized Decontamination; (U) Inhalation Therapy; (U) Nebulizer; (U) Aerosols; (U) Bacterial Contamination

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 extent of contamination of inhalation therapy equipment in our burn unit. To test the effectivenss, in our burn unit, of standard contamination programs. To prevent the generation of bacteria containing aerosols when nebulizers are used in the course of inhalation therapy treatments.

24. (U) Puritan all-purpose heated nebulizers, Ohio deluxe heated nebulizers, and DeVilbiss ultrasonic nebulizers were studied during the course of inhalation therapy treatments. The reservoir chambers from which the fluid was nebulized was sampled aseptically when the instrument was set up for use, and during continusous use at 2, 4, 6, and 24 hours after starting. After determining the level of contamination of these instruments under ordinary therapeutic conditions, a standard program of decontamination was instituted. The study in no way interfered with the routine administration of inhalation therapy as commonly practiced in our burn unit.

25. (U) 71 07 - 72 03 Forty-six per cent of the initial 382 specimens, collected from 15 patients, were contaminated with bacteria. Twenty-four per cent were contaminated with gram negative organisms, and 29 per cent with gram positive organisms. After the institution of the decontaminátion program, utilizing Cidex (gluteraldehyde), ethylene oxide sterilization and daily nebulization of one quarter per cent acetic acid, the degree of contamination of nebulizer chambers was significantly reduced (p less than .001). Of 612 specimens, collected from 27 patients after institution of the decontamination program, 2.8 per cent were contaminated with gram negative organisms, and 0.8 per cent with gram positive organisms.

*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.3A061102B71R-01, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: BACTERIOLOGIC SURVEY OF INHALATION THERAPY EQUIPMENT IN A BURN UNIT--POTENTIAL SOURCE OF AIRBORNE PNEUMONIA IN BURNED TROOPS

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

1 July 1971 - 30 June 1972

Investigator:

Alan H. Morris, MD, Major, MC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

REPORT TITLE: BACTERIOLOGIC SURVEY OF INHALATION THERAPY EQUIPMENT IN A BURN UNIT -- POTENTIAL SOURCE OF AIRBORNE PNEUMONIA 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 1971 - 30 June 1972

Investigator: Alan H. Morris, MD, Major, MC

Reports Control Symbol MEDDH-288(R1)

The degree of contamination of the nebulizing chambers of Puritan all-purpose nebulizers, Ohio deluxe nebulizers, and DeVilbiss ultrasonic nebulizers in use in the US Army Institute of Surgical Research was determined both before and after the institution of a decontamination program. The fluid in the nebulizer chamber was sampled aseptically at the time the instrument was set up for patient use and at 2, 4, 6, and 24 hours after use began. The collection of these specimens in no way interfered with the use of the nebulizers in the routine administration of inhalation therapy as commonly practiced at the US Army Institute of Surgical Research.

Before the institution of decontamination procedures, 46%, of 382 specimens collected from equipment used on 15 patients, were contaminated with bacteria. Twenty-four per cent were contaminated with gram negative rods, and 29% with gram positive organisms. Pseudomonas, Providence species, and Klebsiella aerobacter organisms were the predominant gram negative organisms recovered. After the institution of a decontamination program suggested by the American Thoracic Society, involving the use of Cidex (gluteraldehyde), and nebulized one-quarter per cent acetic acid (American Review of Respiratory Disease 98:3, 1968), the degree of contamination of nebulizer chambers was significantly reduced ($P < 0.001$). Of 612 specimens, collected from 27 patients after institution of the decontamination program, 2.8% were contaminated with gram negative organisms, and 0.8% with gram positive organisms. A decontamination program proved effective in ordinary hospital settings has been shown to effectively eliminate the problem of bacterial contamination of nebulization equipment in the burn unit as well.

Nosocomial pneumonia
Nebulizer decontamination
Inhalation therapy
Bacterial decontamination
Nebulizer
Aerosols

BACTERIOLOGIC SURVEY OF INHALATION THERAPY EQUIPMENT IN A BURN UNIT--POTENTIAL SOURCE OF AIRBORNE PNEUMONIA IN BURNED TROOPS

Since 1955, several physicians have noted an increase in bacterial infections associated with the use of nebulizers. These observations have been made within the larger framework of concern about hospital-acquired infections. In 1968, Dr. Finland reported that approximately 15% of all hospital admissions acquire an infection while in the hospital and that approximately one-third of these, or 5% of all hospital admissions, acquire pneumonias while in the hospital. Most of these pneumonias are due to gram negative bacilli, and a smaller number to staphylococci. Nebulizers, such as the Bird mainstream nebulizer illustrated in Figure 1, can contribute to this serious problem of hospital-acquired pneumonia. As air passes rapidly through the nebulizer jet, it causes water to be drawn up through the small capillary tube from the reservoir, and mechanically broken into a shower of fine particles as the air-fluid mixture is driven against the solid wall. Since the water is mechanically broken into a series of particles to make a mist, any bacteria present in the water are incorporated into the particles so formed, and delivered to the patient to be inhaled. Heated humidifiers, which employ only evaporation and not mechanical disruption of fluid to humidify the gas, do not present this hazard. In 1964, the first serious study of the role of inhalation therapy equipment in hospital-acquired infection was carried out in six major hospitals in Dallas. In all six hospitals, 70 to 90% of the respirators produced a mist containing gram negative bacilli. The source of the bacilli was the large, heated, mainstream nebulizers. Respirators without mainstream nebulizers, and with only small sidestream medication nebulizers, only rarely produced bacterial aerosols. Coincident with the widespread use of nebulizers, and with their widespread contamination with bacteria, there was a significant rise in the incidence of gram negative necrotizing pneumonia. Following the institution of a decontamination program, the incidence of gram negative necrotizing pneumonia fell to a level not distinguishable from that during the years preceding widespread use of inhalation therapy.

Twenty-one months ago, we began a study of the inhalation therapy nebulization equipment at the US Army Institute of Surgical Research, where 4 different nebulizers have been used (Puritan all-purpose heated nebulizer, Ohio deluxe nebulizer, Bird mainstream nebulizer, and DeVilbiss ultrasonic nebulizer). Samples were collected from the fluid in the nebulizer jar, aseptically, by aspiration through a vacutainer which had been inserted through the finger of a sterile glove, so that the outside, as well as the interior, of the apparatus was sterile and the chance of contamination of the nebulizer jar during sampling was thus reduced (Fig. 2). These studies were

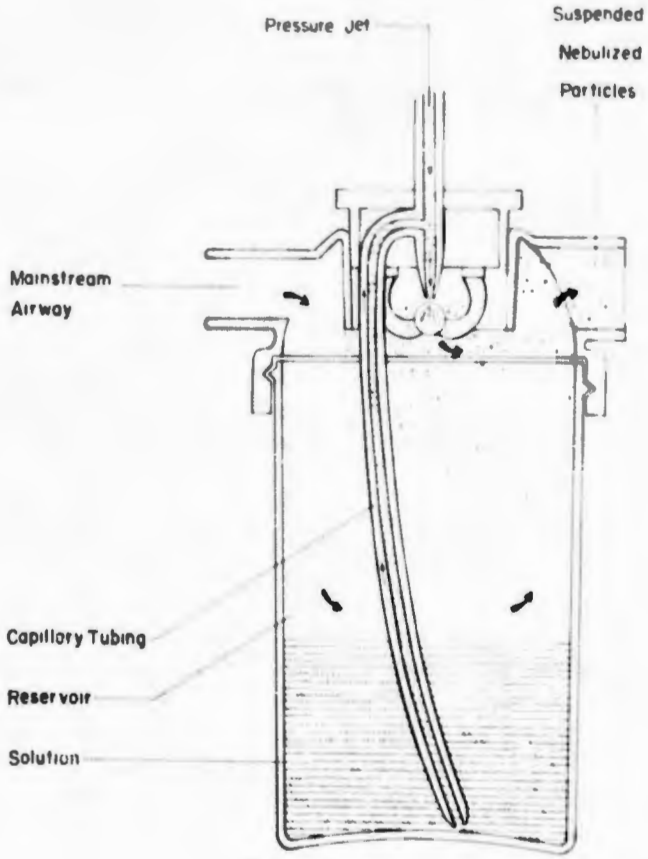

Figure 1

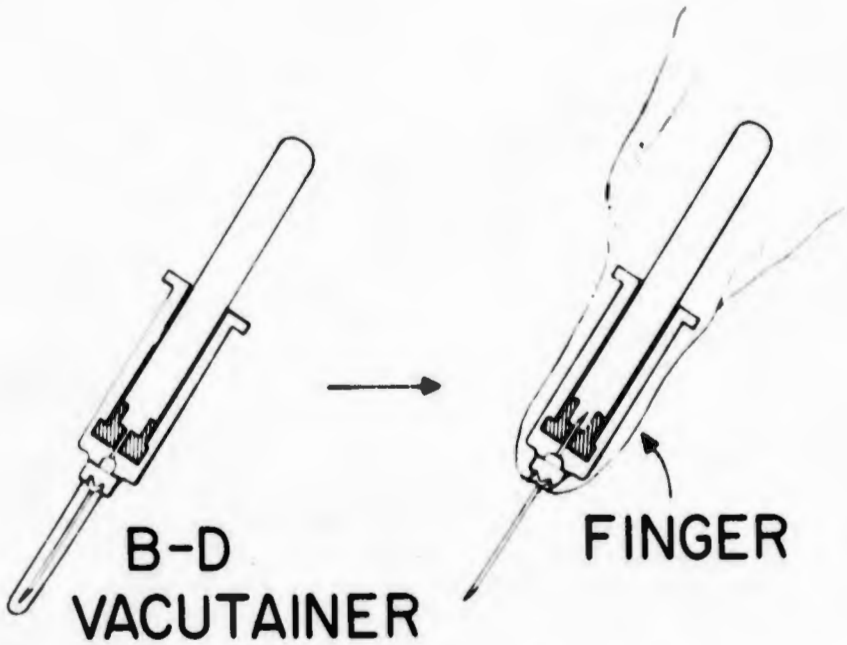

Figure 2

carried out under routine clinical circumstances, without influencing the usual modes of therapy in our Institute. Three hundred eighty two specimens were collected from equipment used on 15 patients (Table 1). A total of 46% were contaminated with bacteria, 24% growing gram negative rods and 29% gram positive organisms. The predominant gram negative organisms were Pseudomonas, Providence species, and Klebsiella aerobacter (Table 2). The contamination was widespread (Fig. 3) and involved all nebulizers studied, including Puritan heated all-purpose nebulizers (indicated by the letter "P"), and DeVilbiss ultrasonic nebulizers (indicated by the letter "U"). The contamination was associated with equipment used on many different patients, as indicated by the results from 4 patients depicted in Figure 4. After the initial level of contamination was determined, an inhalation therapy section was formed, and assumed complete responsibility for the care, preparation, maintenance, and decontamination of all inhalation therapy equipment. A decontamination regimen was instituted according to the recommendations of the American Thoracic Society (Table 3). After use, the nebulizers were completely disassembled, scrubbed with a brush and a quaternary ammonium detergent, and then rinsed with water. They were next soaked in "Cidex" for at least 45 minutes and rinsed with water. They were then dried and placed in clean plastic bags. While in use, the nebulizer was filled with one-quarter per cent acetic acid every morning and the acetic acid nebulized for 15 to 20 minutes. While the nebulizer was in use, the residual fluid in the nebulizer jar was discarded and the jar rinsed with distilled water before each refilling with distilled water. The results of studies of bacterial contamination of nebulization equipment, both before and after the decontamination program was instituted, are presented in Figure 5. Of 382 specimens collected from equipment used on 15 patients during the initial four-month period, approximately 46% were contaminated. Of these 382 specimens, 247 were collected while the nursing personnel of the ward were responsible for the care and maintenance of the equipment. One hundred thirty-five specimens were collected after an inhalation therapist assumed full responsibility for the cleaning, care, and maintenance of equipment, but before any changes in cleaning procedure and before the decontamination program was introduced.

After the institution of the decontamination program, indicated by the heavy vertical line, the incidence of contamination successively fell, and the total contamination of the 612 specimens, collected during nine months following the institution of decontamination procedures, was less than 4%. Both ultrasonic and ordinary nebulizers were successfully decontaminated (Fig. 6). Nebulizers were used continuously for days without contamination as indicated by the results depicted in Figure 7. The days of continuous use without contamination are indicated on the horizontal axis, and the number of patients on the vertical axis. Two patients used nebulizers con-

Table 1

No. of Patients	No. of Specimens	% Gram Negative	% Gram Positive
15	382	24	29
(Total contamination - 46%)			

Table 2

Gram Negative		Gram Positive	
Pseudomonas	64	Bacillus	88
Providence species	26	Staphylococcus (-)	38
Klebsiella aerobacter	20	Streptococcus	1
Serratia	7	Yeast	2
E. Coli	5		
Mima herellea	3		
Achromobacter	4		

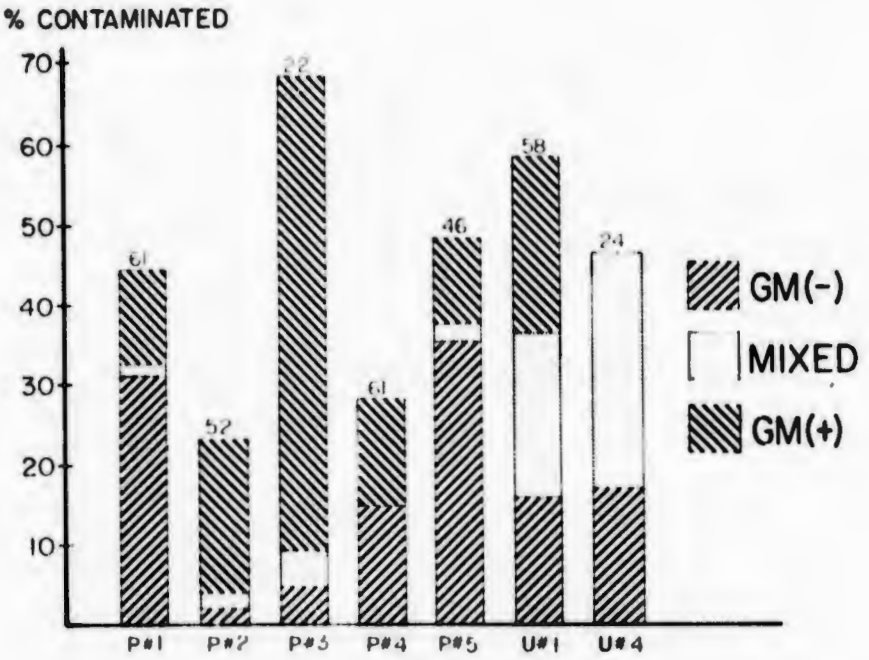

Figure 3

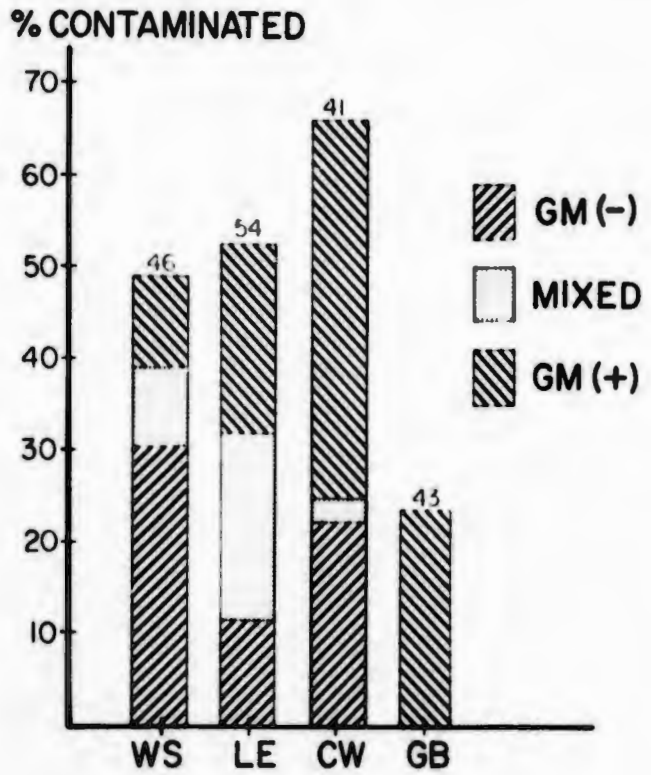

Figure 4

Table 3

Nebulizer Decontamination and Care

Preparation

1. Disassemble, scrub brush, A-33, rinse
2. Cidex (glutaraldehyde) 45 minutes, rinse

In Use

3. Acetic acid 1/4%, nebulize 15-20 minutes daily
4. Discard fluid, rinse jar before refilling

Table 4

No. of Patients	No. of Specimens	% Gram Negative	% Gram Positive	P
	(Before decontamination)			
15	382	23.6	26.8	
	(After decontamination)			<0.001
27	612	2.8	0.8	

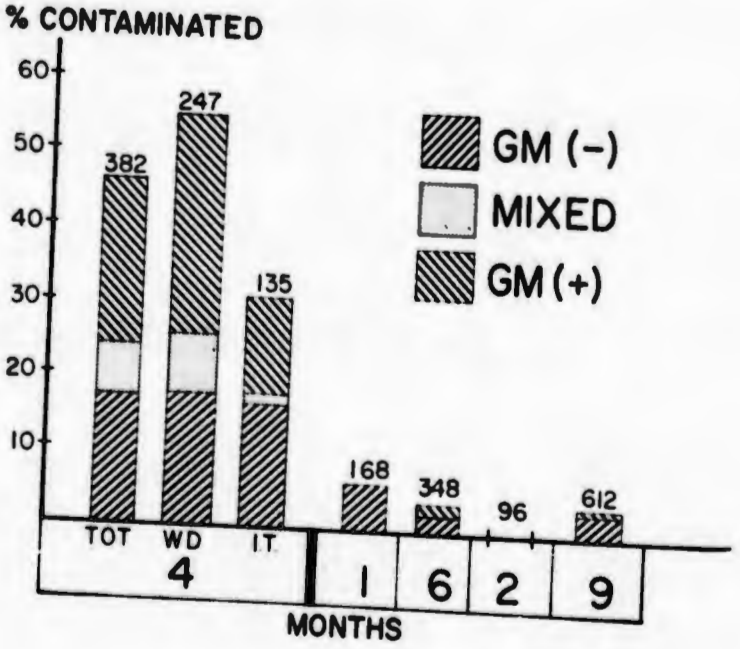

Figure 5

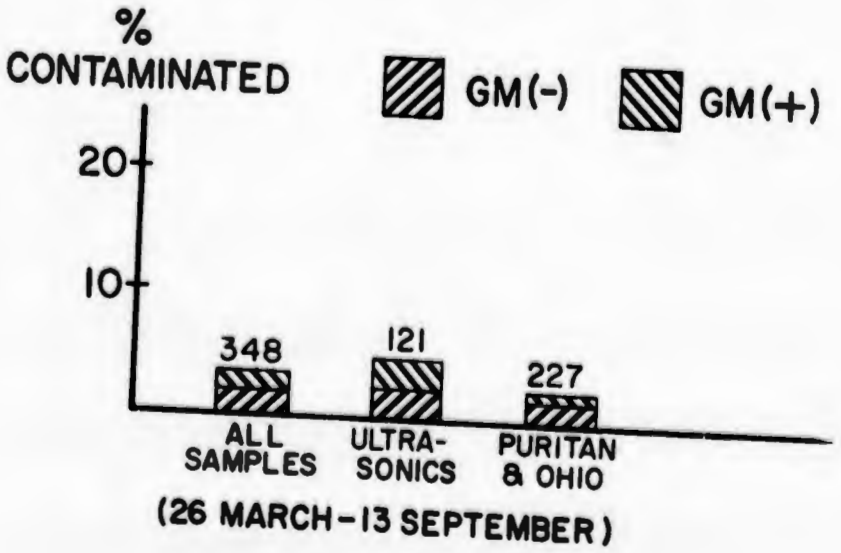

Figure 6

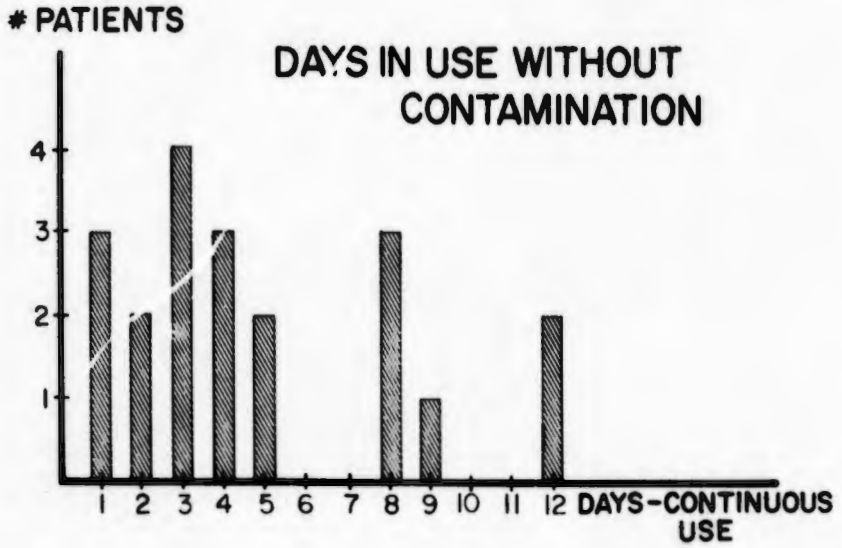
PATIENTS
DAYS IN USE WITHOUT CONTAMINATION
4
3
2
1
1 2 3 4 5 6 7 8 9 10 11 12 DAYS-CONTINUOUS USE

Figure 7

tinuously for 12 days without contamination, one for nine days, three for eight days, and so on. None of the nebulizers represented in Figure 7 became contaminated, and their use was terminated when the patients' physicians discontinued therapy.

The difference between the degree of contamination in the first 382 specimens collected from equipment used on 15 patients, and that found in the next 612 specimens collected from equipment used on 27 patients, was highly significant with a P value of less than 0.001 (Table 4). These data indicate that even in a burn unit, with its very high level of bacterial contamination, inhalation therapy equipment can be successfully decontaminated and made safe for patient use.

PRESENTATION

Morris AH: Presented to the Amer Burn Assn, San Francisco, CA, 7 Apr 72

PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OE 6380	72 07 01	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. GRP'S INSTR'N	9. SPECIFIC DATA - CONTRACTOR ACCESS	10. LEVEL OF SUM
	A. NEW	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

12. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A061102B71R	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 72	0.3	12.6
	CURRENT 73	0.4	15.0

19. RESPONSIBLE DOD ORGANIZATION
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SOCIAL SECURITY ACCOUNT NUMBER:
ASSOCIATE INVESTIGATORS
NAME: Joseph A Moylan, Jr, 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) Intravenous Fat; (U) Intralipid; (U) Parenteral Nutrition; (U) Injured Soldiers

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

23. (U) To evaluate the soybean emultion Intralipid in the thermally injured patient 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. CBC 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) 71 08 - 72 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 adminjstration of fat given at 1, 2, or 3 gm/kg body weight.

* Available to contractors upon requestor's approval.

DD, FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 68 AND 1498-1, 1 MAR 69 (FOR ARMY USE) ARE OBSOLETE.

ANNUAL PROGRESS REPORT

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

REPORT TITLE: SAFETY OF PARENTERAL FAT EMULSION AS A CALORIC SOURCE IN THERMALLY INJURED SOLDIERS

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

1 July 1971 - 30 June 1972

Investigators:

Douglas W. Wilmore, MD, Major, MC
Joseph A. Moylan, Jr., MD, Major, MC
Basil A. Pruitt, Jr., MD, Colonel, MC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A061108B71R-01, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: SAFETY OF PARENTERAL FAT EMULSION AS A CALORIC SOURCE IN THERMALLY INJURED SOLDIERS

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

Period covered in this report: 1 July 1971 - 30 June 1972

Investigators: Douglas W. Wilmore, MD, Major, MC
Joseph A. Moylan, Jr., MD, Major, MC
Basil A. Pruitt, Jr., MD, Colonel, MC

Reports Control Symbol MEDDH-288(R1)

Single unit infusions of fat emulsion, Intralipid, were evaluated in normal and hypermetabolic thermally injured patients. No significant thermogenic response to the fat emulsion 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 measured by carbon monoxide rebreathing test was likewise normal following infusion in 3 patients. Blood gas studies did not change following the infusion of single or multiple units of Intralipid. At present, the soybean emulsion appears to be a promising adjunct as a caloric source in the severely burned patient.

Intravenous fat
Intralipid
Parenteral nutrition

SAFETY OF PARENTERAL FAT EMULSION AS A CALORIC SOURCE IN THERMALLY INJURED SOLDIERS

Extensive weight loss and nitrogen depletion characterize the posttraumatic metabolic response following severe thermal injury. Vigorous nutritional support using combined enteral-parenteral feedings during the catabolic phase of trauma reduces body wasting and often allows early caloric equilibrium and weight stabilization to be achieved (Wilmore et al., 1971[1]). However, use of hypertonic nutritional solutions in the burn patient is not without hazard, and complications of central venous catheterization, associated sepsis and nonketotic hyperglycemic coma have been reported. Fat emulsions contain 1-2 calories/cc and yet are isotonic and may be administered by a peripheral vein, thus preventing many of the hazards associated with central venous feedings. The purpose of this study was to determine the safety of a soybean fat emulsion, intralipid, as a caloric source in thermally injured patients.

METHOD AND MATERIALS

Single 500 ml units of 10/ soybean emulsion were administered over a 4-hour period to 12 hypermetabolic thermally injured patients and 15 healed, convalescing controls. The emulsion, infused after 8 hours of fasting, was administered at a constant rate between the hours of 0700 and 1100 by way of a forearm vein. Body temperature, pulse rate, blood pressure and respiratory rate were monitored prior to the infusion and then serially taken each hour during the infusion and 8 hours following administration. Fat clearance from the blood was determined by measuring plasma optical density by spectrophotometry at 700 mu before the start of the infusion and at 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 cardio-respiratory 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 scintiphotograms and the clearance rate of the gas studied by measuring disappearance rates of the isotope from the lung fields. Following the initial xenon study, a 500 ml unit of 10/ soybean emulsion was administered, and at the end of the 4-hour infusion a repeat ^{133}Xenon lung scan was performed.

Pulmonary diffusion capacity was determined in duplicate in 3 normal, convalescent, fasting individuals sitting in the upright position, using a standard carbon monoxide rebreathing technique.

Following the infusion of 500 ml of 10% fat emulsion over a 4-hour period, the diffusion capacity was again determined using the same technique.

Single or multiple units of intravenous fat were administered to 20 severely burned individuals requiring supplemental or total parenteral nutrition. Arterial blood was drawn at the start of the infusion and at the end of infusion and analyzed for pH, Pco_2 and Po_2.

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 1). 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 were unchanged following the administration of the fat emulsion in the 8 patients studied. No change in 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 change in the 3 patients studied (Table 2). Blood gas measurements carried out in 20 patients demonstrated no significant changes in pO_2, Pco_2 or pH following the infusion of 1 gm/kg body weight, 2 gm/kg body weight, and 3 gm/kg body weight (Table 3). There was no evidence of cyanosis or respiratory insufficiency associated with the fat infusion.

DISCUSSION

Nutrition by vein is a common supportive measure in the burn patient, using solutions containing 5% and 10% glucose. Hypertonic nutrient solutions, containing 20% dextrose and 5% amino acids, administered by central venous catheter supply larger quantities of protein and energy substrate and often allow nitrogen equilibrium and weight stabilization to be achieved in the early catabolic phase of injury. However, sepsis (Boeckman, et al., 1970[2]); hyperosmotic nonketotic hyperglycemia (Wyrick et al., 1970[3]); associated fungemias (Brennan et al., 1971[4]; Ashcraft et al., 1970[5]). and the hazards of central vein catheterization or prolonged cannula placement are complications associated with this technique which discourage frequent use of parenteral nutrition in critically ill patients. Intralipid, the fat emulsion studied, contains

TABLE 1. HEMATOLOGIC AND LIVER FUNCTION STUDIES BEFORE AND 24 HOURS AFTER INFUSION OF 500 ML SOYBEAN EMULSION IN 15 NORMAL INDIVIDUALS (MEAN ± S.D.)

	Before	After
Hematocrit	41 ± 5	42 ± 4
WBC	7,900 ± 2,200	7,700 ± 2,200
Total proteins	7.3 ± 0.5	7.6 ± 0.4
Albumin	3.6 ± 0.6	3.7 ± 0.4
Alkaline phosphatase	13 ± 3	15 ± 4
SGOT	30 ± 11	34 ± 9
Total bilirubin	0.5 ± 0.3	0.5 ± 0.3
Direct bilirubin	0.1 ± 0.0	0.1 ± 0.0

TABLE 2. DIFFUSION CAPACITY BEFORE AND AFTER INFUSION OF 500 ML SOYBEAN EMULSION IN FOUR NORMAL INDIVIDUALS (MEAN ± S.D.)

Pre-Infusion	Immediately Post-Infusion	4 hrs Post-Infusion
33.88 ± 2.42	33.78 ± 2.58	34.49 ± 3.01

TABLE 3. EFFECT OF INTRAVENOUS FAT ON BLOOD GASES (MEAN ± S.D.)

	pO_2		pCO_2		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

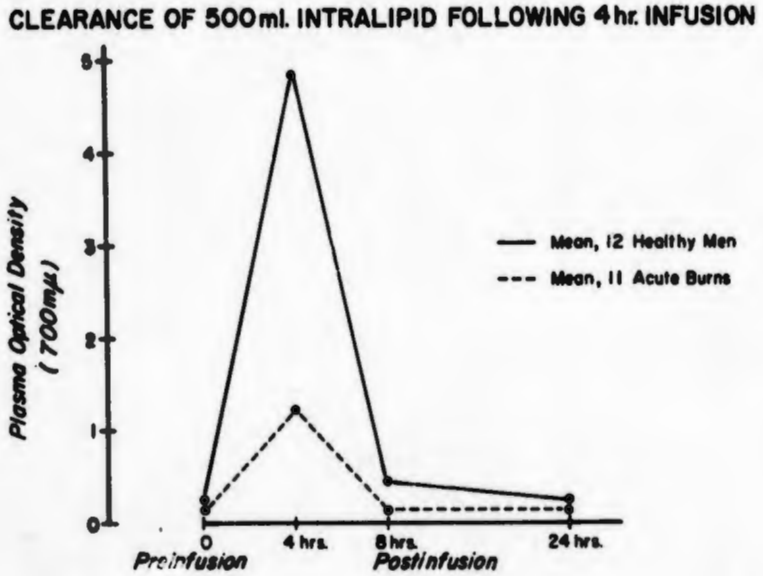

Figure 1. Clearance of 500 ml soybean emulsion (Intralipid) following a constant infusion over 4 hours. Points represent mean values with significant differences between values at 4 and 8 hours.

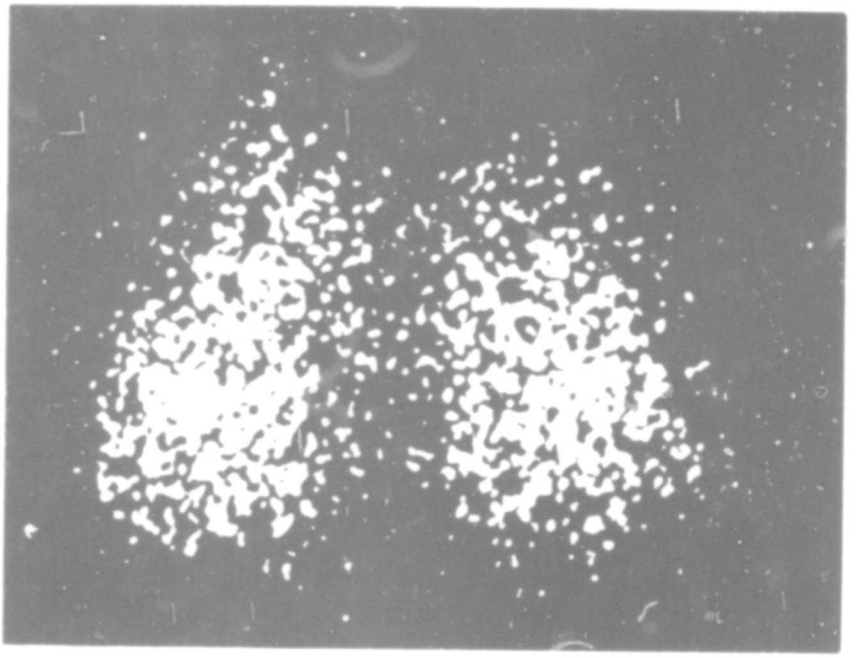

Figure 2A. Normal pulmonary scan following infusion of 500 ml of soybean emulsion demonstrating uniform perfusion.

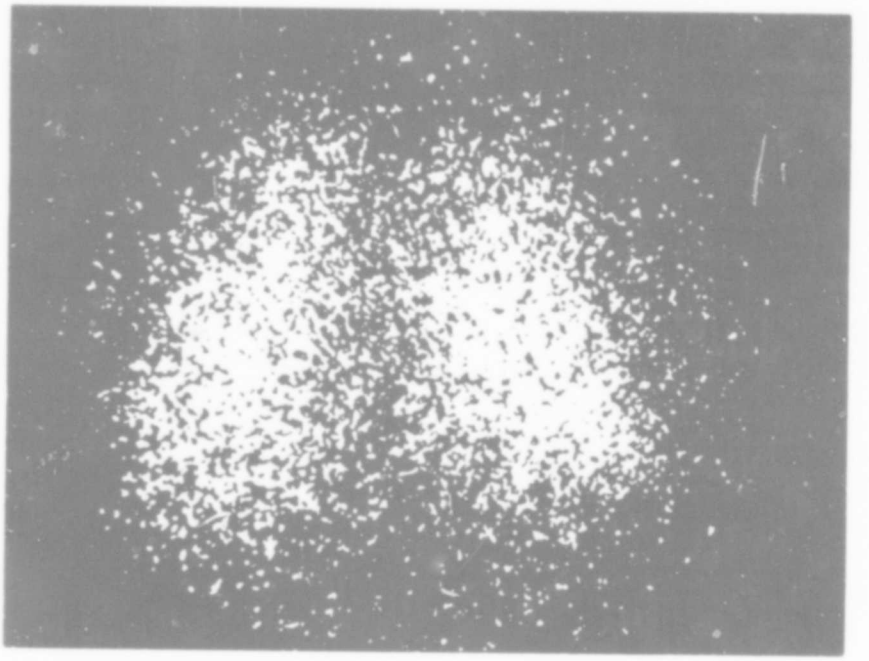

Figure 2B. Normal pulmonary scan following infusion of 500 ml of soybean emulsion demonstrating equal distribution before clearance.

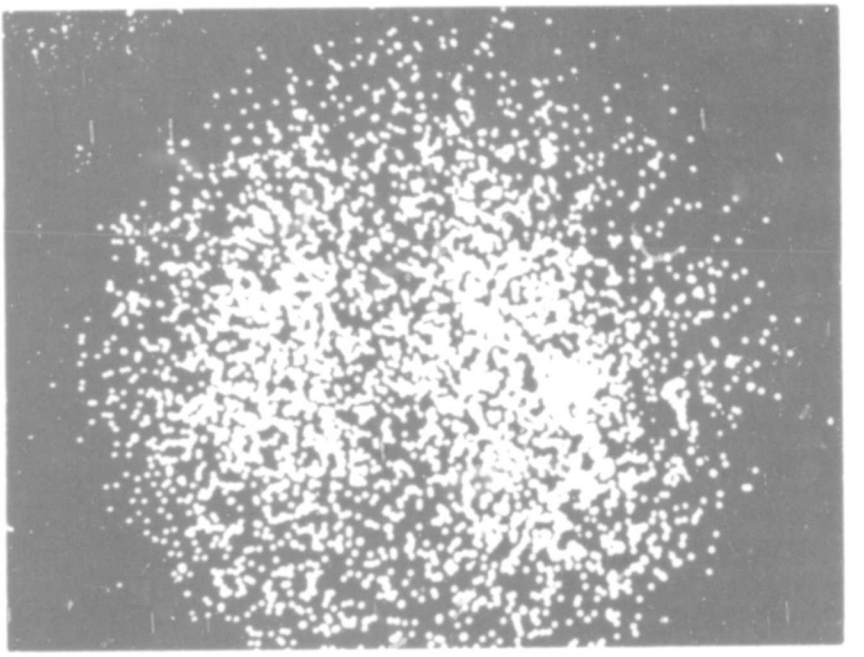

Figure 2C. Normal pulmonary scan following infusion of 500 ml of soybean emulsion demonstrating clearance of isotope almost complete at 88 seconds.

soybean oil, egg yolk phosphatide and glycerol and provides one calorie/cc and may be administered by peripheral vein. Previously, however, complications did occur with fat emulsions, and the immediate side effects included fever, dyspnea, cyanosis, flushing, nausea, vomiting, headache and jaundice. Later complications included hyperlipidemia, alterations in blood coagulation, liver dysfunction, anemia and deposition of intravenous fat pigment (Wretlind, 1962[6]). In this study, the emulsions tested did not demonstrate thermogenic reactions, with units studied taken from 10 different batches of fat emulsion. Blood chemical analysis demonstrates no changes in complete blood counts and liver function tests following one unit of infusion. Further evaluation is being carried out with prolonged infusions to determine safety of the fat preparation.

Previously, fat emulsions have been associated with dyspnea and cyanosis. The frequency of major pulmonary complications following thermal injury emphasized the need to determine the effect of Intralipid on cardiopulmonary function. Our studies to date have demonstrated no changes in the xenon perfusion-diffusion scan or clearance of the isotope from the lung fields after a single unit of fat infusion, no change in pulmonary diffusion capacity in 3 individuals following infusion, and no change in the blood gas values after administration of single or multiple units of the fat emulsion. We are presently continuing these safety studies with multiple unit infusions over an extended period of time.

The burn patient clears intravenous soybean emulsion from the blood stream at an accelerated rate. While clearance cannot be directly equated with fat utilization, previous studies have documented favorable weight gain and nitrogen response to the administration of intravenous fat emulsions in animals and man (Schuberth, et al., 1961[7]). Autopsy and biopsy specimens document that the lipid does not accumulate in tissues, and there is a shift of respiratory quotients toward that of fat oxidation following the administration of the soybean emulsion (Geyer, 1970[8]). Further long-term studies are in progress to evaluate the effect of fat emulsion on the metabolic response to injury and to compare the administration of glucose as a caloric substrate to an isocalorically matched quantity of soybean emulsion.

SUMMARY

Single unit infusions of fat emulsion Intralipid were evaluated in convalescent, healed burn patients and hypermetabolic, unhealed thermally injured patients. No significant thermogenic response to the fat emulsion 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 measured by carbon monoxide rebreathing test was likewise normal following infusion in 3 patients. Blood gas studies did not change following the infusion of single or multiple units of Intralipid. At present, the soybean emulsion appears to be a promising adjunct as a caloric source in the severely burned patient.

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PRESENTATION

Wilmore DW: Safety of parenteral fat emulsion as a caloric source in thermally injured patients. Fourth Anl Mtg of Amer Burn Assn, San Francisco, CA, 7 Apr 72.

PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6978	72 07 C1	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. GROUP NUMBER	8b. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
71 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	3A061102B71R	01	300
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Include 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. END OF AGRD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
FISCAL YEAR PREVIOUS: 72	0.5	17.2
FISCAL YEAR CURRENT: 73	0.5	18.0

19. RESPONSIBLE DOD ORGANIZATION
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NAME: Mary E Spitzer, CPT, AMSC DA

21. GENERAL USE: FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Provide each with Security Classification Code): (U) Gastrointestinal absorption; (U) High protein diet; (U) Trace elements; (U) Humans

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

23. (U) To evaluate the gastrointestinal absorption and nutritional efficacy of a standard high protein hospital diet in extensively burned patients. To see if there are any absolute or relative deficiencies or imbalances of amino acids, essential fatty acids, minerals, or trace elements with this diet for wounded soldiers.

24. (U) Using commercially available chemically defined diets and hospital prepared tube feeding preparations, absorption of fat and nitrogen across the gastrointestinal tract was determined. Stools were collected over 72 hours and analyzed for fecal fat and nitrogen intake. Serum levels were determined for carotene, trace elements, protein, uric acid, vitamins and minerals.

25. (U) 71 07 - 72 06 To date, six patients have been studied. Four of these patients demonstrated significant diarrhea associated with steatorrhea during their clinical course. The diarrhea was not related to the degree of body weight loss, serum protein level or other serum measurement. Further studies are being performed to determine the role of systemic antibiotics used in the burn patient on changes in bacterial flora, the effect of the catabolic phase of trauma on epithelial cell turn-over in the small bowel, and the alterations of absorptive enzymes following thermal trauma. Induction of enzyme systems may be helpful to improve absorption of chemically defined diets in the seriously ill patient.

* Available in unclassified form with 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. 3A061102B71R-01, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: EVALUATION OF GASTROINTESTINAL ABSORPTION AND NUTRITIONAL EFFICACY OF STANDARD HIGH PROTEIN DIET IN BURNED SOLDIERS

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

1 July 1971 - 30 June 1972

Investigators:

Douglas W. Wilmore, MD, Major, MC
Mary E. Spitzer, Captain, AMSC
Wellford W. Inge, Jr., MD, Lieutenant Colonel, MC
P. William Curreri, MD, Lieutenant Colonel, MC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

REPORT TITLE: EVALUATION OF GASTROINTESTINAL ABSORPTION AND NUTRITIONAL EFFICACY OF STANDARD HIGH PROTEIN DIET IN BURNED SOLDIERS

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

Period covered in this report: 1 July 1971 - 30 June 1972

Investigators: Douglas W. Wilmore, MD, Major, MC
Mary E. Spitzer, Captain, AMSC
Wellford W. Inge, Jr., MD, Lieutenant Colonel, MC
P. William Curreri, MD, Lieutenant Colonel, MC

Reports Control Symbol MEDDH-288(R1)

Seven patients with extensive thermal injury and weight loss of greater than 10% of body mass were studied to determine gastrointestinal absorption of the standard hospital diet. Patients received more than 3,000 calories per day by enteral feedings which contained more than 100 grams of fat per 24 hours. In 3 patients studied, stool fat and nitrogen were within normal limits although absorption and excretion of d-xylose were above normal. All of these patients were having normal bowel movements. In 4 patients with extensive thermal injury and diarrhea, abnormal absorption of fat and nitrogen occurred. Symptomatic treatment of the diarrhea resulted in improvement in gastrointestinal function, with return to normal in the absorption of essential nutrients. No trace element abnormalities were found in the patients studied except for a probable zinc deficiency which occurred in a patient on long-term intravenous therapy. Gastrointestinal absorptive function appears normal in injured patients having normal bowel activity. Patients who develop diarrhea demonstrate abnormal absorptive function which seems to be corrected with symptomatic treatment. Etiology of diarrhea in the injured patient is now being studied.

Gastrointestinal absorption
High protein diet
Trace elements

EVALUATION OF GASTROINTESTINAL ABSORPTION AND NUTRITIONAL EFFICACY OF STANDARD HIGH PROTEIN DIET IN BURNED SOLDIERS

Hypermetabolism, negative nitrogen balance and progressive weight loss characterize the metabolic response to thermal injury. Catabolism, associated with utilization of lean body mass and fat stores, generally continues until satisfactory coverage of the burn wound is achieved. Loss of body mass is minimized with enteral feedings, and the standard hospital diet, high in calories and nitrogen, represents the mainstay of dietary therapy for the injured patient. In patients who can receive oral feedings, over two-thirds of the necessary calories required to achieve caloric equilibrium can be administered in the acute phase of injury by vigorous enteral support. As a result, patients with large thermal injuries greater than 40% total body surface may be supported solely by the hospital diet and oral supplements and lose no more than 15% of the total body weight.

In spite of this ability to limit weight loss following thermal trauma, significant alterations in organ function occur during the catabolic phase of injury, with a decrease in many of the body's essential proteins and alteration in cell replication. In the small intestine, epithelial cells divide in the crypts of Lieberkuhn and migrate to the tips of the villi before being shed. The transit time for a cell from crypt to villus tip in normal man is 27-44 hours. The replicating cells in the crypts of the small bowel have the greatest metabolic demands in the body, and cellular activity is greatly affected by alterations in body metabolism. For example, in states of catabolism or starvation, the epithelial cell mytotic rate decreases and the cellular transit time along the villus becomes prolonged (Mönckeberg, 1966[1]). In cases of marasmus, altered replication results in disturbances of normal morphology with fawn and fig leaf villus formation (Tandon et al., 1968[2]). These morphologic changes are associated with alterations in small bowel function, and steatorrhea, abnormal d-xylose and B_{12} absorption have been demonstrated. Absorption returns to normal with the institution of feedings and maintenance of caloric balance.

The purpose of this study was to evaluate the gastrointestinal absorption and nutritional efficacy of the standard high protein hospital diet in extensively burned patients during the catabolic phase of injury.

METHODS

Patients with injury greater than 40% of the body surface and weight loss greater than 10% of body mass were studied. A standard

hospital diet was given daily with caloric count to insure intakes of greater than 3,000 calories per day and more than 100 grams of fat per day. A carmine marker was given at the start and end of a 3-day study period, with stools collected and analyzed for fat and nitrogen. The following day, the patient was fasted and given a 25-gram dose of d-xylose and urine collected for the next five hours. The urine was then analyzed for d-xylose to determine absorption and excretion of this nonmetabolized sugar. Blood was drawn for serum proteins, calcium, phosphorus, cobalt, chromium, magnesium, manganese, zinc, cadmium, iron, nickel and lead.

RESULTS

Severe weight loss was noted in the patients studied, but slight absorption abnormalities were documented in analysis of stool and nitrogen (see table). An abnormal d-xylose absorption test was found in two patients. Three patients demonstrated diarrhea during the enteral feedings, resulting in steatorrhea with stool fat ranging from 8.8 to 27.6 grams per day. Stool cultures taken in these individuals revealed no abnormal fecal flora. Trace element analysis demonstrated no abnormalities except for a low serum zinc which was noted in one patient who had received longterm intravenous feedings for more than three months. In one patient with a 14 kg weight loss, repeat studies following restoration of body mass revealed normal absorption of fat and nitrogen.

DISCUSSION

Weight loss characterizes the post-traumatic metabolic response to thermal injury, but malabsorption from the gastrointestinal tract does not appear to be a significant contributing factor. However, during catabolic states gastrointestinal function is not optimal, as demonstrated in patients 4, 5 and 6 with diarrhea and malabsorption. This absorptive defect seems only temporary and was usually related to the use of tube feedings or synthetic diets. Changes in blood flow, alteration in small bowel epithelium, quantitative and qualitative changes in gastrointestinal flora, and depression of transport enzyme in the small bowel mucosa may all contribute to this malabsorption state.

Further studies are now in progress to study the gastrointestinal flora of the burn patient, analyze the villus structure and function, and quantitatively determine transport enzyme concentration in the gastrointestinal epithelium. The possibility of substrate induction of transport enzymes is being explored as one method of improving gastrointestinal absorption during severe catabolic states.

PUBLICATIONS AND/OR PRESENTATIONS

None

Absorption Studies

Patient	Weight Loss (kg)	A/G	D-Xylose (gm/5 hrs)	Stool Fats (gm/24 hrs)	Stool Nitrogen (gm/24 hrs)
1	16	2.7/5.0	9.0	1.5	1.5
2	13	2.2/5.3	8.3	0.5	0.7
3	15	1.7/3.9	2.9	0.7	1.0
4*	14	1.7/3.7	3.1	8.8	5.7
*Recovery	0	3.1/3.9	5.8	3.5	2.0
5	31	0.6/4.3	1.4	4.7	2.9
6	15	1.4/4.3	3.9	14.4	8.0
7	12	---	---	27.6	4.0
Normal		3.5/4.5	> 5.0	< 5.0	< 2.0

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0D 6979	72 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	3A061102B71R	01	302
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) Metabolic Response of Hypermetabolic Burn Patients to Cooling (44) Improving care of Burned Troops (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
71 11	Cont	DA	C. In-House

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

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 72	0.4	10.5
FISCAL YEAR CURRENT 73	0.4	15.0

19. RESPONSIBLE DOD ORGANIZATION
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NAME:

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

DA

22. KEYWORDS (Precede EACH with Security Classification Code): (U) Energy Expenditure; (U) Ambient Temperature; (U) Critical Temperature; (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) The purpose of this study is to evaluate effect of a negative thermal load on hypermetabolic burn in burned military personnel.

24. (U) Patients with thermal injuries from 20-90% total body surface were studied. Age matched individuals served as controls. Four hours after fasting, the subjects were taken to the tank room on a canvas frame and rested for 30 minutes in ambient temperature of 26-27° C. Using standard spirometry, resting oxygen consumption was determined in duplicate. The subjects were then immersed in cold water bath maintained at room temperature, and after a 10-minute period of equilibration, oxygen consumption again determined. Core temperature was monitored by means of a thermistor probe placed in the rectum. Metabolic rates were measured in duplicate every 10 minutes for a duration of 30 minutes of immersion. The patients were removed from the tank if the core temperature fell below 35.5° C.

25. (U) 71 11 - 72 06 To date, control data has been gained from two normal individuals who have been cooled on three successive occasions. These studies revealed that vasoconstriction remains a prominent portion of the adaptation to the total immersion in the water bath and that shivering and increase in metabolic rate occur but at this temperature the metabolic response is of a cyclic nature. Modification of techniques to measure metabolic rate is presently being made to account for this cyclic phenomenon, which is characterized by short bursts of metabolic energy to maintain core temperature, followed by a return to resting oxygen consumption. Further studies will be carried out in the water bath and a controlled ambient air environment which is presently being installed.

* Applicable 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. 3A061102B71R-01, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: METABOLIC RESPONSE OF HYPERMETABOLIC BURN PATIENTS TO COOLING

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

1 July 1971 - 30 June 1972

Investigators:

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Kenneth W. Spitzer, Captain, MSC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

REPORT TITLE: METABOLIC RESPONSE OF HYPERMETABOLIC BURN PATIENTS TO COOLING

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

Period covered in this report: 1 July 1971 - 30 June 1972

Investigators: Douglas W. Wilmore, MD, Major, MC
Kenneth W. Spitzer, Captain, MSC

Reports Control Symbol MEDDH-288(R1)

The purpose of this study was to evaluate the effect of a negative thermal load on hypermetabolic burn patients. Two fasting subjects rested in a supine position for 30 minutes in an ambient temperature of 26 to 27°C. Using standard spirometric determinations, resting oxygen consumption was determined in duplicate. The subjects were then immersed in a cold water bath, maintained at the same temperature as the room (26-27°C), and after 10 minutes of equilibration, oxygen consumption was again determined. Results in 2 normal individuals demonstrated a cyclic phenomenon in the metabolic response to a negative thermal load. This response demonstrated a dramatic increase in oxygen consumption, followed by a decrease in oxygen utilization to near basal rates. The changes in oxygen consumption were accompanied by periods of violent shivering followed by periods of relaxation while in the cold water. Subjectively, the patients felt quite cold after being submerged, and initially there was marked vasoconstriction. This was followed after one and one-half minutes by uncontrolled shivering, which generated more heat and resulted in a new thermal equilibrium. Further studies will be carried out, carefully monitoring the cyclic nature of this response in normals, to determine the periods of heat storage and loss in human tissue and to further characterize the response of the hypermetabolic thermally injured patient to cooling.

Energy expenditure
Ambient temperature
Critical temperature

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0A 6980	72 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. GRP'S INSTR'N	8B. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
71 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	3A061102B71R	01	165
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE: (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	PRECEDING/SUMMARY	a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
FISCAL YEAR	72	.6	12.8
	73	.6	20.0

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

22. KEYWORDS (Provide KWOC with Security Classification Code)
(U) Protein; (U) Burn; (U) Trauma; (U) Turnover; (U) Rats

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

23. (U) To determine the 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, and treated burned infected rats has been measured. The intravascular/extravascular distribution of albumin and gamma globulin will be determined using immunochemical technics, chromatography, and electrophesis.

25. (U) 71 07 - 72 06 Procedures for extracting proteins from animal tissues and measuring the specific proteins by immunochemical methods have been tested and modified for use with this experimental animal model. This in combination with electrophoretic technics will permit quantitation of the amounts of protein in the extravascular compartments of the test animals. This work is currently in progress and the results obtained will be used to aid in the interpretation of the data obtained by measuring C-14 incorporation into the serum protein fractions.

* Available to contributors upon publication's approval.

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ANNUAL PROGRESS REPORT

PROJECT NO. 3A061102B71R-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

1 July 1971 - 30 June 1972

Investigators:

Wanda L. Brown, MS
Eleanor G. Bowler, PhM
Arthur D. Mason, Jr, MD

Reports Control Symbol MEDDH-288(R1)

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ABSTRACT

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Analytical procedures are being adapted and evaluated for the direct determination of albumin and gamma globulin pool sizes and their distribution after burn injury using an experimental rat model. This information will be correlated with that obtained from our study of glycine-2-^{14}C incorporation into serum proteins of burned rats in an attempt to determine the mechanism responsible for the dysproteinemia observed in man following burn injury.

Protein
Burn
Trauma
Turnover

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

Our previous studies utilizing the incorporation of glycine-2-^{14}C into serum proteins of rats as a measure of protein synthesis or turnover have yielded presumptive evidence that decreased synthesis could not fully explain the marked dysproteinemia which occurs following burn injury (Brown, et al).[1]

Since both the concentrations and relative specific activities of the alpha and beta globulins were increased in all injured animals, the conclusion that there is an increased rate of synthesis of these components is probably valid. Their concentrations in the serum are probably representative of their total pool sizes. However, albumin, after having been secreted from the liver into the intravascular compartment, readily distributes throughout the extravascular space. The apparent rate of albumin synthesis calculated only from the ^{14}C incorporation into plasma albumin would be overestimated if the total body pool size was reduced. A similar error could occur in estimating gamma globulin synthesis from plasma values.

Many models for studies of protein metabolism have been developed. The assumptions involved, and the problems inherent in the use of many of these models have been critically reviewed recently by Bianchi, et al.[2] Sellers,[3] and Katz, et al.[4] made direct measurements of the extravascular albumin pool in control and nephrotic rats. They compared these results with those obtained by calculation from the slopes and intercepts of the plasma disappearance curve of injected labeled albumin and they concluded that the multicompartmental models calculated in this manner greatly underestimated the size of the extravascular albumin pool. Sterling's model,[5] which assumes equal specific activity of albumin throughout the body at equilibrium and is based on extrapolation of the plasma disappearance curve to the ordinate, gave results which were in agreement with those they obtained by direct analysis.

The altered distribution and metabolism of albumin following burn injury makes the multicompartmental models based on the disappearance of injected labeled albumin even more difficult to interpret. For this reason, we decided our next step should be to do direct, specific analyses of albumin in the tissues of control and burned rats to determine its distribution.

MATERIALS AND METHODS

Rats subjected to a full thickness burn of 20% of their body

surface were housed in individual cages and fed ad lib until the sixth day postburn. Stock rats were used as controls. The following procedures, which are essentially the same as those described by Sellers[3] and Katz[4] were performed on the sixth day postburn.

The rats were weighed, shaved, and then 0.2 microcurie ^{131}I-labelled human serum albumin was injected into the tail vein. The rats were immediately anesthetized, the chest cavity was opened, and as much blood as possible was withdrawn from the heart. Care was taken to obtain a sample within 3-6 minutes after injection to be used for determination of the plasma volume. Twenty to 30 ml of isotonic saline were then injected and aspirated to remove as much residual blood as possible from the body. Residual plasma albumin in the tissue extracts was calculated from the ^{131}I content.

The rat's body was divided into viscera, skin, and carcass. The carcass (including bone) was ground in a meat grinder before preparing a 10% homogenate with 0.1% deoxycholate in 0.15 M sodium chloride, pH 8 using a Virtis 45 homogenizer. The other components were treated in the same manner except that the skin was minced, and the viscera were homogenized without preliminary treatment. For these and subsequent steps, the tissues and extracts were kept cold at all times. The homogenates were centrifuged for 30 minutes at 12,000 g using a Type 30 rotor in a Beckman Model L3-50 preparative ultracentrifuge. The supernates were removed, immediately frozen, and stored at -20° C until immediately before analysis. At this time, the extracts were thawed and recentrifuged.

Albumin concentration of the extracts were measured by radioimmunoassay. Plasma albumin concentration was determined both by radioimmunoassay and by cellulose acetate electrophoresis. Plasma volume was calculated from the volume of dilution of the injected ^{131}I-labelled human albumin.

RESULTS AND DISCUSSION

By the sixth day postburn plasma albumin levels in rats subjected to a 20% full-thickness burn have usually reached their minimum levels (Alexander, et al),[6] the rapid transfer of albumin from intravascular to extravascular space which occurs soon after burning has diminished, and plasma volumes have returned to normal. The total body albumin determinations were done at this time because it was felt that the more stable conditions would yield more useful information.

Determinations of total body albumin pool size of 8 rats have been completed at this time. The results indicate that the total

body depletion may not be as severe as one would anticipate from the size of the plasma albumin pool. One cannot draw any definite conclusions from this small number of animals but the study is being continued and will include gamma globulin determinations as well.

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PRESENTATIONS AND/OR PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
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3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. DOWNGRADING	7. REGRADING	8a. DISP'D WHEN	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	3A061102B71R	01	305
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Include with Security Classification Code): (U) Erythrocyte and Plasma Phospholipids in a Military Population with Burn Injuries (44)

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19. RESPONSIBLE DOD ORGANIZATION
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NAME: Douglas W Wilmore, MAJ, MC DA

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22. KEYWORDS (Provide KEYWORDS with Security Classification Code):
(U) Phospholipid; (U) Fatty Acid; (U) Erythrocyte Membrane ; (U) Humans

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

23. (U) The objective of this project is to identify and quantitatively analyze the phospholipids and phospholipid fatty acids present in theerythrocyte membrane and plasma of burn injured soldiers.

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) 71 12 - 72 06 Three burn patients 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. With time and stabilization of their metabolic profile, and perhaps due to administration of intravenous soybean emulsion, two of these patients regained normal lipid fatty acid compositions. The third patient neither received equivalent essential fat supplementation nor returned to an anabolic condition.

* (If available in accordance with originator's approval)

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ANNUAL PROGRESS REPORT

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

REPORT TITLE: ERYTHROCYTE AND PLASMA PHOSPHOLIPIDS IN A MILITARY POPULATION WITH BURN INJURIES

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

1 July 1971 - 30 June 1972

Investigators:

George M. Helmkamp, Jr., Captain, MSC
Avery A. Johnson, BS
Douglas W. Wilmore, MD, Major, MC

Reports Control Symbol MEDDH-288(R1)

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ABSTRACT

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

REPORT TITLE: ERYTHROCYTE AND PLASMA PHOSPHOLIPIDS IN A MILITARY POPULATION WITH BURN INJURIES

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

Period covered in this report: 1 July 1971 - 30 June 1972

Investigators: George M. Helmkamp, Jr., Captain, MSC
Avery A. Johnson, BS
Douglas W. Wilmore, MD, Major, MC

Reports Control Symbol MEDDH-288(R1)

Phospholipid fatty acid patterns have been determined by gas-liquid chromatography on red cells from 12 adult males with burns ranging from 16 to 62% total body surface. Linoleic (18:2), arachidonic (20:4), and docosahexenoic (22:6) acids decreased significantly in the red cells of 4 of these patients (18:2 = 5.9%, 20:4 - 4.3%, 22:6 = 0.4%) when compared with the other burns (18:2 = 9.3%, 20:4 = 13.9%, 22:6 = 2.7%) and controls (18:2 - 9.5%, 20:4 = 17.2%, 22:6 = 3.2%). Simultaneously, plasma phospholipid, triglyceride, and cholesterol ester fatty acid patterns were essentially normal in all patients.

Infusion of a soybean lipid emulsion in 2 patients corrected the fatty acid imbalance in the red cell membrane (18:2 = 10.2%, 20:4 = 15.9%, 22:6 = 4.1%), while a fat-free intravenous diet which provided adequate calories failed to restore the compositional deficiency in a third patient. For the fourth individual, sufficient oral feedings eliminated his fat deficiency (18:2 = 10.7%, 20:4 = 16.4%, 22:6 = 2.46%). Two nonburned patients with enterocutaneous fistulae, receiving fat-free parenteral diets for one month or longer, did not demonstrate significant alterations in their red cell phospholipid fatty acids (18:2 = 7.4%, 20:4 = 13.0%, 22:6 = 2.6%).

Essential fatty acid deficiency in the red cell membrane may result from inadequate caloric or extended fat-free parenteral support in hypermetabolic burn patients, or rapid turnover and utilization of fatty acids following major thermal trauma. The structural and functional abnormalities which occur as a result of thermal injury may be related to these defects in erythrocyte polyunsaturated fatty acids.

Phospholipid
Fatty acid
Erythrocyte membrane

ERYTHROCYTE AND PLASMA PHOSPHOLIPIDS IN A MILITARY POPULATION WITH BURN INJURIES

Alterations in fragility, morphology, survival, and cation concentration characterize red blood cells following severe thermal injury. Of utmost importance is increased osmotic fragility, a condition which leads to hemolysis, hemoglobinuria, and often prolonged anemia.[1,2] On an experimental basis, decreased survival times for rat erythrocytes have been demonstrated.[3] Increased intracellular sodium concentrations have been observed in patients with major burns.[4,5] Taken together, all of the above phenomena suggest modifications of the red cell membrane in these burn patients. The erythrocyte membrane is composed of approximately equal weight proportions of protein and lipid.[6] Phospholipids account for 60% of the total lipid, with the remainder being distributed between free cholesterol (30%) and glycolipid (10%). The present study was undertaken to describe the fatty acid composition of red cell phospholipids, as well as that of various plasma lipids, as a function of thermal injury. Preliminary evidence from this Institute indicated decreased levels of linoleate and arachidonate and increased levels of short-chain saturated fatty acids in burn patients.[7]

METHODS

Blood was drawn 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 then resuspended in saline to a hematocrit of approximately 50% and, together with the plasma, stored under nitrogen at -20°C until extraction.

Lipids were extracted from 3 milliliters of the red cell and plasma fractions by Procedure III of Ways and Hanahan.[8] To retard oxidation of the polyolefinic acids, the methanol used contained 2,6-di-tert-butyl-p-cresol (BHT) at a concentration of 50 mg/L. Highest purity, spectroscopic-grade solvents were used without further treatment.

Phospholipids were separated from total red cell lipids by column chromatography on silicic acid (100-200 mesh).[8] After hydrolysis in 2N HCl at 110-120°C for 18-24 hours, fatty acids were extracted with pentane and converted to their methyl esters using boron trifluoride-methanol reagent.[9]

Plasma lipids were chromatographed on 0.25 mm layers of silica gel G with a development solvent of petroleum ether (b.p. 63-75°)-diethyl ether-acetic acid, 90:10:1 (v/v). Commercially available

standards were employed to identify the phospholipid, triglyceride, and cholesterol ester regions. These lipids were then eluted from the plates[10] and subjected to acid hydrolysis and methylation. The methyl esters derived from cholesterol esters were purified by thin-layer chromatography to eliminate substances which interfere 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° 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° 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.[11] 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 phospholipid fatty acid distributions have been determined on a variety of thermally injured patients (Table 1). In general, the patients can be divided into two classes--those with normal fatty acid patterns (Group I, mean age 35.5, mean burn size 40.9%, mean full thickness 30.4%), and those with significant reductions in long-chain polyunsaturated fatty acids (Group II, mean age 30.8, mean burn size 44.8%, mean full thickness 29.6%). In both groups there are no unusual fatty acids, including the short-chain species reported earlier.[7] When decreases in the polyunsaturated acids (18:2, 20:4, and 22:6) are noted, there are corresponding increases in palmitate (16:0) and oleate (18:1). In comparing the Group II patients with normals, the average decrease in 18:2 was 39%, in 20:4, 75%, and in 22:6, 88%. Simultaneously, 16:0 increased 31% and 18:1 rose 27%. There were also substantial increases in the 24:0 and 24:1 fatty acids. It is noteworthy that all of the diminished fatty acids are biochemically derived from linoleate (18:2) and belong to that class of lipids commonly called essential fatty acids. Except for a few minor variations, our findings for normal humans agree favorably with previously published values.[8,11]

When the weight compositions were converted to molar compositions using appropriate detector response factors, the comparison of controls with Group II burns is given in Table 2. For normals the ratio

TABLE 1. FATTY ACID COMPOSITION OF TOTAL PHOSPHOLIPID FROM RED CELLS OF NORMAL AND BURNED SUBJECTS

Patient	Sex	Age	Burn Size % 3°	Post Burn 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) mean ± SEM					24.9 ±1.3	0.8 ±0.3	16.6 ±0.6	16.5 ±0.4	9.5 ±0.5	17.2 ±0.8	3.2 ±0.5	5.8 ±0.6	5.7 ±0.2
Group I													
5	M	38	40	17	23.0	1.3	15.5	17.3	7.9	14.6	3.3	6.4	10.7
6	M	62	40 41.5	16	21.7	1.2	15.5	17.6	9.2	14.9	4.2	5.6	10.2
7	M	24	24.5 59	11	22.6	2.4	17.0	19.2	10.5	13.8	2.2	5.6	6.8
8	M	31	28 16.5	58	22.6	1.5	16.3	17.4	10.1	15.1	2.5	5.5	9.0
9	M	25	12.5 62	2	27.2	3.1	16.9	18.1	10.3	11.5	3.5	4.6	4.8
10	M	27	47 46	13	25.4	1.7	16.6	17.2	9.4	14.6	2.9	5.5	6.7
11	M	19	46 40	25	22.6	2.6	17.4	19.4	8.1	13.1	1.5	6.3	8.5
12	M	58	24 22.5 21.5	17	24.7	2.5	15.2	18.6	8.7	13.6	1.5	5.2	6.9
Group II													
1	M	40	60 8	17	31.9	2.3	15.6	21.6	6.1	5.5	0.5	7.2	9.3
2	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
3	M	23	55.5 53	96	36.3	0.3	16.7	20.3	5.3	3.7	0.9	8.2	8.3
4	M	41	30 24	2	32.0	2.2	17.8	20.2	6.0	2.2	-	10.3	9.2

TABLE 2. MOLAR FATTY ACID COMPOSITION OF ERYTHROCYTE PHOSPHOLIPIDS FROM NORMALS AND ESSENTIAL FATTY ACID DEFICIENT BURNED PATIENTS

Fatty Acid	Normals (n=5)	Group II Burns (n=4)
	moles/ 100 moles	
16:0	28.5	36.9
16:1n7	0.9	1.7
18:0	16.9	16.7
18:1n9	16.8	21.3
18:2n6	9.7	5.8
20:4n6	15.8	4.5
22:6n3	2.7	0.4
24:0	4.4	5.8
24:1	4.4	6.8
Total saturated	49.8	59.4
Total unsaturated	50.3	40.5
Saturated/unsaturated	0.99	1.47

of saturated to unsaturated fatty acids is 0.99, while the burn population has a ratio of 1.47.

Our findings suggest a unique form of essential fatty acid deficiency among the Group II individuals in that all fatty acids synthetically distal to and including linoleate are reduced. In most other clinical and laboratory cases of essential fatty acid deficiency, linoleate is decreased but arachidonate remains normal. With this in mind it was decided to study the effect of dietary supplementation on the red cell lipid composition. This was accomplished by intravenous infusion of a 10% soybean lipid emulsion particularly rich in linoleic acid (43%). Following multiple units administered over a 6-8 week period, two patients (#1 and #2) exhibited completely normal erythrocyte lipid patterns (Figures 1 and 2). During this period, Patient #1 received a total of 50 units (2.5 kg) of intravenous fat, and Patient #2 a total of 67 units (3.25 kg). A third patient (#3) received a fat-free diet but one of adequate caloric value, maintained a stable but low body weight, yet failed to correct his red cell essential fat deficiency within eight weeks. In contrast, a fourth individual (#4) apparently returned to a normal lipid profile simply as a result of adequate oral intake (Fig. 3).

Three classes of plasma lipids were also investigated. They were the phospholipids, triglycerides, and cholesterol esters, which together account for about 80% of the total lipid. When compared with normals, the Group II burn patients had nearly identical fatty acid compositions (Tables 3-5). Thus, at a time when their erythrocyte phospholipids had markedly reduced levels of 18:2, 20:4, and 22:6 acids, the plasma lipid distribution of these same fatty acids was unchanged or only slightly diminished. When significant deviations from normal did occur, such as low triglyceride linoleate for Patient #1 and Patient #2, there was no consistent pattern among all lipid classes in all patients.

One primary indicator of essential fatty acid deficiency is the presence of 5,8,11-eicosatrienoic acid, a metabolite of oleic acid and a compound usually absent from healthy individuals. Small amounts of 20:3n9 were identified in the plasma phospholipid fractions of the "abnormal" burn patients, ranging in value from 0.6 to 4.2%. Upon administration of intravenous fat to Patients #1 and #2, the level of this trienoate was gradually reduced but not entirely abolished. In addition, Patient #1 responded to fat therapy with increasing proportions of those acids of the linoleate and linolenate series in his plasma phospholipids and triglycerides.

We were fortunate to obtain, during the course of this investigation, blood samples from 2 unburned individuals with enterocutaneous fistulae who were being maintained on fat-free parenteral diets for one month or longer. In comparing their erythrocyte phospholipid fatty acid distribution (Table 6) with the normal and Group II burn patterns,

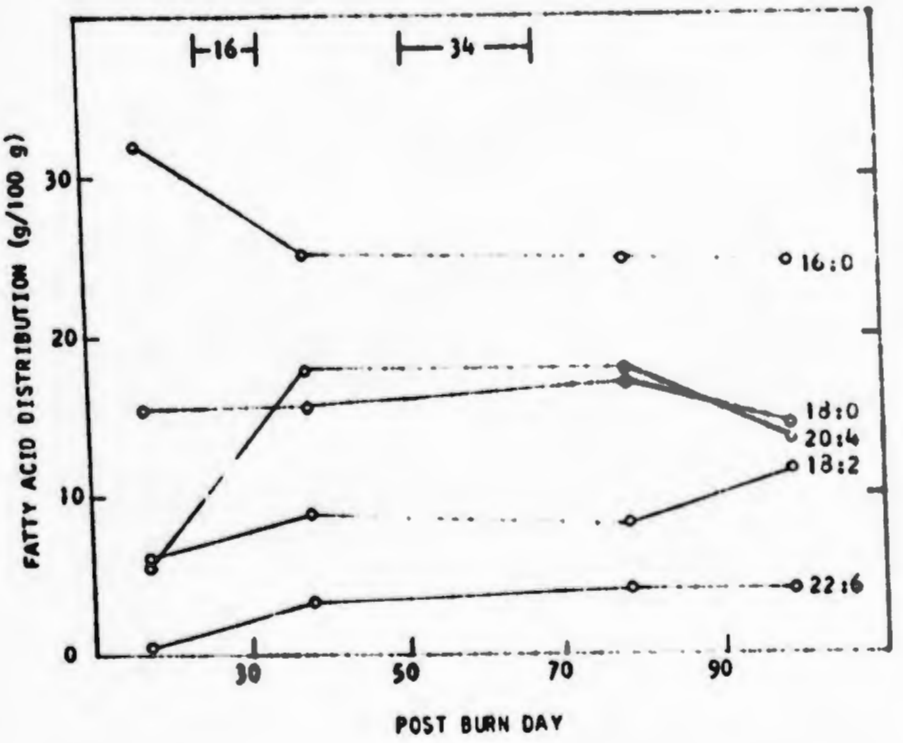

Figure 1. Change in distribution of selected fatty acids from the erythrocyte phospholipids with intravenous fat emulsion in Patient #1, age 40, with a 60% total body surface burn, of which 8% was full thickness. At the top of the figure are the time intervals and total units of soybean lipid infusions.

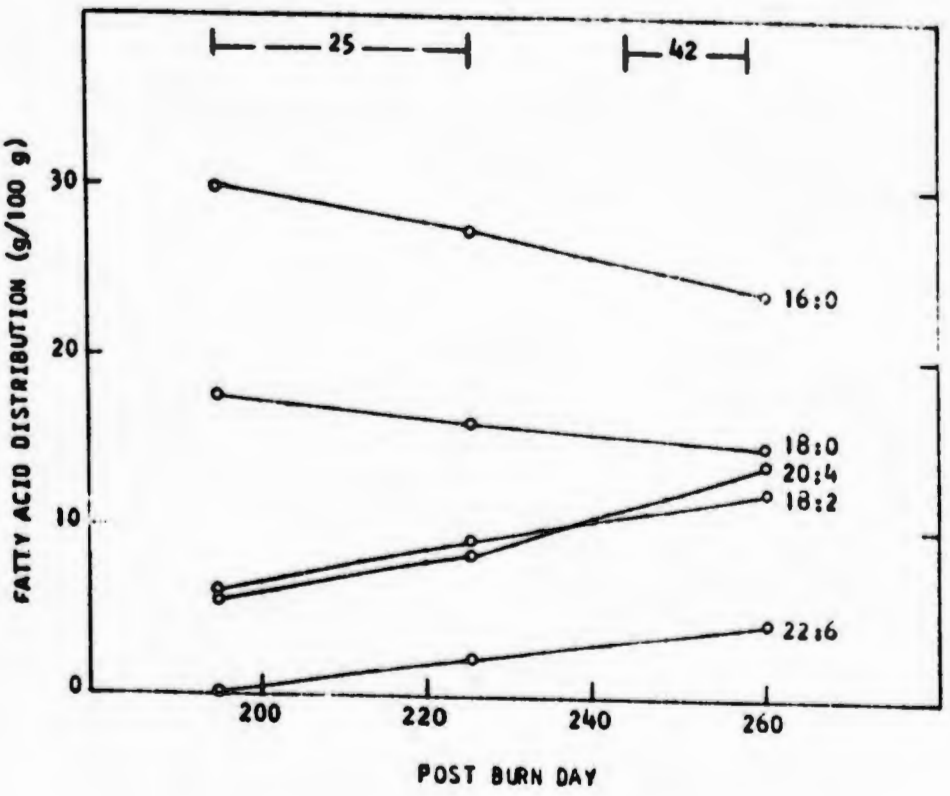

Figure 2. Change in distribution of selected fatty acids from the erythrocyte phospholipids with intravenous fat emulsion in Patient #2, age 19, with a 33.5% total body surface burn, of which all was full thickness. At the top of the figure are the time intervals and total units of soybean lipid infusions.

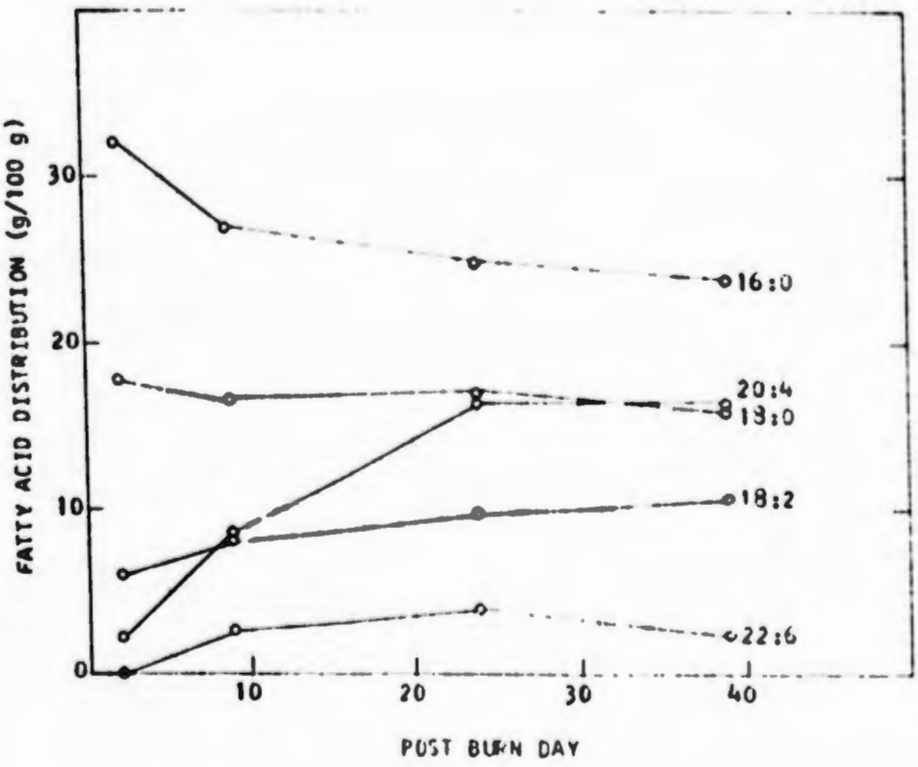

Figure 3. Change in distribution of selected fatty acids from the erythrocyte phospholipids with oral diet in Patient #4, age 41, with a 30% total body surface burn, of which 24% was full thickness.

TABLE 3

FATTY ACID COMPOSITION OF PLASMA PHOSPHOLIPIDS

Fatty Acid	Normals (n=2)	Group II Burns Pt #1	Pt #2	Pt #3
		g/100 g		
16:0	30.0	29.6	26.6	26.0
16:1n7	0.6	2.5	1.9	0.4
18:0	16.0	14.2	16.4	18.1
18:1n9	12.3	16.9	16.5	9.7
18:2n6	22.8	16.9	15.0	25.9
20:3n9	-	1.8	4.2	1.1
20:3n6	2.6	4.7	2.1	3.5
20:4n6	9.6	9.5	8.2	9.5
22:5n3	0.6	0.4	0.9	0.8
22:6n3	2.0	1.5	2.1	1.7
24:0	1.4	0.5	2.1	3.9
24:1n9	2.2	2.8	3.9	1.9

TABLE 4

FATTY ACID COMPOSITION OF PLASMA TRIGLYCERIDES

Fatty Acid	Normals (n=2)	Group II Burns Pt #1	Pt #2	Pt #3
			g/100 g	
14:0	1.2	1.2	2.1	4.4
16:0	25.1	27.5	27.8	17.4
16:1n7	5.2	6.3	6.0	4.2
18:0	4.9	5.0	5.2	4.0
18:1n9	40.6	50.4	47.2	26.4
18:2n6	22.8	7.5	8.6	32.8
18:3 (?)	-	-	-	1.8
20:4n6	1.2	1.2	2.1	1.8

TABLE 5

FATTY ACID COMPOSITION OF PLASMA CHOLESTEROL ESTERS

Fatty Acid	Normals (n=2)	Group II Burns Pt #1	Pt #2	Pt #3
		g/100 g		
16:0	10.4	13.4	15.1	7.1
16:1n7	3.6	12.1	8.2	2.3
18:0	2.9	2.6	6.6	5.8
18:1n9	19.5	30.6	23.9	12.5
18:2n6	53.9	34.8	36.6	59.7
18:3 (?)	-	-	3.2	5.0
20:4n6	9.6	6.5	6.3	7.6

TABLE 6. EFFECT OF LONG-TERM FAT-FREE DIETS ON RED CELL PHOSPHOLIPID FATTY ACID DISTRIBUTION

Fatty Acid	Normals (n=5)	Pt # 12	Pt # 13 (2/2/72)	Pt # 13 (10/3/72)
		g/ 100 g		
16:0	24.9	26.8	24.0	24.2
16:1n7	0.8	0.9	3.0	2.0
18:0	16.6	14.3	13.9	17.2
18:1n9	16.5	18.0	18.1	20.6
18:2n6	9.5	10.3	5.8	6.2
20:4n6	17.2	11.8	14.7	2.5
22:6n3	3.2	1.7	3.9	2.3
24:0	5.8	7.9	7.4	4.7
24:1n9	5.7	8.2	9.2	10.3

it is apparent that, except for slightly lower linoleate levels, these patients differ little from controls. Analysis of the plasma lipids of one of these subjects (#5, 10 March 1972) also yielded an essentially normal fatty acid profile. In particular, none of the 20:3n9 species could be detected.

DISCUSSION

The fatty acid composition of erythrocyte phospholipids from 4 individuals with burns covering 30 to 60% of their bodies has been shown to be consistent with essential fatty acid deficiency. That is to say, significant reductions in the acids of the linoleate and linolenate families were observed. However, this is not a general characteristic of thermal injury, since other patients with apparently equally severe trauma display normal fatty acid profiles. Those factors which predispose selected burn patients to these red cell lipid abnormalities remain obscure. While burn size and postburn day appear to be of minor importance, a more reliable guideline would probably be the overall metabolic state of the patient. Under the extreme catabolic conditions which are often encountered in burn patients, essential fatty acid deficiency might indeed be suspected.

Correction of essential fat deficiency was achieved in 2 patients (#1 and #2) through multiple infusions of soybean lipid emulsion and in a third (#4) by extensive oral feedings. Yet a fourth patient (#3), who received no intravenous fat and remained essentially catabolic, still exhibited lipid abnormalities after two months. (His discharge from this institute prevented continuing analysis of the deficiency.) It would be premature to ascribe too vital a role to intravenous fat therapy. Rather, the nutritional value of the emulsion (110 calories/100 ml) and the patients' improved eating habits and body weights suggest that a supranormal caloric regimen is perhaps the key factor in eliminating an apparent red cell essential fatty acid imbalance. In support of this argument is the demonstration by Curreri et al.[5] that glucose-sustained intravenous hyperalimentation is sufficient to correct the elevated intracellular sodium levels in these burn patients.

Collins et al[12] have reported the use of soybean fat emulsion in a patient who had been maintained on a fat-free intravenous diet for over three months. Prior to fat therapy the patient developed a skin rash, and the proportion of 5,8,11-eicosatrienoate in his plasma phospholipids rose to 14%, with corresponding decreases in linoleate and arachidonate. With the onset of fat infusions there was a dramatic return to a normal fatty acid distribution and disappearance of the skin rash. In this case, at least, the skin pathology and trienoic acid levels were consistent with the classical picture of essential fatty acid deficiency seen in infants[13] and some experimental animals.[14]

None of the patients examined in this survey had skin lesions characteristic of essential fat deprivation, nor did they have particularly abnormal plasma lipids. The observed fatty acid alterations were confined almost entirely to the phospholipids of the red cell membrane. The decreases in polyunsaturated acids are similar to those described for a variety of other red cell disorders. Neerhout[15] has recently reviewed such findings and, in general, reduced erythrocyte linoleate levels are associated with the following diseases: hereditary spherocytosis, elliptocytosis, acquired hemolytic anemias, and acanthocytosis. But only in the manifestation of acanthocytosis (abetalipoproteinemia) is there also a concomitant decrease in arachidonate.[16] Another striking similarity between these clinical states and that induced by thermal injury is the physiologic modification of the red cell membrane. Acanthocytes undergo distinct morphologic changes,[15] while hereditary spherocytes became more fragile and exhibit elevated cation fluxes.[17] As noted earlier, thermally traumatized red cells are distinguished by increased osmotic fragility and supranormal sodium concentrations.

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PUBLICATIONS AND/OR PRESENTATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

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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
71 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	3A061102B71R	01	307
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Procede 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

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71 05	Cont	DA	C. In-House

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

18. RESOURCES ESTIMATE	PRECEDING	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	72	0.6	16.8
	CURRENT 73	0.5	16.0

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

22. KEYWORDS (Procede 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. Procede text with security classification code.)

23. (U) The general metabolic state or erythrocytes from burned humans and rats will be evaluated in an effort to account for the elevated sodium concentrations associated with these cells.

24. (U) Subjects will include those patients in the severe burn category and rats with inflicted 30% scald burns, as well as appropriate controls. Areas of investigation will include activities of the various membrane-bound adenosine triphosphatases, intracellular hexokinase activity, the level of 2,3-diphosphoglyceric acid, and the overall glucose-to-lactate conversion.

25. (U) 71 07 - 72 06 In the analysis of erythrocyte membrane ATPases, no differences were noted between normal and burned humans. Burned rats, however, exhibited nearly twice the total ATPase activity of unburned animals as reflected in maximum velocity values, without significant alteration of the enzymes' binding capacity for substrate. While burned humans showed a significantly greater hexokinase activity than their controls, rats had identical enzyme levels before and after thermal injury. When overall rates of glycolysis were measured in terms of glucose utilization and lactate production, neither humans nor rats demonstrated changes related to thermal injury. As for intracellular levels of 2,3-diphosphoglyceric acid, burned rats show an initial decrease during the early postburn phase bur normal concentrations by the fifth postburn day and thereafter.

*Available to contractors upon contractor's approval

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ANNUAL PROGRESS REPORT

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

REPORT TITLE: THE METABOLIC STATE OF THE RED CELL IN BURNED SOLDIERS AND IN A LABORATORY MODEL

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

1 July 1971 - 30 June 1972

Investigators:

George M. Helmkamp, Jr., Captain, MSC
Jerl P. Blackwell, BS
Ysidro Villarreal, BS

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 1971 - 30 June 1972

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A general picture of the metabolic state of red cells in thermally injured humans and laboratory rats has been obtained. For both groups there is no change in the overall conversion of glucose to lactate through the normal glycolytic pathway, when intact cells are incubated in a glucose-containing medium. In hemolysates, however, humans show an elevated hexokinase activity in the postburn period. Rats, on the other hand, exhibit normal levels of hexokinase as well as 2,3-diphosphoglycerate.

In related experiments, the adenosine triphosphatase activities of isolated erythrocyte membranes were determined. Humans showed no difference between controls and burned patients when enzyme activity was measured at a constant substract concentration. However, when the kinetic parameters, V_{max} and K_m, were calculated, small increases were found in the Na,K-independent and Na,K-dependent ATPase rates. With the rat model these increases following thermal injury were considerably greater.

The above observations are discussed with respect to the elevated intracellular sodium levels characteristic of burned humans but not of burned rats.

Membrane adenosine triphosphatase
Hexokinase
2,3-Diphosphoglyceric acid
Glycolysis

THE METABOLIC STATE OF THE RED CELL IN BURNED SOLDIERS AND IN A LABORATORY MODEL

Several laboratories, including this institute, have reported elevations of erythrocyte sodium concentration associated with severe thermal injury, the magnitude of which is 40-45%.[1,2] It was also demonstrated by Curreri et al. that supranormal caloric intake by these patients was sufficient to correct this cation imbalance. The abnormal influx of sodium into the cell could be explained by a defective active transport system, an altered passive permeability barrier, or changes in the intracellular energy-generating process. A more complex situation would involve several of these factors.

We, therefore, decided to investigate the various adenosine triphosphatase activities of the red cell membrane, of which the Na, K-dependent species is directly associated with the active cation pump. In addition, several barometers of erythrocyte glucose metabolism were considered, including overall glycolysis, hexokinase activity, and 2,3-diphospho-glycerate concentrations.

METHODS

Adenosine Triphosphatase Activity. Erythrocyte membranes were prepared from heparinized human blood by lysis in 15 mOsM Tris-HCl, pH 7.5, as described by Ronseberg and Guidotti.[3] Protein concentration was determined with the biuret method. With minor modifications the procedure of Mircevova and Simonova was followed in the measurement of stromal ATPase activity.[4] The composition of the basic incubation medium was 250 mM Tris-HCl, 75 mM NaCl, 12.5 mM KCl, 5 mM $MgCl_2$, 2,5 mM ATP, and approximately 1-2 mg of stromal protein at a pH of 7.4 at 37°. To distinguish between Na,K-dependent and Na,K-independent activities, 5×10^{-5} M ouabain was added to inhibit specifically the former. The release of inorganic phosphate was ascertained according to Fiske and Subbarow[5] or more easily by the automated method of van Belle.[6]

For the preparation of rat erythrocyte ghosts it was necessary to employ the phosphate buffer systems of Dodge et al.[7] for washing and lysis. Tris buffers were ineffective in removing all hemoglobin from the red cell membranes. However, once the ghosts were essentially colorless, they were washed extensively with hypotonic Tris buffer to remove as much interfering phosphate as possible. The assay of rat erythrocyte ATPase also required modification of the human procedure; the incubation medium contained 50 mM imidazole, 0.1 mM disodium-EDTA, ATP and $MgCl_2$ in a molar ratio of 0.67, and sufficient glycyglycine for a final pH of 7.55 at 37°. Since

ouabain is not an effective inhibitor of the rat Na,K-dependent ATPase,[8] 75 mM NaCl and 20 mM KCl were added to the above buffer to activate this enzyme.

Glucose Metabolism. Blood was collected in heparinized syringes and the red cells separated and washed several times in ice-cold saline. The cells were then suspended in a medium described by Keitt:[9] Na^+ 145 mM; K^+ 5mM; Cl^- 127 mM; HCO_3^- 25mM; Mg^{2+}, 1 mM; and glucose, 1 mM. Using approximately 10^9 cells per ml, the samples are incubated at 37° for a proscribed length of time, after which the reaction is terminated by trichloroacetic acid and brief sonication. Centrifugation yields a clear, colorless supernatant which is assayed for glucose and lactate by a dual-channel autoanalyzer procedure. Red cell counting was done either by hemocytometer or with a CoulterCounter, Model S.

Hexokinase Activity. Lysates of saline-washed rat erythrocytes were prepared according to Brewer et al,[10] except that freezing and thawing were done with equal volumes of packed red cells and 0.1 M Tris-HCl, pH 8.0, containing 1mM disodium-EDTA. These changes eliminated the crystallization and precipitation of hemoglobin that readily occur at neutral pH. Hemoglobin remained soluble and was measured spectrophotometrically after conversion to cyanmethemoglobin. The lysate was then diluted to a hemoglobin concentration of 10 mg/ml. Enzyme assays were performed at 25° and pH 7.2, as outlined by Brewer et al;[10] results were expressed in μmoles of glucose phosphorylated per hour per gm of hemoglobin. Human red cells were examined in a similar manner.

2,3-Diphosphoglycerate. The enzymatic hydrolysis of 2,3-DPG to phosphoglycerate and inorganic phosphate, as described by Rose and Liebowitz,[11] was used to determine 2,3-DPG levels in lysates of washed erythrocytes from humans and rats. These lysates could be frozen for at least two weeks with no appreciable loss of activity. The released phosphate was assayed colorimetrically.[6]

RESULTS

Erythrocyte membrane ATPase activities have been determined on a wide variety of burned individuals, as well as numerous controls. In the actual procedure one obtains a total ATPase activity and, by selective inactivation, the Na,K-independent ATPase activity; by difference one calculates the Na,K-dependent ATPase. In Table I are listed the results of our normal and burn populations. Despite the lack of rigorous statistical analysis, it is obvious that there is no difference between the two groups. In these experiments an ATP concentration of 2.5 mM was used.

Upon variation of substrate concentration and measurement of

Table 1. Erythrocyte Adenosine Triphosphatase Activities

Activity	Normal (10)	Thermally Injured (14)
Na^+,K^+-ATPase	0.048 ± 0.005*	0.054 ± 0.007
Mg^{++}-ATPase	0.099 ± 0.011	0.087 ± 0.011
Total ATPase	0.148 ± 0.013	0.141 ± 0.016

*ATPase values represent micromoles of phosphate released per hour per milligram of membrane protein.

subsequent enzyme activity, one can examine enzyme kinetics in the Michaelis-Menten fashion and establish the maximum velocity (V_{max}) and Michaelis constant (K_m) for the particular enzyme. The latter parameter is roughly equivalent to the binding constant of the enzyme for its substrate. Typical results of this treatment are shown in Figure 1. The linearity of the double reciprocal plots conforms to a hyperbolic relationship between activity and substrate concentration and also to the absence of substrate inhibition or subunit cooperativity. From the axial intercepts, V_{max} and K_m are calculated; these results are summarized in Table 2. In general, thermal injury led to increases in V_{max} for all ATPase activities in both humans and rats. K_m values, on the other hand, changed less dramatically and in no consistent manner. The most remarkable alteration was noted in the Na,K-dependent ATPase activity in the group of 5-day post-30% burned rats. Not only was there a greater than twofold increase in V_{max} in the burned animals, but the K_m for ATP was substantially reduced.

As for the human ATPase activities, the observed normal K_m's compared favorably with values reported by Godin and Schrier,[12] although the V_{max} figures of these authors are three to four times those recorded here.

The general metabolic state of red cells should be reflected in overall glycolysis. As these cells are devoid of any respiratory apparatus, glycolysis proceeds to the accumulation of lactate. Very little glucose (less than 10%) is diverted through the oxidative pentose shunt. Thus, the rate of lactate production should be approximately twice the rate of glucose utilization. That this is indeed the case is illustrated in the experiment described in Figure 2. The linear portions of the curves yield a rate of glucose utilization of 0.19 μmoles per hour per 10^9 cells and a rate of lactate formation of 0.36 μmoles per hour per 10^9 cells.

When glycolysis was followed in this manner as a function of postburn day, it was found that with the exception of decreased rates at the fourth day, values were generally normal (Table 3). Admittedly, it will be necessary to examine the 1-3 day postburn period. Although no human burned patients have been investigated to date, mean values for 8 control subjects are 0.18 μmoles of glucose utilized and 0.35 μmoles of lactate produced per hour per 10^9 cells.

To probe further the pathway of glucose metabolism in the red cell, the primary enzyme in this process was studied. Hexokinase activity was determined as a function of hemoglobin concentration in hemolysates from burned and normal humans and rats (Table 4). In the human patient population, with a mean burn

Table 2. Kinetic Parameters of ATPase from Control and Burned Humans and Rats

	K_m	V_{max}
	mM	μmole $P_i hr^{-1} mg^{-1}$
I. Humans, control (n=3)		
Total ATPase	0.23	0.20
Na,K-independent ATPase	0.16	0.13
Na,K-dependent ATPase	ND	0.07
Humans, burned (n=5)		
Total ATPase	0.28	0.27
Na,K-independent ATPase	0.17	0.17
Na,K-dependent ATPase	ND	0.10
II. Rats, control (n=5)		
Total ATPase	0.48	0.84
Na,K-independent ATPase	0.37	0.45
Na,K-dependent ATPase	0.73	0.45
Rats, Burned (n=5)		
Total ATPase	0.41	1.59
Na,K-independent ATPase	0.59	0.71
Na,K-dependent ATPase	0.40	0.99

ND, Not determined

Table 3. Erythrocyte Glycolysis in rats with 30% Total Body Surface Thermal Injury

Post-burn Day	Number of Determinations	Glucose Utilization	Lactate Production
		μmole per hour per 10^9 cells	
control	5	0.33	0.68
4	2	0.18	0.37
5	4	0.32	0.59
6	2	0.30	0.59
7	3	0.41	0.78
10	4	0.27	0.55

Table 4. Hexokinase Activity from Control and Burned Humans and Rats

	Number of Determinations	Activity (mean ± SEM)	Significance
		μmoles glucose-P per hr per g Hb	
I. Humans, control	8	13.7 ± 0.6	$0.02 < p < 0.05$
Humans, burned (46%; 24 PBD)	13	23.9 ± 3.4	
II. Rats, control	16	100.8 ± 3.7	N.S.
Rats, burned (30%; 5 PBD)	8	93.0 ± 4.5	

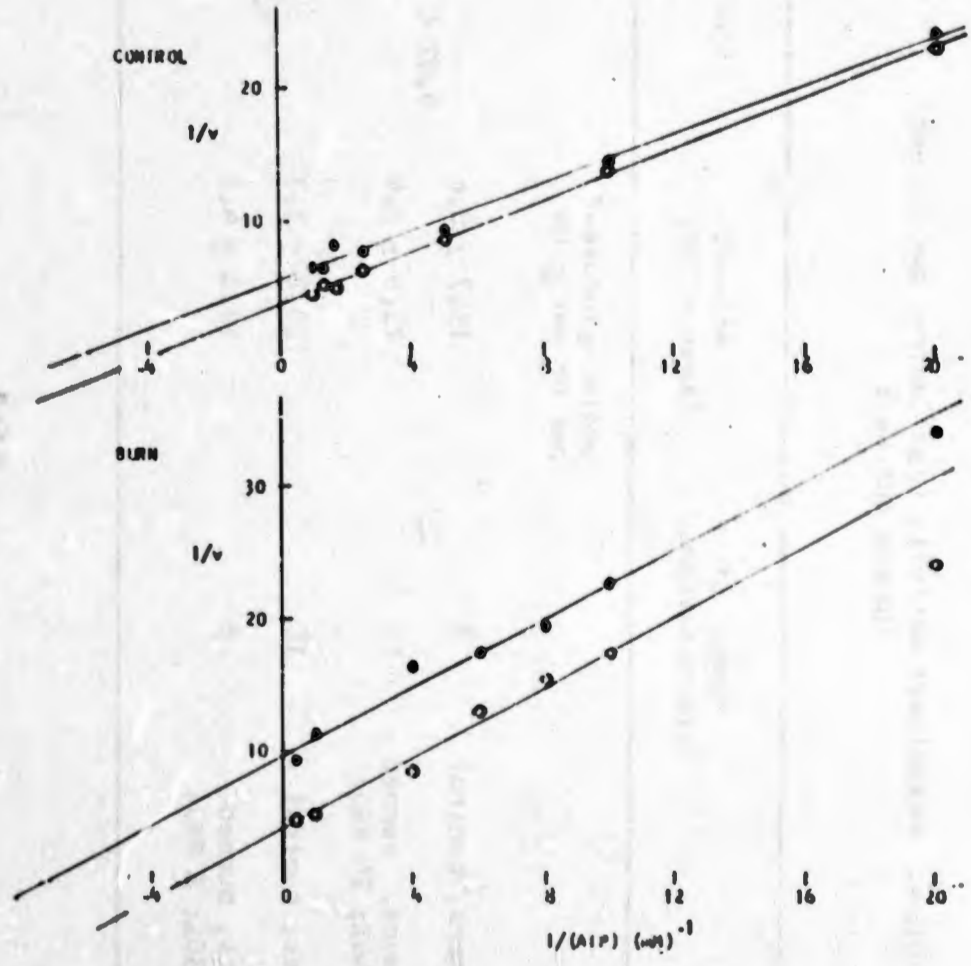

FIGURE 1. Double reciprocal plots of total ATPase (O) and Na,K-independent ATPase (●) activities of erythrocyte membrane ghosts from control and burned humans. See text for details.

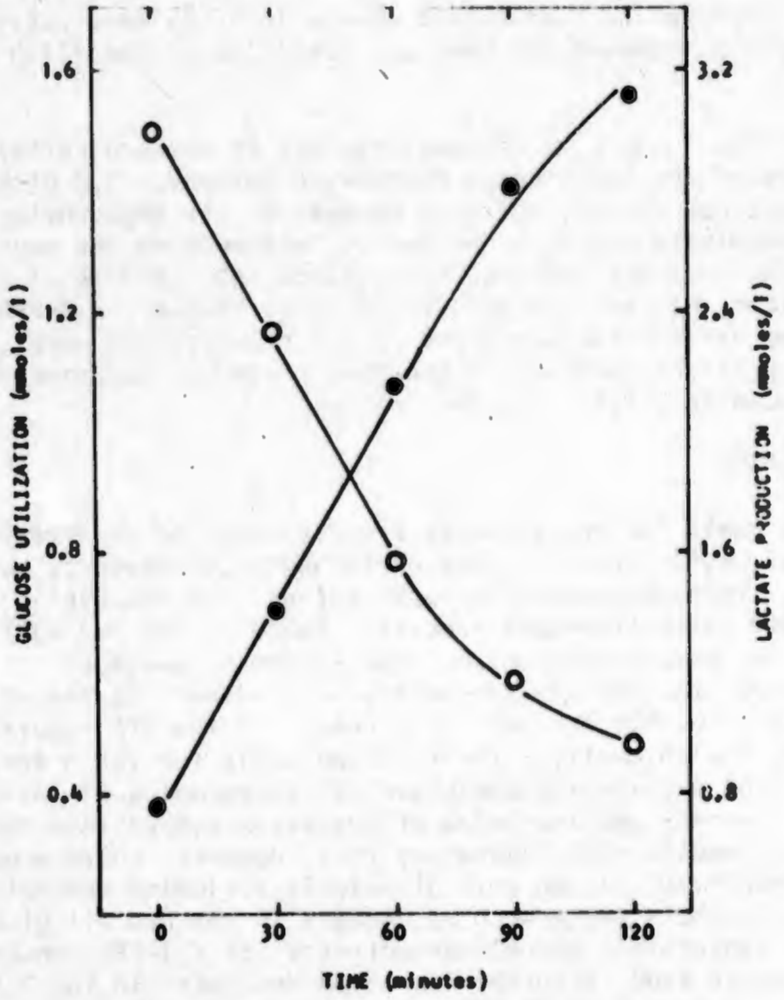

FIGURE 2. Glycolysis in erythrocytes from a burned rat. On the fourth day following a 30% total body surface thermal injury, the red cells of this animal were washed and incubated at 37° in a buffered medium containing 0.90 mM glucose; the red cell count was 1.92×10^9 per ml. At the indicated times, protein-free filtrates were assayed for glucose (O) and lactate (●).

size of 46% and analysis at 24 days postinjury, there was a 74% increase in hexokinase activity. Increases of variable magnitude were observed as early as one week postburn and as late as 8 weeks postburn; in addition, burn size (21-85%) appeared to bear no simple relationship to enzyme levels. Rats, on the other hand, displayed no significant change in hexokinase activity following a standard 30% burn and sacrifice at the fifth postburn day.

We also thought it of some interest to measure certain intermediates of the erythrocyte glycolytic pathway. 2,3-Diphosphoglycerate was chosen, not only because of its high-energy phosphate chemistry but also due to its influence on the oxyhemoglobin dissociation equilibrium and the activity of several glycolytic enzymes. As seen in Table 5, however, 2,3-DPG levels remained essentially unchanged in our standard rat model. Following a slight decrease at one-hour postburn, measurements taken up through the eighth day were normal.

DISCUSSION

The basis for the above experiments was the observation of elevated sodium levels in the erythrocytes of severely burned humans. This phenomenon no doubt arises from changes in one or more of the cation transport processes found in the red cell membrane. The active pumping mechanism, that is the movement of sodium out of the cell against its concentration gradient, is linked to glucose utilization within the cell as a result of the ATP requirement. In fact the stoichiometry of the reaction calls for the hydrolysis of one mole of high-energy phosphate for three moles of sodium pumped out of the cell and two moles of potassium pumped into the cell.[13] From our results with laboratory rats, however, there appears to be no impairment of red cell glycolysis following thermal injury. In these animals there were no changes in the overall glucose-to-lactate conversion, hexokinase activity, or 2,3-DPG concentration. On the other hand, Arturson has noted decreases in the 2,3-DPG levels in humans with 40-50% full-thickness burns, but normal or slightly elevated levels in those patients with smaller injuries.[4] Of particular interest at this point is our inability to demonstrate changes in the intracellular sodium concentration with the laboratory model. In burned rats (30% full thickness) the concentration is 12.4 mEq/liter (n=18), compared with a normal value of 12.2 mEq/liter (n=10).

Active cation transport also depends on an intact membrane-bound enzyme system, the Na,K-dependent ATPase. When this enzyme was measured under optimal conditions in isolated rat erythrocyte ghosts, the burned animal had nearly twice the activity of the control group. It is interesting to speculate that this twofold

Table 5. Erythrocyte 2,3-Diphosphoglycerate Concentration in Rats with 30% Total Body Surface Thermal Injury

Post-burn Day	Number of Determinations	2,3-Diphosphoglycerate Concentration
		μmoles/100 ml packed cells
Control	9	546
One hour	4	490
1	4	566
2	8	587
3	4	531
6	6	526
8	8	534

increase in the rate of ATP hydrolysis is sufficient to correct deficiencies, if any, in the passive diffusion of sodium as a result of thermal injury.

Altered passive diffusion of sodium indeed appears to play a major contributing role in the greater-than-normal intracellular levels in human burn patients. ATPase activities are unchanged, while hexokinase activity is nearly twice normal. Thus, there is most likely an abundant supply of energy to operate the active sodium pump. Actual cation flux measurements, soon to be conducted with red cells, will allow a more complete description of sodium imbalance.

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PUBLICATIONS AND/OR PRESENTATIONS

None

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	A. NEW	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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72 04	Cont	DA	C. In-House

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c. TYPE: d. AMOUNT:
e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 72	0.4	14.4
	CURRENT 73	0.2	7.0

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

22. KEYWORDS (Precede each with Security Classification Code)
(U) Thermal Injury; (U) Wound Healing; (U) Rats; (U) Epithelialization

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

23. (U) To establish an experimental model to study grossly and histochemically open granulating wounds in the burned animal to improve care of burned troops.

24. (U) Ten adult rats were divided into two groups and anesthetized with ether. In group A, 5 rats, a 30% third degree burn was inflicted upon the back by immersion into 85° C of water for 30 seconds. A 2.5 x 4.5 cm full thickness defect was then made on the upper back with a scalpel. In group B, unburned controls, a similar defect was created. Tebdec marking sutures were placed at the corners and midpoints of all wounds. Using planemetry, rate of epithelialization was recorded at 3, 7, 14, 21 days. Wound contraction was evaluated by measuring the distance between sutures on two sides and multiplying them. The product, a decreasing area, indicated rate of contracture. All animals were sacrificed at 21 days. Wound biopsies were taken at that time. Significantly cultures of all wounds were taken at 3, 7, 14 and 21 days.

25. (U) 72 04 - 72 06 Preliminary results suggest that burn injury impairs wound healing but this may be a non-specific effect of postburn nutritional changes.

(Available to contractor, upon established approval)

DD FORM 1 MAR 69 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 68 AND 1498-1 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

ANNUAL PROGRESS REPORT

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

REPORT TITLE: EFFECT OF THERMAL INJURY ON WOUND HEALING

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

1 July 1971 - 30 June 1972

Investigators:

Roger E. Salisbury, MD, Major, MC
Douglas W. Wilmore, MD, Major, MC
Glenn D. Warden, MD, Major, MC
F. D. Foley, MD

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

REPORT TITLE: EFFECT OF THERMAL INJURY ON WOUND HEALING

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

Period covered in this report: 1 July 1971 - 30 June 1972

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Reports Control Symbol MEDDH-288(R1)

So many systemic and local factors affect healing in the burn patient that the wound is difficult to clinically evaluate. The purpose of this study was to establish an experimental model to study, grossly and histochemically, open granulating wounds of the burned animal.

Ten adult rats were divided into 2 groups. Group A, 5 animals, received a 30% third-degree burn of the back. A full thickness defect was then created in the unburned back and neck, 5.5 x 3.5 cm. In Group B, the unburned animals, the same sized defect was also created in the upper back and neck. At 3, 7, and 14 days postinjury, cultures, pictures and circumferential measurements were made of all wounds. The animals were sacrificed on the 21st day postburn. Pictures of all wounds were taken and the wounds were sent for histologic evaluation.

Surprisingly, the burned animals healed more rapidly and in 10 days the wounds were one-half in circumference those of the unburned animals. By 21 days, 4 out of 5 of the burned animals had healed their wound, whereas only one out of 5 of the unburned animals had healed.

Thermal injury
Wound healing

EFFECT OF THERMAL INJURY ON WOUND HEALING

Except for several studies by Levenson on healing laparotomy incisions, controlled studies of wound healing in the burned animal are rare. The open granulating wound has received less attention. Yet it is the granulating wound that determines our ability to successfully graft the patient and effect healing. The purpose of this study was to establish an experimental model to study, grossly and histochemically, open granulating wounds in the burned animal.

METHOD

Ten adult rats were divided into 2 groups. After light ether anesthesia their backs were shaved and prepped. Group A, 5 animals, were dipped into water 85° C for 30 seconds producing a 30% third degree burn of the back. A full thickness defect was created with a scalpel in the unburned upper back and neck measuring 5.5 x 3.5 cm. In Group B, 5 animals were not burned but the same full thickness defect was created in the upper back and neck. Tevdec marking sutures were placed at the corners and midpoints of all wounds. Using planimetry, rate of epithelialization was to be recorded at 3, 7, 14, and 21 days. Wound contraction was determined by measuring the distance between the midpoint sutures on opposing sides of the wound and computing the product of these 2 measurements. Measurements of the circumference of the wounds were likewise made at 3, 7, 14, and 21 days. All animals were sacrificed at 21 days. All wounds were excised and sent for histological evaluation. Significantly, all rats were maintained on a normal cage diet. All open wounds were cultured at the time of picture taking.

RESULTS

All animals in both groups survived to 21 days. Both burned and unburned rats had staphylococci present in their wounds. Interestingly, the burned rats healed faster than the unburned group. Unfortunately, many of the Tevdec sutures pulled through or were eaten out during the course of the 21 days. Therefore, rates of epithelialization and contraction could not be measured. However, the circumference of the wounds were measured and at 10 days the average wound circumference in the unburned rats was 5.5 cm as opposed to 2.4 cm in the burned group. At 21 days, 4 out of 5 of the burned animals had completely healed their wounds whereas only one out of 5 of the unburned animals had healed their wounds. Histological evaluation revealed no differences between the 2 groups in regard to epithelialization, quantity of collagen or granulation tissue.

CONCLUSION

In this small series the burned animals appeared to heal more rapidly than their unburned counterparts. These results are certainly contrary to the findings of other investigators. The bacterial flora of the surgical wounds in both burned and unburned animals was similar with staphylococci the predominant organism. One would have anticipated that the proximity of the burn wound to an open granulating bed would have produced an infected wound resulting in decreased wound healing but such was not the case. It is possible that placing the open granulating wound next to the burn wound produced a false but apparently beneficial effect on wound healing in that the burn wound may have contracted during the ensuing 21 days, hastening contraction of the granulating wound. Therefore, the experiment will be repeated but the full thickness defect will be placed distant to the burn wound. Likewise, heavier sutures will be placed at the corners and midpoints of the wound in order to measure rate of epithelialization and contraction.

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5. Ramirez AT, et al: Experimental wound healing in man. Surg Gynecol Obstet 128:283, 1969.

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'S	8b. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
71 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	3A061102B71R	01	191
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) Pathogenesis of Burn Wound Infection: Bacterial Flora of Burn Wounds of Military Personnel Receiving Sulfamylon Treatment (44)

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PRECEDING FISCAL YEAR 72	0.3	8.4
CURRENT FISCAL YEAR 73	0.3	9.8

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21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede each with Security Classification Code)
(U) Burns; (U) Staph aureus; (U) Providencia stuartii; (U) Sepsis; (U) Humans

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

23. (U) Soldiers in combat incur thermal injury at a high rate; in its treatment, suppression of 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) 71 07 - 72 06 Continued epidemic Staph aureus, Type 84, was observed. Previous 84,85 and (group) 83A patterns disappeared completely in 1971; at the same time, antibiotic resistance rose steadily; at least 50% of recent isolates exhibit broad cross-resistance, not necessarily related to penicillinase production. Only nafcillin of the methecillin-type antibiotics remain moderately effective (i.e. with 30% of isolates). Providencia stuartii was the most common cause of septicemia; typing systems to classify this enteric form are under preparation, so that its role in burns can be better understood.

* Available to contractors upon established need-to-know.

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ANNUAL PROGRESS REPORT

PROJECT NO. 3A061102B71R-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
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1 July 1971 - 30 June 1972

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ABSTRACT

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Sepsis due to bacterial infection has remained a major problem in burn treatment despite the introduction of effective topical therapy. The major pathogenic species, as revealed in cultures of wounds, blood, sputum, biopsies, urine, and intravenous catheter tips are Staphylococcus aureus, Pseudomonas aeruginosa and Providencia stuartii. Klebsiella sp, Enterobacter sp, Proteus mirabilis and Escherichia coli played a decreased role in 1971 in contrast to earlier years. An extremely diverse mixed infection problem with gram positive cocci and gram negative enteric species was observed. Sequential septicemia with multiple invaders has become more apparent in the last 2 years. The primary infection problem involves increasing presence of staphylococci in the burn patient in 1971. These strains were most commonly antibiotic resistant.

Burns
Staph aureus
Providencia stuartii
Sepsis

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

Effective control of Pseudomonas burn wound sepsis has continued to be based on the use of topical Sulfamylon therapy in this Institute. Although classical invasive Pseudomonas burn infection has been reduced to an acceptably low incidence, infection in the severely burned patient has continued to serve as a major cause of morbidity and mortality. Previous observations have made it clear that the burn wound flora is not a stable entity from year to year, but one that fluctuates, modified by such variables as the severity of injuries, variations in therapeutic regimens, and probably by such imponderables as changes in ward personnel and physical alterations in the burn ward (Lindberg, et al).[1] The clinical condition of a patient obviously dictates the nature of the specimens submitted to the laboratory, so that the microbial flora recorded here is not a random sample. The bacteriologic findings summarized here reflect the principal agents affecting burn patients and major sites of involvement.

ANTEMORTEM BACTERIOLOGY IN BURN PATIENTS

The overall bacteriologic picture of burn wound flora for 1971 is shown in Table 1. This summary of isolates is obviously weighted by the many cultures taken on extremely ill patients and the paucity of samples from those less severely injured. However, the proportions of isolates are not altered if the compilation is made solely on the number of patients positive at least once for a given species. The identity of major species and in some instances the minor role played by organisms that have been reputed to act as opportunistic invaders can be seen.

There were 27 major genera or species differentiated in this resume. Staphylococcus aureus, Klebsiella pneumoniae, Pseudomonas aeruginosa and Providencia stuartii were the significant groups numerically. These 4 species made up 51% of all strains recovered from clinical specimens. Precise differentiations between Klebsiella sp and Enterobacter sp, replacing the obsolete Klebsiella-aerobacter differentiation, was implemented in February 1971. Thus, a residual K-E group (not differentiated between Klebsiella and Enterobacter) is listed for the first part of 1971. The overall incidence of Klebsiella sp (virtually all K. pneumoniae on the basis of sample strains tested) would probably be 11% instead of 9%. The K-A category used prior to 1971 probably included a preponderance of Klebsiella sp.

TABLE 1, Bacteriology of Antamartan Burn Patients
ISR - 1971

Organism	Source and No. of Isolates							Total Isolates
	Wounds and Surfaces	Blood	Lesions and Sputum	Urine and Foley	IV Cath.	Biopsy	Stool	
Staphylococcus aureus	204	85	78	34	16	56	4	477
Staphylococcus epidermidis	91	6	34	39	3	17	10	200
Strep. alpha hemo.	65	2	102	0	0	0	0	169
Strep. beta hemo.	6	0	5 (1 gp A)	3	0	0	0	14
Strep. non-hemo.	17	4	11	10	1	6	11	60
Bacillus sp.	5	1	4	6	0	2	1	19
Corynebacterium sp.	11	5	1	6	0	1	0	24
Pseudomonas sp.(1)	157	32	90	48	5	52	7	395
Klebsiella sp. (2)	117	13	67	52	5	17	39	310
Enterobacter aerogenes (3)	23	1	25	18	1	7	6	81
cloacae	19	3	12	12	0	13	5	64
K-E group (4)	17	3	10	12	0	14	2	58
Escherichia coli	103	7	57	103	0	18	64	352
Serratia sp.	5	0	6	2	0	3	0	16
Citrobacter sp.	8	5	10	2	0	1	3	29
Salmonella typhimurium	0	4	0	0	0	0	0	4
Proteus mirabilis	98	2	12	68	0	11	23	214
rettgeri	5	1	1	5	1	0	0	13
morganii	5	0	0	6	0	2	2	15
vulgaris	4	0	1	4	0	2	1	12
Providencia stuartii (5)	156	50	89	89	31	65	5	485
Mima-Herellea sp.	5	0	2	2	0	0	0	9
Neisseria sp.	33	0	33	0	0	0	0	66
Candida sp.	18	9	10	40	8	8	0	93
								3179
No. specimens	622	2662	287	597	312	197	68	
No. patients	180	179	94	263	123	76	35	

(1) rare P. maltophila and P. stutzeri seen
(2) only K. pneumoniae found
(3) 5 strains of E. hafniae and E. liquefaciens
(4) K-E group listed Jan 1971 only
(5) rare Prov. alcalifaciens found

Bacteriology of the Burn Wound and Other Surface Areas.

There were 23 groups or major species recovered from burn wounds. The predominant strains are listed in Table 2.

The wound and surface flora of the 147 patients who were cultured resembled, in many respects, the overall predominant pattern seen when all flora of all patients was summarized. Staph. aureus, in 61.2% of all samples, was the most common organism. The other gram-positive cocci, primarily coagulase-negative staphylococci and alpha streptococci, were found in from 26 to 30% of samples. Among the gram-negative flora, Klebsiella, Escherichia coli, and Proteus mirabilis occurred in similar frequency. The E. coli incidence was unexpectedly high, since it almost never is recorded as a potential invader of the burn wound. Klebsiella, which is a prominent feature of respiratory infection, was less common than was expected, and Proteus mirabilis, regarded as almost ubiquitous in healing wounds, was also less often found than had been anticipated. The other 3 species of Proteus were found in only 12 samples all together. Enterobacter sp would appear to have opportunity to colonize as readily as Klebsiella but they were not numerous. Genera seen only in 4 to 5 patients were Citrobacter, Serratia, Mima-Herellea, Bacillus, and Corynebacterium sp. They were inconsequential in wound infection. The high level of alpha-streptococci observed represents primarily group D streptococci on the basis of sample strains which were grouped. The presumption of fecal flora contamination is made. The wound flora was essentially heterogeneous, and most colonizing strains were relatively inocuous. Tissue invasion appeared to occur only with Staph., Pseudomonas and Providencia.

Septicemia in the Burn Patient, Institute of Surgical Research, 1971.

Positive blood cultures occurred in over 30% of the patients cultured. One hundred seventy-nine patients were cultured, and 63 were positive. This was 22% of all admissions, a decrease from 25% of admissions in 1970, but the septicemia rate was much higher than that which occurred in 1966 and 1967. A total of 1331 cultures were drawn for an average of 7.5 cultures per patient on whom at least one culture was taken. The total bacteriologic picture of blood stream infection in burns is summarized in Table 3. Seventeen genera were recovered. Predominant organisms were Staph. aureus, Providencia stuartii, Ps. aeruginosa and Klebsiella sp (these strains were undoubtedly pneumoniae, but speciation was done only on a sample of the isolates). The lethal implications of blood culture positive for one of the gram-negative species were much higher than for the staphylococci, but even with Staph. aureus,

TABLE 2. Predominant Burn Wound Flora in 147 Burn Patients
Institute of Surgical Research - 1971

Species	No. Patients Positive	No. Strains Isolated	% of Patients Positive
Staphylococcus aureus	90	204	61.2
Staph. coagulase-negative (=S. epidermidis)	44	93	29.9
Strep. alpha-hemolytic	39	65	26.5
Klebsiella sp.	37	117	25.2
Enterobacter			
aerogenes	20	28	13.6
cloacae	13	19	8.8
Escherichia coli	43	103	29.3
Proteus mirabilis	41	98	27.8
Providencia stuartii	50	156	34.0
Pseudomonas aeruginosa	52	157	35.2

19-4

TABLE 3. Blood Culture Isolates from 63 Burned Patients, 1971: Relation of Species to Mortality in Septicemia

Organism	No. Patients Positive	No. of Blood Cultures Positive	No. Patients Expired	% of Patients with Positive Cultures Who Expired
Staphylococcus				
aureus	27	85*	12	44
epidermidis	6	6	1	17
Streptococcus				
alpha hemolytic	2	2	0	0
non hemolytic	3	4	0	0
Corynebacterium sp.	4	5	3	75
Bacillus sp.	1	1	0	0
Providencia stuartii	23	50	20	87
Pseudomonas sp.	20	32	17	85
Klebsiella sp.	9	15	7	77
Enterobacter				
aerogenes	1	1	1	100
cloacae	3	4	2	66
Escherichia coli	5	7	5	100
Proteus				
mirabilis	1	2	1	100
rettgeri	1	1	0	0
Citrobacter sp.	1	5	0	0
Salmonella typhimurium	1	4	0	0
Candida sp.	5	9	4	80

* 28 isolates were collected from one patient

44%, of patients with a positive culture expired, although not necessarily with staphylococcal sepsis. When septicemia is compared with total burn flora, the lower level of opportunistic invasion by such genera as Serratia, Escherichia and Mima-Herellea is noteworthy. However, the frequency of occurrence of a given species in the blood stream does not convey the actual sequence of events which occurs in most of these septicemic episodes. To show the multiple invasive episodes that occur, the individual patients were listed with the total of species which occurred in each patient. This information is summarized in Table 4.

Single species blood stream invasion occurred in 14 patients with Staph aureus, 6 with Pseudomonas, 5 with Providencia stuartii, 3 with Klebsiella, 2 with Candida and one with E. coli. Thirty out of 63 patients had 2 or more species recovered in successive cultures. One-fourth of all positive patients had 2 species, one-eighth had 3, and 5 out of 63 had 4 or 5 species recovered during the course of illness. There was no predominant succession: the episodes show a distribution which parallels the predominant species seen in the overall bacteriologic flora of burned patients. Multiple infections occurred more often among fatally burned patients, but there was little evidence that a particular sequence of species was more lethal than any other. Providencia stuartii and Ps. aeruginosa occurred sequentially in 5 patients, and Staph aureus with Klebsiella occurred twice. All other sequences occurred as single episodes.

Out of 57 deaths, 35 had a positive blood culture while 22 did not. Out of 105 survivors, 27 had a positive blood culture. Enteric gram-negative bacilli and Ps. aeruginosa were the most frequent offenders in septicemia with lethal outcome.

Respiratory Tract Bacteriology.

Sputum and Lukens tube aspirate specimens were collected from 94 patients, with 287 samples, an average of 3 specimens per patient. In each of the 2 preceding years, about twice as many samples were collected. Why this decrease in sampling of the pulmonary flora occurred is not known.

The flora is divided into 2 groups: the alpha hemolytic streptococci, Neisseria and coagulase negative staphylococci, and the Staph aureus and gram-negative bacilli. The former constitute normal upper respiratory tract flora; there is no reason to ascribe to these organisms a pathogenic role in pulmonary infection. The status of Staph aureus is equivocal; it undoubtedly is frequently found in the nasopharynx, but in the proportions in which it was found here, and in view of the fact that almost all isolates

TABLE 4. Blood Cultures Positive in 63 Burned Patients: Species Recovered from Individual Patients - 1971

Species Recovered	No. Patients
Staphylococcus aureus	14
Staphylococcus aureus, Prov.stuartii	1
Staph.aureus, Prov., non-hemo. strep.	1
Staph.aureus, Entero. cloacae	1
Staph.aureus, Pseudomonas, Coryne.	1
Staph.aureus, Pseudomonas	1
Staph.aureus, Klebsiella, Prov.,Pseudo.	1
Staph.aureus, Klebsiella	2
Staph.aureus, non-hemo. strep.	1
Staph.aureus, Staph.coag.neg., Pseudo, Coryne., alpha-strep.	1
Staph.aureus, Klebsiella, E. coli, Pseudo., Prov.	1
Staph. aureus, Klebsiella, Prov.	1
Staph.aureus.,Staph.coag.neg.,Bacillus, Candida sp.	1
Providencia stuartii	5
Prov., Pseudomonas	5
Prov., Pseudomonas, Klebsiella	1
Prov., Entero.cloacae, E.coli	1
Prov., Pseudomonas,Candida	1
Prov., Entero., Pseudomonas	1
Prov., Staph. coag. neg.	1
Prov., Proteus mirabilis	1
Prov., Coryne.	1
Prov., Coryne., E. coli, Candida	1
Prov., Proteus rettgeri	1
Klebsiella sp.	3
Pseudomonas aeruginosa	6
Pseudomonas, Candida sp.	1
Citrobacter, non-hemo.strep.,Salmonella typhimurium	1
E. coli	1
Staph.,coag.neg.,alpha-strep.	1
Staph, coag.neg.	2
Candida sp.	2

		% of all Positives
No. of patients with one species recovered	33	52
No. of patients with two species recovered	17	26.9
No. of patients with three species recovered	8	12.9
No. of patients with four species recovered	3	4.7
No. of patients with five species recovered	2	3.0
	63	

TABLE 5. Predominant Species of Bacteria Recovered from the Respiratory Tract (94 Patients Cultured), Institute of Surgical Research - 1971

Species	No. Patients Positive	% of Cultured Patients Positive
alpha-hemolytic strep	58	61
Neisseria sp	26	27
Staph. coagulase negative	27	27.2
Staph. coagulase positive	41	43
Pseudomonas sp	37	39
Providencia stuartii	31	33
Klebsiella sp	43	45
E. coli	27	27.2
Enterobacter aerogenes	13	13
Enterobacter cloacae	11	11

were phage type 84, rather than the heterogeneous type distribution which is seen in healthy individuals, it must be regarded as an active pathogenic species. Among gram-negative flora, the Pseudomonas, Providencia and Klebsiella predominated. Klebsiella was found in almost half the patients whose sputa were cultured. These 3 genera, none of which are prominent in the normal nasopharynx, account for a major part of morbidity and mortality due to pneumonia in the burn patient. In contrast to Klebsiella, the closely related Enterobacter sp apparently play a minor role in pulmonary bacteriology in the burn patient. E. coli occupied an intermediate level of frequency of occurrence. It did not, however, play a conspicuous part in the bacterial picture seen at autopsy.

Biopsy Cultures and the Burn Patient.

As a diagnostic tool, wound biopsy has become a routine procedure. Surface culture reflects the bacterial flora found in the tissue but cannot offer a precise quantitative assessment nor can it, of course, offer information about the histology of the burn. There were 77 patients on whom biopsies were done; 197 samples, or 2.5 per patient were collected. Qualitative bacteriologic results are shown in Table 6. Comparison with comparable data for 1969 is given.

TABLE 6. Burn Wound Biopsies on 77 Patients
Institute of Surgical Research - 1971

Species	No. Patients Positive	% of Patients Positive		% of Patients Positive Who Expired	
		1969	1971	1969	1971
Staph aureus	34	42	44	22	38
Prov. stuartii	31	51	40	14	58
Ps.aeruginosa	23	30	30	39	57
Klebsiella sp	13	20	17	50	31
E. coli	15	14	19	47	33
Prot. mirabilis	10	34	13	38	40
Candida sp	17	6	22	-	53

The incidence of predominant species in tissue samples is shown as the percentage of patients positive for the species. In 1971, the major species were Staph aureus, Providencia stuartii, and Ps. aeruginosa. E. coli and Proteus mirabilis were found in significant numbers, and Candida sp was recovered from one-fifth of the patients. The incidence of Staph., Providencia, Pseudomonas, Klebsiella, and E. coli varied little from similar collections in 1969; Proteus was much less common and Candida sp were more frequently recovered.

The per cent of patients who expired with a history of harboring a given pathogen in the burn tissues may contribute to understanding the significance of such invasion. A marked rise in the death rate of those with Providencia was noted. Fluctuations with other tissue-invading strains were not as marked; a rise in deaths in patients with Staph aureus and with Ps. aeruginosa was contrasted with a drop in those with Klebsiella and E. coli. More patients were positive for Candida in 1971, and the death rate in such patients had no counterpart in 1969.

Catheter Tip Cultures.

A source of sepsis of continued concern is infection localized at the site of an indwelling intravenous catheter. One hundred twenty-three patients had catheter tips cultured on removal from the vein; there were 312 catheter tips examined. The bacterial flora was not a reflection of the wound, blood stream or pulmonary tract flora. Species and incidence of recoveries are shown in Table 7.

TABLE 7. Bacterial Flora of I.V. Tips From 123 Burn Patients
Institute of Surgical Research - 1971

Species	Total Patients Positive	No. of Strains Isolated
Staph aureus	11	16
Strep, non-hemolytic	2	2
Coagulase-negative Staph	3	3
Bacillus sp	1	1
Klebsiella sp	4	5
Enterobacter aerogenes	1	1
Proteus rettgeri	1	1
Providencia stuartii	22	31
Pseudomonas sp	6	7
Candida sp	6	8

Staph aureus, not unexpectedly, was one of the 2 species found with relative frequency, in 8% of the patients. Providencia stuartii was recovered from 16% of patients. Pseudomonas, otherwise a prominent part of the burn flora, was found in very small numbers. It is suggested that Providencia stuartii here shows, once more, a propensity for invading tissues of the burn patient in a degree not exhibited by other Enterobacteriaceae.

DISCUSSION

The proportion of burn patients who developed sepsis has remained essentially unchanged since 1964 with only the predominate organisms varying year by year. Burn wound sepsis due to Ps aeruginosa remained at a low incidence, and bacteria in wounds, lung and blood stream remain the obvious incitants of this state. The septicemia rate remained high, and was conspicuous for its display of a wide variety of distinct species appearing in succession in the burned patient. Routine admission blood cultures, taken after manipulation of the patient in the Hubbard tank, were rarely positive.

Staph aureus, Providencia and Pseudomonas remained the major septicemic problem. Klebsiella sp was the other gram-negative enteric form which was found in the blood in significant numbers. The respiratory tract flora showed Staph., Pseudomonas and Providencia as prominent as they were in septicemia, but the Klebsiella incidence was proportionately far greater than was its role in septicemia. Tissues also had a bacterial population that paralleled the lung and blood stream flora. The bacterial flora of both burn tissue and lung suggest that both sites are plausible sources of bacteria for invasion.

SUMMARY

The relation between bacterial colonization and sepsis in the burned patient appears to be more complex as the bacteriologic picture is scrutinized in detail. The extreme diversity of causative organisms in septicemia does not accord with a specific etiology for sepsis, and the variation in the lung flora as revealed in sputum cultures also indicates that the bacterial pneumonia which may occur is an opportunistic phenomenon related to diminished host resistance and the bacterial flora of the individual patient. The offending categories of greatest numerical importance are Staph aureus, Providencia stuartii and Ps aeruginosa. Klebsiella pneumoniae and E. coli have shown an increasing degree of involvement of the respiratory tract in the past year. No promising new developments in control of these organisms have occurred.

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1. Lindberg RB, et al: Pathogenesis of burn wound infection: Bacterial flora of burn wounds receiving Sulfamylon treatment. USA Institute of Surgical Research Ann Prog Rpt FY 1970, BAMC, Ft Sam Houston, Tx. Section 16.

PRESENTATIONS AND/OR PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
	DA OE 6955	72 07 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISB'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
	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	3A061102B71R	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

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
69 06	Cont	DA	C. In-House

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a. DATES/EFFECTIVE: EXPIRATION:
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18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 72	0.1	1,0
	CURRENT 73	0.2	3.5

19. RESPONSIBLE DOD ORGANIZATION
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21. GENERAL USE
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22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Staphylococcus; (U) Burns; (U) Septicemia; (U) Burn Infection; (U) Humans

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

23. (U) The observation of increasing rates of sepsis due to methicillin-resistant staphylococci in burn 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) Staph phage typing with standard WHO-phages, tube-dilution sensitivity tests, detailed qualitative and quantitative bacteriologic study of series of patients in this burn ward, and possible special technics to assess virulence mechanisms of resistant strains are to be used.

25. (U) 71 07 - 72 06 The emergence of a monotype Staph aureus type 84 has been revealed, with a uniquely high level of methicillin resistance. Seventy per cent to 85% of isolates were resistant. The single phage type followed a multi-type epidemic of group III staphylococci. Continued type and sensitivity studies clarify the epidemic pattern, although as yet no antibiotic prophylaxis has been found.

*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. 3A061102B71R-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 1971 - 30 June 1972

Investigators:

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Ruth L. Latta, BS
Evan T. Thomas, Lieutenant Colonel, MSC*
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Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A061102B71R-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

Period covered in this report: 1 July 1971 - 30 June 1972

Investigators: Robert B. Lindberg, PhD
Ruth L. Latta, BS
Evan T. Thomas, Lieutenant Colonel, MSC*
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Staphylococcus aureus is an important pathogen in burn wounds, but in the Institute of Surgical Research it played a minor role from 1960 through 1967. Phage types showed the population to be heterogeneous, and antibiotic resistance was not conspicuous. In 1968, however, a sharp rise in type 84,85 incidence occurred; by 1969, this pattern was seen in 60% of the isolates. In 1970 a related but distinct strain, with phage pattern 47,54,75,84,85 reached epidemic proportions until September 1970 when it was replaced by type 84 in what could be termed a monotype epidemic. During the 1968-1971 period, antibiotic resistance of staphylococci increased rapidly; methicillin-resistance increased to the point where 85% of isolates are now resistant. The most effective remaining agents are gentamycin and cephalothin but these 2 are decreasing in effectiveness.

Staph aureus septicemia increased markedly in 1970, with type 84 the predominant cause. No effective measures for eliminating staphylococci from a burn ward population exist, and thus a monotype, highly resistant epidemic strain has become a major problem.

Staphylococcus Septicemia
Burns Burn infection

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

The history of therapeutic use of antibiotics has been marked by continued appearance of resistant forms among pathogens originally susceptible, with the counter-stroke of discovery of new antibiotics which are once more effective. The semi-synthetic penicillins of the methicillin pattern were hailed as the ultimate weapons in the search for anti-staphylococcal antibiotics. However, recent reports have announced a change of predominant phage types from group 1 to group III strains in Europe (Bulow,1971[1]; Jessen, et al, 1969[2]) and more recently in the United States (Barrett, et al)[3]. In addition to increases in the proportion of penicillin and tetracycline resistant strains, these new phage types showed, beginning in 1967, a slow but significant rise in methicillin-resistant strains. This trend appears to be occurring in many areas in which sensitivity monitoring is maintained. This report summarizes the results of a continuing study on antibiotic sensitivity of burn wound flora and of strains causing septicemia in burned patients.

Staphylococcus aureus, long regarded as a major pathogen in burn wounds, played a relatively minor role in infections at the Institute of Surgical Research from 1963 through 1968. However, since 1968 a dramatic rise in the rate of sepsis due to Staph aureus has occurred. At the same time, antibiotic resistance has increased, and staphylococci in the burn wards have changed from a heterogeneous to an increasingly homogeneous group. Today they constitute a single predominant phage type in this Institute, devoted mainly to burn injuries.

These recent changes in antibiotic sensitivity and in the epidemiology of Staph aureus are recorded for the US Army Institute of Surgical Research, but the observations noted here present fundamental implications for future management of traumatic wounds in which a high rate of bacterial colonization occurs.

The type identity of pathogenic staphylococci would be an academic problem if it were not for evidence of a rapid increase in infection rates which has accompanied the change in sensitivity and the emergence of a monotype population. In the categories of, staphylococcal bacteremia, presence of staphylococci in biopsies, and recovery of staphylococci from veins in thrombophlebitis, there has been a relative increase in positive cultures in this Institute. At the same time an increase in the proportion of sputum and tracheal aspirate cultures yielding staphylococci has occurred. An illustration of this trend is shown in the increase of staphylococcal

bacteremia in the annual total blood cultures from patients in our Institute.

This table summarizes the incidence of staphylococci in blood cultures from 1963 through 1970. There was a steady drop in the rate of positives, from 23% of all patients with bacteremia in 1963 to 6% in 1968. The dramatic reversal which occurred in 1969 has continued since then. More than half of patients with positive blood cultures harbored staphylococci in the blood in 1971.

There were no major changes in therapy nor in source of patients which might have accounted for this increase in infection. In consequence, the staphylococci isolated from these patients were scrutinized more closely. Antibiotic sensitivity of staphylococcus strains recovered in this Institute during the past 5 years were compared on an annual basis. These data are summarized in Table 2.

This table compares the sensitivity to 6 antibiotics beginning in 1967. Gentamycin and Keflin were added only in the last 3 years. Testing was done by tube dilution. More than half of the strains tested each year were recovered from blood cultures. The limiting level for sensitivity was set at 6.25 µg/ml or less of antibiotic, a level which has been widely used to denote sensitivity for staphylococci.

Kanamycin and tetracycline had, in earlier years, been highly effective anti-staphylococcal agents. In 1967 and 1968, these 2 were effective against 1/5 to 1/3 of the strains tested, but in 1969 tetracycline dropped from a 38% to a 13% inhibitory level. In 1970 and 1971, this antibiotic became virtually ineffective against staphylococci. Kanamycin remained active against staphylococci during 1969, but its effect was negligible in 1970 and 1971. Lincomycin was highly effective in 1967, but resistance to this compound increased steadily up to 1970. A residue of 28% of isolates were still sensitive in 1971. Most disturbing of all were the changes toward methicilin and 2 other semi-synthetic analogous antibiotics, oxacillin and nafcillin. Each of these was initially highly active against all strains tested. In 1967, oxacillin and nafcillin were still almost completely effective, but methicillin-resistant strains began to appear. In 1968, oxacillin and nafcillin-resistant strains appeared, and all 3 were comparable in activity. Then, in 1969, a sudden drop occurred with each of these antibiotics. Methicillin was most affected, and only 25% of isolates tested in that year were sensitive. Oxacillin and nafcillin were slightly more active. During the next 2 years, oxacillin dropped to a 20% effective range and methicillin to 15%, while nafcillin was still active against 33% of the strains tested. A major category of antibiotics thus became of

Table 1. Incidence of Staphylococcus aureus Bacteremia in Burn Patients, ISR, 1963-1970

Year	No. Patients With Positive Blood Cultures	No. Patients With Staph. aureus Recovered	Incidence of Staph. aureus Among Bacteremias %
1963	81	19	23.4
1964	33	10	30.3
1965	17	3	17.6
1966	29	6	20.6
1967	21	2	9.5
1968	50	3	6.0
1969	79	36	45.5
1970	81	46	56.7

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

Year	Antibiotic and % Inhibited by 6.25 µg/ml							
	K	L	Ps	Sc	U	T	G	Kf
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	50.0	56.4

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

minimal value in therapy or prophylaxis.

Gentamycin, which was first used extensively in 1969, has remained effective against approximately one-half of the staphylococci. Keflin was only tested in 1971; it is still, in this population, the most effective antibiotic for staphylococci, although the record of developing resistance to cephalothins elsewhere offers little assurance that it will continue to be of great value (Finland)[4].

More disturbing than resistance to individual antibiotics is the appearance of strains which show complete cross-resistance to all antibiotics. This event has, of course, been reported from various parts of the world. Changes observed in our Institute since 1969 are presented in Table 3.

This table shows the change in cross-resistance from 1969 through 1971. The observations are limited in number, but the upward trend of this phenomenon is nevertheless disturbing.

Beginning in 1963, staphylococci were differentiated by bacteriophage typing of a random sampling of isolates from the burn ward. This was primarily a precaution to establish a baseline for comparison in the event that more detailed information became desirable. The results of this program are summarized, in reference to predominant types, in Table 4.

During the 3 years before 1967, no single type had made up more than 10% of the strains isolated. The population was typically heterogeneous, and the number of individual types was large. Starting in 1967, there were still a large number of types, each of which occurred only in small numbers. There were 33 different phage patterns. The 2 predominant types, 42E,53 and 6,42E,47,54,were present as 15% and 8% of all strains tested. The proportion of patients harboring them was of the same order.

In 1968, type 84 was for the first time predominant, and made up 20% of all strains. It appeared in 19% of patients harboring staphylococci. In 1967, both predominant types were in group III of phage types. In 1968, a group I pattern, 52,22A,80,81, appeared as a major part of the staphylococcus population. Group I strains included the classical type 80,81, which for a decade was regarded as the "hospital strain" of pathogenic staphylococcus. This reappearance in 1968 was the last time that this or any other group I pattern has been prominent in this Institute.

In 1969, a striking increase in staphylococcal sepsis occurred, and at the same time there came a rise in antibiotic resistance. Type

Table 3. Proportion of Staphylococcus aureus Exhibiting Complete Cross-Resistance to all Antibiotics Tested: ISR

Year	No. Strains Tested	% of Isolates Showing Cross-Resistance
1969	63	16.0
1970	97	40.3
1971	100	24.0

Antibiotics: Kanamycin, Lincocin, Methicillin, Oxacillin, Nafcillin, Tetracycline, Gentamycin (since 1969), Keflin (1971).

Table 4. Predominant Phage Types of Staphylococcus aureus ISR, 1967-1971

Year	Strains of S. aureus			Patients	
	No. Typed	Predominant Type	% of Total	No.	% with Predominant Type
1967	124	42E,53	15.3	68	17.6
		6,42E,47,54	8.0		8.8
1968	108	84	20.3	63	19.1
		52,52A,80,81	17.5		14.2
1969	328	84	28.2	141	34.7
		84,85	21.1		32.6
1970	593	47(54)(75)			
		84,85	50.0	169	55.6
		84	26.1		31.3
1971	564	84	74.6	159	89.3

84 increased to 28% of isolates, and was found in 34% of patients harboring staphylococci. Type 84,85, the other predominant organism, made up one-fifth of the isolates and was found in almost as many patients as type 84.

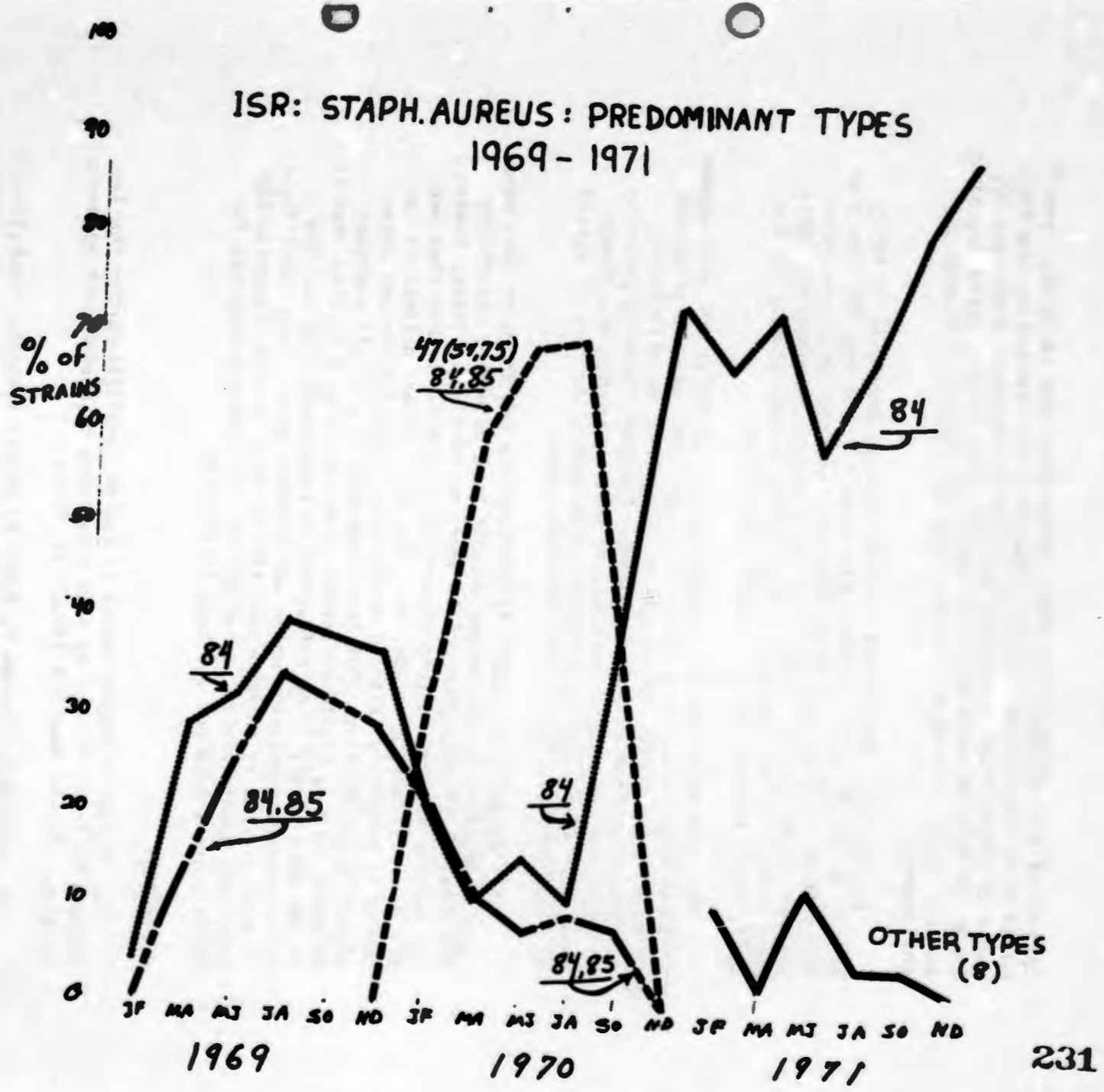
In 1970, type 84 persisted as did type 84,85, but for 8 months these two were a minority of the isolates. A closely related group of strains, designated as type 47,84,85, with less frequent reactions with combinations of phages 54 and 75, appeared abruptly and became the vastly predominant type. These 3 types made up the whole staphylococcus population; there were no other types recovered from patients during 1970. At the end of 1970, type 84,85 disappeared abruptly and completely, as did 47,84,85. In terms of patients seeded, types 47, 84,85 and 84 together appeared on 87% of those with positive cultures in 1970. In 1971, only type 84 appeared in significant numbers. This sequence of events is more readily envisioned when portrayed in graphic form. It is shown here in the figure, in terms of per cent of all staphylococcus strains which made up the predominant types in 2-month intervals. In 1969, the 2 predominant type, 84 and 84,85, were relatively close together in incidence. It is impossible categorically to separate these 2 patterns as unequivocally separate types. However, type 84 fluctuated between 8 and 25% in 1970, while type 84,85 fell to zero incidence between 3 low peaks of occurrence. In September type 84,85 disappeared, while type 84 suddenly became the predominant type. It appears plausible that these 2 were indeed separate types. The epidemic of type 47,84,85 began and ended abruptly as is shown here. There were 8 other types recovered during 1971, but each of them occurred in low incidence. The group shown here constitutes the total of these infrequent types.

Type 84 was recovered in 92% of staphylococcus-positive blood cultures in 1971; it was present in 84% of biopsies and in 93% of autopsy wound samples in which staphylococci were present. It constituted 92% of all strains recovered from vein biopsies and catheter tips, and was found in 79% of all sputum specimens positive for staphylococci.

Nontypable strains totalled 20% to 25% of all staphylococci tested. In the peak epidemic period of 1970, only 8% of isolates were nontypable.

DISCUSSION

The staphylococcus population now present (in 1972) in the burn wards of the Institute of Surgical Research has been designated as a monotype epidemic. Type 84, once established in the ward in 1968, increased in incidence steadily until the virtually explosive episode


ISR: STAPH. AUREUS: PREDOMINANT TYPES
1969 - 1971
% of STRAINS
100
90
80
70
60
50
40
30
20
10
0
47 (54,75)
84,85
84
84
84,85
84
84,85
OTHER TYPES (8)
JF MA MJ JA SO ND JF MA MJ JA SO ND JF MA MJ JA SO ND
1969
1970
1971

episode of type 47,84,85 in 1970. After that type vanished, type 84 moved in to reestablish complete predominance. Meanwhile, the incidence of staphylococcal sepsis increased strikingly. Both type 47, 84,85 and type 84 were recovered readily from floors, surfaces, sinks and air in the burn ward. These types undoubtedly permeated the environment.

In contrast, when nasopharyngeal surveys were made in 1971, only 2 out of 60 ward attendant personnel carried type 84. Most of the carrier strains were untypable; the remainder were a heterogeneous collection of types, with all 3 groups represented. Evidently this epidemic type does not readily become planted in the upper respiratory tract of healthy individuals.

The problem of similar staphylococcus epidemics may well appear in other hospitals, since group III phage types are now prominent throughout the world. There are no procedures for eliminating staphylococci from an open burn ward. Further, incoming patients in 1971 harbored type 84 strains, so that re-seeding was always possible. While aseptic procedures can reduce the rate of spread of staphylococci, they cannot eliminate them.

This sequence of events illustrates the way in which this monotype staphylococcus has become a major burn pathogen, resembling the behavior of staphylococci 10 years or more ago. Today, however, our weapons for controlling staphylococci are far weaker than were the ones available a decade ago. The best current defense is embodied in carefully selected methicillin, cephalothins and aminoglycoside antibiotics. But the appearance of a single dominant epidemic strain, highly resistant to antibiotics, indicates that it is far more likely to be refractory to management than was the heterogeneous population which was formerly seen. Type identification of strains is an important tool to use, if such transmissible resistant agents are to be recognized. Effective procedures for their control have not yet been forthcoming.

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types and antibiotic susceptibility of Staph aureus. Arch Int Med 125:867-873, 1970.

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PRESENTATION

Lindberg RB: The Changing Role of Staphylococcus aureus in burn wound infection. Amer Burn Assoc, 4th Annual meeting. San Francisco, Calif, April 7, 1972.

PUBLICATION

Lindberg RB, Thomas ET, Latta RL, Pruitt BA,Jr: Epidemic methicillin-resistant staphylococcus type 84 in burn patients. Bact Proc 1972, pp 113.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0A 6397	72 07 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISP'N INSTR'N	9. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
71 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	3A061102B71R	01	132
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)

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

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
54 07	Cont	DA	C. In-House

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a. DATES/EFFECTIVE: EXPIRATION:

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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 72	0.2	5.6
	CURRENT 73	0.2	6.0

19. RESPONSIBLE DOD ORGANIZATION

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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 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) 71 07 - 72 06 Broad spectrum resistance of strains of Enterobactereaceae to antibiotics has increased since 1970. Resistance-transfer factors were present in several strains of Klebsiella and Enterobacter sp. Providencia stuartii strains were 94% resistant to gentamycin, 98.6% resistant to tetracycline and to Kantrex, and completely resistant to all other antibiotics tested. Staph aureus, almost entirely type 84, was resistant to methicillin in 85% of isolates tested. The antibiotic-resistance problem increased in 1971 over previous years.

* Available to contractors upon established DDC criteria.

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ANNUAL PROGRESS REPORT

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

REPORT TITLE: ANTIBIOTIC SENSITIVITY OF CURRENT MILITARY BURN PATIENT FLORA

US ARMY INSTITUTE OF SURGICAL RESEARCH
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1 July 1971 - 30 June 1972

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Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ANTIBIOTIC SENSITIVITY OF CURRENT MILITARY BURN PATIENT FLORA

Despite the development of effective topical therapy, the role of infection in burn patients has remained an important one. Sepsis, frequently with bacteremia, is a major feature of severe burns in the Institute of Surgical Research. Its control depends as a matter of course on antibiotic treatment, and selection of such antibacterial agents can be aided by a knowledge of current antibiotic sensitivity patterns. There has been a continued trend toward increasing resistance to antibiotics among the major groups of organisms infecting burns, and a resume of the sensitivity patterns of recent years gives information not otherwise available. The accelerating rate of appearance of antibiotic-resistant gram-negative bacilli has been explained by the growing incidence of resistance transfer factors. Multiple RTF have been described, which can explain the extremely rapid appearance of resistance in previously sensitive strains.

TECHNIC

Strains tested in the Institute of Surgical Research were primarily from blood cultures and from other sites in gravely septic patients. The battery of test antibiotics was revised in February 1971. Chloramphenicol was dropped and ampicillin introduced. Chloramphenicol had been progressively less effective in the previous 2 years; it had become virtually ineffective antibiotic by *in vitro* criteria. The gram negative organism battery in 1971 consisted of the following:

Symbol	Antibiotic
T	Tetracycline
A	Ampicillin
G	Gentamycin (Garamycin)
K	Kanamycin (Kantrex)
Co	Colymycin (Colistimethate sulfate)
Kf	Keflin (Cephalothin)

In addition, all *Pseudomonas aeruginosa* strains were tested with carbenicillin. *Proteus mirabilis* strains have been tested for 2 years with Penicillin G, in view of reports that this antibiotic is effective against the species. However, only rarely has a sensitive strain been observed.

The gram positive organisms have been tested against the following battery in 1971:

Symbol	Antibiotic
K	Kanamycin (Kantrex)
L	Lincocin (Lincomycin)
Ps	Prostaphlin (Oxacillin)
Sc	Staphcillin (Methicillin)
T	Tetracycline
U	Unipen (Nafcillin)
G	Gentamycin (Garamycin)
Kf	Keflin (Cephalothin)

Gentamycin was added to the battery in 1971, after the trend toward increasing antibiotic resistance was recognized in the sensitivity data collected in 1970.

The tube dilution assay technic has been described previously (Lindberg, et al)[1]. The number of strains of each species tested, and their sources are shown. The samples were selected for test because they were of importance clinically, thus the bacterial population tested is weighted toward strains actively involved in infection. Further, serial isolates were tested while the patients were, in some instances, under intensive antibiotic therapy, so that selective recovery of resistant clones may have been more likely to occur.

Table 1 summarizes the strains tested and their sources.

RESULTS

Percentage of strains sensitive are shown as a cumulative total, starting with the lowest, 0.78 µg/ml, level. The upper limit for "sensitive" strains is 12.5 µg/ml for gram negative bacilli. For gram positive cocci and bacilli, the upper limit is 6.25 µg/ml.

Sensitivity of 99 strains of Staphylococcus aureus are shown in Table 2.

The cumulative progression of sensitivity as antibiotic concentration rises shows not only the absolute level for sensitivity, i.e., 6.25 µg/ml, but the pattern of increase. Thus, Kanamycin was of little potential value even at 12.5 µg/ml, as was true also of Tetracycline. Lincocin was probably potentially usable for about one-third of isolates; the 3 semi-synthetic penicillins (Prostaphlin, Staphcillin and Unipen) differed markedly in degree of potential usefulness above the 6.5 level. Staphcillin (Methicillin) was relatively ineffective at 12.5 µg/ml, but Prostaphlin and Unipen, at the 12.5 µg/ml level inhibited over 40% of isolates and still deserved consideration. Gentamycin and Keflin were the most effective antibiotics in terms of in

ABSTRACT

PROJECT NO. 3A061102B71R-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 1971 - 30 June 1972

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

Sepsis in burn patients is a major cause of morbidity and death, and control of opportunistic bacterial invaders relies extensively on antibiotics. Periodic resumes of sensitivity of strains collected in the Institute of Surgical Research offers the most reliable guide to selection of antibiotics when isolation and testing of invading flora have not been completed. Examination of 335 strains of Staphylococcus aureus, Staphylococcus epidermidis, Klebsiella, Enterobacter, Proteus mirabilis, Providencia stuartii, Escherichia coli, Citrobacter sp, and Pseudomonas aeruginosa was carried out using tube dilution technic to determine MIC. A relatively high degree of resistance to most antibiotics was found, but sensitivity of several genera of gram-negative bacteria had not altered markedly over the past 3 years. The most serious emergence of resistant clones has occurred with Staph aureus and with Providencia stuartii. For the latter, complete resistance has now developed. Staphylococcus strains are becoming more resistant; no effective solution has been found for this trend.

Burn wound flora
Antibiotic sensitivity
Pseudomonas
Providencia

Table 1. Organisms From Burn Patients Tested For Antibiotic Sensitivity, ISR - 1971

Species	No. Strains	Sources				
		Blood	Sputum	Urine	Biopsy	Wound
Staph aureus	99	68	10	3	2	16
Staph coag neg	9	5	2	2	0	0
Klebsiella	32	8	8	5	3	8
Enterobacter	13	2	9	2	0	0
Proteus mirabilis	35	3	2	13	2	15
Providence	68	29	19	4	5	11
E. coli	18	4	5	5	0	4
Citrobacter	6	2	4	0	0	0
Pseudomonas	5	18	16	8	4	9
Total	335	120	75	42	16	63

Table 2. Staph aureus: Cumulative Inhibitory Levels for 104 Strains Isolated in ISR - 1971

MIC/µg/ml	Antibiotic and % of Strains Inhibited							
	K	L	Ps	Sc	U	T	G	Kf
> 25	100	100	100	100	100	100	100	100
25	19.4	42.1	50.0	46.6	61.0	5.9	83.5	84.7
12.5	11.6	39.2	41.3	17.4	45.0	3.9	72.1	65.8
6.25	3.8	28.4	20.1	15.5	33.0	2.9	50.0	56.4
3.12	1.9	8.8	13.4	6.7	16.0	2.9	17.3	30.5
1.56	1.0	1.9	6.7	1.0	9.0	1.9	3.8	17.6
0.78	1.0	1.9	5.7	1.0	5.0	1.0	1.9	5.8
< 0.78	0	1.0	3.8	0	3.0	0	1.9	4.7
Total Tested	103	102	104	103	100	101	104	85

vitro test, for Staph aureus in 1971.

Coagulase negative staphylococci were seldom found in circumstances that merited antibiotic sensitivity testing. Nine strains were tested. Kanamycin and Lincocin were effective at 6.25 µg/ml; for 2 out of 9 strains; the semi-synthetic penicillins (Ps, Sc, U) inhibited from 37 to 50 % of 8 strains; tetracycline inhibited one-third, Gentamycin 62% and Keflin 85% of strains tested.

Ps. aeruginosa , 55 strains were tested and the results are summarized in Table 3. Ps. aeruginosa strains were virtually all resistant to tetracycline, Kanamycin, Keflin and Ampicillin. The latter was completely inactive. Colymycin and Gentamycin were virtually equivalent in activity; at 12.5 µg/ml over 70% of tested strains were inhibited. This level of activity has persisted for at least 3 years; no increase in resistant forms was seen.

Cross resistance of Pseudomonas strains between Gentamycin and Colymycin has been viewed as a potential disadvantage resulting from continued use of these antibiotics. Comparison of this phenomenon showed that differences between the 2 are equally distributed (Table 4). It was apparent that linked cross-resistance to the 2 antibiotics does not commonly occur.

Carbenicillin sensitivity varied widely. The upper limit for indication of clinical feasibility has not been unequivocally defined. If the level of 312 µg/ml were taken, 38% of strains were sensitive. At 78 µg/ml, only 12% were sensitive.

Klebsiella sp infections, as reflected by sources of strains tested, were uniformly distributed between blood stream, pulmonary, wound and urinary tract involvement. The range of sensitivity was within achievable levels for 4 antibiotics. Table 5 summarizes these levels.

Tetracycline was not effective with Klebsiella sp. Kanamycin at 12.5 µg/ml inhibited 31% of strains and Keflin 24%. Colymycin inhibited 31% of strains at 12.5 µg/ml but 28% were inhibited at the 6.2 µg level. Gentamycin, the most effective antibiotic, inhibited 71.8% of strains tested at 12.5 µg. Ampicillin was devoid of activity.

Enterobacter aerogenes was the only Enterobacter species which came to hand. Thirteen strains were tested. Tetracycline was active against 7 of these, Colymycin against 6, and Gentamycin against 10 at the 12.5 µg/ml level. None were inhibited by Ampicillin at this level.

There were 36 strains of Proteus mirabilis tested for sensitivity.

Table 3. Pseudomonas aeruginosa: Cumulative Inhibitory Levels for 56 Strains Isolated, ISR - 1971

MIC µg/ml	Antibiotic and % of Strains Inhibited							
	T	K	Kf	Co	G	Amp	Carbenicillin	
> 25	100	100	100	100	100	100	>1250	100
25	26.7	5.3	5.8	79.2	75.0	0	1250	56
12.5	12.5	0	5.8	73.3	71.4	0	625	46
6.25	1.7	0	3.9	58.4	64.2	0	312	38
3.12	0	0	0	32.0	44.6	0	156	30
1.56	0	0	0	0	7.1	0	78	12
0.78	0	0	0	0	1.7	0	39	4
< 0.78	0	0	0	0	0	0	19	2
Total	56	56	51	53	56	34		50

21-6

Table 4. Gentamycin and Colymycin Sensitivity to Antibiotics, 53 Strains, ISR - 1971

Resistant to 12.5 µg/ml of Co and of G	Sensitive to Co Resistant to 12.5 µg/ml G	Resistant to Co. Sensitive to G	Sensitive to Both Co and G
7 strains	8 strains	7 strains	31 strains

Table 5. Klebsiella (pneumoniae): Cumulative Inhibitory Levels for 32 Strains Isolated, ISR - 1971

MIC µg/ml	Antibiotic and % of Strains Sensitive					
	T	K	Kf	Co	G	Amp
> 25	100	100	100	100	100	0
25	15.6	37.5	41.3	43.7	96.6	0
12.5	9.3	31.2	24.1	31.2	71.8	0
6.25	0	3.1	6.8	28.1	71.8	0
3.12	0	0	0	6.2	43.7	0
1.56	0	0	0	0	9.3	0
0.78	0	0	0	0	0	0
< 0.78	0	0	0	0	0	0
No. tested	32	32	29	32	32	21

In this case Penicillin G was included in the testing battery. Results are presented in Table 6.

Proteus mirabilis strains were virtually resistant to tetracycline and Kanamycin, Colymycin and Penicillin G. The latter observation is at variance with several reports of sensitivity to Penicillin G but the findings were reached with no variation occurring. Ampicillin was active against one-third of the strains tested. The two optimal antibiotics were Keflin and Gentamycin. Keflin was obviously the antibiotic of choice.

Providencia stuartii. With this exotic opportunistic pathogen the patients in the Institute of Surgical Research have experienced a degree of colonization and of infection without parallel in reports from other burn research centers. During the past years, the incidence of completely resistant strains has increased, although the resistance level was very high in the first strains tested. The sensitivity pattern observed in 1971 is shown in Table 7. Previous reports (Lindberg, et al)[1] had pointed out the extremely refractory state of Providencia stuartii, so that infections with this opportunistic invader were beyond the reach of any antibiotic. The incidence of Providencia in burn wounds, sputum and blood cultures was, if anything, higher in 1971 than it had previously been.

There was little change in the sensitivity levels for Providencia in 1969 and 1970. There were, at the 12.5 µg/ml level, 3 antibiotics that inhibited from 15% to 23% of the isolates tested. However, in 1970 the sensitivity level dropped abruptly to a negligible degree of susceptibility. A comparison of the 1969-1970 and the 1971 sensitivity curves is presented in Table 8. The 5 antibiotics shown are those with which continued observations have been made. Chloramphenicol was dropped and Ampicillin added; neither offered any inhibitory action against this organism.

The number of Providencia strains tested annually has not varied significantly. Thus, the number of patients with infections which occasion sensitivity testing has not materially altered. But the antibiotic resistance, which had previously been regarded as a problem, has been compounded to a level at which there is no in vitro basis for using any given antibiotic.

DISCUSSION

The organisms of major importance in sepsis in burned patients in 1971 were, as they have been in the recent past, Staph aureus, Pseudomonas aeruginosa and Providencia stuartii. Inhibitory effect

Table 6. *Proteus mirabilis*: Cumulative Sensitivity Levels for 36 Strains From Burn Patients, ISR - 1971

MIC/µg/ml	Antibiotic and % Sensitive						
	T	K	Kf	Co	G	Amp	Pen G
> 25	100	100	100	100	100	100	100
25	8.3	18.8	87.3	2.7	94.1	33.3	13.0
12.5	8.3	8.3	76.4	0	58.8	33.3	8.7
6.25	8.3	2.7	58.8	0	29.4	14.2	4.3
3.12	5.5	2.7	20.5	0	8.8	14.2	0
1.56	0	0	0	0	2.9	9.5	0
0.78	0	0	0	0	0	0	0
< 0.78	0	0	0	0	0	0	0
Strains Tested	36	36	34	36	34	21	23

Table 7. Providencia stuartii: Cumulative Sensitivity Levels for 68 Strains From Burn Patients, ISR - 1971

MIC/µg/ml	Antibiotic and % Sensitive					
	T	K	Kf	Co	G	Amp
> 25	100	100	100	100	100	100
25	1.4	1.4	0	0	13.2	0
12.5	1.4	1.4	0	0	7.3	0
6.25	1.4	0	0	0	2.9	0
3.12	1.4	0	0	0	1.4	0
1.56	0	0	0	0	0	0
0.78	0	0	0	0	0	0
< 0.78	0	0	0	0	0	0
No. Strains Tested	68	68	66	66	68	58

Table 8. Providencia stuartii: Comparison of Cumulative Antibiotic Sensitivity for 1969-1970 and for 1971

MIC/µg/ml	T		K		Kf		Co		G	
	69-70	71	69-70	71	69-70	71	69-70	71	69-70	71
> 25	100	100	100	100	100	100	100	100	100	100
25	21.1	1.4	16.4	1.4	6.7	0	17.2	0	49:2	13.2
12.5	17.4	1.4	7.8	1.4	3.7	0	15.7	0	23.4	7.3
6.25	11.9	1.4	4.2	0	1.4	0	10.5	0	12.1	2.9
3.12	7.7	1.4	2.1	0	0.7	0	4.5	0	5.3	1.4
1.56	6.3	0	1.4	0	0	0	0	0	2.2	0
0.78	1.4	0	0.7	0	0	0	0	0	1.5	0
< 0.78	0.7	0	0	0	0	0	0	0	0	0
No Tested	142	68	140	68	133	66	137	68	132	68

of antibiotics fell noticeably for Staph aureus in this year. There was no change, or possibly a slight increase in the number of Ps. aeruginosa inhibited by Colymycin and Gentamycin, the only antibiotics with impressive degrees of effectiveness. With the extremely prevalent Prov. stuartii, there was abrupt disappearance of antibiotic sensitive strains. No significant number of isolates were inhibited by any of the battery of gram negative antibiotics used.

There has been a continued drop in the incidence of severe infections due to Proteus mirabilis. There has been no increase in resistant strains of Proteus; the sensitivity level has remained constant for the past 3 years. This situation also prevails for Klebsiella, which has not shown an increase in resistant forms since 1969.

Thus, the prospect of a constantly increasing number of resistant strains has not materialized in the burn population of this Institute for species including Klebsiella, Proteus, Escherichia and Ps. aeruginosa. The resistance level may be relatively high in the latter species, but it is not increasing. Increasing resistance is, however, seen in Staph aureus and in Prov stuartii. For the latter, resistance in 1971 was virtually complete. Survey of new antibiotics for an effective agent is indicated, although such solutions are inherently temporary.

REFERENCE

1. Lindberg RB,Contreras AA, Townsend CE, Pruitt BA, Jr: Sensitivity of Burn Wound Flora to Antibiotics, 1969-1970. US Army Institute Surgical Research Ann Prog Rpt FY 1971, BAMC, FSHT, 30 June 1971, Sec 4.

PRESENTATION

Lindberg RB: A Changing Role of Staphylococcus aureus in Burn Wound Infection. Amer Burn Assoc meeting, San Francisco, 6-8 Apr 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. GROUP NUM'R	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
71 07 01	D. CHANGE	U	U	NA	NL	☐ YES ☐ NO	A. WORK UNIT

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a. PRIMARY	61102A	3A061102B71R	01	267
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11. TITLE (Include with Security Classification Code): (U) Sensitivity of Pseudomonas Aeruginosa Recovered from Burned Soldiers to Sulfamylon (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 Clinical Medicine

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18. RESOURCES ESTIMATE

	FISCAL YEAR	a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
PRECEDING	72	0.3	10.1
CURRENT	73	0.3	10.5

19a. RESPONSIBLE DOD ORGANIZATION
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NAME: Virginia C English, MA
NAME: DA

20. GENERAL USE: FOREIGN INTELLIGENCE NOT CONSIDERED

21. KEYWORDS (Provide KWIC and Security Classification Code): (U) Pseudomonas; (U) Burns; (U) Sulfamylon; (U) Topical Therapy; (U) Humans

22. TECHNICAL OBJECTIVE, 23. APPROACH, 24. PROGRESS (Provide 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) 71 07 - 72 06 Sulfamylon sensitivity has remained within previously recognized limits in the series of over 300 strains of P. aeruginosa monitored in 1971. The absence of emergence of in vitro resistance to this drug is extremely unusual; no other topical medication, even including silver nitrate has been so consistently effective in the range which was established as early as 1964. Continued monitoring is needed to assure the safety of this treatment modality.

* Available to contractors upon originator's approval.

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ANNUAL PROGRESS REPORT

PROJECT NO. 3A061102B71R-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 1971 - 30 June 1972

Investigators:

Robert B. Lindberg, PhD
Virginia C. English, MS
Ruth L. Latta, BS
Arthur D. Mason, Jr., MD

Reports Control Symbol MEDDH-286(R1)

UNCLASSIFIED

ABSTRACT

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

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Reports Control Symbol MEDDH-288(R1)

Two hundred and eighty strains of Pseudomonas aeruginosa, collected essentially at random from 95 patients who had at least one positive Pseudomonas culture, were tested for sensitivity to Sulfamylon. There was a slight rise in resistance to Sulfamylon in comparison to the results of cultures in 1970; the median sensitivity level was 0.125% in 1971, and 0.108% in 1970. However, medians for 1968 and 1969 were higher than the 1971 level, and no sign of Sulfamylon-resistance was found. A group of strains from one phage type, F 3, constituted almost all of the strains requiring the highest dose of Sulfamylon for inhibition, and this strain persisted in the burn ward for 7 months. No excess of clinical illness or indication of treatment failure was noticeable in patients with the most tolerant F 3 strain. Sulfamylon continued to exhibit absence of drug-resistance in Ps aeruginosa strains.

Pseudomonas
Burns
Sulfamylon
Topical therapy

SENSITIVITY OF PSEUDOMONAS AERUGINOSA RECOVERED FROM BURNED SOLDIERS TO SULFAMYLON

Since the development of Sulfamylon burn cream as an effective prophylactic for Pseudomonas burn wound sepsis, the possibility of the emergence of resistant Pseudomonas aeruginosa strains has been considered. A close and continuing monitoring of the strains of Ps aeruginosa recovered from the patients in the burn ward has been maintained. Although the drug inhibits growth of other bacteria as well as Pseudomonas, the emphasis has been placed on Pseudomonas. Without control of this organism, topical therapy would undoubtedly permit a recrudescence of burn wound sepsis. The 10% concentration of Sulfamylon in the burn cream reflects the relatively weak action of the drug.

Since relatively tolerant strains would find intervals during which a treated patient has a fall in surface concentration of Sulfamylon, such strains could produce treatment failure.

METHODS

The technic for testing for Sulfamylon sensitivity has been described (Lindberg, Calvert, Brame, Dent).[1] Dilution of Sulfamylon ranging from 0.19% to 1.25% in nutrient agar were poured in grid marked plates. Inoculum was a drop of 4-hour broth culture with 200 ± 50 colonies per drop. A 10^{-3} dilution of broth culture was routinely used, subject to confirmation by a control plate. The endpoint was a barely visible granular appearance; inhibition is reflected in diminished growth over a dilution range, rather than a sharp endpoint.

RESULTS

Two hundred and eighty strains of Ps aeruginosa from clinical (and autopsy) specimens were tested. Inhibiting concentrations are presented from 1967 through 1971 (Table 1). Note that the upper limit of tolerance of Sulfamylon remained at 0.625%; this figure has not been exceeded since 1967, when 15 strains which tolerated 0.625% were found. However, there had been from 1968 through 1970 a continued fall in the upper limit of tolerance for Sulfamylon. Indeed, in 1970 there were no strains tested which required as much as 0.625% of Sulfamylon. In 1971, this pattern changed. Seventeen per cent of the strains tested required 1.25% for inhibition. This percentage was greater than that seen in 1967, 1968, and 1969.

The relationship of Sulfamylon to the sensitivity of Ps

Table 1.

Inhibiting Concentrations of Sulfamylon for Pseudomonas aeruginosa, 1967-1971

Year	Concentration of Sulfamylon in % and Number Inhibited							
	2.5	1.25	0.625	0.312	0.156	0.078	0.039	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
Total (1726)	0	15	116	416	367	378	334	100
% of Total	0	.008	6.7	24.0	21.2	21.8	19.4	5.7

Table 2.
CUMULATIVE SENSITIVITY TO SULFAMYLON OF PSEUDOMONAS AERUGINOSA, 1971

Year	No. of Strains	Concentration and % of Total Strains Inhibited						
		1.25	0.625	0.312	0.156	0.078	0.039	0.019
1967	471	100	96.8	87.6	81.7	61.3	46.4	15.6
1968	294	100	100	95.4	60.4	45.8	14.3	1.7
1969	385	100	100	96.5	50.0	26.9	7.7	0.5
1970	296	100	100	100	79.0	49.9	21.9	2.0
1971	280	100	100	92.9	68.3	48.3	27.9	4.7

aeruginosa for this drug is shown in the cumulative sensitivity levels for the strains tested. These values are shown in Table 2. The total of strains inhibited up to 0.078 µg/ml had not changed markedly from 1970 to 1971. The increase in strains requiring more Sulfamylon for inhibition is shown in the totals for 0.156 µg/ml and 0.312 µg/ml. This shift was not of a magnitude to suggest that the drug would be ineffective against such strains, but it was still a significant change in the sensitivity pattern.

As a means of recognizing the relative susceptibility of groups of Ps aeruginosa strains selected on the arbitrary basis of annual increment, the median Sulfamylon level, which would inhibit one half of the total tested is shown in Table 3.

Table 3. Median Value of Pseudomonas aeruginosa Sensitivity to Sulfamylon

Year	No. of Strains	Median Inhibitory Value (%)
1967	471	0.083
1968	294	0.136
1969	385	0.176
1970	296	0.008
1971	280	0.125
Total of 5 Years	1726	0.119

The earliest year shown was also the year with lowest median value. Sulfamylon was, by 1967, well-established as a treatment modality. During the 2 following years the median increased, but in 1970 it dropped sharply, and the increase in 1971 still gave a median well below the 2 highest years, 1968 and 1969. Fluctuations obviously occurred on both sides of the median value of 0.119% for the entire 5-year period.

Ps aeruginosa strains are differentiated in this Institute by phage typing, which has offered a precise and reliable means of characterizing individual strains (Latta, Lindberg, Brame, Mason, Pruitt).[2] This continuing operation furnishes assurance that the

infection pattern of Ps aeruginosa has not altered. The population of Ps aeruginosa was sorted for predominant phage types, and these were listed with the sensitivity level which their isolate exhibited. Table 4 summarizes the sensitivity distribution for 4 predominant phage types.

Type 31,119X (Coded F 3), was recovered from 17 patients. Thirty-eight isolates, the entire lot of this type recovered from these 17 patients, were assayed for sensitivity. It is apparent that the major part of the strains which required 0.625% for inhibition were included in type F 3. They were collected from these patients between the first admission, Nr. 105,on 25 May, and the last, Nr. 229,admitted 15 October 1971. The type was recovered through November; one strain was recovered in December. Patient incidence was highest from July through November, but there was no abrupt peak of incidence which would correspond to a rise in rate of cross-infection or unusual persistance of this type. There were 4 isolates that occupied a range of sensitivity from 0.312% to 0.039%.

Three other predominant types were similarly assessed. Type 1214,68,119X, Coded H 3, had sensitivity among 18 isolates ranging from 0.312% to 0.039%, with 8 strains inhibited at the 0.156% level. In 1970, H 3 was a predominant type, again with inhibition at 0.156 µg/ml. Type 119X, Coded M 2, was represented in 8 patients with one strain each. One strain was inhibited at 0.625%; the other 7 ranged from 0.156% to 0.039%. Type 119X, F7, Coded M 4, appeared in 7 patients; 14 isolates ranged from 0.312% to 0.078% in sensitivity. Thus, only with F 3 was there a loading of sensitivity at the 0.625% level. It appeared plausible that this strain was perpetuated on the burn ward by cross-infections.

There was no unusually high incidence of wound invasion due to type F 3. Out of 7 deaths there were 6 autopsies of patients who had yielded F 3 in culture. Autopsy bacteriology showed burn wound sepsis in one, a 20-month-old child with a 54% body surface burn. Of the other 5 autopsies, 4 had no significant concentration of Pseudomonas in the tissues; one had Pseudomonas in several blocks of tissue but the organism was not predominant. The remaining 4 did not have Pseudomonas in the burn at autopsy.

DISCUSSION

On the basis of several years of monitoring, there has been no recovery of a Pseudomonas strain resistant to Sulfamylon. Neither have patients who have been under prolonged topical therapy been a source of increasingly resistant forms. The appearance of a single type F 3, which required 0.625% Sulfamylon for inhibition was the

Table 4.
SULFAMYLON SENSITIVITY REACTIONS OF Four PREDOMINANT PHAGE TYPES

		PATIENT ISOLATES WITH INHIBITING CONCENTRATIONS* AT					
		0.625	0.312	0.156	0.078	0.039	0.019
Type F-3	105	4					
	137	1					
17 Patients	117	1					
	135	4					
	148	1					
	170	1					
38 Isolates	162	5					
	169	7					
	166	2					
	168	1					
	196	1	1				
	209	1					
	229				1		
	167		1				
	192	1				1	
Total-each inhibiting strain	213	1					
	174	1					
		34	2		1	1	
Type 4-3	14			1			
	17				1		
17 Patients	27			1			
	30				1		
	42				3		
18 Isolates	44					2	
	26				1		
Total-each inhibiting strain	74		1				
	29			1			
	104			3			
	160				1		
	103			2			
			1	8	7	2	
Type H-2	78				1		
	76			1			
9 patients	86					1	
8 Isolates	95			1			
	26					1	
Total-each inhibiting strain	163					1	
	211	1					
	140				1		
		1		2	2	2	
Type H-4	50			2			
	145			2	1		
7 patients	91			1			
	73			2			
14 Isolates	122				1		
	107		5				
Total-each inhibiting strain	144		1				
			6	7	2		

* All concentrations in Gms.%

most striking example yet seen of persistance over several months of a single strain that was at the upper end of the tolerance curve for Sulfamylon. In 1970, a distinctive phage type presented a group of strains all sensitive at 0.312 µg/ml. This was the same phenomenon as in 1971, but at a lower level. This does not mean that all F 3 strains are of necessity Sulfamylon-tolerant. Since this attribute persisted in a succession of cultures, the most plausible explanation is that the strain, once established, persisted in a series of successive cross-infections. The Sulfamylon sensitivity thus served, as did the F 3 type structure, as a recognition marker.

Continued monitoring of Sulfamylon sensitivity is essential for effective use of this antebacterial agent. Since no resistant forms have been recovered in 7 years of study, it is possible that Sulfamylon suppression of Pseudomonas is comparable to the universal sensitivity of group A streptococci to penicillin, although the suppressive level is very much higher.

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2. Latta RL, Lindberg RB, Brame RE, Mason AD,Jr, Pruitt BA, Jr: Bacteriophage types of Pseudomonas aeruginosa found in burned patients. US Army Institute of Surgical Research Ann Prog Rpt FY 1971, BAMC, Ft Sam Houston, Texas, Section 5.

PRESENTATION

Lindberg RB: Topical Chemotherapy and the Microbiology of Burns. Symposium on Clinical Microbiology & Hospital Infections, Am Pub Health Assoc, Minneapolis, Minn., 19 Oct 1971.

PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
				DA 0B 6397	72 07 01	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8A. DOD'D INSTR'N	8B. SPECIFIC DATA CONTRACTOR ACCESS	9. LEVEL OF SUM
71 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	3A061102B71R	01	188
b. CONTRIBUTING				
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11. TITLE (Include with Security Classification Code)

(U) Bacteriophage Types of Pseudomonas Aeruginosa Found 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
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18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 72	0.3	8.0
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19. RESPONSIBLE DOD ORGANIZATION

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NAME: R L Latta, BS

NAME: DA

21. GENERAL USE

FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Provide those with Security Classification Code)

(U) Pseudomonas; (U) Phage Typing; (U) Burn Wounds; (U) Topical Chemotherapy; (U) Humans

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

23. (U) 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 phagetyping, permits effective monitoring of therapy, emergence of resistance, and environmental contamination recognition and control.

24. (U) Phagetyping system, developed in this Institute is used to type P. aeruginosa isolates.

25. (U) 71 07 - 72 06 A major change in source of incoming strains of P. aeruginosa incurred with diminished input from the Vietnam conflict. The nontypable percentage simultaneously rose markedly: 40% of strains tested were nontypable by bacteriophage, using the ISR-developed system. Such changes are inevitable in any typing system: phage, serologic, or pyocin. A screening of nontypable strains yielded several lytic agents, from which two phages are being processed to re-establish a high level of coverage for this typing set.

*Available to contractors upon arbitrator's approval.

DD FORM 1 MAR 65 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498-1 NOV 64 AND 1498-1, 1 MAR 65 (FOR ARMY USE) ARE OBSOLETE.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0D 6387	72 07 01	DD-DR&E(AR)636

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71 07 01	D. CHANGE	U	U	NA	NL	☐ YES ☐ NO	A. WORK UNIT

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c. CONTRIBUTING				

11. TITLE: (U) Stability of Phage Typing Systems for Pseudomonas Aeruginosa as Recovered from Burned Military Patients (44)

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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 72	0.3	8.3
	CURRENT 73	0.3	10.0

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NAME:

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS: (U) Pseudomonas; (U) Phage Typing; (U) Burns; (Humans

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 a continuing threat in surface and systemic infections in burned soldiers. Definitive characterization of this opportunistic pathogen is essential for rational therapy, prophylaxis, and understanding of the mechanisms of these infections.

24. (U) A phage typing system was derived and standardized, and has been used effectively. The stability of these phages and the spectrum of reactivity have been periodically assessed to be sure of the significance of the type patterns.

25. (U) 71 07 - 72 06 Emergence of increasing numbers of non-typable strains occurred. The typing set was shown to be stable; thus the non-typables represent evolutionary changes in this labile species. Using lysogeny and phage isolation, new phages which type the otherwise non-typables have been collected. Enhancement of titer is being done, and a series of type identities of former non-typables is being obtained.

*(If available in accordance with agency guidance)

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1497A, 1 NOV 68 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

ANNUAL PROGRESS REPORT

PROJECT NO. 3A061102B71R-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 1971 - 30 June 1972

Investigators:

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

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

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

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

Period covered in this report: 1 July 1971 - 30 June 1972

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Reports Control Symbol MEDDH-288(R1)

Bacteriophage typing revealed a uniquely high proportion of nontypable strains which included 40% of the 637 strains typed in 1971. These were recovered from 121 patients, or 45% of all admissions. Out of all admissions, 72% were from the United States in contrast to the past 5 years when most cultures came from patients injured in Vietnam. This change may be related to the high rate of nontypables now being seen. There were 7 types distinguished among the typable strains. Out of the 3 predominant types, 38 patients harboring the strain were United States patients; only 3 were from patients injured in Vietnam. The persistence of phage types from 1970 was higher than had previously been noted; out of 7 major types, only 2 had not been present in 1970. One of these, F 3, was the most common typable strain in 1971; the other, B 35, was the least common. Septicemic and pneumonic strains were distributed by type in accord with the overall pattern; no unique invasive types could be differentiated.

Pseudomonas
Phage typing
Burn wounds
Topical chemotherapy

BACTERIOPHAGE TYPES OF PSEUDOMONAS AERUGINOSA FOUND IN BURNED SOLDIERS

The desirability of precise identification of strains of Pseudomonas aeruginosa is receiving increasing emphasis in many hospitals throughout the world as the problem of nosocomial infections with gram-negative bacilli grows. In this Institute, with the major role played by pseudomonads in burn wounds, the approach to specific recognition of different strains has been the use of phage typing. The phage set developed here (Lindberg, Latta, Moncrief, Brame;[1] Lindberg, Latta, Moncrief, Brame, Mason[2]) has been used successfully to differentiate strains for over 10 years. During this time the role of Ps aeruginosa has changed as effective topical therapy with Sulfamylon has reduced the incidence of burn wound sepsis to an extremely low level. However, despite the control of this syndrome, the incidence of Pseudomonas in burned patients has not fallen markedly, and clinical infections, often as part of a mixed flora, are a prominent part of the sepsis which often occurs in burned patients. The type differentiation of Ps aeruginosa in burn patients is presented in this report with some indications of epidemiologic significance.

METHODS

The technics for phage typing of Ps aeruginosa have been described (Lindberg, Latta, Brame, Moncrief).[3] Cultures from which strains were collected included all usual clinical sources, plus biopsies and autopsy tissue samples. The striking decrease of Vietnam casualties which occurred in 1971 altered the source of material markedly.

RESULTS

Predominant Phage Types, 1971.

The source of patients altered abruptly in 1971. In the 2 previous years, 50% of admissions were injured in Vietnam in 1969, and 53% in 1970. In 1971, Vietnam casualties were 33% of all admissions, and the admissions in 1971 were 268, in contrast to 325 in 1970 and 301 in 1969. During the period of heavy input of Vietnam casualties, they offered a constant source of seeding of the burn wards, in some groups of 15 admissions, all would be seeded with Ps aeruginosa when they arrived. In 1969 and 1970, out of all Vietnam arrivals, 53% harbored Ps aeruginosa on the burn when they arrived.

Out of 39 Vietnam arrivals in 1971, 12 were positive for Ps

aeruginosa when they arrived. This seeding rate of 33% is lower than was seen previously. Smaller patient loads and fewer severe burns undoubtedly contribute to the lessened seeding.

During 1971, 268 patients were admitted to the Institute of Surgical Research Burn Ward. Six hundred thirty-seven Pseudomonas strains were derived from 121 patients during the year. Six of these were 1970 patients positive in 1971. Thirty-three patients were Vietnam evacuees, and 88 were patients from places other than Vietnam. Sources of Pseudomonas strains included the burn wound, wound contact cultures, biopsies, catheters, blood, urine, stool, and postmortem tissues.

The predominant phage types from this series of Pseudomonas strains are listed in Table 1 in order of frequency. Approximately 75% of all strains fell into one of the 8 listed categories. On the left side is the Phage Type Code designation along with the specific Phage Type to which it refers. Next is the incidence of each phage type (number of patients - number of strains) in Vietnam and non-Vietnam patients, and finally the total patients and strains. As in 1970, nontypable strains predominated in this collection. The high incidence of nontypable strains is naturally very disturbing. Efforts are being made, however, to find new phages by which these strains may be further delineated. Thirteen Vietnam patients had 32 nontypable strains while 51 non-Vietnam patients accounted for 224 strains. Approximately 40% of all strains were nontypable.

31,119x (Phage Type Code F 3). Strains of this type, while not unfamiliar as a "burn" type, did not predominate in the 1970 collection and actually were not observed at all in 1971 until the latter half of the year. A very high incidence occurred during this period. The incidence of this type and the remaining listed types in patients was considerably lower than was that of nontypable strains. Seventeen patients had 65 strains of Type F 3. Strangely, it was found only in non-Vietnam patients. The sudden upsurge of strains of this type is typical of what has been characterized as a micro-epidemic.

Incoming Vietnam patients cannot be designated as the source of this strain, since it was not found in this group. In view of the fact that less common types were detected in both patient groups, it may be reasonably concluded that type F 3 was either non-existent or at least was relatively rare in patients from Vietnam.

119X,F 7 (Phage Type Code M-4). Strains of this type were collected in 1970, but only from August onward. It continued to occur sporadically in the first 6 months of 1971, but since June

Table 1. Predominant Pseudomonas Phage Types in ISR Burn Ward Patients, 1971

Phage Type Code	Phage Type	Patient Origin		Total No.	
		Other than Viet Nam	Viet Nam		
		No. of Patients - Strains		Patients	Strains
NT	non-typable	51-224	13-32	64	256
F 3	31,119X	17-65		17	65
M 4	119X,F7	11-85	1-2	12	97
H 3	1214,68,119X	10-28	2-5		33
M 2	119[illegible]	5-7	3-5	8	12
F 12	31	5-7	2-2	7	9
I 1	68	5-7	1-1	6	8
R 35	7,119X	1-1	3-4	4	5

475

23-3

1971, only 2 patients have harbored this strain. Type M 4 was the fifth in frequency of occurrence in 1970 and increased to third in incidence in 1971. There was thus a real peak of occurrence. It was found mainly in non-Vietnam patients, of whom 11 were the source for 85 strains. One Vietnam patient yielded 2 strains, for the highest number of a single type, 87 strains.

1214,68,119X (Phage Type Code H 3). Strains of this type have been a conspicuous part of the Institute of Surgical Research burn patients' flora in previous years. It was the most common typable strain recovered in 1969 and in 1970. At that time only nontypable strains were more frequent. It fell markedly in numbers in 1971, when it was the fourth most commonly encountered form. This phage type had, in the past, been found at the time of admission on patients injured in Vietnam. During 1971, it occurred only during a 6-month period. This time, 10 patients from various parts of the United States harbored it for a total of 28 strains. There were 2 Vietnam returnees with 5 strains, for a total of 12 patients positive with 33 strains. The decline in numbers of Vietnam patients may account for the reduced numbers of strains of type H 3.

119X (Phage Code M 2). Strains of this type were the third most common in 1970, but in 1971, they fell to a much lower incidence. In previous years type M 2 was traceable to incoming patients injured in Vietnam, but in 1971 it was found in 5 United States patients with 7 strains, and in 3 Vietnam returnees with 5 strains for a total of 8 patients and 12 strains.

31 (Phage Type Code F 12). At one time phage type F 12 was numerically predominant. It was the second most common strain in 1968. It did not disappear completely but has since been a minor part of the flora. It was fifth most common in 1970 and in 1971, sixth. Five non-Vietnam patients harbored 7 strains, and 2 from Vietnam, one strain each in 1971.

68 (Phage Type Code I). This type, which has been detected in collections from various areas of the United States, was present in relatively low numbers until 1968 when it was the predominant type. It has subsequently become a minor part of the flora. It was sixth most common in 1970 and in 1971 was the seventh most common type. Six patients (one from Vietnam) harbored a total of 8 strains.

Type 7,119X (Phage Type Code B 35). This is a relatively uncommon type. It was not found at all in 1970 and in 1971 was recovered only during one month (June). Three Vietnam returnees harbored the strain, and one patient who was injured in the United States.

In summary, all the predominant phage types listed for 1971, with the exception of 31,119X (Phage Type Code F 3) and 7,119X (Phage Type Code B 35), were also found among the predominant types during 1970. There were minor shiftings in the order of incidence of each type. The percentage of nontypable strains doubled in 1971; for 1970, the percentage of these strains was 19.4% while in 1971 it was 40%.

Phage Types of Pseudomonas from Blood Cultures of ISR Burn Ward Patients, 1971.

Because of the close association between Pseudomonas bacteremia and fatal endotoxin shock, the nature of this complication is of particular interest. A close watch is maintained to detect the emergence of a particular invasive strain.

During 1971, 31 strains of Pseudomonas were isolated from blood cultures of 21 burn patients. The phage types of this particular collection of strains are given in Table 2. Listed in chronologic sequence are the Phage Type Code and Phage Type of strains from each individual with the number of strains for each patient. In the far right column is given the number of strains of each type from each patient. With those patients who expired, the patient number is underlined. In this particular study, only 3 out of 21 patients with septicemia survived.

Nontypable strains were the most numerous, 16 strains having been found in 10 patients. Strains of Phage Type Code M 4 (119X, 57) with 7 strains from 5 patients, were second in prevalence. Phage Type Code D93 appeared in 2 patients with 2 strains. The remaining Phage Type Codes, A187, C19, D24,F 3, H 3, and M 2 each appeared in a single patient with one strain each. The first 2 listed patients had more than one type recovered.

As was found in strains from all sources, nontypable strains predominated in this collection. Phage Type M 4 strains were second most prevalent. This was the third most common type from all sources.

The nontypable strains could well reflect the high incidence of nontypables in the whole population. With the strains which could be typed, the most common types among all strains for 1971 were F 3, M 4, H 3 and M 2 in that order. There were 5 patients with M 4, and one each with F 3, H 3 and M 2. The possibility exists that the M 4 strains were more invasive, but this is not established.

Table 2. Phage Types of Pseudomonas from Blood Cultures, 1971

Patient Number	Month	Phage Type Code	Phage Type	Number of Strains
324	Jan	M 4	119X,F7	2
			NT	1
10		M 4	119X,F7	1
			NT	2
26	Feb		NT	1
14		M 3	1214,68,119X	1
	Mar			
47	Apr		NT	1
45		M 4	119X,F7	2
91	May	M 4	119X,F7	1
92		D93	21,44,1214,68,109,352,119X,F8	1
90		D93	21,44,1214,68,109,352,119X,F8	1
107	Jun	M 4	119X,F7	1
131	Jul		NT	1
163	Aug	D24	21,1214,68,109	1
169	Sep	C19	16,21,1214,68,109,119X	1
171			NT	1
194	Oct		NT	3
218 VN			NT	3
229		F 3	31,119X	1
206		A187	2,7.16,21,73,109,352,119X	1
216			NT	1
233			NT	2
211		M 2	119X	1

Phage Types of Pseudomonas from Postmortem Lung Tissues.

Increasing importance has been ascribed to pneumonia as a cause of death in burn patients, and a scrutiny of Pseudomonas phage types recovered from postmortem lung tissues was made to determine whether any particular phage type was associated with the development of pneumonia.

Listed in Table 3 are the phage types of 51 strains of Pseudomonas collected from lung tissues of 17 patients.

There were 23 nontypable strains of Ps aeruginosa, from 10 patients. This is the same rate of nontypable strains that was found for all strains examined in 1971. Type 119X,F 7 (M 4) was found in 3 patients, and 31,119X(F 3) in another 3 patients. Type 68 (I 1) was found in 2 patients. Three others had other phage types: D 42, F 12, and H 3. Only the latter was common enough to have been found frequently in the total of cultures for 1971. Four patients had nontypable strains as well as typable strains. The incidence of Phage Type Code M 4, F 3 and I 1 is consistent with the overall distribution of phage types in the entire collection. No specific type could be incriminated as being associated specifically with pneumonia in the burned patient. The situation was consistent with the concept of the bacterial component of pneumonia as secondary to the pulmonary damage that paved the way for bacterial involvement.

Sequential Distribution of Predominant Phage Types in ISR Burn Patients, 1971.

The shifts of individual phage types (i.e, strains) of Ps aeruginosa in a burn ward population is most readily understood by setting down the predominant types by months, as a convenient time interval. This incidence pattern is shown in Table 4. The phage type (and code designation) for each predominant type are listed. The number of isolates and the number of patients positive for each type are set down in a box outline. The heavily outlined box denotes the numerically predominant type during that month. A double outlined box denotes the next most common type, a single line the third most common, and dotted outlines indicate the less common strains which ordinarily occurred in only one patient in a month.

The outstanding feature of interest, and one which calls for much further study, was the remarkable upsurge of nontypable strains. These strains, which fail to react with the ISR typing set, made up 40% of the annual total. Previous years had exhibited nontypables up to 20% of isolates, but 10% to 15% was more typical. The

Table 3. Phage Types of Pseudomonas from Post-Mortem Lung Tissues, 1971

Patient Number	Month	Phage Type Code	Phage Type	Number of Strains
323	Jan	M 4	119X,F7	1
324		M 4	119X,F7	4
			NT	4
10			NT	3
	Feb			
28	Mar	D42	21,31,68	4
		F12	31	4
47	Apr		NT	2
73	May		NT	4
71		H 3	1214,68,119X	1
		I 1	68	1
107	Jun	M 4	119X,F7	3
136	Jul		NT	2
166	Aug	F 3	31,119X	4
		I 1	68	1
160			NT	1
169	Sep	F 3	31,119X	4
171			NT	4
176			NT	1
209	Oct	F 3	31,119X	1
216			NT	1
233			NT	1

Table 4. Monthly Distribution of Predominant *Pseudomonas* Phage Types

Phage Type Code	Phage Type	Month											
		Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
NT	non-typable	5-33	4-9	3-5	8-19	6-28	4-5	14-56	7-11	6-27	13-47	3-7	4-9
F 3	31,119X						1-2	4-7	5-12	4-23	7-13	3-7	1-1
M 4	119X,F7	5-50			1-7	4-19	3-9		2-2				
M 3	1214,6R,119X		2-3	4-6	3-9	1-7	3-6		1-2				
M 2	119X		1-1	1-1	3-3	2-3	.	1-1	1-2		1-1		
F 12	31			1-3			3-3	2-2				1-1	
I 1	68			1-1		1-3	3-3		1-1				
9 35	7,119X						4-5						

stability of the individual bacteriophage preparations in the typing set was initially suspect, although in view of the stability of these phages for up to 10 years of constant use there seemed little reason to assume that several of them had mutated all at once. In any event, the possibility of change in the phages was excluded when cross-reaction patterns to confirm phage identity were run. This cross-reaction procedure, using each of the 18 typing phages against 18 propagating strains, has been used to monitor the stability of the phages since 1964. The individual spectra of activity has not changed as shown by a consistent cross-reaction pattern for each phage (Latta, Lindberg, Pruitt).[4]

Since the typing system has not changed, the conclusion must be that the population of Ps aeruginosa is different from that which has been observed over the period from 1962 through 1970. The predominance of nontypable strains was reflected in a preponderance of nontypable strains in blood cultures, lung and tissue cultures. It is assumed that there are multiple types, but they must be sorted out for the same reasons that Ps aeruginosa required strain differentiation a decade ago.

Among the typable strains, type 31,119X (F 3) was most numerous. It first appeared in June, and rapidly spread to become the predominant type. This sudden incursion of a new type has been designated as a micro-epidemic. Its source was not detected, but it was most plausibly present on an incoming patient. This type included almost all of the strains requiring 0.625% Sulfamylon for inhibition.

Type 119X, F 7 (M 4) was the most common type in January; it disappeared for 2 months, then reappeared at a lower incidence over a 5-month mid-year period.

Type 1214,68,119X (H 3) was the most common type in March and persisted on until August. This type was very numerous in 1969 and in the first 8 months of 1970; it then disappeared until its re-emergence in February 1971.

Type 119X (M 2) strains persisted at a low incidence throughout the year; it was most common in April together with type H 3.

Type 31 (F 12) appeared in 7 patients over a 4-month period. In 1967 and 1968 type 31 was very numerous and a predominant strain in burn infections. After virtually disappearing for 2 years, it reemerged in a minor role in 1970, and has continued in that role since.

Type 68 (I 1) was found in 6 patients during a 4-month period.

Its highest incidence (3 patients) occurred in June.

Type 7,119X (B 35) strains were only found in June. During that month they were the predominant typable strain. The strain apparently arrived on 2 Vietnam evacuees who were admitted at the beginning of the month. The strain appeared to start a rapid spread, then stopped abruptly.

DISCUSSION

The strain distribution of Ps aeruginosa, as shown by phage typing, resembled the patterns established in previous years in the presence of distinctive types which would enter the ward population, persist for some months in successive seedings of patients, and then, in the case of at least 4 types, disappear. Types reappeared in 1971 that had been negligible in incidence in recent years. A major type, F 3, was predominant for 7 months during which virtually all isolates of this type were uniquely tolerant of Sulfamylon. The inhibiting concentration was 0.625% in a population of which the median inhibitory level for all strains was 0.1250%. No specific septicemic or invasive types could be distinguished.

The unique change in the epidemiology of Ps aeruginosa in the Institute of Surgical Research burn ward was the appearance of a very large number of nontypable strains. This unique development was shown to be a function of the strains themselves, not of the phage typing set. To recover adequate typing capability, new phages, able to lyse these strains, are being sought. Since all Ps aeruginosa are lysogenic, the initial attempt is being made with temperate phages recovered from strains of the propagating series. Several phages, in the main represented by multiple isolates, have been recovered by the serial passage technic. Of these, 2 separate bacteriophages offer promise of a range of activity that will encompass the untypable strains which are now the dominant part of the Ps aeruginosa population.

The specificity of the bacteriophage typing system is its principal virtue. When strains lacking specific susceptibility sites appear, this same virtue becomes a flaw that can be surmounted only by recovering new phages to cover the refractory population. The source of these strains is not known. Clearly they arrived on the ward with incoming patients from CONUS, but there is no obvious reason why such a population should suddenly alter its phage susceptibility. It is, however, a biological fact to be handled as expeditiously as possible.

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PRESENTATION

Lindberg RB: The Present Status of Bacteriophage Typing of Pseudomonas aeruginosa. Amer Soc of Microbiologists Symposium on Taxonomy of Pseudomonadacese. Philadelphia, Pa, 26 Apr 1972.

PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
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71 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	3A061102B71R	01	135
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE: (U) Evaluation of Anti-Pseudomonas Hyperimmune Globulin in Pseudomonas Sepsis 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
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FISCAL YEAR	PRECEDING 72	0.3	4.0
	CURRENT 73	0	0

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21. GENERAL USE
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22. KEYWORDS (Provide EACH with Security Classification Code)
(U) Pseudomonas Sepsis; (U) Hyperimmune Globulin

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

23. (U) To evaluate hyperimmune human anti-Pseudomonas globulin in the treatment of Pseudomonas sepsis.

24. (U) Two groups of patients are to be studied: (a) burn patients with clinical Pseudomonas burn wound sepsis with biopsy confirmation and/or a positive blood culture; (b) burn patients with clinical pneumonia with positive blood culture for Pseudomonas as well as in the sputum. There is a control group in each group. Human anti-Pseudomonas hyperimmune globulin is administered i.m. at a dosage of 0.5 cc/kg/day, given in two divided doses for 5 to 7 days. Serial blood cultures, sputum cultures, burn wound cultures and serum immunoglobulin determinations are performed.

25. (U) 71 07 - 72 04 No new patients have entered the study since the last report. Internal problems within the manufacturer's organization have precluded a further supply of globulin for this study, and accordingly it has had to be suspended.

* 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.

FINAL REPORT

PROJECT NO. 3A06112B71R-01, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: EVALUATION OF ANTI-PSEUDOMONAS HYPERIMMUNE GLOBULIN IN PSEUDOMONAS SEPSIS IN THE BURNED SOLDIER

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

1 July 1971 - 30 June 1972

Investigators:

Wellford W. Inge, Jr., MD, Lieutenant Colonel, MC
Basil A. Pruitt, Jr., MD, Colonel, MC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

REPORT TITLE: EVALUATION OF ANTI-PSEUDOMONAS HYPERIMMUNE GLOBULIN IN PSEUDOMONAS SEPSIS IN THE BURNED SOLDIER

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

Period covered in this report: 1 July 1971 - 30 June 1972

Investigators: Wellford W. Inge, Jr., MD, Lieutenant Colonel, MC
Basil A. Pruitt, Jr., MD, Colonel, MC

Reports Control Symbol MEDDH-288(R1)

Infection continues to be a major cause of death in patients who have been severely burned. Gram-positive organisms such as Streptococcus and Staphylococcus have been controlled with penicillin and penicillinase-resistant antibiotics. Gram-negative bacteria are still the major offending organisms of infection in burn patients. While topical chemotherapy of the burn wound has brought many of these gram-negative infections under control, in patients with large burns sepsis of other than burn wound origin, especially with Pseudomonas aeruginosa, has been extremely difficult to control by any treatment regimen. Studies with the use of heptavalent Pseudomonas antigen have shown a direct relationship between antibody titer for specific Pseudomonas serotypes and resistance to infection. Vaccination with the polyvalent Pseudomonas antigen, however, is effective only if given within the first six days postburn. In established infection or in patients received after the sixth day postburn, it would seem that the institution of passive immunity with anti-Pseudomonas hyperimmune globulin might be effective in the treatment and prevention of Pseudomonas sepsis whether of burn wound or nonburn wound origin in the burned soldier.

Only 3 patients have received anti-Pseudomonas globulin on a life-saving emergency basis. This is too small a number for drawing any conclusions. Internal problems within the manufacturer's organization have precluded a further supply of globulin for this study, and accordingly it has had to be suspended.

Pseudomonas sepsis
Hyperimmune globulin

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
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71 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	3A061102B71R	01	243
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) Bacteriophage Types of Serratia Marcessens from Burn Wounds of Military Personnel (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 Clinical Medicine

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67 07	Cont	DA	C. In-House

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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 72	0.4	8.0
	CURRENT 73	0.3	10.0

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21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede 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 and its complications, a continued threat to military personnel, require additional delineation of opportunistic invaders in the program controlling nosocomial infection.

24. (U) A phage typing set, devised for this purpose, is propagated to give high potency typing fluids for delineating wound, biopsy, lung and autopsy strains.

25. (U) 71 07 - 72 06 The incidence of Serratia marcessens in burn patients decreased in 1971, during a time when a purported "outbreak" was occurring in the adjacent General Hospital population. Typing of these isolates from several hospital wards (both medical and surgical) showed that there was no evidence of a hospital wide seeding with one or even with several strains. The isolates were heterogeneous and only in local instances was evidence of one-time transmission seen. This finding is the kind of specific information that can clarify much unfounded speculation about "epidemic" outbreaks.

*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 66 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE.

ANNUAL PROGRESS REPORT

PROJECT NO. 3A061102B71R-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 1971 - 30 June 1972

Investigators:

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

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

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

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

Period covered in this report: 1 July 1971 - 30 June 1972

Investigators: Virginia C. English, MA
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Reports Control Symbol MEDDH-288(R1)

From 68 patients, 107 strains of Serratia marcessens were collected for phage typing. There were 23 burn ward patients with 50 strains, and 45 general hospital patients with 57 strains. Seeding of the burn ward was less striking with the decrease in admission of patients from Vietnam than it had been when most admissions were from the Far East. The predominant type in burn patients was 3,5,7, 11,15,18; in general hospital patients, the predominant type was 5, 7,11,15. In neither instance was there evidence of extensive epidemic spread of a given phage type; the Serratia population was heterogeneous and was continually reinforced by new strains from incoming patients. The exclusion of a purported Serratia epidemic was achieved by use of phage typing. The importance of precise epidemiologic markers in assessing transmission of nosocomial infections is further substantiated.

Burns
Serratia
Bacteriophage

BACTERIOPHAGE TYPES OF SERRATIA MARCESSENS FROM BURN WOUNDS OF MILITARY PERSONNEL

The flora of the burn wound is today predominantly a gram negative population of Enterobacteriaceae. Probably this predominance has been enhanced by the extensive use of Sulfamylon burn cream, which selectively suppresses Pseudomonas aeruginosa. Although antibacterial action of Sulfamylon can be demonstrated with Klebsiella, Enterobacter, Escherichia and Proteus spp, it requires a higher concentration than is needed for Pseudomonas. The median inhibitory concentration may vary from 0.6% to 2%, in contrast to a median inhibitory level of 0.12% for Pseudomonas. Evidently the enteric bacilli are not inhibited from growing on the Sulfamylon-treated wound. Among the genera of enteric bacilli which have been described, with increasing frequency, as an opportunistic pathogen is the genus Serratia. Although it has not risen in incidence in recent years in the Institute of Surgical Research, it has, on occasion, caused unequivocal burn wound sepsis. Isolates of Serratia marcessens (the only species encountered here) have also been shown to be capable of producing invasive burn wound sepsis in the burned rat model, although such virulence has been erratic and difficult to reproduce.

S. marcessens has appeared in an episodic manner, with a flurry of isolates followed by disappearance of the strain. This capability of being rapidly disseminated in a ward population lends urgency to the need for a means of recognizing strains and tracing their progression through a susceptible population. In general, the experience of the past decade has been one of increasing pathogenicity and invasiveness of gram negative bacilli formerly regarded as harmless commensals. S.marcessens is an opportunistic pathogen, and the extent of its potential activity cannot be foreseen. Continued surveillance of this species is hence being carried out using a bacteriophage typing system developed in this laboratory (English, Latta, Brame, Lindberg)[1].

METHODS

Seven bacteriophages comprise the group used in the typing system. These were originally isolated from sewage effluent. The procedures follow those described by Adams.[2] The typing system has repeatedly been challenged with a variety of strains of Klebsiella, Enterobacter and Providencia species. No lysis has yet occurred with any strain of these coliforms.

The classical brick-red pigmented isolates and those which fail

to manifest this property show no other difference in metabolic mechanisms. Since the majority of strains isolated from patients in the Institute of Surgical Research have been of the achromogenic variety, careful differentiation from other enteric bacilli and confirmation of identity is a necessity.

S. marcessens is a non-lactose fermentor. A small percentage of strains may ferment lactose after prolonged incubation. If gas is produced from glucose, it is observable only in liquid medium containing a Durham tube which contains one small bubble. The ability to liquefy gelatin is an important factor in the differentiation of other enteric bacilli from Serratia. Gelatin is rapidly liquefied by Serratia (24-48 hours); the few Enterobacter cloacae and Enterobacter aerogenes that possess this capability do so very slowly (greater than 5-7 days), while the Hafniae group do not liquefy gelatin at all. The rapidly gelatin-liquefying Enterobacter liquefaciens can be differentiated from Serratia by use of arabinose, a pentose and the trisaccharide, raffinose. All three Enterobacter species are fermentors of these carbohydrates, whereas Serratia does not possess this capacity.

Once identity of Serratia is confirmed, phage typing is accomplished using the same techniques and methods as are employed for Pseudomonas at this Institute (Lindberg, Latta, Moncrief, Brame).[3]

During 1971 the incidence of Serratia was not as high as it has been in previous years. Fifty strains were isolated from 23 patients; in 1970 there were 117 strains from 49 patients.

The first strains were recovered in 1971 on 4 January from 4 of 8 admissions from Vietnam. There were, during the year, 11 different phage patterns recognized, plus 6 isolates (from 4 patients) which were nontypable. The nontypable rate was thus 12%; in previous years it has been in the range of 6%. The number of isolates is not great enough to be able to recognize whether this nontypable rate is due to new factors appearing in Serratia, or simply variations due to a small sample. Further monitoring will answer this question.

Phage patterns recognized throughout the year are recorded in Table 1. Organization into ascending numerical order permits easy recognition of groups as a particular phage reaction falls out of the pattern, or is replaced by another. Type 3,5,7,11,15,18 was predominant both with regard to number of patients harboring the strain and the number of isolates recovered. It is of interest that this type was the only one in which the number 3 phage reacted. Types 5,7,9,11,15,18 and 15 were found in 3 patients each, the former with 7 isolates, the latter with 3 isolates.

TABLE 1.

Phage Types of *Serratia marcescens*
Recovered from 23 Burn Patients

Phage Type	Number of Patients	Number of Isolates
3,5,7,11,15,18	5	11
5,7,11,18	2	3
5,7,9,11,15,18	3	7
5,9,11,18	1	1
7	1	1
11	2	2
11,15	2	3
11,15,18	1	1
11,18	2	5
15	3	3
18	1	7
Nontypable	4	6

The predominant phage types are seen in a more meaningful relationship if the patterns observed for the past 5 years (since the system was developed) are viewed. This information is presented in Table 2. Type 5,7,9,11,15,18 was predominant in 1968, 1970 and 1971. It was found in the greatest number of patients and made up the largest number of isolates. In 1969, type 15 was recovered from 8 patients as opposed to 7 for 5,7,9,11,15,18.

Type 3,5,7,11,15,18 was the fourth most common type in 1968. It almost disappeared after that; only one strain was found in 1969 and none in 1970-1971. In 1972, it has reappeared as a predominant type.

The two predominant phage types for 1971, 3,5,7,11,15,18 and 5,7,9,11,15,18 are shown in detail in Table 3 to convey the transmission potential of this organism. The first, type 3,5,7,11,15,18, was recovered from 5 patients, with 9 strains isolated. It was brought in on 4 January on the burn of a patient from Vietnam, with 3 strains recovered from a leg wound. Three months later another Vietnam returnee brought in a strain on his burns and in his urine. This strain persisted for at least 2 weeks. Two patients from the United States acquired this strain after they were admitted. In one, sputum was positive; in the other, an autopsy tissue sample. The last appearance of this strain in 1971 was in a patient from Vietnam who died with Pseudomonas bacteremia. Although no antemortem cultures yielded Serratia, it was found in lung and burn wound tissue samples at autopsy.

The second most common type of 1971, 5,7,9,11,15,18 was isolated from 2 patients in January and one in August. One strain was collected from a Vietnam evacuee on arrival, 4 January. Five days later a United States burn patient was found positive, one week after his arrival on the burn ward. The plausible interpretation was a patient to patient transfer of this strain, since his cultures on arrival, prior to his "exposure" to the Vietnam carrier, were negative for Serratia.

Mixed infections, either with more than one type or with a type and a nontypable strain, occurred in 4 patients. This sequence is summarized in Table 4. Patient Nr. 36 initially harbored type 11,18. On 28 February, this type and a nontypable strain were both recovered. The nontypable strain may have been acquired after a week's residence on the ward. The two strains persisted in subsequent cultures. Patient Nr. 156 had no Serratia recovered during life, but at autopsy 2 different phage types were found in separate tissue blocks. A similar sequence occurred with patient Nr. 207 and with Patient Nr. 218.

TABLE 2.

Comparsion of Yearly Predominant Phage Types

Year	Phage Type	Number of Patients	Number of Isolates
1972	3,5,7,11,15,18	5	11
	5,7,9,11,15,18*	3	7
1971	5,7,9,11,15,18*	10	29
	5,7,9,15,18	8	9
	15	8	13
1970	5,7,9,11,15,18*	10	19
	5,7,9,15,18	4	4
	7,9,15	7	5
	15	5	5
1969	5,7,9,11,15,18*	7	18
	5,7,15	7	7
	11,15	7	16
	15	8	16
1968	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 was among predominant each year, though in a significantly less number of patients in 1972

TABLE 3.

Distribution of Two Predominant *Serratia marcescens* Types in the ISR Burn Ward, 1971

Phage Type 3,5,7,11,15,18			
Patient Number	Admission Date	Date Isolated	Source
3	1-4-71	1-4-20	Leg
39	3-8-71	3-9-27	Urine
		3-9-29	R.thigh
		3-9-34	L.leg
		3-22-16	R. palmer surface
182	9-4-71	11-20-4	Sputum
207	9-21-71	9-24-4	A # 10
218	10-11-71	10-21-25	A # 11
		10-21-35	LUL

Phage Type 5,7,9,11,15,18			
Patient Number	Admission Date	Date Isolate	Source
1	1-2-71	1-9-5	Arm
6	1-4-71	1-4-22	Chest
163	8-15-71	8-15-6	R.scapular area
		8-15-7(1)	R. hand
		8-16-15(2)	L.forearm

TABLE 4.

Serratia Phage Types in Patients with Multiple Types
ISR - 1971

Patient Number	Admission Date	Phage Type	Date Isolated	Source of Culture
36	2-22-71	11,18	2-23-26	R. thigh
			2-26-17	R. thigh
			2-28-6	R. thigh
			3-3-11	R. thigh
		Nontypable	2-28-6	Throat
			3-2-14	Throat
156	8-3-71	11	8-14-11	A # 8
		11,15	8-14-21	A # 26
207	9-21-71	3,5,7,11,15,18	9-24-4	A # 10
		11,15,18	9-24-10	A # 2
218	10-11-71	3,5,7,11,15,16	10-21-25	A # 11
			10-21-35	LUL
		Nontypable	10-21-21	A # 5

25-7

Patients injured in Vietnam accounted for one-third of the patient population in which Serratia was found. Surveillance of patients admitted from Vietnam who harbored Serratia is recorded in Table 5. Half of a group of patients admitted 1-4-71 harbored Serratia in their burn wounds as ascertained by contact cultures. Three phage types were distributed in that group of patients. Among these were 3 isolates of the type that proved to be predominant during the year.

Two patients transferred from Vietnam were admitted harboring type 15. Type 15 appeared again in a follow-up contact culture from a patient injured in Vietnam 7 days following his admission.

Type 3,5,7,11,15,18 was found on admission from patient Nr. 39 who was injured in Vietnam; isolates were cultured from burned areas of the thigh and leg and from urine. Subsequent contact cultures showed the type to persist.

Another patient from Vietnam brought type 11 into the ward at his admission. Serratia was cultured from burned areas of the thigh. The type was seen only once again in another patient at autopsy.

Chronologically, the last patient from Vietnam from which Serratia was isolated was in October 1971. At autopsy type 3,5,7, 11,15,18 was found in tissue specimens from the lung and burn wound. Another tissue specimen yielded a nontypable isolate. There had been no antemortem cultures positive for Serratia.

For the first time since phage typing was begun, the major portion of Serratia marcessens strains were found in patients admitted from the United States rather than Vietnam. Two dominant types emerged, while the remainder of the types were seen sporadically throughout the year. This pattern is typical of the genus Serratia as it has been observed here. Predominance of a type has not yet appeared to pose a threat of epidemic proportion. The increasing incidence of Serratia found in autopsy specimens, both of burned tissue and viscera,has been noted. The unpredictable character of the organism with reference to its potential for epidemic spread merits continued close study utilizing the bacteriophage technique.

Phage Typing of Serratia from Brooke General Hospital, 1971.

During 1971 an increase in cultures of S. marcessens from the patients in Brooke General Hospital prompted concern that an epidemic of Serratia might be present. After a consultation, the strains collected by the hospital laboratory were sent to our laboratory for phage typing, in order to determine whether an epidemic was indeed

TABLE 5.
Survey of Serratia Phage Types from Patients Transferred from Vietnam

Patient Number	Admission Date	Admission Cultures		Subsequent Cultures	
		Phage Type	Source	Phage Type	Source
2	1-4-71	15	?	-	-
3	1-4-71	3,5,7,11,15,18 (3 specimens)	Leg	-	-
4	1-4-71	15	Upper arm	-	-
6	1-4-71	5,7,9,11,15,18	Chest	-	-
5	2-1-71	-	-	15	?
36	2-22-71	-	-	11,18 (4 cultures)	R.thigh
				Nontypable (2 cultures)	Throat
39	3-8-71	3,5,7,11,15,18	Urine R.thigh L.leg	3,5,7,11,15 8	R.palmar surface
86	4-26-71	11	R.thigh	-	-
218	10-11-71			3,5,7,11,15,18 Nontypable	A # 11 LUL A # 5

occurring. This was the first opportunity to test the phage typing system on a set of strains from an outside installation.

From 45 patients, 57 Serratia strains were recovered. Ten strains were from patients in one hospital building; the remaining 47 strains were from patients in another hospital building. Typing was done by the procedures developed in our laboratory.

Table 6 summarizes the findings on strains collected from 22 separate ward areas. Ward 42-H (General Medical) produced the largest collection of strains. Twelve Serratia were recovered from 8 patients. Type 11,18 was found in the urine of 2 patients. Two other patients each had a nontypable strain. But every remaining patient had a type unique to himself on this ward. Thus, there was no evidence of transmission of one type to several patients.

Ward 12-A had two patients positive with the same type, 5,7,9, 11,18. These were both urinary tract strains. No other isolates appeared from this ward.

Ward 43-E had 2 strains from the blood of a patient with sepsis. These were both type 5,7,11,15. Three other patients had isolates each of a distinctive type. On 43-E ICU, 2 cutdown site isolates were of the same type; another patient with a positive urine had a distinctly different type. This diversity of types within individual areas refuted the idea of an epidemic spread of a single strain; each of these isolates was epidemiologically a distinct entity.

Table 7 shows the number of different phage types and their distribution throughout the hospital. Note that nontypable strains were isolated, one each from 7 different ward areas. Type 5,7,11,15 was found on 6 different wards with 8 strains recovered. One of 2 wards in which 2 patients carried a single type was 12-A, where each of 2 patients had a type 5,7,9,11,18. The second was Ward 42-H, where 2 patients each had type 11,18. All the other types were scattered throughout the ward in small numbers for each locus. Absence of multiple infections with any one type further refuted the picture of a Serratia epidemic.

DISCUSSION

The initial request for aid in characterizing these strains was couched in terms indicating that the Hospital Infections Committee regarded the presence of a high rate transmission problem, if not a frank epidemic, an established fact. Indeed, if one contemplated the rate of recovery of S. marcessens during brief periods in the hospital as a whole, this was a convincing picture. But precise strain typing

TABLE 7.
Distribution of 19 Phage Types Isolated from Wards at Brooke General Hospital - 1971

Type	Ward	Number of patients	Number of Isolates	Type	Ward	Number of patients	Number of Isolates
5,7,9,11,19	12-A	2	5	9,11,15,18	42-B	1	2
	42-H	1	2		436	1	1
					43-H	1	1
5,7,11,15	42-A	1	1				
	43-F	1	2	9,11,18	43-E	1	2
	43 EICU	1	2				
	43 ECCU	1	1	11	42-[illegible]	1	1
	Med.Cl.#1	1	1				
	43 WIS	1	1	11,15	15-B	1	1
					42-C	1	1
5,7,9,15,19	42-A	1	1		43-A	1	1
					43-E	1	1
5,7,9,11,15	42-H	1	2				
				11,15,19	42-H	1	4
5,7,11,15,19	43-C	1	1				
				11,19	42-H	2	3
5,7,9,11,15,19	43 EICU	1	1		[illegible].Cl.	1	1
	Derm.Cl.	1	2				
				15	13-A	1	1
5,9,11,19	43-E	1	1		42-A	1	1
					[illegible].Cl.	1	1
5,9,11,15	43 ECCU	1	1				
				Nontypable	13-A	1	1
5,11,15	43-F	1	1		15-A	1	1
	436	1	1		42-A	1	1
					42-H	1	1
7,11	13 ABB	1	1		43-A	1	1
					43-C	1	2
7,9,11,19	42-H	1	1		ER	1	1

25-11

25-12

TABLE 6.

Survey of Serratia Phage Types Found on Wards of Brooke General Hospital - 1971

Ward	Culture Number	Source	Phage Type
12-A	024404	Urine	5,7,9,11,18
	024404A	?	5,7,9,11,18
	024384	?	5,7,9,11,18
	023867	?	5,7,9,11,18
	20426	Urine	5,7,9,11,18
13-A	14864	Urine	15
	5154	Abscess	Nontypable
13-A##	2775A	Blood	7,11
15-A	7915	Wound	Nontypable
15-B	1935A	Blood	11,15
42-A	28024	Blood	Nontypable
	3904B	?	5,7,11,15
	10815	Endo tube	5,7,9,15,18
	5969	Trachea	15
42-B	4970	Trachea	9,11,15,18
	5155	Trachea	9,11,15,18
42-C	019767	Throat	11
	022827	Urine	11,15
42-H	6475	Wound	5,7,9,11,15
	6959	Wound	5,7,9,11,15
	19909	Urine	5,7,9,11,18
	23765	?	7,9,11,18
	11231	Trachea	11,15,18
	11264	?	11,15,18
	11314	Trachea	11,15,18
	019795	Sputum	11,15,18
	2043	Urine	11,18
	024184	Urine	11,18
	5396	Trachea	Nontypable
	19804	Foley Cath.	Nontypable

Ward	Culture Number	Source	Phage Type
43-A	11135	Wound	11,15
	11229	R.knee	Nontypable
43-C	5125	Wound	5,7,11,15,18
	7689	?	Nontypable
	7689A	Wound	Nontypable
43-E	254382	Blood	5,7,11,15
	?	Blood	5,7,11,15
	13213	Throat	5,9,11,18
	19870	Urine	9,11,18
	20119	Urine	9,11,18
	7905	Cath.tip	11,15
43-F	2536A	Blood	5,11,15
43-H	21276	Urine	9,11,15,18
43 PICU	7902	Cutdown	5,7,11,15
	?	Cutdown	5,7,11,15
	20397	Urine	5,7,9,11,15,18
43 PCCU	2544A	Blood	5,7,11,15
	5930	Cath.tip	5,7,11,15
43 4IS	27548	Urine	5,7,11,15
436	7801	?	5,11,15
	023540	Urine	9,11,15,18
Med.Cl.#1	010022	Wound	5,7,11,15
Derm.Cl	77944	Wound	5,7,9,11,15,18
	77944	Wound	5,7,9,11,15,18
GU Cl.	21072	Wound	11,18
	5360	?	15
ER	11139	Ear	Nontypable

Note: Culture numbers enclosed in brackets are different specimens from the same patient

corrected this illusion. There was not an epidemic, but instead a low, steady rate of input of Serratia infections from a wide variety of sources. These were most plausibly fecal carriers, and probably, in some instances, the source was endogenous. But the critical point is that these were 19 different types, not a single transmissible strain.

The ability of the Institute of Surgical Research typing set to differentiate between strains in detail validated further the stability and sensitivity of this phage typing system. There are all too few nosocomial infecting agents in which this differentiation is possible. Its use should be continued.

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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
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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
71 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	3A061102B71R	01	304
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) Cell Mediated Immunity in the Experimental Burn - A Laboratory Analogue of Changes Occurring 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 08	72 04	DA	C. In-House

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FISCAL YEAR	PRECEDING 72	0.3	13.0
	CURRENT 73	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) Delayed Hypersensitivity in Burns; (U) Lymphocyte Transformation; (U) Immunity

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

23. (U) To examine quantitatively the cellular immune response of rat lymphocytes after thermal injury, using lymphocyte transformation as an indicator, and phytohemagglutinin (PHA) and tuberculin and the mixed lymphocyte reaction as non-specific and specific immunologic stimuli.

24. (U) In vitro lymphocyte cultures of rat lymph node lymphocytes in 20% fetal calf serum and tritiated thymidine. Effect of burn sera and steroid levels.

25. (U) 71 07 - 72 04 Enchancement of PHA transformation in lymphocytes from 4 and 10 day old burns, not explained by differences in cell viability, rate of culture cell death, hypercorticosteroidism or burn sera factors. The presence of lymphoid hyperactivity in a model heretofore considered suppressed is of interest, and suggests that delayed hypersensitivity suppression may be selective for certain cell type or cell functions.

*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. 3A061102B71R-01, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: CELL MEDIATED IMMUNITY IN THE EXPERIMENTAL BURN - A LABORATORY ANALOGUE OF CHANGES OCCURRING IN BURNED SOLDIERS

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

1 July 1971 - 30 June 1972

Investigators:

Karl Eurenius, MD, Major, MC
Richard F. Mortensen, MS, SP 5

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

REPORT TITLE: CELL MEDIATED IMMUNITY IN THE EXPERIMENTAL BURN - A LABORATORY ANALOGUE OF CHANGES OCCURRING 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 1971 - 30 June 1972

Investigators: Karl Eurenius, MD, Major, MC
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Reports Control Symbol MEDDH-288(R1)

It is a well-established clinical impression that cellular immunity is suppressed after thermal injury. Cutaneous anergy and skin graft tolerance have been demonstrated in laboratory animals following such trauma. We have examined the response of draining rat cervical lymph node lymphocytes to phytohemagglutinin (PHA) after infliction of a 30 per cent third degree scald burn. At one hour and one, 4, and 10 days post injury lymphocyte suspensions were established in medium 199 with 20% heat inactivated fetal calf serum at a concentration of 1 x 10^6 cells per ml. Cells were incubated at 37° with and without optimal PHA (Difco PHA-P) and harvested 48, 72, and 96 hours after culture, following a 16-hour pulse of tritiated thymidine (1 microcurie/culture tube). Results indicate increased basal thymidine incorporation at 4 and 10 days post injury and a marked enhancement in the PHA response in these same cells. This enhancement was not associated with changes in cell viability, endogenous corticosterone levels or the addition of sera from burned rats to the cultures. Electronic particle sizing analysis indicated an increase in mean cell volume of lymphocytes from the 4 and 10 day post injury populations due mainly to the appearance of a population of large cells. We believe these results are most compatible with either increased cell growth of the original node population or the immigration of PHA sensitive cells.

Delayed hypersensitivity in burns
Lymphocyte transformation
Immunity

CELL MEDIATED IMMUNITY IN THE EXPERIMENTAL BURN - A LABORATORY ANALOGUE OF CHANGES OCCURRING IN BURNED SOLDIERS

Knowledge of the status of delayed hypersensitivity in the burned subject is crucial, because this defense mechanism is challenged by the many pathogens and grafts to which the subject is exposed during both his acute insult and chronic convalescence. It is generally believed that cellular immunity is suppressed during this period. The clinical impression exists that skin graft tolerance is increased following burns while cutaneous anergy has been demonstrated in guinea pigs after burn injury. The effects of a burn on host lymphocyte function are not yet clear.

We have studied the response of rat lymph node lymphocytes, removed from burned animals at varying periods after injury, to the mitogen phytohemagglutinin (PHA). Included in this study are basal and PHA stimulated DNA synthesis, cell sizing, and serum and corticosteroid effects.

METHODS

Sprague-Dawley rats were inflicted with a 30% third degree scald burn under pentobarbital anesthesia. Cervical lymph node cell suspensions were prepared from animals 1, 24, 96, and 240 hours after injury, and from control, unburned animals. Cell counts were performed, and suspensions cultured for 24, 48, or 72 hours in the presence or absence of phytohemaglutinin (PHA-P, Difco) 0.01 ml/ml culture. Tritiated thymidine (H^3Tdr) incorporation by the cells was measured in all cultures following a 16-hour pulse label, by liquid scintillation. Cell sizing was performed with an electronic particle counter, and cell viability by trypan blue dye exclusion.

RESULTS

1. Suspension cell viability was not affected by burn injury

2. Lymphocyte mean cell volume increased by the fourth post-burn day, and returned to normal by the 10th postburn day.

3. H^3Tdr incorporation (DNA synthesis) increased in proportion to cell size changes in non-PHA cultures.

4. H^3Tdr incorporation (DNA synthesis) increased in excess of cell size changes in PHA-stimulated cultures.

CONCLUSIONS

The response of lymphocytes to PHA includes protein synthesis and mitosis. The degree of response has been used as a measure of the general status of delayed hypersensitivity. That PHA transformation is increased in the burned rat is of considerable interest. First, the possibility exists that either a new population of lymphocytes, normally more sensitive to PHA, enters the draining lymph node, or a depletion of PHA insensitive cells occurs after thermal injury. Such migration studies have not been done. Second, it is possible that lymphocytes from the burned subject are, because of mechanical or enzymatic damage, more susceptible to the cytotoxic effects of PHA and other nonspecific agents, but less capable of response to a specific antigen. Third, the possibility exists that such cells are immunologically paralyzed by newly formed antigen from the burn, and that PHA acts in this setting as an adjuvant. Finally, it is possible that the burned rat has an intact or hyperimmune response which is reflected by these data. The increases in cell size and basal DNA synthesis observed in these experiments are consistent with all of these possibilities.

Until the influence of burn injury or stress upon the complex immunologic mechanisms of the host are better understood, or until the relationship between the PHA response and the specific cellular immune response is clarified, no further conclusions regarding the significance of this response can be drawn.

PUBLICATION

Eurenius K, Mortensen RF: The Phytohemagglutinin (PHA) response in the thermally injured rat. Int Arch Allergy 40: 707-718, 1971.

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. SMSF'N INSTR'N	8b. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
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FISCAL YEAR	PRECEDING 72	0.3	8.1
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19. RESPONSIBLE DOD ORGANIZATION
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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) A systemic comparison of sulfamylon, sulfadiazene, silver-sulfadiazene and gentamicin, each in topical aqueous gel vehicle, was carried out on burned rats challenged with 5 selected strains of P. aeruginosa of increasing virulence. The tests were run simultaneously on groups of 5 to 8 rats treated with the test agents.

25. (U) 71 07 - 72 06 A detailed study of 5% sulfamylon burn cream as a prophylactic and therapeutic agent was carried out after reports from other laboratories had raised the question of whether this more dilute medication might be used in place of 10% sulfamylon. Diminished discomfort to the patient was a major reason for this observation. The earlier findings (1962-1965) were strongly affirmed; 5% sulfamylon is much less effective than 10% sulfamylon in control of experimental Pseudomonas burn wound sepsis. Eight different challenge strains were used; the survival patterns were parallel with all of these.

*Available to contractors upon originator's approval.

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ANNUAL PROGRESS REPORT

PROJECT NO. 3A061102B71R-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 1971 - 30 June 1972

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ABSTRACT

PROJECT NO. 3A061102B71R-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

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Reports Control Symbol MEDDH-288(R1)

Question of the minimum concentration of Sulfamylon in burn cream which could control Pseudomonas burn wound sepsis has been raised due to desire to make a less painful formulation when applied to second degree burns. One report on an animal trial stated that 5% Sulfamylon equalled 10% in preventing death in the seeded, burned rat model. A re-investigation of this question was made; 7 challenge strains, highly lethal for untreated animals, were used to give a broader indication of range of therapeutic effectiveness. Survival ranged from 8.9% with the most lethal to 68% for the least lethal strain. Parallel values for 10% Sulfamylon were 20% and 100%. A definite therapeutic effect was exerted by 5% Sulfamylon, but as shown in the developmental stage for this drug, it was not as effective as the 10% cream.

Pseudomonas
Sulfamylon-Sulfadiazene
Burn wound

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

Topical Sulfamylon burn cream, 10%, is now an accepted clinical method, and is in world-wide use. Continued interest in improved formulations for controlling burn wound sepsis has been evident, and comparative studies using the Institute of Surgical Research burned, seeded rat model have been called for. The original Sulfamylon therapy was developed with the objective of having a therapeutically effective agent present on the wound. The determination of the maximally effective concentration of Sulfamylon was made in 1962 and 1963 on the burned rat model and the 10% concentration of Sulfamylon HCl was selected. Lower concentrationa, while effective in controlling infection in the burned, seeded rat, still permitted some deaths that could be prevented by use of a 10% concentration (Lindberg, Moncrief, Mason).[1] A factor that has become of concern since the use of Sulfamylon topical therapy became widespread is the transient pain that accompanies application of the burn cream to areas of partial thickness burn (Moncrief, Lindberg, Switzer, Pruitt).[2] This effect was noted as soon as clinical experimental trials were undertaken in this Institute, but was not considered a problem of consequence in view of the major problem of controlling invasive burn wound sepsis. Pain is primarily a problem in second degree burn areas, lasts usually 20 to 30 minutes following application and in 6 to 10 days postburn the healing process results in marked decrease in severity of pain. However, with more widespread use, the pain factor has occasioned complaint and re-consideration of use of a lower concentration of Sulfamylon has been urged. The 10% concentration is painful in part because of it hypertonicity; a 5% concentration would be more benign. A recent report stated that a 5% concentration was as effective as 10% in preventing invasive sepsis in the rat using a single challenge strain. The original derivation of the 10% Sulfamylon burn cream formula compared all strengths from 2% up to 14%; and 5% cream, although it was effective in controlling Pseudomonas burn wound sepsis in the experimental animal model, was less effective than the 10% strength. However, another study of the 5% cream in the acetate form was made to assess the validity of the proposal that burn wound care could safely be done with 5% Sulfamylon.

MATERIALS AND METHODS

The experimental wound and treatment method have been described previously (Lindberg, Brame, Moncrief, Mason).[3] In this series, the 20% scald burn (10 seconds) on the 200 gram rat was seeded with 10^8 Pseudomonas aeruginosa at 90 minutes postburn. Treatment began

24 hours later with topical application of 3.5 gm of the indicated cream, applied once daily. (The lessened protection of once daily treatment was reflected in increased mortality over twice-daily treated animals). Treatment was continued for 10 days. Seven different strains of Ps aeruginosa, varying in virulence, were used as challenge organisms.

RESULTS

The challenge of the burned rat with 5 different strains of Ps aeruginosa, and the effect of 5% Sulfamylon acetate on the death rate, is shown in Table 1. The effect on survival was least noticeable with the extremely virulent strain VA 134. Ninety-one per cent of the treatedd animals, and 98% of the controls, died. At the other extreme, strain 12-4-4 killed 81% of the controls, and 32% of the treated animals. With strain 3-24-5, as with VA 134, the difference in survival rates was not significant. With strains 5-23-8 and 8-28-3, significant increases in survival rate occurred.

Retrospective comparison of 10% Sulfamylon acetate cream, which had been studied extensively in 1966, indicated the relative survival potential of the 5% and 10% compound (Table 2). The comparison with 3 strains reflects the pattern with other challenge strains: 10% Sulfamylon acetate saved more animals than 5% Sulfamylon acetate. With the extremely virulent strain VA 134, few survived on the on-treatment per day schedule. If 2 treatments per day were used, survival rates for strain VA 134 could be as high as 80%.

The behavior of Sulfamylon acetate and Sulfamylon hydrochloride in treating experimental burn wound sepsis was scrutinized carefully at the time when the relative effectiveness of these two drugs was being compared (Lindberg, Moncrief, Brame, Mason).[4] Since, at the 10% concentration, the relative merits of introducing the acetate as opposed to the chloride radical were weighed against the relative effectiveness of the 2 forms in controlling sepsis, the decision was made to use the acetate. However, at 5% concentration, the halving of the chloride input makes it once more pertinent to consider whether a 5% hydrochloride form might merit consideration in therapy. Five per cent Sulfamylon acetate and 5% Sulfamylon hydrochloride were made up in the water-dispersible base used for compounding topical therapeutic formulations in this laboratory (Lindberg, Moncrief, Mason)[1] and were tested in parallel. The results are summarized in Table 3.

It was evident that the survival rates were higher with 5% Sulfamylon hydrochloride than with 5% Sulfamylon acetate. The survival rates with strains VA 134 and 8-28-3 were not statistically

Table 1. 5% Sulfamylon Acetate in Control of Experimental Burn Wound Sepsis

Strain	5% Sulfamylon Acetate (ISR) Died/Total	Ave.Day Died	% Died	Controls Died/Total	Ave.Day Died	% Died	No. Groups Run
VA-134	41/45	5.5	91.1	43/44	5.4	97.7	7
3-24-5	25/32	9.7	78.1	28/29	7.5	96.5	4
5-23-E	6/15	10.2	40.0	10/11	10.0	90.9	2
8-28-3	21/51	7.6	41.1	42/48	8.3	87.5	9
12-4-4	17/53	10.8	32.0	37/46	9.6	80.8	10
Total of all Strains							
	110/196		56.1	160/178		89.8	

27-3

Table 2. Relative Survival Rates of Burned Rats Seeded with Strains of *Pseudomonas aeruginosa* and Treated with 10% and 5% Sulfamylon Acetate Burn Cream

Challenge Strain	Drug and % of Challenged Rats Surviving	
	10% Sulfamylon Acetate	5% Sulfamylon Acetate
12-4-4	96.2	79.2
8-28-3	82.4	60.0
VA 134	10.1	4.2

Table 3. Comparison of 5% Sulfamylon Acetate (ISR) and 5% Sulfamylon HCl (ISR) in Control of Experimental Burn Wound Sepsis

Challenge Strain	Treatment				Controls	
	5% Sulfamylon acetate		5% Sulfamylon HCl			
	died/total	% died	died/total	% died	died/total	% died
VA 134	41/55	91.1	24/36	66.6	17/17	100
8-28-3	12/30	40.0	9/51	17.6	49/51	96.0
12-4-4	17/53	32.0	0.30	0.0	27/31	87.0

27-5

different with the 2 drug forms; they were highly significant with strain 12-4-4. The differences were always in the direction of increased survival with the hydrochloride form.

A further comparison between 5% and 10% Sulfamylon acetate, using drug preparations from Sterling Laboratories, was made with 6 strains of Ps aeruginosa. In this series, each strain was 100% virulent. Results are summarized in Table 4.

Here again, although the groups were small, the overall survival was greater with 10% than with 5% Sulfamylon.

DISCUSSION

The indications are that 5% Sulfamylon acetate could be effective in reducing Pseudomonas burn wound sepsis, but that it would be less effective than the 10% preparation. However, there are limits to the level of extrapolation possible from animal to human conditions. It is not impossible that the 5% cream would serve adequately to protect burn patients from invasive sepsis. It would appear desirable that the comparison, if it were attempted, would include detailed monitoring of surface colonization and invasion as detected by serial biopsies. The strong possibility is present that a more dilute treatment than the 10% burn cream could significantly increase the risk of burn wound sepsis. The data presented herein has discouraged the inauguration of a clinical trial.

REFERENCES

1. Lindberg RB, Moncrief JA, Mason AD,Jr: Control of experimental and clinical burn wound sepsis by topical application of Sulfamylon compounds. Ann NY Acad Sci 150: 950-960, 1968.

2. Moncrief JA, Lindberg RB, Switzer WE, Pruitt BA, Jr: The use of a topical sulfonamide in the control of burn wound sepsis. J Trauma 6: 407-415, 1966.

3. Lindberg RB, Brame RE, Moncrief JA, Mason AD,Jr: Biopsy of the burned seeded rat as a means of evaluating burn therapy and pathogenesis of infecting microorganisms. US Army Surgical Research Unit Ann Prog Rpt FY 1966, BAMC, Ft Sam Houston, Texas, Section 41.

4. Lindberg RB, Moncrief JA, Brame RE, Mason AD, Jr: Comparison of Sulfamylon hydrochloride and Sulfamylon acetate in control of burn wound infections. US Army Surgical Research Unit Ann Prog Rpt FY 1966, BAMC, Ft Sam Houston, Texas, Section 40.

PRESENTATIONS AND/OR PUBLICATIONS

None

Table 4. Comparison of 5% Sulfamylon acetate (Sterling) and 10% Sulfamylon acetate (Sterling) in Control of Experimental Pseudomonas Burn Wound Sepsis

Challenge Strain	Treatment and Deaths/Total		
	5% Sulfamylon Acetate	10% Sulfamylon Acetate	Controls
VA 134	6/7	2/7	6/6
12-4-4	3/7	1/7	6/6
5-23-8	4/6	4/5	5/5
9-30-11	6/6	4/5	5/5
4-18-9	6/7	2/7	6/6
3-24-5	5/7	2/6	5/5
Total of all strains	30/40	15/37	33/33
Mortality rate	75%	40.5%	100%

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0E 6382	72 07 01	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. GRP'N INDIC'N	8A. 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	3A061102B71R	01	317
b. CONTRIBUTING				
c. CONTRIBUTING				

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

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
71 10	Cont	DA	C. In-House

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

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 72	0.3	11.4
	CURRENT 73	0.3	14.0

19. RESPONSIBLE DOD ORGANIZATION
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NAME: William F McManus, MAJ, MC
NAME: DA

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede each with Security Classification Code)
(U) Burn; (U) Eschar Separation; (U) 5% Sulfamylon Acetate Solution; (U) Humans

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

23. (U) Ten per cent Sulfamylon acetate burn cream is an effective topical agent when applied to burn wounds to control bacterial population. During the latter stages of eschar separation where residual eschar is interspersed between areas of open granulation, the application of the cream is made difficult and the cream adheres poorly to areas of open granulation tissue. 5% Sulfamylon acetate solution is used to facilitate development of the residual non-viable tissue in wounded soldiers.

24. (U) 5% Sulfamylon acetate is used as a debriding agent by appling 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

25. (U) 71 10 - 72 06 By using 5% Sulfamylon acetate solution as wet soaks residual non-viable tissue can be ramoved 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.

Available to contractors upon originator's approval.

ANNUAL PROGRESS REPORT

PROJECT NO. 3A061102B71R-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 1971 - 30 June 1972

Investigators:

John L. Hunt, MD, Major, MC
Joseph A. Moylan, Jr., MD, Major, MC
Basil A. Pruitt, Jr., MD, Colonel, MC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A061102B71R-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 1971 - 30 June 1972

Investigators: John L. Hunt, MD, Major, MC
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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

Eighty patients have been included in this study 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 4x8-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 reinstitution of Sulfamylon burn cream therapy.

White blood counts with differentials and hematocrits were followed biweekly during treatment. At no time was the white blood 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 16.8% 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 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 0E6950	72 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'N INDIC'N	8A. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
	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	3A061102B71R	01	080
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) Subeschar Antibiotic Infusion to Prevent Burn Wound Invasion in a Laboratory Model Simulating Burn Patients (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
71 11	72 04	DA	C. In-House

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

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
FISCAL YEAR	PREVIOUS 72	0.4	13.2
	CURRENT 73	0	0

19. RESPONSIBLE DOD ORGANIZATION
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NAME: Paul Silverstein, 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) Burn Wound Sepsis; (U) Subeschar Infusion

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

23. (U) To evaluate the efficacy of subeschar antibiotic infusion in the prevention and treatment of burn wound sepsis in the laboratory animal burn model as a possible treatment for burned soldiers.

24. (U) Two burned and 17 Sprague-Dawley rats were used. Fifth rats were antibiotic controls and 167 rats were given a 20% scald burn and infected with a lethal strain of Pseudomonas aeruginosa. Twenty-seven rats were infection controls and received no antibiotics. Thirty rats received carbenicillin, 20 gentamycin, 20 neomycin and 20 colistin subeschar daily for 10 days. Ten burned infected rats received carbenicillin subcutaneously, at a distance from the burn wound daily for 10 days, ten were given carbenicillin intravenously and 10 intraperitoneally twice daily for 10 days.

25. (U) 71 11 - 72 04 We found no unique advantage in the subeschar route of injeccion of antibiotics in a reproducible animal burn model. Only carbenicillin was effective in reducing mortality and this effect appeared to be independent of route of administration.

*Available in contractor's own 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.

FINAL REPORT

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

REPORT TITLE: SUBESCHAR ANTIBIOTIC INFUSION TO PREVENT BURN WOUND INVASION IN A LABORATORY MODEL SIMULATING BURN PATIENTS

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

1 July 1971 - 30 June 1972

Investigators:

William F. McManus, MD, Major, MC
Paul Silverstein, MD, Major, MC
Harrel L. Walker, MS
Arthur D. Mason, Jr., MD
Basil A. Pruitt, Jr., MD, Colonel, MC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

REPORT TITLE: SUBESCHAR ANTIBIOTIC INFUSION TO PREVENT BURN WOUND INVASION IN A LABORATORY MODEL SIMULATING BURN PATIENTS

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

Period covered in this report: 1 July 1971 - 30 June 1972

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

Reports Control Symbol MEDDH-288(R1)

The subeschar infusion of antibiotics has been proposed as an effective method to prevent and treat burn wound invasion. One hundred and sixty-seven Sprague-Dawley rats were given a 20% scald burn and within one hour postburn were infected with a broth culture of an LD_{100} of Pseudomonas aeruginosa. Infection controls received no antibiotics. All antibiotic controls survived and all infection controls died. All burned, infected animals receiving subeschar carbenicillin survived and those receiving colistimethate, gentamycin and neomycin died. All burned infected animals receiving subcutaneous carbenicillin at a distance from the burn wound survived. Sixty per cent of burned infected animals receiving single daily intraperitoneal carbenicillin injections died. Thirty per cent of the burned infected animals receiving carbenicillin in divided doses twice daily intraperitoneally died and 30 per cent of those animals receiving single daily doses of carbenicillin intravenously died. Subeschar infusion of antibiotics with the exception of carbenicillin failed to protect the animal from death via burn wound invasion. Subeschar carbenicillin did protect the animal, however, when given the same antibiotic subcutaneously at a distance from the burn wound, protected the animal equally well and it was felt that it was the function of this antibiotic, not the route of injection, that protected the animals.

Burn wound sepsis
Subeschar infusion

SUBESCHAR ANTIBIOTIC INFUSION TO PREVENT BURN WOUND INVASION IN A LABORATORY MODEL SIMULATING BURN PATIENTS

Historically bacterial invasion of viable subeschar tissue was the major cause of septicemia and death in burn patients. Effective topical chemotherapeutic agents have significantly reduced but not eliminated the occurrence of burn wound sepsis. Subeschar infusion of antibiotics has been proposed as a technique to prevent and treat burn wound invasion (Baxter).[1] This route of administration has a theoretical advantage of delivery of a high concentration of antibiotic solution directly into and beneath the burn wound which because of thromboses may be inaccessible to systemically administered antibiotics. The effectiveness of subeschar antibiotic infusion has been evaluated in a reproducible animal burn model.

One hundred eighty-seven Sprague-Dawley rats weighing 180-210 grams were anesthetized with intraperitoneal pentobarbital. Twenty rats served as antibiotic controls, 5 received carbenicillin, 500 mg per kg, 5 colistimethate, 5 mg per kg, 5 gentamycin, 5 mg per kg, and 5 neomycin, 15 mg per kg subcutaneously daily for 10 days. One hundred sixty-seven rats were given a 20% scald burn (Walker).[2] Within one hour postburn the wounds were infected with a broth culture of a lethal strain of Pseudomonas aeruginosa containing 10^8 organisms per ml. This organism was sensitive by the tube dilution technique to carbenicillin at 312 units, colistimethate at 6.2 µg/ml, gentamycin at 3.1 µg/ml and neomycin at 12.5 µg/ml. Twenty-seven burned infected rats served as infection controls and received no antibiotics.

All antibiotic controls survived, all infection controls died, with their average day of death being 5.3 days. Thirty burned infected rats received subeschar carbenicillin daily for 10 days. Twenty burned infected rats received subeschar colistimethate daily for 10 days, another similar group received subeschar gentamycin and a third group subeschar neomycin daily for 10 days. All burned infected animals receiving carbenicillin subeschar survived. All animals receiving colistimethate, gentamycin or neomycin died. The average day of death for those animals receiving colistimethate was 6 days, gentamycin 8.3 days, and neomycin 6.3 days.

Since carbenicillin was the only antibiotic which when given subeschar protected the burned infected animal, its effectiveness when given subcutaneously at a distance from the burn wound was evaluated. Ten burned infected animals were given carbenicillin subcutaneously in the anterior abdominal wall at a distance from

the burn daily for 10 days. All infected burned animals receiving subcutaneous carbenicillin at a distance from the burn wound survived. The effectiveness of the carbenicillin when given intravenously or intraperitoneally also was examined. Ten burned infected rats were given a single daily injection of carbenicillin intravenously and 20 infected animals received carbenicillin in a single daily intraperitoneal injection for 10 days. Ten burned infected rats received 250 mg/kg carbenicillin intraperitoneally twice daily for 10 days. Sixty per cent of those animals receiving single daily intraperitoneal injections died. Thirty per cent of the burned infected animals receiving carbenicillin in divided dosage intraperitoneally twice daily,died. Thirty per cent of those receiving single daily doses of carbenicillin intravenously died.

We found no unique advantage in the subeschar route of injection of antibiotics in a reproducible animal burn model. Neomycin, colistimethate and gentamycin failed to protect the animals when given subeschar, despite the fact that the organism was sensitive in vitro to each of these drugs. Only carbenicillin when given subeschar protected the animals, but protected the animals equally well when given subcutaneously at a distance from the burn wound.

Carbenicillin like the penicillins, has an unusual ability for diffusion and also is a potent agent for the control of Pseudomonas infection. Carbenicillin when given in a single injection intravenously, intraperitoneally or in divided injections intraperitoneally, afforded some protection even though some animals expired. The rapid excretion of the drug when given intravenously or intraperitoneally prevents maintenance of sustained blood levels of carbenicillin. The subcutaneous and subeschar deposition of carbenicillin appears to provide a depot from which an effective blood level is maintained. The protection afforded by subcutaneous or subeschar administration of carbenicillin appeared to be a function of the antibiotic itself and not a specific result of the injection into the burn wound.

REFERENCES

1. Baxter CR: Personal communication.

2. Walker HL, Mason AD,Jr: A standard animal burn. J Trauma 8:1049-1051, 1968.

PRESENTATION

McManus WF: Subeschar Antibiotic Infusion to Prevent Burn Wound Invasion. Am Burn Assoc, San Francisco, Cal, 7 Apr 1972.

PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 08 6978	72 07 01	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY OCTY	6. WORK SECURITY	7. REGRADING	8A. ENTRY MATH	8B. SCIENTIFIC DATA - FOREIGN/CONTRACTOR ACCESS	9. LEVEL OF WU
71 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	3A061102B71R	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	PRECEDING/CURRENT	a. PROFESSIONAL MAN YRS	b. FUNDS ($ in thousands)
FISCAL YEAR	72	0.2	6.0
	73	0.2	6.5

19. RESPONSIBLE DOD ORGANIZATION
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NAME: H D Smith, Jr, SP6 DA

21. GENERAL USE: FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Provide KEY1 KEY2 KEY3 Security Classification Code): (U) Military Burn Unit
(U) Fungi; (U) Mucor; (U) Candida; (U) Rhizopus; (U) Burns; (U) Phycomycosis

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

23. (U) To determine the species of fungi in burn patients and determine the importance of such opportunistic invaders.

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) Recovery rates on fungi have been improved by more prompt planting of carefully selected tissue slices and use of optimal substrate - gas ratios in culture bottles. Predominant species have changed: Fusarium replaced Geotrichum in prominence as the burn population shifted from a predominantly Far East casualty group to a Continental U.S. population.

* Available in computerized form and explanation of codes.

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. 3A061102B71R-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
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1 July 1971 - 30 June 1972

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Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

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

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

Period covered in this report: 1 July 1971 - 30 June 1972

Investigators: Robert B. Lindberg, PhD
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Peter M. Kirchgessner, SP 5
Basil A. Pruitt, Jr., MD, Colonel, MC

Reports Control Symbol MEDDH-288(R1)

Continued scrutiny of burn tissues at autopsy and in biopsies has been made for presence of yeasts and fungi in order to better understand the part played by mycotic infection in burn wound pathogenesis. Sixty-one patients were studied at autopsy, with spleen, liver, lung, kidney and burn wound samples cultured. Visceral cultures were positive for Candida in 22.9% of autopsied cases. Fungi included 16 genera; phycomycetes were uncommon, while predominant genera included Fusarium, Cephalosporium, Penicillium and Nigrospora. Specific infections could not be associated with these strains. Biopsy samples were positive for fungi in 37% of patients; predominant genera resembled those seen in autopsies. Improved recovery of fungi was achieved by technical changes, but at least one-third of microscopically positive strains do not yield fungi. The role of presumably saprophytic contaminants is to be investigated in view of their appearance in invaded wounds.

Fungi
Mucor
Candida
Rhizopus
Burns
Phycomycosis

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

The part played by fungi and yeasts in burn wound infection has apparently been increasing in recent years (Bruck, Nash, Foley, Stein, Lindberg).[1] In conferences on nosocomial infections, reports of more and more infections due to fungi have appeared, and the species incriminated are largely those regarded as inocuous saprophytes. In this Institute fungi have increased as colonizers and invaders of the burn wound (Lindberg, Townsend, Contreras, Pruitt;[2] Nash, Foley, Pruitt[3]). Cultures of autopsy tissues and of wound biopsies have, for the past 5 years, routinely included cultures for yeasts and fungi. This procedure has been reviewed to achieve the maximum recovery of fungi from excised tissue, since not infrequently tissues have been positive microscopically but have not yielded fungi on culture. This report summarizes the species and numbers of fungi recovered from biopsy and autopsy tissues in 171.

MATERIALS AND METHODS

Biopsy samples were collected as indicated by the clinical condition of the patient and the appearance of the burn wound. Special emphasis was placed on the darkening of the surface of the burn that has,on occasion, presaged development of fungal wound sepsis. Samples from biopsies are usually small, since the initial specimen is limited by the need for minimal trauma and that specimen must be shared between pathology and bacteriology. The median weight of biopsies received in microbiology is approximately 200 mg per sample. For fungus culture, a sliver of tissue was removed aseptically with either blade or scissors, and was planted on Sabouraud's agar. The remainder of the sample was homogenized and cultured for bacteria qualitatively and quantitatively. Recovery of fungi was found to be more successful when a tissue slice was cultured than when the ground tissue homogenate was used as inoculum. Another factor that appeared to enhance opportunity for recovery of fungi was planting samples as soon as possible after their collection. In contrast to bacterial counts, which change little in the first 24 hours when held in the cold after collection, fungi diminish in recoverable numbers when held in the cold for a day before planting on media.

Autopsy tissue samples were collected in the manner long established in the Institute of Surgical Research. Blocks of over one gram of tissue were collected, dipped in 70% alcohol and flamed. The outer one mm or more of the block was removed asepti-

cally in the laboratory, and a sliver of tissue from this exposed surface was planted on Sabouraud's agar. Incubation was at 26° C.

RESULTS

The recovery of fungi and yeasts from autopsy tissues is set down in Table 1.

The yeast population, although it is grouped with fungi, really constitutes a separate entity. Yeast burn wound sepsis is not as clearcut an entity as fungal wound sepsis, and in the great majority of cases, yeasts on the burn wound behave as a colonizing contaminant rather than as an invading pathogen. The predominant species on burn patients was Candida albicans, as it has been whenever species differentiation is done. Candida stutzeri has been recognized on burns but it has not been common in our patients. Candidal organisms recovered from lung, liver, or spleen may connote pneumonia or systemic sepsis. The 11 patients with positive cultures in viscera fall in this category. Since systemic and wound infection would imply more severe involvement, the patients were separated into those with only wounds positive and those with systemic involvement as well. This localization is shown in Table 2.

The wound was obviously the most common site for finding Candida. Ten patients did not have it elsewhere. Five had lung and wound positive. One patient had Candida in all viscera plus wound, one in spleen, liver and wound, and 2 had liver and wound cultures positive for Candida. Three patients had only positive lung cultures, one had only spleen positive and one had spleen and liver positive at autopsy. Systemic infection would thus have been consistent with the findings in 6 patients (liver and/or spleen positive) and the lung findings implicated that site in 10 patients.

With regard to fungi, 13 genera were recovered from autopsies. This number has not changed in 3 years, although changes in frequency have occurred. In 1971 the most common form encountered was Fusarium. It was found in viscera of 7 patients and in the wounds of 9. In terms of total varieties recovered, there were 11 genera from viscera and 14 from the burn wound. Strains found only in viscera included Paecilomyces and Geotrichum, each in one strain from one patient. The change in frequency of Geotrichum was striking; in 1969, there were 75 strains recovered from 59 autopsies. There was no recognizable change in morbidity due to this genus; its relationship to burn wounds is obviously not as direct as seemed the case in 1969. As to most common genera in 1971, the proportion of total strains recovered reflects their incidence. Principal genera and frequency of occurrence were:

Table 1. Fungi Recovered From Burn Patients at Autopsy, ISR 1971

Genus	Source and No. Patients Positive		No.Strains Recovered
	Lung, Liver, Spleen	Burn Wound	
Mucor	0	3	7
Rhizopus	1	1	2
Absidia	0	1	3
Aspergillus	3	6	13
Penicillium	5	8	23
Paecilomyces	1	0	1
Alternaria	0	2	3
Cephalosporium	1	8	23
Fusarium	7	9	31
Helminthosporum	0	4	5
Nigrospora	3	9	18
Scopulariopsis	1	5	10
Sepedonium	1	1	2
Diplosporium	0	1	3
Geotrichum	1	0	1
Fansecaea	2	2	6
Candida sp	11	13	49

No. patients positive for fungi or yeast	46:	75.4%
No. patients negative for fungi or yeast	15:	24.6%
Total patients cultured	61	

Table 2. Tissue Localization of *Candida* sp in Autopsy Specimens
ISR - 1971

Total Patients Positive	Sites Positive on Culture			
	Lung	Spleen	Liver	Burn Wound
3	+	0	0	0
1	+	+	+	0
5	+	0	0	+
1	+	+	+	+
1	0	+	0	0
1	0	+	+	+
2	0	0	+	+
10	0	0	0	+
24				

Genera	% of all Isolates
Fusarium	20.5
Cephalosporium	15.2
Penicillium	15.2
Nigrospora	11.2
Aspergillus	8.6

Mucor and Rhizopus spp have been incriminated in this Institute in most of the cases of invasive fungal burn wound sepsis. Thus, their relative scarcity in autopsy tissues is noteworthy. Since they occur but rarely in the burn not clinically diagnosed as having invasive Phycomycosis, there is a strong implication of selectivity in the occurrence of these forms in invasive infection.

Other changes of numerical significance between 1969 and 1971 were evident. In 1969 one strain of Fusarium was recovered; in 1971, 31 strains. Cephalosporium increased from 7 to 23 isolates; Nigrospora from 0 to 18, and Penicillium strains from 7 to 23 isolates.

The results of biopsy cultures for fungi resembled the species recovery which was obtained from autopsy samples. Table 3 summarizes these findings. A total of 197 samples were collected from 77 patients. The incidence of positive fungal and yeast cultures was only one-half of the recovery rate for autopsy tissues. Samples, of course, were confined to the burn wound.

Mucor and Rhizopus spp were recovered in low incidence from biopsy samples. These species do not invariably cause invasive sepsis, but their presence indicates that the possibility for Phycomycosis exists. Genera found in both autopsy and biopsy tissures also included Alternaria, Cephalosporium, Fusarium, Helminthosporium, Nigrospora, and Fonsecaea spp. The numbers were too small for a high level of significance, but the predominant genera were probably Nigrospora and Fusarium. As with autopsy specimens, the most frequently encountered genus was Candida. Fourteen, or 18% of patients biopsied,harbored Candida, usually albicans.

DISCUSSION

The search for fungi has reached a plateau of effectiveness of technic in the 1969-1971 period. There were 18 genera recovered in 1969, 17 in 1971. This is exclusive of Candida spp. The predominant genera in 1971 included Fusarium, Cephalosporium, Penicillium, Nigrospora and Aspergillus. Neither Mucor nor Rhizopus, both of which have been recovered from severe or fatal cases of

Table 3. Fungi Recovered From Biopsy of Burn Patients
ISR - 1971

Genus	No. Patients Positive	No.Strains Isolated
Mucor	1	1
Rhizopus	2	2
Aspergillus	2	2
Penicillium	1	1
Alternaria	3	3
Cephalosporium	1	1
Fusarium	4	8
Helminthosporium	1	2
Nigrospora	7	8
Fonsecaea	2	2
Trichoderma	1	1
Candida sp	14	17

No. patients cultured	77	
No. samples submitted	197	
No. patients positive for yeast or fungi		29 - 37.6%
Patients with one species	20	
Patients with 2 species	8	
Patients with 3 species	1	
No. patients negative for yeasts or fungi		48

Phycomycosis, were frequently seen. The predominant genera include such common saprophytes as Penicillium and Aspergillus. Dermatophytes were rare; one strain of Trichoderma was recovered.

There is little evidence that, up to now, indicates seeding of the burn wound by any dominant strain. The increased recovery of Fusarium spp could refute this observation if it continues, but this is not a new phenomenon. A major difference in the ward population was the smaller number of Vietnam casualties in contrast to the 1969 experience. Such a marked change in source of patients, and the drop-off of patients with immediate experience in a warm humid environment where dermatomycosis is a major medical problem, would have been expected to result in a marked change in fungus content of burn wounds. In fact, this did not occur. Except for the drop in incidence of Geotrichum and a rise in Fusarium, there was little change in the fungi found in burns. It appears that the seeding effect of fungi on burns is not highly specific for geographical area; on the basis of this set of observations, one would be justified in concluding that fungal seeding is relatively uniform in widely separated geographical areas.

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2. Lindberg RB, Townsend CH, Contreras AA, Pruitt BA,Jr: Role of fungi in burn wound infections. US Army Institute of Surgical Research Ann Prog Rpt FY 1970, BAMC, Ft Sam Houston, Texas, Section 26.

3. Nash G, Foley FD, Pruitt BA,Jr: Candida burn wound invasion. A cause of systemic Candidiasis. US Army Institute of Surgical Research Ann Prog Rpt FY 1970, BAMC, Ft Sam Houston, Texas, Section 25.

PRESENTATION

Lindberg RB: Burn Wound Flora and the Problem of Wound Disinfection. Annual mtg, Chemical Specialties Manufacturing Assoc, NY, Dec 6, 1971.

PUBLICATION

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
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	A. NEW	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

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a. PRIMARY	61102A	3A061102B71R	01	303
b. CONTRIBUTING				
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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

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	CURRENT 73	0.1	2.0

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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 will 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 per cent total body surface burn; Group II 30 to 60 per cent total body surface and Group III greater than 60 per cent 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) 71 09 - 72 06 Thirteen 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. At present the numbers are too small and the study too incomplete to draw any definite conclusions.

*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. 3A061102B71R-01, RESEARCH IN BIOMEDICAL SCIENCES

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

US ARMY INSTITUTE OF SURGICAL RESEARCH
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1 July 1971 - 30 June 1972

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Reports Control Symbol MEDDH-288(R1)

ABSTRACT

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

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

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

Period covered in this report: 1 July 1971 - 30 June 1972

Investigators: Wellford W. Inge, Jr., MD, Lieutenant Colonel, MC
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Reports Control Symbol MEDDH-288(R1)

Since topical chemotherapy has been shown to effectively control bacterial burn wound sepsis, fungal and yeast colonization or invasion of the burn wound might be prevented with topical antifungal agents such as nystatin. Nystatin mixed with Sulfamylon cream, 10,000 units per gram of Sulfamylon,has been applied to 13 patients who had biopsy proved fungal infection or invasion of the burn wound. Nine of the 13 patients expired, 8 of them having evidence at autopsy of fungal invasion of the burn wound. None of the patients were treated prophylactically but for established infection. No control groups have been studied. Numbers of patients admitted to the study are too small to draw any definite conclusion.

Fungus
Topical chemotherapy
Topical nystatin
Burn wound

PREVENTION OF FUNGAL AND YEAST COLONIZATION AND INFECTION OF THE BURN WOUND WITH TOPICAL NYSTATIN (MYCOSTATIN^{R})

In the 3 years from June 1967 through December 1970, 30 cases of invasive infection of the burn wound with fungi (Phycomycetes and Aspergillus sp), have been documented in patients treated at the US Army Institute of Surgical Research and a mortality rate of 50% recorded (Bruck, Nash, Foley, et al).[1] Forty-six per cent of survivors in this group required an amputation. In 1969, a prospective study of the incidence of colonization of the burn wound with fungi and yeasts demonstrated fungal colonization in 37% of all burn wounds studied. The peak incidence of fungal colonization occurred during the second and third weeks postburn. In no case were fungi seen in burn wound biopsies obtained during the first week postburn. When related to depth of burn, 16.7% of second degree burns and 60% of third degree burns were noted to be colonized by the end of the second week postburn. In this group, 3 massive fungal infections occurred, an incidence of 4.2%. While the mycologic flora included Phycomycetes, Aspergillus and Geotrichum sp, the predominant organism by far was Candida sp, which was present in almost every biopsy specimen.

Since topical chemotherapy effectively controls bacterial burn wound sepsis, it seems possible that a topical anti-fungal agent used prophylactically prior to fungal seeding of the burn wound could control or eliminate fungal colonization of the burn and perhaps prevent fungal invasion which often leads to loss of life or limb. Nystatin is a polyene anti-fungal agent which interferes with glycolysis within the cells of fungi by binding to a sterol in the cell membrane thus altering cellular permeability (Oster, Woodside).[2] The efficacy of topical nystatin for the treatment of vaginal, oral and cutaneous moniliasis is well established (Brown, Hazen).[3] Nystatin causes essentially no side effects when used topically, and no instances of allergy or contact dermatitis have been reported with this drug. Even when used intramuscularly in doses as high as 12 grams daily for periods as long as 6 months, no side reactions other than transient nausea and vomiting were noted (Newcomer, Wright, Sternberg, et al.).[4] Absorption studies in animals utilizing 2 to 3 times the maximum dose of nystatin proposed for use in human patients demonstrated no blood levels when nystatin was applied topically to surgical wounds or placed in the peritoneal cavity of rabbits (Lindberg).[5]

METHODS

Patients admitted to the study were placed in one of 3 groups according to burn size. Group I were patients with less than 30%

total body surface burn. Group II patients had from 30 to 60% total body surface burns and Group III patients had burns greater than 60% of the total body surface. Mycostatin Sulfamylon cream was prepared using the Mycostatin powder so that there were 10,000 units of Mycostatin per gram of Sulfamylon. This cream was applied twice daily or more frequently if necessary to keep the wound covered. All wounds were treated exposed. Each patient had a burn wound biopsy for histologic and bacteriologic examination prior to being placed in the study and then weekly thereafter. Weekly white counts, BUN, SGOT, and urinalysis were obtained, to monitor possible side effects of the Mycostatin.

RESULTS

At the present time only 13 patients have been admitted to the study. One patient in the less than 30% total body surface burn group, 8 in the 30-60% group and 4 in the greater than 60% group. All were treated because of evidence of fungal infection or invasion. No one received treatment as prophylaxis. Six of the 8 patients in the 30-60% total body surface burn group expired, 5 of them having evidence of fungus infection or invasion at post. Two of these patients received topical Mycostatin for only 2 and 4 days before expiring; the rest received it for at least a week. Three of the 4 patients in the greater than 60% group succumbed and all had evidence of fungal burn wound infection or invasion at post. These patients received topical Mycostatin therapy for 4, 8 and 10 days. No alteration in WBC, BUN or urine sediment was seen in any of the patients treated with topical Mycostatin Sulfamylon.

DISCUSSION

This study has too few patients at this time to draw any definite conclusions. It would seem that once invasive fungus infection has become established topical Mycostatin in the concentration used in this study does little to alter its course. Two of the patients diagnosed with invasive fungus shortly before death also received I.V. Amphotericin with no alteration in the final result. Both had invasive fungus at post.

A group of patients is to be studied in which the Mycostatin Sulfamylon cream will be applied prophylactically to determine whether fungal colonization and subsequent invasion can be prevented.

SUMMARY

Thirteen patients have been treated with topical Mycostatin Sulfamylon cream for mycotic infection or invasion of the burn

wound. One had a burn of less than 30% total body surface, 8 had 30-60% total body surface burns and 4 had burns of greater than 60% of the total body surface. Nine of the 13 died and 8 of these had invasive fungal infection at post. The combination Mycostatin Sulfamylon cream does not appear to be effective in the treatment of established fungal invasion. The prophylactic effectiveness of the cream is to be evaluated.

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5. Lindberg RB: Unpublished data.

PRESENTATIONS AND/OR PUBLICATIONS

None

32

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
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a. PRIMARY	61102A	3A061102B71R	01	118
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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

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19. RESPONSIBLE DOD ORGANIZATION
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22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Burns; (U) Fungi; (U) Rats; (U) Hyperglycemia

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

23. (U) The significance of fungi in the burn wound as well as successful modalities of treatment are to be elucidated. Experimental model of fungal burn wound infection is required to perfect therapy of fungal infection in burned soldiers.

24. (U) Rats burned and seeded with suspension of spores of Rhizopus so develop superficial burn wound infection. Rats made diabetic by treatment with alloxan develop deep burn wound infection when seeded with Rhizopus sp. Rats made hyperglycemic with alloxan are not a suitable animal model because of the high mortality associated with the use of the drug. Unipen, another hyperglycemic agent, is investigated for the purpose of producing burn wound infection with fungi.

25. (U) 72 01 72 06 A reproducible model has been developed and the proper dosage of Streptozotocin determined. Histology of infected animals reveals direct extension of the fungus from the burn wound into subjacent viable tissue.

* Available to contractors upon originator's approval.

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ANNUAL PROGRESS REPORT

PROJECT NO. 3A061102B71R-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
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ABSTRACT

PROJECT NO. 3A061102B71R-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 1971 - 30 June 1972

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6. Reports Control Symbol MEDDH-288(R1)

Sprague-Dawley rats were made hyperglycemic by the use of intravenous Streptozotocin. After hyperglycemia was verified by laboratory means, the animals were given a standard 20% total body surface scald burn at which time they were seeded with a suspension of mucor species containing 10^6 spores per ml. The animals were followed to death at which time histologic evaluation was carried out. Initial results revealed that deep burn wound invasion was produced in this animal model.

Further studies are to be carried out with various other fungi.

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.[1] Significance of fungi in burn wounds as well as the modalities of treatment still remain to be elucidated. Bruck et al[2] used the alloxan treated rat as a model to establish and evaluate fungal burn infection. Streptozotocin, derived from Streptomyces achromogens, possesses the distinct biological activity of producing hyperglycemia when given to various animals as well as humans. The only success so far in establishing invasive fungal infection in an animal has been when a hyperglycemic state exists in the animal.

Streptozotocin possesses antibiotic, antitumor and a hyperglycemia action. The development of frank hyperglycemia in rats and dogs treated with this drug, was first reported by Rakieten et al.[3] Streptozotocin has a highly effective beta-cytotoxicity similar to alloxan but more specific and with a wider margin of safety than alloxan. Sprague-Dawley rats were made hyperglycemic by the use of intravenous streptozotocin. The hyperglycemia was verified by determining blood sugar levels four days after the injection. A dose of 65 mg/kg produced an average blood sugar of 470 mg%. The urine was tested for acetone but none was found in any of the animals.

After the hyperglycemic state was produced, the animals were then given a standard 20% total body surface scald burn, at which time they were immediately seeded with a suspension of Rhizopus spores 10^6 per ml. Twenty-one animals were seeded with 10^6 Rhizopus spores per ml. Twenty animals died, a mortality rate of 95.2% with the average time of death 18 days postburn, ranging from the 14th to the 23rd day. All animals were autopsied. All animals exhibited deep invasive infection of the burn wound with involvement of the back musculature, and organs such as the liver and spleen. Hematogenous spread was not identified.

The progression of infection in these animals was followed by histologic examination of sections of the burn wound, liver, spleen, kidney and back muscles. Infecting organisms were recovered by culture in the animals.

Further studies will be carried out in order to elucidate the pathogenesis of the fungal burn wound invasion. Attempts will also be made to establish burn wound invasion by various other fungi.

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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 OD 6380	72 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	9. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
71 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	3A061102B71R	01	308
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Procede with Security Classification Code): (U) An Evaluation of the Use of Enzymatic Debridement of Burn Wound Eschar to Decrease Morbidity in Burned Troops (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
70 01	Cont	DA	C. In-House

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

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS.	b. FUNDS (In Thousands)
FISCAL YEAR	PRECEDING 72	0.5	16.5
	CURRENT 73	0.3	10.0

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21. GENERAL USE: FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Procede EACH with Security Classification Code): (U) Enzymatic Debridement; (U) Eschar; (U) Thermal Injury; (U) Rats

23. TECHNICAL OBJECTIVE, 24. APPROACH, 25. PROGRESS (Furnish individual paragraphs identified by number. Procede 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 third degree scald burn.

25. (U) 71 07 - 72 06 Initial evaluation of Bromelain, a proteolytic enzyme derived from the pineapple, has shown it unsuitable for use in its present form because of systemic toxicity and incomplete debridement. Laboratory and clinical trials with Sutilains the proteolytic enzyme of the bacteria B. subtilis, is continuing. Investigation is also under way in the laboratory exploring the possibilities of combining proteases with lipases and phospholipases in an effort to achieve more complete debridement of full thickness eschar. Sutilains, the most promising of the enzymatic agents tested in vitro, is being studied with a goal of creating a new dosage form compatible with a variety of currently employed topical antibacterial agents.

*Available to contractors upon originator's approval.

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

ANNUAL PROGRESS REPORT

PROJECT NO. 3A061102B71R-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

1 July 1971 - 30 June 1972

Investigators:

Paul Silverstein, MD, Major, MC
George M. Helmkamp, Jr., Captain, MSC
Robert A. Lincoln, Jr., SFC
Arthur D. Mason, Jr., MD

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A061102B71R-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 1971 - 30 June 1972

Investigators: Paul Silverstein, MD, Major, MC
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Reports Control Symbol MEDDH-288(R1)

A laboratory evaluation of 2 proteases was continued,using a spectrophotometric method for analysis of enzyme-mediated protein hydrolysis. A unique in vitro model permitted quantification of proteolysis of natural and thermally denatured skin preparations, including human burn eschar. Subsequent to last year's report of basic dose-response curves with various enzymes and substrates, this year's research included additive and sequential applications of proteases and of protease with elastase.

Enzymatic debridement
Eschar
Thermal injury

AN EVALUATION OF THE USE OF ENZYMATIC DEBRIDEMENT OF BURN WOUND ESCHAR TO DECREASE MORBIDITY IN BURNED TROOPS

Expeditious eschar removal with prompt closure of the burn wound results in decreased morbidity and earlier recovery of patients with thermal injuries. The eschar can be easily excised from patients with small burns, but surgical removal of massive amounts of tissue from extensive burns imposes severe physiologic stress and blood loss on an already traumatized patient. It is now customary to wait for such eschar to separate by natural processes before applying grafts. With topical antibacterial therapy, eschar may remain adherent for a much longer period of time than when dressings or simple exposure methods were used, making suitable methods for rapid removal of eschar that will permit immediate skin grafting desirable for optimum care of the burn patient. Enzymatic debridement of the burn wound represents one possible approach to this goal.

METHODS

The standard incubation media for both nonspecific proteolysis and collagenolysis of skin preparations was either 0.1 M potassium phosphate buffer at pH 7.0, or 0.03 M Tris-HCl, pH 7.4, containing 0.15 M NaCl. Substrates tested included:

Bovine serum albumin (5 mg/ml)
Microcrystalline collagen (5 mg/ml)
Split-thickness viable porcine skin (0.8 cm^2/ml)
Full-thickness human eschar (1.5 x 2.7 cm/30 ml)

Eschar samples were freshly excised and used immediately or stored at 4° C. in 1% Wescodyne solution. A special holder was designed for skin and eschar specimens that permitted incubation medium contact with only the epidermal surface by means of a window.

After the specimen was tightly secured in the holder, hydrolysis was initiated by submersion in 30 ml of incubation medium containing the enzyme(s) to be tested. Enzyme concentrations employed were 0.25 mg/ml for soluble substrates (pigskin gelatin and bovine serum albumin) and 1.5 mg/ml for microcrystalline collagen, pigskin, and eschar. The experiments were performed at 37° C. in a shaker bath. At timed intervals, aliquots of the incubation medium were withdrawn and analyzed for products of hydrolysis, as described below.

Peptide and Amino Acid Analysis. Nonspecific hydrolysis of substrate resulting in the release of peptides and amino acids into

the buffered medium was measured by the ninhydrin method. Samples (0.1 ml) were mixed with 1.0 ml ninhydrin reagent, heated at 100° C. for exactly 15 minutes, and then cooled to room temperature. Fifty per cent Ethanol (5 ml) was added and the absorbance of the mixture was measured at 570 nm in a Gilford Model 240 Spectrophotometer. Leucine was used as the reference standard.

Hydroxyproline Analysis. Hydrolysis of collagen was monitored by hydroxyproline release. Samples (1.0 ml) of the incubation medium were hydrolyzed by addition of 12N HCl (1.0 ml) and heating at 120° C. for 12-18 hours in a teflon-lined screw cap test tube. The medium was then neutralized with NaOH (1 N) and adjusted to a final volume of 10 ml with water; 1.0 ml of this solution was assayed for hydroxyproline, with results determined spectrophotometrically at 550 nm. Hydroxyproline standards were carried through the entire procedure.

RESULTS

A comparison of protein hydrolysis by sutilains, bromelain, and clostridial collagenase was reported in detail in last year's Annual Report (Section 36). Sutilains and bromelain were approximately equally effective in hydrolyzing collagen, although the former showed slightly greater nonspecific proteolytic activity. Collagenase, on the other hand, was approximately 6 times more effective than either sutilains or bromelain in digesting collagen, though the nonspecific proteolytic activity of collagenase was only one-half that of the other enzymes.

These results suggested that combination or sequential treatment with a nonspecific protease, such as sutilains, and a substrate-specific protease, such as collagenase, might enhance protein breakdown and produce more rapid debridement. The result of simple combination of sutilains and collagenase on the digestion of skin protein is shown in Table I. The ratio of the two enzymes was varied in an attempt to determine the most effective combination. In no instance, however, was enhancement of nonspecific proteolysis observed. The mean rate of hydrolysis by the combination of enzymes was only slightly greater than the rates of the individual enzymes measured separately and added together.

The enzymes were then applied sequentially to human eschar with contact only at the outer skin surface. When eschar was incubated with sutilains or collagenase for 3 hours, washed briefly in saline, and reincubated with a second aliquot of the same enzyme for an additional 3 hours, the rate of nonspecific protein hydrolysis decreased during the second period (Fig. 1), suggesting that available substrate became a limiting factor. (It should be

TABLE I

EFFECT OF COMBINATION OF ENZYMES ON PROTEASE ACTIVITY DURING ENZYMATIC TREATMENT OF SPLIT-THICKNESS PIGSKIN

PROTEASE ACTIVITY (nmoles leucine/ml/min)			
SUTILAINS	COLLAGENASE	SUTILAINS + COLLAGENASE	CALCULATED ACTIVITY
A	B	C	A + B
5 mg	10 mg	5 + 10 mg	
29	6	39	35
7.5 mg	7.5 mg	7.5 + 7.5 mg	
34	5	44	39
10 mg	5 mg	10 + 5 mg	
50	3	56	53

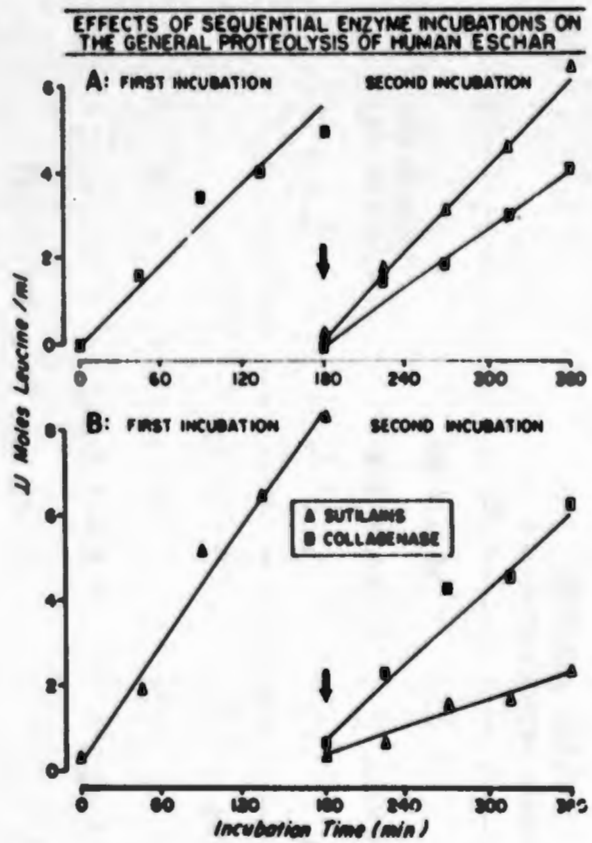

Figure 1. Effects of sequential protease incubation on the general proteolysis of human eschar. Specimens of human eschar were incubated in 0.03 M Tris-HCl, pH 7.4, containing 0.15 M NaCl and the following enzymes: Collagenase, 3 mg/ml; Sutilains, 6000 units/ml. After 3 hours (↓), the eschar substrates were washed briefly in 0.15 M NaCl and reincubated with fresh enzyme. Each point has been corrected for ninhydrin values of eschar incubated without enzymes (buffer blank) and enzyme incubated without eschar (autolysis). In the 0-3 hour incubation period, the points represent the average of 3 simultaneous eschar digestions.

noted that at lower enzyme concentrations, the proteolysis rate remained constant during both incubations.) When the alternate enzyme was employed for the second incubation, the release of leucine equivalents more closely approached the kinetics of the initial treatment period. For example, when eschar was treated first with sutilains and then with either sutilains or collagenase (Fig. 1B), the rate of protein hydrolysis by collagenase during the second period was nearly 2 times that by sutilains and was comparable to that associated with an initial collagenase treatment.

The effects of sequential enzymatic hydrolysis of human eschar are also reflected by the quantity of hydroxyproline released into the medium (Fig. 2). Skin incubated twice with sutilains or collagenase showed no appreciable change in the rate of collagen digestion between the first and second incubation periods. Skin exposed initially to sutilains and subsequently to collagenase (Fig. 2B) exhibited a marked increase in the release of hydroxyproline during the second incubation period. However, when treatment with sutilains followed treatment with collagenase (Fig. 2A), no significant difference between the results of the two incubations was observed.

The rates of product formation associated with sequential hydrolysis treatments, calculated from the data in Figures 1 and 2, are listed in Table II. Included in these values is the hydrolytic rate measured during second incubation intervals with buffer alone. (This rate had already been considered in the curves of Figures 1 and 2.) It is noteworthy that despite the removal of collagenase from the incubating medium at the termination of the first period, collagenolytic activity was also measured throughout the second 3-hour period, suggesting that this enzyme adhered rather tenaciously to the eschar, perhaps imbedded or attached to receptor sites on the collagen fibers.

Though eschar is composed of predominantly denatured collagen, other proteins, such as elastin, are present in the tissue and may contribute to delayed eschar separation. Histologically, the persistence of elastic tissue in the burn wound eschar has been noted (Vistness LM, Hogg GR: Plast Reconstr Surg 48:56-60, 1971). Elastin fibers are seen to traverse the zone between the eschar and underlying viable tissue, and, as a result, retard separation of the necrotic component. It was therefore of interest to examine the efficacy of elastase as a means of more rapid debridement.

Using human eschar as substrate and elastase (25 units/mg) at a concentration of 1.5 mg/ml, proteolysis was followed by the release of ninhydrin-sensitive peptides and amino acids. Due to the more alkaline conditions required for optimum elastolytic activity, the

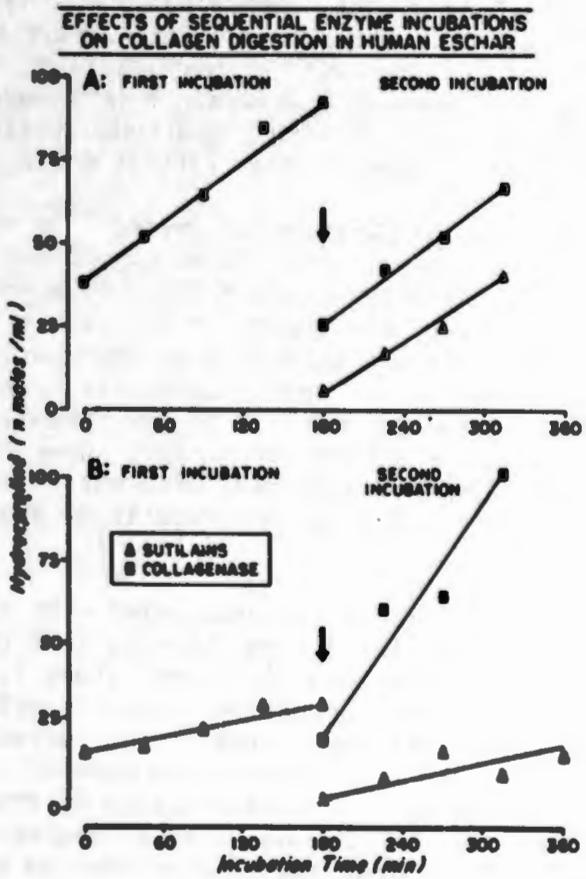

Figure 2. Effects of sequential protease treatment on collagen breakdown in human eschar. The conditions, enzymes, and eschar specimens were identical to those described in Figure 1. Each point has been corrected only for ninhydrin values of eschar incubated without enzyme buffer blank.

TABLE II

KINETICS OF PROTEIN HYDROLYSIS DURING SEQUENTIAL ENZYMATIC TREATMENT OF HUMAN ESCHAR

ENZYME		LEUCINE EQUIVALENTS	HYDROXYPROLINE
1st Treatment (0-3 hr)	2nd Treatment (3-6 hr)	(μmole/ml/hr)	(nmole/ml/hr)
Collagenase		1.95	18.7
	Buffer	0.75	9.2
	Sutilains	2.75	23.9
	Collagenase	2.12	28.2
Sutilains		2.74	5.5
	Buffer	0	0
	Collagenase	2.27	35.4
	Sutilains	1.17	5.2

pH of the standard Tris-HCl buffer was increased to 8.6. Elastase was also tested in combination with sutilains (5333 units/ml). The results of these experiments, run in duplicate,are given in Fig. 3 and Table III.

TABLE III

Protease Activity (nmoles leucine hr/ml)

Elastase	Sutilains	Elastase + Sutilains
0.77	2.57	3.10

At the enzyme level used, elastase was only 30% as effective as sutilains in digesting eschar protein. Unfortunately, there is no method for measuring specific elastin hydrolysis. Furthermore, the combination of elastase and sutilains resulted in no initial enhancement of proteolysis, although together these 2 enzymes allowed a more sustained digestion rate in that the activity remained linear for the duration of the 3-hour incubation. Perhaps the increase in pH, from 7.4 to 8.6, could account for the unusual curvature of the sutilains digestion rate.

CONCLUSIONS

Pretreatment of eschar with sutilains enhanced the subsequent interaction with collagenase. This observed two-fold increase in collagenolytic activity may have been related to the fact that collagen is found in the deeper dermal layers of skin, and pretreatment with a nonspecific protease facilitated the subsequent contact of a collagen-specific enzyme with its substrate. It was of interest, however, that overall collagenolysis by the sutilains → collagenase sequence was no better than the collagenase → collagenase sequence. Yet when overall nonspecific proteolysis was considered during a 6-hour period, the sequential treatment by sutilains → collagenase resulted in 50% more protein digestion than the collagenase → collagenase regimen.

Although sutilains also manifested collagenolytic activity, it did not preferentially degrade exposed collagen. Thus, 2 sequential

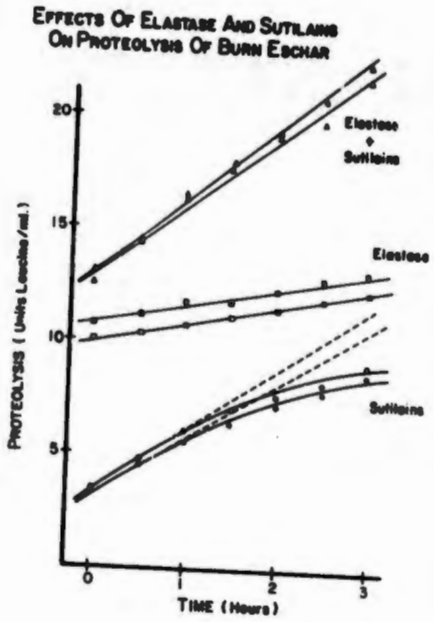

Figure 3. Effects of elastase and sutilains on proteolysis of burn eschar.

treatments of eschar with sutilains did not result in increased hydroxyproline release during the second incubation.

When elastase was combined with sutilains, the resulting rate of proteolysis was not enhanced, indicating that little therapeutic value might be expected. However, the release of ninhydrin-positive products of digestion was sustained in linear fashion for a longer period than when sutilains was used alone.

PRESENTATION

Silverstein P: Enzymatic Debridement of Burn Wound Eschar. Amer Burn Assoc, April 1972, San Francisco, Calif.

PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OD 6967	72 07 01	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. ESS'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
71 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	3A061102B71R	01	184
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE: (U) Evaluation and Preparation of Formalin Fixed Cutaneous Grafts as a Temporary Wound Cover for Burned Soldiers (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
70 01	72 06	DA	C. In-House

17. CONTRACT/GRANT: Not Applicable
a. DATES/EFFECTIVE: EXPIRATION:
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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 72	0.4	12.4
FISCAL YEAR CURRENT 73	0	0

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

22. KEYWORDS (Provide EACH with Security Classification Code)
(U) Formalin; (U) Cutaneous Grafts; (U) Wound Cover

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

23. (U) The advantages of fresh allograft on granulating wounds have been well demonstrated. However, inadequate supply of cadaver donors has stimulated a search for allograft substitutes. Formalinized skin is an inexpensive, simple product to produce and has demonstrated previously its ability to function as an allograft substitute.

24. (U) Fresh allograft and porcine xenograft will be fixed with formalin and studied as to optimum time of fixation and adequacy of formalin washout by quantitative determinations of formaldehyde content in skin and wash water. Fixed skin will be stored in 0.5% formalin until ready for use. It will be evaluated in an animal model with a projected clinical trial, if successful.

25. (U) 71 07 - 72 06 Clinical trials of formalin fixed split thickness graft have been started based on the success of previously reported evaluation in an animal model. Twelve patients have received this preparation on granulating wounds. While good adherence has been achieved in some cases, problems due to stiffness of the grafts and poor initial adherence have been encountered. Methods are currently being sought to soften the skin by pre-soaking in various agents, but with little success to date.

*Available to contractors upon originator's approval.

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FINAL REPORT

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

REPORT TITLE: EVALUATION AND PREPARATION OF FORMALIN-FIXED CUTANEOUS GRAFTS AS A TEMPORARY WOUND COVER FOR BURNED SOLDIERS

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

1 July 1971 - 30 June 1972

Investigators:

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Harrel L. Walker, MS
James Hines, MD
William F. McManus, MD, Major, MC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

REPORT TITLE: EVALUATION AND PREPARATION OF FORMALIN-FIXED CUTANEOUS GRAFTS AS A TEMPORARY WOUND COVER 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 1971 - 30 June 1972

Investigators: Paul Silverstein, MD, Major, MC
Gilbert L. Raulston, Colonel, VC
Harrel L. Walker, MS
James Hines, MD
William F. McManus, MD, Major, MC

Reports Control Symbol MEDDH-288(R1)

Formalin-fixed split-thickness heterograft or homograft has been found to provide adequate wound coverage for up to 30 days when applied to 30% total body surface area wounds on the backs of white male rats.

Serial biopsies of 50 grafted wounds demonstrate excellent adherence of the formalinized graft to the wound bed with moderate granulation in-growth and proliferation of fibroblasts, minimal inflammatory reaction, and absence of suppuration. When the graft is stripped from the wound, the bed readily accepts autograft.

Repeated grafting with formalinized skin does not produce sensitization or accelerated rejection. Formalin-fixed skin stored for more than 5 years had no bacterial growth on culture. No external dressing is required, and motion of the grafted part is not restricted. A limited clinical evaluation of formalinized skin as a substitute for fresh homograft has been conducted in 15 patients.

Formalin
Cutaneous grafts
Wound cover

EVALUATION AND PREPARATION OF FORMALIN-FIXED CUTANEOUS GRAFTS AS A TEMPORARY WOUND COVER FOR BURNED SOLDIERS

The attributes of viable cutaneous allografts used as physiologic dressings include decrease in wound pain, evaporative water and heat loss, stimulation and protection of granulating tissue, and suppression of bacterial flora.[1] Unfortunately, the limited viability and difficulty of matching supply and demand restrict the use of such allografts. Previous laboratory investigation[2] has indicated that the simple, inexpensive maneuver of formalinization decreased the antigenicity and increased the shelf life of cutaneous allograft or xenograft. The current study documents the effectiveness of such materials as temporary wound coverings and discloses the histologic basis for their nonreactivity.

METHODS

Skin grafts, 0.010 - 0.020 inch thick, harvested aseptically from recently deceased human donors or anesthetized laboratory animals, were spread on fine mesh gauze, immersed for 16 hours in 10% buffered formalin, washed in distilled water for 24 hours, and then stored at room temperature in sealed containers filled with 0.5% formalin. Prior to use, the skin is washed in physiologic saline for 30 minutes to reduce extractable formalin content to 0.016% as determined by the method of Tanenbaum and Bricker.[3] Specimens produced by this method and stored for over 5 years have been proved sterile by cultures for bacteria and fungi.

Grafts were evaluated as temporary physiologic dressings on freshly excised 30% total body surface wounds on the backs of 200 g white, male, Sprague-Dawley rats from which skin and panniculum carnosus had been removed. Grafts were subjectively scored daily for wound adherence. The end point was that day after application when 50% or more of the graft was no longer adherent. Comparisons were made between fresh skin and grafts fixed in formaldehyde, paraformaldehyde, and gluteraldehyde. The latter two agents were subsequently discarded because they rendered the grafts too inflexible.

RESULTS

Wound coverage by formalinized skin when compared in the same animal model to fresh human and porcine xenograft and rat allograft (Long-Evans, Sprague-Dawley) revealed that satisfactory wound coverage was provided for 9.3 $\pm$ 0.6 days by fresh rat allograft, 8.2 $\pm$ 0.7 days by fresh porcine and human xenograft, and 30.0 $\pm$ 5 days by formalinized xenograft. All wounds readily accepted autograft from the rat's abdomen after removal of the physiologic temporary grafts,

indicating that the test materials had adequately protected the granulating wound bed from desiccation and infection.

The histopathology of the graft-host wound interface was studied by injection of 5 cc of India ink into the rat tail vein 3 minutes prior to sacrifice on day 3, 6, 9, 12 and 20 postapplication of the test graft. Autografted animals demonstrated 100% graft take with free circulation of carbon particles in both granulating wound bed and graft on all days biopsied. Viable homograft demonstrated initial graft take with free circulation of ink particles on day 3, but by day 9, carbon particles were no longer evident in the graft and a classic rejection sequence had begun, with thrombosis of graft vasculature and mononuclear round cell infiltration. Neither fresh porcine or human xenograft nor any of the formalinized grafts ever exhibited uptake of ink particles, despite excellent adherence to the wound and invasion of host fibroblasts with minimal polymorphonuclear or monocytic infiltration into the lower dermal collagen fibril networks of the grafts.

Second and third set application of viable xenograft or formalinized graft failed to manifest accelerated rejection customarily seen with immunologically active tissue. Absence of clinical immune response and lack of revascularization in the formalinized grafts resulted in their longer survival as effective wound covers and suggest that their physical properties are of critical importance in their function as "physiologic" dressings.

Encouraged by the laboratory evaluation, a clinical trial was undertaken, and 15 patients received formalinized human or porcine skin to portions of their granulating burn wounds during the period between eschar separation and autografting. Each patient received between 1 and 4 applications of formalin-fixed skin. Skin was changed only because of poor adherence, subgraft suppuration, or preparation for autografting. When applied to second degree burns, the formalinized skin could be left in place until re-epithelialization occurred beneath it, provided good initial adherence was achieved.

At the time of graft removal, wounds were graded on a 1 - 4 scale:

Grade I. No graft adherence with subgraft suppuration.
Grade II. No graft adherence without subgraft suppuration.
Grade III. Good adherence without granulation bleeding.
Grade IV. Good adherence with granulation bleeding.

By the time Grade IV reading was recorded, the wounds were ready for autografting. Formalinized skin was left in place on debrided wounds for 1 - 8 days and changed only for the criteria mentioned

above. Wound biopsies at the time of autografting noted good graft adherence with minimal inflammatory reaction, moderate ingrowth of granulation tissue into the lower dermal elements of the graft, and no foreign body or mononuclear immune reaction elicited by the graft.

The major problems experienced with the clinical use of formalinized allo- and xenografts were (1) poor initial adherence, and (2) stiffness of the graft due to formalin fixation. Initial adherence could be improved by wrapping the grafts for 24 hours in saline-soaked dressings, after which time they were usually softer and better hydrated. Residual stiffness remains as an insurmountable problem and has been responsible for discontinuation of the project.

A search for other fixatives with the same antibacterial properties of formalin has proved unsuccessful. Presoftening of the graft by soaking in urea solution has not alleviated the problem. Since formalinized skin does not handle as well clinically as fresh cadaver allograft or fresh and frozen porcine xenograft, the project has been terminated.

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1. Artz CP, Becker JM, Sako Y, et al: Postmortem skin homografts in the treatment of extensive burns. Arch Surg 71:682-687, 1955.

2. Raulston GL, Teplitz C, Walker HL, et al: De-antigenization of skin of heterogenous origin. US Army Surg Res Unit Ann Prog Rpt, 30 June 1964, BAMC, Ft Sam Houston, Texas, Section 55.

3. Tanenbaum M, Bricker CE: Microdetermination of free formaldehyde. Anal Chem 23:354-357, 1951.

PUBLICATION

Silverstein P, et al: Evaluation of formalin-fixed skin as a temporary dressing for granulating wounds. Surg Forum 22:60-62, 1971.

PRESENTATION

Silverstein P: Evaluation of Formalin-Fixed Skin as a Temporary Dressing for Granulating Wounds. Amer Coll Surgeons Surg Forum, Atlantic City, NJ, October 1971.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0E 6390	72 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. SRDR'N HISTORY	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
	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	3A061102B71R	01	318
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) A Study of Immune Response to Split-Thickness Cutaneous Xenograft to Improve Care of Burn Wounds 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 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 72	0.3	10.4
FISCAL YEAR CURRENT 73	0.3	10.0

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

22. KEYWORDS (Precede each with Security Classification Code): (U) Immune; (U) Cutaneous; (U) Xenograft; (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) The purpose of this study is to determine the antigenic potential of split thickness cutaneous xenograft used as biologic dressings in the treatment of burn wounds. Preliminary investigation demonstrated an inability to produce accelerated rejection of these grafts by sequential application to excised wounds on Sprague Dawley rats. High priortty military need exists for burn wound covering.

24. (U) Fischer rats of known immune background will receive 400 rads 48 hours prior to being grafted with viable split thickness porcine xenograft. Histologic and clinical evaluation will attempt to determine if there is any immune response to this xenograft.

25. (U) 71 10 - 72 06 Results have been clinically difficult to evaluate because of the wide range of "adequate wound coverage" measurements and screening individual responses to radiation. Histologic conclusions will be presented in the text.

*Available to contractors upon originator's approval.

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

FINAL REPORT

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

REPORT TITLE: A STUDY OF IMMUNE RESPONSE TO SPLIT-THICKNESS CUTANEOUS XENOGRAFT TO IMPROVE CARE OF BURN WOUNDS IN MILITARY PERSONNEL

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

1 July 1971 - 30 June 1972

Investigators:

Paul Silverstein, MD, Major, MC
Glenn D. Warden, MD, Major, MC
William F. McManus, MD, Major, MC
Harrel L. Walker, MS

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

REPORT TITLE: A STUDY OF IMMUNE RESPONSE TO SPLIT-THICKNESS CUTANEOUS XENOGRAFT TO IMPROVE CARE OF BURN WOUNDS 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 1971 - 30 June 1972

Investigators: Paul Silverstein, MD, Major, MC
Glenn D. Warden, MD, Major, MC
William F. McManus, MD, Major, MC
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Reports Control Symbol MEDDH-288(R1)

Recent investigations of porcine and canine cutaneous xenograft have demonstrated the efficacy of these products as biologic wound covers, but have failed to elucidate the nature of the interaction that occurs between graft and host. Classical accelerated second set rejection of viable porcine split-thickness cutaneous xenograft cannot be produced in the 20% total body surface excised rat model. Indeed, in most experiments, second set grafts applied to either the excised recipient bed of the first set graft or to a new site persisted longer than did the first-set graft. Moreover, vascularization of cutaneous xenograft in the same rat model cannot be demonstrated by injection of carbon particle suspension intravenously into a rat tail vein, or by the in vivo stereomicroscopic technique of Taylor and Lehrfeld. Lastly, delayed hypersensitivity does not develop in either animals or human patients after repeated applications of xenograft.

These phenomena may be due to disruption of either the afferent or efferent limbs of the immune arc, or the grafts may be immunologically inactive because of their failure to vascularize. The purpose of this study was to further study the graft-host relationship of split-thickness cutaneous xenograft systems.

Immune
Cutaneous
Xenografts

A STUDY OF IMMUNE RESPONSE TO SPLIT-THICKNESS CUTANEOUS XENOGRAFT TO IMPROVE CARE OF BURN WOUNDS IN MILITARY PERSONNEL

The inability to demonstrate an immune response to split-thickness porcine cutaneous xenograft has been described in previous reports. Absence of accelerated second-set graft rejection and delayed cutaneous hypersensitivity raise the question of whether cutaneous xenografts may be inactive immunologically because of failure of either antigenic transfer, antibody synthesis, or antigen-antibody interaction.

Considering the disparity between porcine and human species, it is difficult to conceive of total immune tolerance between such divergent tissues. Nevertheless, clinical observations imply that xenograft behaves as an inert dressing with no significant immunologic relationship to its host.

Another possible explanation of the clinical inertness of cutaneus xenograft may involve a massive immunologic attack immediately upon graft application and prior to vascularization, based on pre-existing anti-species antibodies. In an effort to demonstrate this type of reaction, an irradiated rat model was used to block immune response and determine whether or not vascularization would then occur.

METHOD

Fisher rats were subjected to 400-450 rads total body irradiation from a cobalt source. Forty-eight hours after irradiation, baseline white blood counts were obtained to document radiation effect. Rats with WBC < 2000 cells/mm^3 received 2x3 cm split-thickness xenograft and 2x2 cm split-thickness autografts applied to excised dorsal wounds. Grafts were biopsied after injection of 5 cc of carbon particle suspension intravenously and examined histologically for evidence of graft vascularization and cellular immune response. Clinical day of graft rejection was also measured by the criterion of 50% epithelial slough.

RESULTS

While the WBC was consistently reduced from $\sim$ 20,000 to < 2000 cells/mm^3 by 450 rads of gamma radiation, the clinical results demonstrated no consistent differences in duration of adequate coverage between the control and xenograft test animals in 3 experiments, each employing 10 test and 5 controls. Average duration of xenograft on both irradiated and nonirradiated controls was 10-15 days.

A more predictable system was then tested, using true rat allograft (Sprague-Dawley donor $\rightarrow$ Fisher recipient). In the allograft experiments, graft survival was > 30 days in most of the test animals

as compared to 8 days in the nonirradiated controls. Whereas the allografted animals developed vascularization in their grafts prior to rejection, the xenografted animals demonstrated no carbon particles in the blood vessels of their grafts, as had been noted in earlier reports (Annual Report 1971). All autograft controls took successfully, vascularized, and were not sloughed or rejected.

DISCUSSION

An important consideration in the efficacy of porcine cutaneous xenograft as a biologic wound dressing is the demonstrated absence of vascularization after application to open wounds. The possibility exists that failure of vascularization is due to massive immunologic attack by "natural" humoral antibodies already present in the graft recipient. This experiment was designed to destroy the "natural" antispecies antibodies that may exist, and to paralyze the immune response of the recipient by total body irradiation to see whether or not graft vascularization would then occur.

From the results of these experiments, it is obvious that the above hypothesis was not supported, since graft prolongation and vascularization were not observed in the xenograft system. The validity of utilizing 450 rads of gamma radiation to paralyze the immune response of the 200 gm Fisher rat was confirmed in the allografted animals.

CONCLUSIONS

This project did not provide evidence supporting the hypothesis that failure of vascularization of split-thickness porcine cutaneous xenograft was due to massive immune reaction occurring at the time of graft application and mediated by naturally occurring antispecies antibodies. However, the possibility still exists that humoral antibodies may play a role in rejection of such skin grafts.

PUBLICATIONS AND/OR PRESENTATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0E 6386	72 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
	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	3A061102B71R	01	119
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) Effect of Prior Trauma on Donor Site and Recipient Site Healing - A Study to Develop Improved Technics of Care 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 04	Cont	DA	C. In-House

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

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 72	0.3	5.4
	CURRENT 73	0.2	7.0

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

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Prior Trauma; (U) Donor Site; (U) Recipient Site Healing; (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) To discover if stimulation of a prospective donor site by controlled trauma will result in acceleration of healing in burned military personnel.

24. (U) Three days prior to surgery, symmetrical donor and recipient sites (3" x 2") on the backs of adult dogs will be outlined with Methylene Blue. One donor site will serve as control, the other infiltrated with 1% Xylocaine, and 25 punctures per square inch into the subcutaneous tissue will be made with an 18 guage needle. The procedure will be repeated 48 hours before surgery. At surgery, split thickness grafts 10/1000 inch thick will be taken from the back and placed on a prepared defect on the back. Donor sites will be covered with fine mesh gauze. Biopsies will be taken four days later and evaluated for epithelialization and maturity of connective tissue. When the gauze separates, gross visual evaluation of the sites will be made for color and pliability. Biopsies of graft and recipient site and adherence studies will be done to evaluate the effect of prior trauma on graft take.

25. (U) 72 04 - The study has recently begun and insufficient data are available for analysis at present.

*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. 3A061102B71R-01, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: EFFECT OF PRIOR TRAUMA ON DONOR SITE AND RECIPIENT SITE HEALING - A STUDY TO DEVELOP IMPROVED TECHNICS OF CARE OF BURNED SOLDIERS

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
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1 July 1971 - 30 June 1972

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Gary W. Welch, MD, Major, MC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

REPORT TITLE: EFFECT OF PRIOR TRAUMA ON DONOR SITE AND RECIPIENT SITE HEALING - A STUDY TO DEVELOP IMPROVED TECHNICS OF CARE 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 1971 - 30 June 1972

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Reports Control Symbol MEDDH-288(R1)

The split thickness graft donor site is one type of clean surgical wound. Normal wound healing usually occurs within 10 to 14 days. Accelerated healing to shorten recropping time would be desirable in a patient with extensive burns and limited donor sites. The purpose of this study was to evaluate the effect of prior trauma on the healing of a donor site. Carefully controlled studies reveal that maximum acceleration of healing (as measured by wound tensile strength) occurs on day 3 but is present as long as 40 days after the initial wound. The acceleration is thought to be due to local wound environment rather than a systemic agent.

In 3 adult dogs, symmetrical donor sites were outlined on the lower back with methylene blue dye. One-half of the donor sites were traumatized with 25 needle punctures per square inch 72 and 48 hours prior to surgery. At surgery split thickness grafts of 10/1000th inch thick were removed and placed on recipient sites on the upper back. Four days later biopsies were taken of the donor sites and healing was clinically evaluated.

There was no evidence, grossly or microscopically, that prior trauma to prospective donor site either enhanced or hindered wound healing.

Donor site
Healing
Trauma

EFFECT OF PRIOR TRAUMA ON DONOR SITE AND RECIPIENT SITE HEALING - A STUDY TO DEVELOP IMPROVED TECHNICS OF CARE OF BURNED SOLDIERS

Investigators of wound healing have tested innumerable methods and substances to accelerate healing with questionable results. Likewise, the effect of previous trauma on the healing of a second wound is controversial. A clear example of accelerated wound healing has been demonstrated in the destruction and resuturing of a sharply excised surgical wound. Carefully controlled studies revealed that maximum acceleration of all subsequent healing (as measured by wound tensile strength) occurs if the primary wound is disrupted on day 3, but is present as long as 40 days after the initial wound. The acceleration is thought to be due to local wound environment rather than a systemic agent.

PURPOSE

The purpose of this study was to determine if previous traumatic stimulation to a prospective donor site would result in acceleration of healing of the donor site. Accelerated healing of the limited donor sites of patients with extensive burns would be of clinical importance since repeat harvesting of grafts from those sites could be carried out at more frequent intervals.

METHOD

Three adult dogs were selected and 72 and 48 hours prior to surgery symmetrical donor and recipient sites on the back, 3" x 2", were outlined with methylene blue. One donor site served as control and the other was punctured 25 times per square inch into the subcutaneous tissues with an 18-gauge needle. Pressure was applied for 3 minutes to prevent bleeding. At surgery, split-thickness grafts, 10/1000th inch thick, were taken from the donor site and clipped on the recipient sites on the back. The donor sites were then covered with fine mesh gauze. Four days later all donor sites were evaluated grossly for healing and biopsies taken.

RESULTS

There were no differences in wound healing among the donor sites by visual inspection or microscopic evaluation. Grossly the fine mesh gauze had separated from all donor sites and all showed signs of early healing. Microscopically all donor sites exhibited early epithelialization 6 to 10 cell layers thick with underlying immature granulation tissue.

CONCLUSIONS

In this small series sharp trauma to a prospective donor site neither accelerated nor hindered wound healing. In future studies other physical and chemical stimuli to prospective donor sites will be tested in an attempt to accelerate healing.

REFERENCES

1. Prudden JF, et al: The acceleration of healing. Surg Gynec Obstet 128:1321, 1969.

2. Savlov ED, Dunphy JE: The healing of the disrupted and resutured wound. Surgery 36:363, 1954.

3. Sandblom PH, Muren A: Differences between the rate of healing wounds inflicted within a short time interval. Ann Surg 140:449, 1954.

PUBLICATIONS AND/OR PRESENTATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0D 6969	72 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-FOR CONTRACTOR ACCESS	9. LEVEL OF SUM
71 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	3A061102B71R	01	187
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Procede with Security Classification Code): (U) Hypertrophic Scarring - Etiology and Control of A Disabling Complication 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
70 07	72 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 72	0.4	10.4
	CURRENT 73	0	0

19. RESPONSIBLE DOD ORGANIZATION
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NAME: Timothy R Stone, CPT, VC DA

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Procede EACH with Security Classification Code)
(U) Hypertrophic Scar; (U) Steroids; (U) Thermal Injury

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

23. (U) To create an experimental model of hypertrophic scarring in a laboratory animal in which new modes of therapy can be evaluated; to evaluate current modes of therapy in a clinical burn population.

24. (U) Attempts to induce hypertrophic scarring in an animal model by creating deep second degree burn wounds or partial-thickness skin loss with chronic healing have been applied to rats, dogs and pigs. Current clinical therapy includes evaluation of intradermal injection of steroids, and chronic compression devices applied to hypertrophic burn scars.

25. (U) 71 07 - 72 06 Attempts at creating a hypertrophic scar have resulted in a successful prototype in the New Jersey red Duroc pig. Clinical evaluation is proceeding with good results from the use of compression devices and fair to poor results with the steroid applications. Evaluation of 40 patients treated with custom made elastic garments (26) and Dermajet injection of steroids (14) has been completed. Use of steroids has been restricted to small scars in selected cases. Elastic compression garments are currently prescribed for all major second and third degree burn patients on a prophylactic or therapeutic basis as soon as skin healing is complete. A six month - one year therapy period is advised.

*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.

FINAL REPORT

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

REPORT TITLE: HYPERTROPHIC SCARRING--ETIOLOGY AND CONTROL OF A DISABLING COMPLICATION IN BURNED SOLDIERS

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

1 July 1971 - 30 June 1972

Investigators:

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Malcolm N. Goodwin, Jr., Major, MC
Gilbert L. Raulston, Colonel, VC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

REPORT TITLE: HYPERTROPHIC SCARRING--ETIOLOGY AND CONTROL OF A DISABLING COMPLICATION 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 1971 - 30 June 1972

Investigators: Paul Silverstein, MD, Major, MC
Malcolm N. Goodwin, Jr., Major, MC
Gilbert L. Raulston, Colonel, VC

Reports Control Symbol MEDDH-288(R1)

This project was designed to study hypertrophic scarring by developing an animal model in which scarring could be examined and by analyzing human scar hypertrophy histologically and therapeutically.

Results of animal experimentation have been encouraging. Histologically confirmed hypertrophic scar has been created in 7 Duroc pigs. Attempts are now being made to reproduce this model in other species.

Therapeutic trials of Dermajet infusion of triamcinolone and hyaluronidase were carried out, with limited success. Topical steroid application using Cordran tape was also evaluated. The most promising therapy to date has been application of Jobst compression garments for six months after completion of grafting. Forty patients have been studied in this project.

Hypertrophic scar
Steroids
Thermal injury

HYPERTROPHIC SCARRING--ETIOLOGY AND CONTROL OF A DISABLING COMPLICATION IN BURNED SOLDIERS

Since the word keloid was introduced by Alibert in 1806, the surgical literature has been replete with descriptions, hypotheses, and treatments reflecting the frustration of medical practitioners who attempted to deal with this enigma of wound healing. The diverse modalities of proposed attack with oral, topical, intralesional and systemic drugs, radiation and pressure devices, emphasize the inadequacy of current management. Many of the testimonials to each form of therapy are poorly controlled retrospective analyses unsuitable for statistical evaluation. Despite elucidation of the structural and biosynthetic steps of collagen synthesis, little is known of the metabolic derangements in wound healing leading to scar hypertrophy.

The influence of age, race, sex, heredity, and regional susceptibility of different body areas have been described, but their mechanisms of affecting wound healing are still undisclosed. Investigators now have the knowledge and tools necessary to dissect abnormal scar formation to its molecular source. Only one fundamental prerequisite is lacking--a convenient and reproducible animal model.

Hypertrophic scar has been described in horses, cattle, dogs, and the feet of vultures and eagles. However, none has withstood the test of reproducibility,histologic scrutiny, and natural history. The knob-like lesion noted on the traumatized legs of several horses has not proved to be hypertrophic scar, but a massive proliferation of granulation tissue with thick-walled blood vessels enclosed in an epidermal envelope.

Recent research in our laboratories with biologic dressings of split-thickness cutaneous porcine xenograft has led unexpectedly to the appearance of densely fibrotic hypertrophic dermal scar in donor swine. Easy duplication of the process and histologic evaluation of the resulting tissue suggest that this porcine scar may provide an in vivo model for further investigation of the dynamics of collagen metabolism and abnormal wound healing, and evaluation of proposed therapeutic modalities. This report describes the method by which the scar is created and presents the morphologic characteristics of such scars.

The scars are created in 6-week old, 20-pound, female Duroc pigs by repeated stripping of the dorsal and flank skin with an air dermatome set at 20/1000 of an inch,until only a basal layer of dermal collagen reticulated by small subcutaneous fat lobules remains. Usually three or four strippings are required to create a 6x6-inch

wound, depending on the size of the animal and the thickness of its hide. A 3-inch wide single strip donor site control wound is created in the paravertebral area opposite the study wound.

After recovery from their Innovar-nitrous oxide anesthetic, the animals are returned to their individual pens. The wounds are allowed to granulate and re-epithellalize without application of topical agents or dressings, since the pig is remarkably resistant to invasive sepsis. Four to six weeks are required for healing and scar formation, and the longer the convalescent period, the thicker is the resulting scar. Postoperatively, the pigs are fed standard dog chow and water ad lib, and usually attain weights in the range of 150 pounds, making long-term observation in a laboratory setting difficult. However, skeletal growth progresses from 30% to 80-90% of adult maturation during the 4-month postoperative period. This phase approximates a span from 8-15 years of age in the human.

RESULTS AND DISCUSSION

While the control wounds usually repigment and grow bristles, the study wounds remain hairless and frequently devoid of pigment. None of our pigs have been observed for more than four months, and therefore no comment can be made in reference to further persistence, spread, or reabsorption of the scar.

The thick scar observed in the Duroc pig closely resembles human hypertrophic scar in that it occurs in young animals due to full-or partial-thickness dermal injury. It is potentially disfiguring, and microscopically distinguishable from normal dermis. The maturation cycle progresses from a densely cellular stage with sparse collagen deposition to a sparsely cellular stage with deposition of enlarged collagen fibers. The orientation of the collagen fibers is predominantly parallel to the wound surface, but scattered areas of disorganized collagen are common. Elastic fibers are absent from the scar until late in its maturation cycle. The thickest scars have been biopsied from areas slowest to heal, and are characterized by dermal depths of up to 15,000 μ (1.5 cm). These areas contrast with normal porcine dermis and ordinary scar thickness of 2500 to 3000 mμ. Hair is present in the uninjured skin and reappears in the ordinary flat scar. It is consistently absent from areas of thick scar. Cut surfaces demonstrate translucent zones that correspond histologically and chronologically with relatively immature scar.

In addition to ordinary transmitted light microscopy with hematoxylin and eosin stains, porcine scar tissue has been studied with special stains for collagen and elastic tissue. Polarized light microscopy is most useful in evaluating, qualitatively, collagen deposits and fiber patterns.

Normal porcine skin is composed of an epidermis measuring 25-40 μ in thickness, not including rete pegs. The dermis is composed

of loosely woven collagen fibers generally arranged parallel to the epithelial surface. Layers of fibers cross each other at approximately right angles.

Young hypertrophic scars are characterized by a thick, hyperkeratotic, stratum corneum and a hyperplastic epithelium measuring up to 400 μ in depth, exclusive of rete pegs, extensions of prickle cell hyperplasia or hyperkeratoses. Epidermal thickness in the most mature tissue is 75 μ. None of the tissue in this series is old enough to have completely remodelled to normal epidermal architecture and dimensions. Beneath the epidermis is a markedly thickened dermis, packed with small basophilic-staining fibroblasts and eosinophilic young collagen fibers.

Scar maturity varies directly with the ratio of collagen fibers to fibroblasts. As the scar ages, there are fewer fibroblasts and more collagen fibers. Since collagen is birefringent, polarizing microscopy is valuable in assessing tissue maturity; the most mature area is located just below the epidermis. Another zone of early maturation is found at the deeper scar margin at its junction with subcutaneous fat. The dermis is thereby observed to mature in a centripetal pattern, proceeding from its most superficial and deep margins towards the center.

The configuration of collagen fiber deposition in the thick scar never approximates the loose, regular, interdigitating style of normal dermis. Two patterns are found that are easily distinguishable and irregularly dispersed through the hypertrophic tissue. First, there are closely packed fibers lying parallel to skin surface but not grouped into distinct bundles. These fibers are immediately obvious under polarized light. The areas of brightly polarized parallel collagen fibers are consistent with the eosinophilic zones demonstrated in the H & E preparations. The central immature region transmits no polarized light and correlates with the basophilic area of small fibroblasts in which no collage has yet been deposited.

The second configuration recognized in the hypertrophic porcine scar is distinguished by disorganized deposition of collagen fibers similar to that described in human hypertrophic scar. The polarized light pattern is also diagnostic.

These two collage configurations may occur separately or together. While the determining factor for each of these patterns is unknown, they may be conceivably linked with the degree and direction of linear stress (or lack of it) present in the vicinity of the healing wound.

The mature porcine hypertrophic scar has compact bundles of collagen fibers with a structural morphology somewhat suggestive of human keloid by light microscopy. However, polarized-light microscopy and higher power examination reveal strongly eosinophilic, brightly polarizing, hypertrophic, collagen fibers and fail to demonstrate the hyaline, glassy, pale staining, faintly refractile giant fibers typical of keloid.

Elastic fibers in porcine hypertrophic scar have been characteristically absent in all but the most mature specimens, where they are present in minimal quantities.

In summary, a technique has been described by which hypertrophic scar formation has been induced in small female Duroc pigs. Grossly and histologically, the morphologic structure and maturation cycle of this porcine scar resembles that of human hypertrophic scar. Both human and porcine scar share several etiologic prerequisites such as dermal trauma, delayed healing, and subacute infection leading to intense fibroblastic proliferation and collagen deposition in excess of that expected in normal scar formation and normal healing.

CLINICAL STUDY

In the last annual report, preliminary results were presented from a clinical project to study efficacy of two modes of therapy of hypertrophic burn scars: (1) steroid therapy--intralesional Dermajet (intradermal) injections or application of Cordran tape, and (2) pressure therapy using the Jobst pressure device, custom made to fit body segments or extremities. Since burn scars often cover large body surface areas, surgical excision and radiotherapy were not usually feasible except for very small lesions. The two methods chosen for evaluation were more safely applicable to large wounds. Unfortunately, there are no drugs yet available to control collagen synthesis directly, although several are being tested in other laboratories.

Both Cordran tape and Jobst stockings can be used by the patient on convalescent leave, while the Dermajet treatments are given at the hospital at two to four week intervals. Jobst pressure devices are not used about the chest and have been limited to one limb of bilaterally burned extremities so the other extremity can serve as a control. Periodic photographs are the major means of evaluation. Biopsies are sparingly employed and confined to the limbs.

Forty patients with extensive second- and third-degree burns were treated by either continuous pressure to their burn scars or by local injection of a triamcinolone hyaluronidase mixture. Pressure therapy was applied to 26 patients by means of custom-made elastic

garments (Jobst) which apply 40 mmHg pressure to the wounds. Fourteen patients received steroid therapy via air-gun injection of up to 200 mg triamcinolone diacetate and 150 N.F. units of hyaluronidase diluted in 0.5% Xylocaine. Periodic wound biopsies were obtained in selected patients to follow the evolution of the burn scar.

Results of therapy were graded photographically, by biopsy, and by subjective evaluation of scar pain, pruritis, softening, and flattening. Intralesional steroid therapy was of necessity limited to small areas of the face and hands, and was conducted during an average treatment period of 3.2 months. While no complications of subcutaneous tissue atrophy or skin depigmentation were encountered, subjective and functional results were poor, and the pain experienced during therapy contributed significantly to the discontinuation of this technique in 6 patients.

Twenty-eight legs, 8 arms, and 5 hands in 26 patients were treated with compression sleeves and stockings over an average treatment period of six months. Results were uniformly excellent in patients commencing therapy within three months of injury who diligently wore the devices as instructed.

Histological evaluation of wound biopsies demonstrated no consistent evidence of foreign body reaction, chronic inflammation, or differences in collegen maturation and configuration. No dense large collagen bundles or invasion of normal tissue were observed.

CONCLUSIONS

1. A reproducible animal model for the study of hypertrophic scar formation has been described. Future research will involve examination of collagen metabolism within this model to determine if it is truly representative of human scar.

2. Of the various modes of therapy evaluated clinically, a compression using elastic custom-made garments has been found most successful in shrinking already formed hypertrophic scars and in preventing hypertrophic scar formation in susceptible individuals with slow healing deep second- and third-degree burns.

PRESENTATION

Silverstein, P: Hypertrophic Scarring--Etiology and Control. Plastic Surgery Research Council, Boston, MA, 4 May 72.

PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0D 6950	72 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
71 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	3A061102B71R	01	269
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) Anemia Following Thermal Injury - Laboratory Study of Changes Occurring 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 02	72 04	DA	C. In-House

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

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 72	0.4	14.2
	CURRENT 73	0	0

19. RESPONSIBLE DOD ORGANIZATION
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NAME: Ysidro Villarreal, BA DA

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Burn anemia; (U) Red Cell Lifespan; (U) Iron Utilization

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

23. (U) (a) To measure red cell lifespan, the kinetics of RBC destruction and distribution of RBC's following thermal injury with the use of chromium-51 labeled RBC's. (b) To measure plasma iron clearance, erythron iron uptake, and red cell utilization of iron by standard techniques employing 59 FE-plasma. (c) To measure plasma-iron levels following burn injury.

24. (U) To utilize these measurements (a, b, c) to identify the source of the prolonged anemia that follows a burn injury.

25. (U) 71 07 - 72 04 The shortened survival of RBC's is directly related to the size of the burn injury. Plasma iron turnover is increased postburn. No latent anemia was detected. Correlation studies showed no significant effect of sepsis on red cell production.

*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. 3A061102B71R-01, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: ANEMIA FOLLOWING THERMAL INJURY - LABORATORY STUDY OF CHANGES OCCURRING IN BURNED SOLDIERS

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

1 July 1971 - 30 June 1972

Investigators:

Karl Eurenius, MD, Major, MC
Richard F. Mortensen, MS, SP 5

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

REPORT TITLE: ANEMIA FOLLOWING THERMAL INJURY - LABORATORY STUDY OF CHANGES OCCURRING 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 1971 - 30 June 1972

Investigators: Karl Eurenius, MD, Major, MC
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Reports Control Symbol MEDDH-288(R1)

The hemolytic anemia immediately following a burn has been attributed to direct thermal action on red cells. Measurements of ^{51}Cr labelled red cell survival and distribution revealed not only a shortened lifespan and intravascular hemolysis, but also significant sequestration of red cells at the burn site and slight hepatic and splenic sequestration.

Measurement of ^{59}Fe turnover and serum iron demonstrated a transient increase in iron metabolism until the red cell mass was restored. Iron distribution patterns demonstrated increased red cell iron utilization and higher splenic iron uptake. In contrast to other investigations, neither defective erythropoiesis nor chronic anemia were detectable in our uninfected burn model.

Burn anemia
Red cell lifespan
Iron utilization

ANEMIA FOLLOWING THERMAL INJURY - LABORATORY STUDY OF CHANGES OCCURRING IN BURNED SOLDIERS

Following burn injury patients experience an acute hemolysis at the time of injury and a later poorly documented red cell marrow failure. The early hemolytic anemia has been related to direct thermal injury, spherocytosis and increased osmotic fragility. The later anemia has been related to defective hemoglobin synthesis.

The present studies were directed toward evaluation of these anemic states in rats after a standardized scald burn.

Male Sprague-Dawley rats were inflicted with 20, 30, 40 or 50% total body surface scald burns under pentobarbital anesthesia and sequential examination of hematocrit, red cell mass, using a ^{51}Cr labelling technique, and ^{59}Fe plasma clearance and red cell incorporation.

Results indicate that following a 30% burn injury, 50% of the red cell mass is lost in the first 24-48 hours as a result of intravascular hemolysis and trapping by the burned skin. Plasma iron turnover in these animals was brisk, and red cell mass was nearly restored by 6 days. No late anemia was observed, even with large burns, suggesting that marrow function is not suppressed.

PUBLICATION

Mortensen RF, Eurenius K: Erythrokinetics and Ferrokinetics Following Thermal Injury in the Rat. Clin Res 20:45, 1972 (Abstr).

PRESENTATION

Eurenius K: Erythrokinetics and Ferrokinetics Following Thermal Injury in the Rat. San Antonio Research Club, San Antonio, Texas, October 1971.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OD 6385	72 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
71 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	3A061102B71R	01	313
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)

(U) Coagulation Abnormalities in Thermally Injured Soldiers (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
69 01	Cont	DA	C. In-House

17. CONTRACT/GRANT

a. DATES/EFFECTIVE: Not Applicable EXPIRATION:

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c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 72	0.3	12.4
	CURRENT 73	0.3	10.0

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) Coagulation; (U) Platelets; (U) Thrombocytopenia; (U) Disseminated Intravascular Clotting; (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) To ascertain the clotting factor changes with time following uncomplicated thermal injury and to evaluate departures from these expected "norms" in burn patients with hemorrhagic complications.

24. (U) Fifty patients admitted to the US Army Institute of Surgical Research with greater than 30% total body surface burns have been included in this study. Serial assays of the blood clotting parameters in this group of patients were obtained. In addition, blood from all burn patients with bacterial septicemia or clinical evidence of spontaneous subcutaneous or gastrointestinal bleeding was similarly examined.

25. (U) 71 07 - 72 06 Five patients with Disseminated Intravascular Coagulation have been observed. In contrast to the postburn "hypercoagulability" seen in the general burn population, these patients exhibited classical clotting factor consumption, and restoration of hemostasis and factor activity followed heparin therapy. Fibrinogen contraction was the most reliable indicator of DIC, while thrombocytopenia was seen both in this syndrome, and also in patients dying of sepsis. Secondary fibrinolysis, as evidenced by increased fibrin split product titres and decreased plasminogen levels, was seen in association with DIC.

*Available to contractors upon originator's approval.

ANNUAL PROGRESS REPORT

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

REPORT TITLE: COAGULATION ABNORMALITIES IN THERMALLY INJURED SOLDIERS

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

1 July 1971 - 30 June 1972

Investigators:

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William F. McManus, MD, Major, MC
Dwight D. McEuen, SP 4

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

REPORT TITLE: COAGULATION ABNORMALITIES IN THERMALLY INJURED SOLDIERS

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

Period covered in this report: 1 July 1971 - 30 June 1972

Investigators: Karl Eurenius, MD, Major, MC
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Reports Control Symbol MEDDH-288(R1)

Serial coagulation studies in human burn patients and in experimentally scald burned rats were performed to evaluate clotting dynamics. These studies indicate that following burn injury there is a rapid consumption of clotting factors associated with evidence of fibrinolysis. This is followed by a period of "hypercoagulability" as defined by elevated in vitro clotting activity and further evidence of fibrinolysis. Five patients developed disseminated intravascular coagulation (DIC) during this period of "hypercoagulability". A close correlation between rat and human studies was observed, and it is suggested that this model be used to evaluate specific clotting changes which occur with thermal injury.

Coagulation
Platelets
Thrombocytopenia
Disseminated intravascular clotting

COAGULATION ABNORMALITIES IN THERMALLY INJURED SOLDIERS

Hemorrhage and thromboembolic phenomena are frequently seen after major burn injury, and have been documented elsewhere. We have studied in serial fashion, clotting parameters in 35 burn patients and in experimentally scald burned rats.

Studies included platelet concentration, fibrinogen concentration (turbidometric and thrombin clottable), prothrombin time (P.T.), activated partial thromboplastin time (P.T.T.), quantitative thrombin time (T.T.), plasminogen concentration (casein digestion) and fibrin degradation product titer (F.D.P. - staphylococcal clumping technique).

Patients were divided retrospectively into 17 survivors with a mean burn size of 42% and 18 non-survivors with a mean burn size of 55%. Rats received a 30% third-degree scald burn after pentobaribtal anesthesia.

The follcwing observations were made. Following burn injury in patients, there is a prompt elevation in fibrinogen and FDP titre, and a fall in plasminogen, suggesting fibrinolysis. In rats, this same response is preceded by a brief episode of clotting factor consumption. The data suggest that intravascular coagulation occurs immediately after injury, followed by a period of "hypercoagulability". Evidence of persistent fibrinolysis, either primary or secondary to a mild but persistent DIC, continues during the first 3 weeks of convalescence.

Early postburn thrombocytopenia in burned humans and rats persists in "non-survivors" until death. In this group, there is also a significant elevation of P.T. and P.T.T. Mixing studies suggest that this is not the result of a circulating anticoagulant, and is more likely a reflection of a deficiency state. In contrast, fibrinogen concentrations are identical and high in both surviving and non-surviving patients, and have been noted to fall only with clinically significant DIC. A drastic fall in fibrinogen concentration may be the best indication of DIC which has now been observed in 5 burn patients.

PRESENTATION

McManus WF: Disseminated Intravascular Coagulation in Burns. American Burn Assoc. San Francisco, Calif. 1972.

PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OD 6399	72 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
71 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	3A061102B71R	01	268
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) Granulocyte Kinetics in the Burned Rat - A Model of Changes in Burned Military Patients (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 Clinical Medicne

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
70 11	72 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 72	0.4	15.2
	CURRENT 73	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) Granulocytic; (U) Granulopoiesis

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

23. (U) Assess rates of production and destruction of granulocytes in burned rats, as a model of changes occurring in burned humans.

24. (U) Total circulating and marginating granulocyte pools. Bone marrow, total granulocyte counts and differential distribution.

25. (U) 71 07 - 72 05 Immediate postburn granulocytosis, due to cell demargination, is followed by a gradual increase in the circulating blood granulocyte concentration. This apparent granulocytosis is in fact an absolute peripheral granulocytopenia since marginations is absent. The bone marrow response to either the burn injury or the absolute granulocytopenia, is a depletion or release of mature cells with a gradual return to normal pool sizes.

*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. 3A061102B71R-01, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: GRANULOCYTE KINETICS IN THE BURNED RAT - A MODEL OF CHANGES IN BURNED MILITARY PATIENTS

US ARMY INSTITUTE OF SURGICAL RESEARCH
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1 July 1971 - 30 June 1972

Investigators:

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Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

REPORT TITLE: GRANULOCYTE KINETICS IN THE BURNED RAT - A MODEL OF CHANGES IN BURNED MILITARY PATIENTS

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

Period covered in this report: 1 July 1971 - 30 June 1972

Investigators: Karl Eurenius, MD, Major, MC
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Reports Control Symbol MEDDH-288(R1)

A 30%, third-degree, scald burn in rats was used to study granulocyte kinetics after thermal injury. Early postburn leukocytosis, a result of granulocyte demargination and early bone marrow release, was followed by an absolute granulocytopenia, despite elevated concentrations of circulating cells. The total peripheral granulocyte pool was slowly restored to normal by a combination of increased marrow production and release, and the reestablishment of a marginating pool. These studies are related to the leukocytosis observed in thermally injured patients.

Granulocytic
Granulopoiesis

GRANULOCYTE KINETICS IN THE BURNED RAT - A MODEL OF CHANGES IN BURNED MILITARY PATIENTS

Leukocytosis is a well-known clinical observation in burned patients. The relatively short lifespan of granulocytes in the circulation makes comprehensive study of the dynamics of this cell difficult. Isotope labelling has been used to assess cell turnover in normal, leukemic, and infectious states. Since host defense against bacterial invasion is crucial in the burned subject, we examined granulocyte kinetics in a scald-burned rat model, and present data which describes the status of the circulating, marginating and marrow pools following thermal injury.

MATERIALS AND METHODS

Patients. White blood counts were collected sequentially in trisodium citrate anticoagulant from 25 consecutive human burn patients with greater than 30% total body surface burns. At least 7, and as many as 26 samples were taken from each patient.

Animals. Male, Sprague-Dawley rats were anesthetized with pentobarbital and a 30%, third-degree, scald burn was produced using a fiberglass mold and immersion of the clipped dorsal body surface in water at 100° C for 10 seconds. Twenty-five animals served as controls, while 20 rats were followed sequentially after burn injury. Invasive sepsis does not occur in such animals in the absence of deliberate bacterial seeding.

Blood, collected by aspiration from a small distal tail vein incision, was assessed for leukocyte concentration with an improved Neubauer ruled counting chamber.

Marrow suspensions were obtained from rat femurs which were dissected, cleaned of surrounding tissue, and transected. Marrow contents were flushed into 10 ml of normal saline and 3.2% trisodium citrate (9:1). Nucleated marrow cells were counted in 2% acetic acid. After counting, the suspension was centrifuged at 300 x g and the cell button resuspended in a small amount of fetal calf serum. Smears of the resuspended cell pellets were made with a rounded cover glass, and 200-cell differentials were performed by each investigator, using a May-Grunwald Giemsa stain.

Epinephrine, 0.1 ml, 1:10,000, was injected into the dorsal tail vein of rats at various periods postburn, and into unburned controls. White counts and tail vein blood smears for differential counts were taken at 5 minute intervals, and the maximal granulocyte count was used as the value of maximal demargination.

RESULTS

In 26 burned patients, a mean leukocyte count of 24,000 WBCs/mm^3 was observed from 24 hours through day 3 postburn. High normal values were recorded from day 4 through day 6, when a second elevation occurred peaking at day 9 and persisting until day 18.

White blood counts in 20 burned rats showed an initial rise during the first 4 hours, and a subsequent fall, a second sustained elevation began on the 5th postburn day and persisted for almost 3 months.

When total circulating granulocytes were calculated, the early peak contained $105 \pm 49 \times 10^6$ cells per 200 g rat as compared to a value of 41 in normal animals. These values dropped to near normal by 24 hours, remained there for 8 days, and then rose again. The mean contribution of myelocytes and metamyelocytes to the circulating pool was 10%, demonstrating a significant addition by immature cells which lasted for 2 months. There was no significant contribution to this pool of promyelocytes or blast forms.

Next, the total peripheral granulocyte pool (circulating cells plus marginated cells) was examined. A significant, absolute granulocytopenia occurred by 4 hours postburn and was maximal at 3 days postburn with approximately 120×10^6 cells per 200 g rat, or 65% of normal. This was followed by a slow return to control values. This apparent granulocytosis in the presence of actual granulocytopenia, was the result of almost complete depletion of the marginating pool which fell from 131×10^6 cells/200 g rat to 14.3 at 4 hours, 70.0 at 5 days, and rose to only 126 by 90 days.

Changes in marrow granulocytes indicate nondividing mature cells (segmented forms, bands, metamyelocytes) vacating the marrow during the first 28 hours postburn. This compartment was restored by 3 to 5 days postburn and remained hyperplastic through day 30. The dividing compartment (myelocytes, promyelocytes, blast forms) was significantly increased by the first day after burning and remained significantly elevated throughout the convalescent period, returning toward normal by 60 days postburn.

DISCUSSION

It is a clinical impression that leukocytosis occurs after extensive thermal injury. We observed both an early increase and a subsequent sustained elevation of circulating granulocyte concentrations in burn patients. Similar elevations have been observed after exposure to cortisol, epinephrine, and bacterial endotoxin. We also demonstrated an early elevation in granulocyte concentration

in burned rats, and our data suggest that this response is due both to an acute cell demargination and to a sudden release of marrow granulocytes. Both catecholamine and cortisol concentrations are drastically increased after burn injury, and may be responsible for this demargination and subsequent marrow release.

While circulating granulocyte concentrations were elevated during convalescence, total peripheral content values indicated an absolute granulocytopenia, despite the significant contributions of dividing and non-dividing cells from the marrow. This absolute granulocytopenia suggests a shortened cell survival.

The decrease in total peripheral content during the first 24 hours indicates a decrease in the production/destruction ratio, which reached equilibrium during the next 5 days, and then increased. The restoration of total peripheral granulocytes, in the presence of circulating immature cells, and a shift toward immaturity in the marrow indicates increased marrow granulocyte production and release. The absolute granulocytopenia masked by circulating leukocytosis in these studies may contribute to the incidence and severity of bacterial infections in burn patients.

PRESENTATIONS AND/OR PUBLICATIONS

None

41

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OD 6983	72 07 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMAR/ SCTY	6. WORK SECURITY	7. REGRADING	8. DISP'N INSTR'N	9A. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
	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	3A061102B71R	01	301
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code) (U) Suppression of Granulocyte and Platelet Production in Burn Wound Infection - A Laboratory Model of Sepsis 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
71 08	72 03	DA	C. In-House

17. CONTRACT/GRANT: Not Applicable
a. DATES/EFFECTIVE: EXPIRATION:
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c. TYPE: d. AMOUNT:
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18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 72	0.6	20.0
FISCAL YEAR CURRENT 73	0	0

19. RESPONSIBLE DOD ORGANIZATION
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22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Blood Cell Kinetics; (U) Infection; (U) Platelets; (U) Granulocytes

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

23. (U) Define blood cell kinetics in rats with burn wound infection.

24. (U) Femoral bone marrow and circulating cell concentrations, differentials, and 51 Chromium labeled platelet and red cell survival and distribution were serially examined in rats with 25% third degree scald burns and lethal Pseudomonas burn wound infection.

25. (U) 71 08 - 72 03 Completed studies revealed peripheral granulocytopenia and thrombocytopenia related to selective suppression of the marrow granulopoiesis and thrombopoiesis which are normally stimulated by burn injury. Peripheral cell destruction was not additionally enhanced by infection.

* Available to contractors upon originator's approval.

DD, FORM 1 MAR 66 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65 AND 1498-1, 1 MAR 66 (FOR ARMY USE) ARE OBSOLETE.

FINAL REPORT

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

REPORT TITLE: SUPPRESSION OF GRANULOCYTE AND PLATELET PRODUCTION IN BURN WOUND INFECTION--A LABORATORY MODEL OF SEPSIS IN BURNED TROOPS

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

1 July 1971 - 30 June 1972

Investigators:

Thomas W. Newsome, MD, Major, MC
Karl Eurenius, MD, Major, MC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

REPORT TITLE: SUPPRESSION OF GRANULOCYTE AND PLATELET PRODUCTION IN BURN WOUND INFECTION--A LABORATORY MODEL OF SEPSIS 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 1971 - 30 June 1972

Investigators: Thomas W. Newsome, MD, Major, MC
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Reports Control Symbol MEDDH-288(R1)

In uncomplicated burn injury in rats, postburn blood cell kinetics are characterized by shortened survival due to cell destruction, enhanced bone marrow activity, and a resultant granulocytosis, thrombocytosis, and rapid restoration of the circulating red cell mass. In patients with systemic gram-negative infection, granulocytopenia and thrombocytopenia often develop. This response to acute infection has been studied by following granulocyte, platelet, and red cell changes in the burned rat with Pseudomonas burn wound infection.

Peripheral granulocytopenia in the infected, burned animals was associated with depletion of both mitotic and non-mitotic myeloid marrow cells. This contrasted to a markedly stimulated myelopoiesis in the rats with uninfected burns. Peripheral thrombocytopenia persisted in the burned, infected rat and reflected the absence of the sustained megakaryocyte proliferative response which following uninfected burn injury. Platelet survival and label distribution were identical in infected and uninfected animals. The levels of circulating red cells and marrow erythroid cells did not differ significantly in the two groups. Thus, granulocytopenia and thrombocytopenia in the infected burned rat were related to selective suppression of the marrow myeloid and megakaryocyte response to burn injury.

Blood cell kinetics
Infection
Platelets
Granulocytes

SUPPRESSION OF GRANULOCYTE AND PLATELET PRODUCTION BURN WOUND INFECTION--A LABORATORY MODEL OF SEPSIS IN BURNED TROOPS

Using a murine model, recent studies[1-3] have defined the alterations of hematologic cell kinetics that follow burn injury.[4,5] Post-burn blood cell kinetics are characterized by shortened survival due to cell destruction, followed by increased bone marrow activity with resultant granulocytosis, thrombocytosis, and rapid restoration of red cell mass.

In a clinical burn population, granulocytopenia and thrombocytopenia often develop coincident with systemic gram-negative infection.[6] This response to acute infection has been further studied by following granulocyte, platelet and red cell changes in the burned rat with a superimposed Pseudomonas burn wound infection.[7] The results have been compared with those obtained previously in uninfected rats with similar burns.[1-3]

MATERIALS AND METHODS

Animals. Male, Sprague-Dawley rats, 225 ± 15 g, were anesthetized with pentobarbital (1 mg/225 g I.P.), clipped over the dorsal surface, and burned over 25% of the body surface by scalding.[8] With the exception of an anesthetic mortality of less than 5% such animals have a normal life span and do not develop burn wound infection. After burning, these rats were seeded with a broth culture of Pseudomonas aeruginosa, USAISR strain 12-4-4, containing 10^8 organisms per ml. They were housed separately and offered feed and water ad lib.

Cell Counts. The hematocrit, leukocyte count and differential, and platelet count were determined daily from tail vein blood. Red cell mass was determined by a ^{51}Chromium-labelled red cell dilution technique.[3] Total circulating granulocytes were calculated as the product of the leucocyte concentration, granulocyte differential and blood volume. Blood volumes were calculated from the red cell mass using the central venous hematocrit corrected for peripheral dilution.[9] Platelet counts were determined by phase microscopy and were also corrected for blood volume changes.

Survival Studies. Red cell survival and platelet survival were determined with ^{51}Chromium-labelled normal rat erythrocytes and platelets which were injected into the hearts of recipient rats one hour after burning.[1,3] The activity of 0.1 cc of recipient tail vein blood was monitored daily with a standard well counter. Label disappearance was expressed as a per cent of the activity measured one hour postinjection and was corrected for daily blood loss (2%), label decay, and background, as described elsewhere.[1] Platelet label

distribution four days postinfusion, expressed as a per cent of injected label, was determined in spleen, liver, kidney, lung, and both burned and unburned skin.

Bone Marrow Studies. At daily intervals, rat femurs were dissected, transected, and flushed with a 10 cc of citrated Ringer's lactate. The cell suspension obtained was centrifuged and the cells resuspended in 1% acetic acid for determination of total nucleated cell counts and megakaryocyte counts with hemocytometer microscopy.[1] Differential counts of 200 cells were made after May Grunwald-Giemsa staining of cells suspended in fetal calf serum. Cells flushed from the marrow in this manner represent 41.7 ± 2% of total femoral marrow cells.[1] Counts were expressed as flushable cells per femur. Myeloid cells were divided into mitotic (myeloblast, promyelocyte, and myelocyte) and nonmitotic (metamyelocyte, band form, and segmented form) compartments, and the myeloid mitotic index (mitoses per 100 marrow myeloid cells) was determined.

RESULTS

Pseudomonas infection of the 25% burn wound was lethal to all rats between the third and sixth days after seeding. Postmortem examination revealed severe pulmonary as well as burn wound infection.

Circulating Cell Counts. Immediately after injury, total circulating granulocytes increased dramatically as seen in Figure 1. This probably reflects postburn granulocyte demargination.[2] In contrast to animals with uninfected burns, the burned infected animals subsequently developed a severe circulating granulocytopenia (Fig.1), accompanied by a shift toward granulocyte immaturity (Table 1). The circulating platelet count fell soon after burning in both groups (Fig. 2), but thrombocytopenia persisted only in the infected group. In contrast, early postburn loss of red cell mass (Fig. 3a) was followed by gradual restoration in both groups.

Cell Survival. The survival curve of ^{51}Chromium-labelled normal platelets infused into acutely burned, infected rats was exponential. Platelet survival was shortened, but identical to that in rats with uncomplicated burns (Fig. 4). Four days after infusion, platelet label distribution in the spleen, liver, kidney, and lung was comparable in control, burned, and burned, infected animals (Fig. 5). In both burned and burned, infected animals, however, there was a 50-fold increase in label accumulation in the burn wound over that in an equal surface area of unburned or control skin. ^{51}Chromium-labelled red cell survival was similar in burned and burned, infected animals (Fig. 3c) and differed from control animals by an early loss of red cells.

Bone Marrow Content. Burned, infected rats displayed a progressive fall in total marrow nucleated cells (Table 2), reflecting progressive

Table 1. Circulating White Blood Cells in Burned, Infected Animals

	Preburn	Time Postburn				
		4*	1	2	3	4
Total count x 10^3	18.0	20.2	6.3	2.5	4.5	13.3
Differential %						
Granulocytes:						
Immature *	0.2	2.5	5.0	4.0	3.6	3.0
Band forms	2.0	7.7	12.9	5.0	7.6	12.8
Segmented	14.0	35.7	18.7	4.3	11.2	14.0
Lymphocytes	81.2	49.7	59.4	82.5	73.3	69.0
Monocytes	1.6	4.1	2.5	3.6	2.6	1.0
Eosinophils	1.0	0.3	1.5	0.6	1.7	0.2
Basophils	0	0	0	0	0	0
Total	100.0	100.0	100.0	100.0	100.0	100.0

* Myelocytes and metamyelocytes

Table 2. Flushable Marrow Cells

		Preburn	Day Postburn			
			1	2	3	4
Total nucleated x 10^8	Burn	1.80 ± 0.12	2.07 ± 0.19	2.12 ± 0.23	1.94 ± 0.20	2.16± 0.33
	Burn+infection		1.52 ± 0.11	1.13 ± 2.24	0.74 ± 0.20	0.68± 0.17
Erythroid x 10^6	Burn	55.4 ± 5.0	66.4 ± 10.4	70.8 ± 8.2	62.6 ± 7.1	71.8 ± 15.9
	Burn+infection		52.0 ± 10.9	46.5 ± 17.6	55.6 ± 6.0	55.2 ± 2.5
Myeloid x 10^6	Burn	63.4 ± 7.8	69.4 ± 8.8	85.2 ± 8.6	71.0 ± 6.7	86.7 ± 9.2
	Burn+infection		23.5 ± 7.3	9.0 ± 3.0	2.9 ± 2.2	3.3 ± 0.4
E:M ratio	Burn	0.9:1	1:1	0.8:1	0.9:1	0.8:1
	Burn+infection		2.2:1	5.3:1	20:1	16.7:1

Data are expressed with 95% CL.

41-4

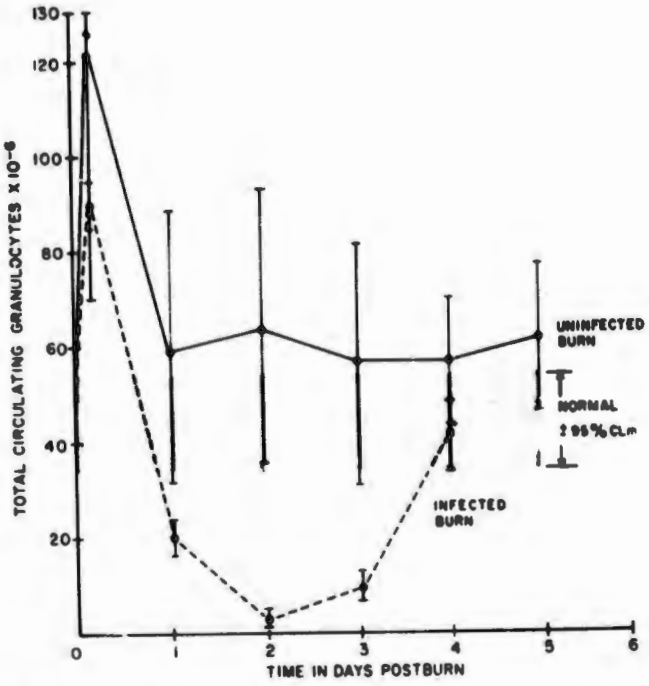

Figure 1. Total circulating granulocytes in burned rats with and without Pseudomonas burn wound infection. Data are expressed as means ± 95% CL.

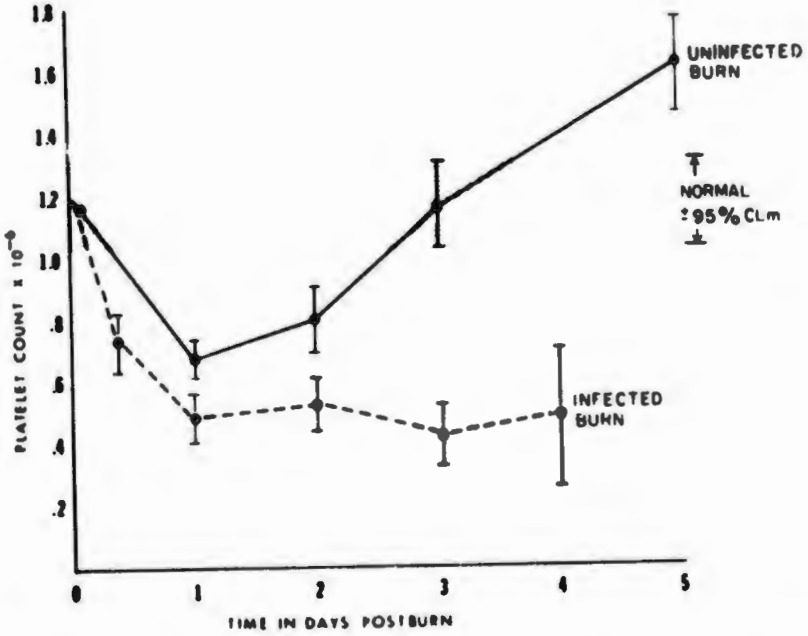

Figure 2. Platelet counts in burned rats with and without Pseudomonas burn wound infection. Counts are corrected to pre-burn blood volumes (see text) and expressed as means ± 95% CL.

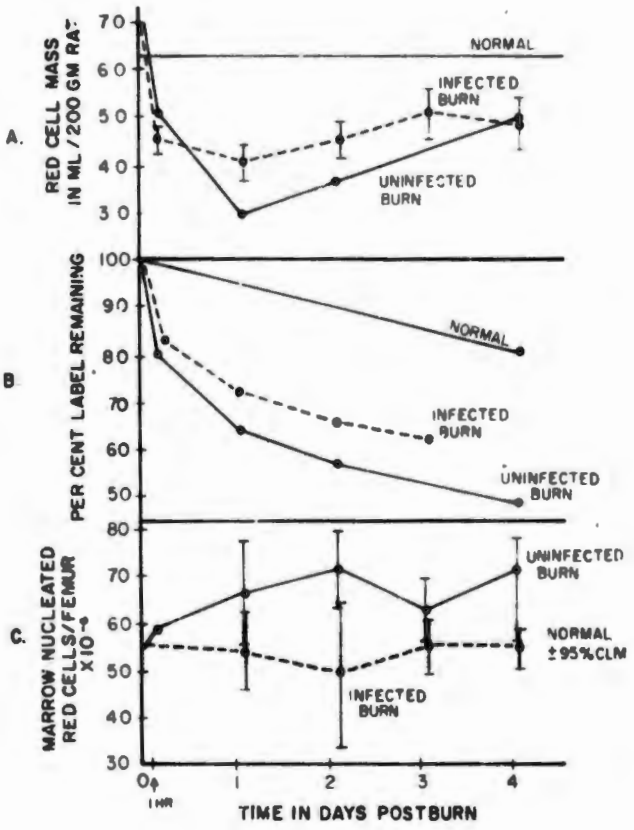

Figure 3a. Red cell masses per 200 g rat in burned rats with and without Pseudomonas burn wound infection. Data are expressed as means ± 95% CL.

Figure 3b. Flushable marrow nucleated red cells per femur in burned rats with and without Pseudomonas burn wound infection. Data are expressed as means ± 95% CL.

Figure 3c. Per cent survival of ^{51}Chromium labelled normal rat red cells infused one hour postburn into burned rats with and without Pseudomonas burn wound infection.

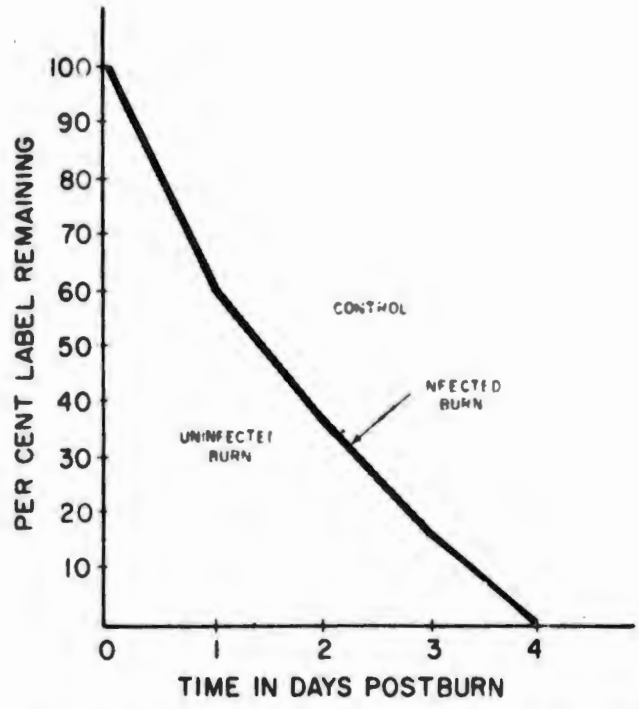

Figure 4. Per cent survival of ^{51}Chromium labelled normal rat platelets infused one hour postburn into burned rats with and without Pseudomonas burn wound infection. Survival in control rats and in rats with uninfected burn are depicted by shaded curves of the means ± 95% CL.

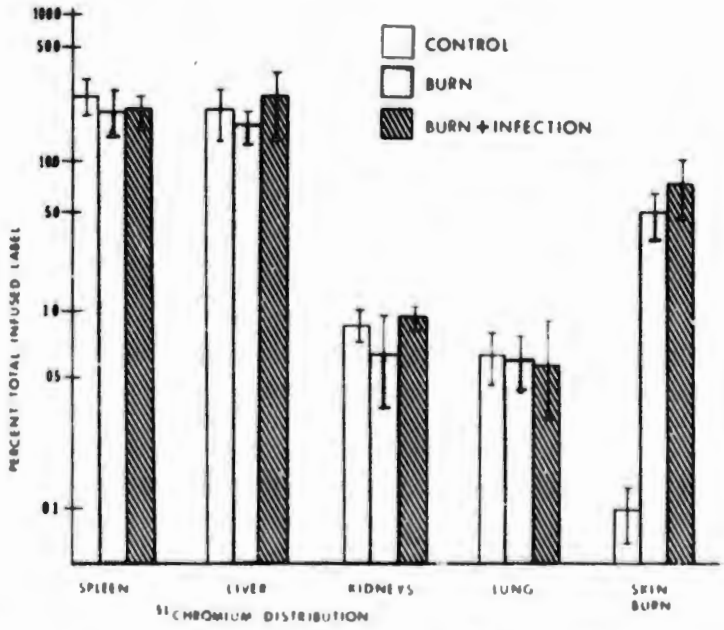

Figure 5. Organ distribution of ^{51}Chromium platelet label four days postinfusion in control rats, burned rats, and burned, infected rats.

depletion of both mitotic and nonmitotic myeloid cell compartments (Fig. 6). The myeloid mitotic index was slightly lower than the preburn level (Table 3) and contrasted with the brisk mitotic response in rats with uninfected burns. Since marrow erythroid precursors were not significantly diminished (Fig. 3b, Table 2), the erythroid:myeloid (E:M) ratio changed from 0.9:1 to 20:1 by the third postburn day in the infected group (Table 2). In contrast to the proliferative response of marrow megakaryocyte precursors to uncomplicated burn injury, no sustained megakaryocyte response developed in the presence of infection (Fig. 7).

DISCUSSION

Granulocytopenia and thrombocytopenia, known to occur in burn patients with gram-negative infections,[6] occurred in this laboratory burn model in association with Pseudomonas infection of the burn wound.

Following initial demargination manifested by a granulocytosis in both groups, total circulating granulocytes fell to significantly lower levels in animals with infected burns than in animals with uninfected burns. This was directly related to severe depletion of the myeloid marrow, including both mitotic and non-mitotic compartments, in infected animals. In marked contrast, increased mitotic activity, precursor cell proliferation, and rapid restoration of marrow granulocyte reserves were seen following burn injury alone.

The persistence of thrombocytopenia in infected, burned animals was related to depression of the marrow megakarocyte response normally associated with burn injury. Postburn platelet destruction was not enhanced, nor was organ sequestration of platelets significantly altered by acute infection.

In contrast, restoration of the circulating red cell mass postburn was equally prompt in infected and uninfected animals following early red cell destruction. Although the erythroid marrow counts in rats with infected burns were lower than in rats with standard burns, the difference was not statistically significant.

Thus, Pseudomonas infection selectively suppresses the myeloid and megakarocyte marrow response to burn injury. The mechanism of this suppression has not been defined by this study, but the apparent selectivity suggests that marrow function is altered distal to the uncommitted stem cell. This could involve a general depression of marrow function, or it could represent a more specific inhibition of the proliferative response of granulocytes and megakaryocytes to burn injury.

In this study Pseudomonas cell fractions and endotoxin were not

Table 3. Myeloid Mitotic Index

	Preburn	Postburn Day			
		1	2	3	4
Burn	2.2 ± 0.9	12.4 ± 4.3	7.0 ± 1.9	3.1 ± 0.9	1.8 ± 0.9
Burn + infection	2.2 ± 0.9	0.4 ± 0.8	0.6 ± 0.8	0.6 ± 0.6	1.4 ± 1.7

Data are expressed with 95% CL.

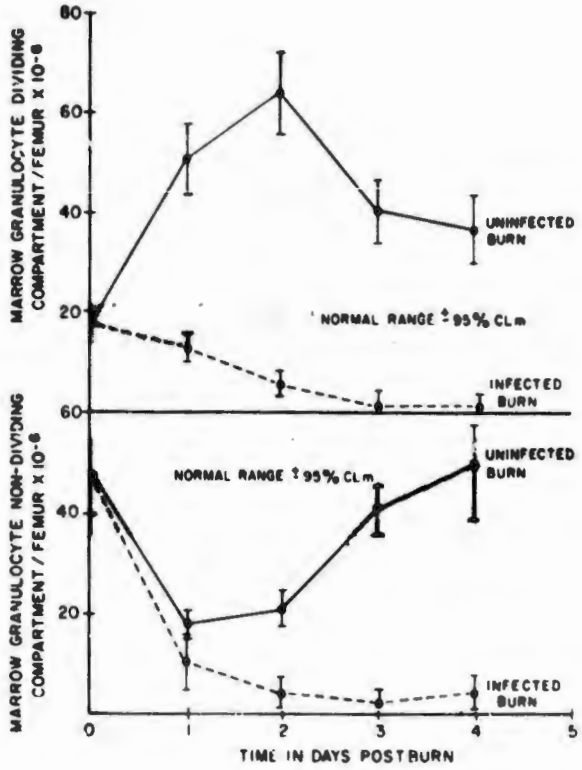

Figure 6. Flushable marrow granulocytes per femur separated into compartments of dividing cells (myeloblasts, promyelocytes, myelocytes) and nondividing cells (metamyelocytes, band forms, and segmented cells). Comparisons are made of burned rats with and without Pseudomonas burn wound infection. Data are expressed as means ± 95% CL.

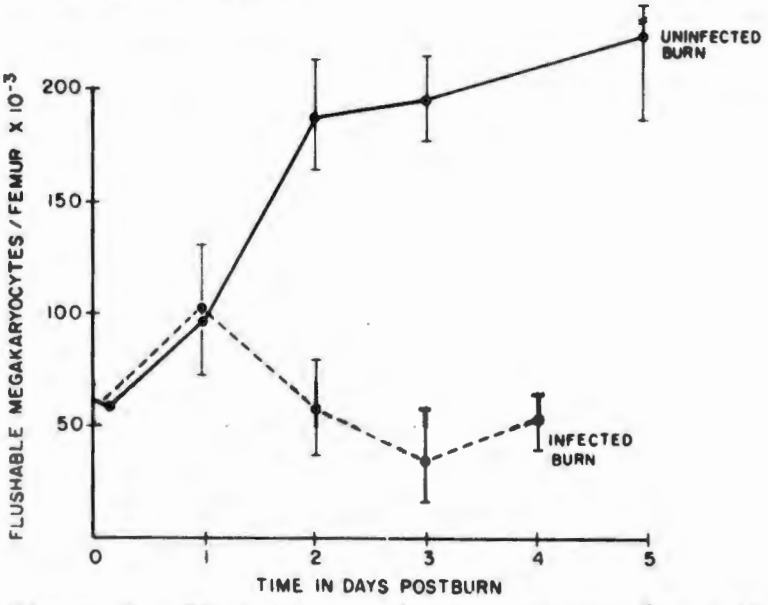

Figure 7. Flushable megakaryocytes per femur in burned rats with and without Pseudomonas burn wound infection. Data are expressed as means ± 95% CL.

administered to burned rats to determine the agent responsible for marrow suppression. Others have studied the response to endotoxin, which usually elevates the circulating granulocyte count by inducing release of mature marrow cells[10,11] and stimulating precursor proliferation.[12-14] There is some recent evidence, however, that larger endotoxin doses may have an inhibitory effect on myeloid cell colony stimulation in the mouse.[15] In that study the *in vitro* application of endotoxin to agar cultured colonies also depressed growth. Platelet counts are usually depressed by endotoxin, which has been shown to alter the structural integrity of the platelet.[16] Increased platelet destruction did not occur in the infected, burned rats in our study.

It has been previously reported that granulocyte function is impaired following burn injury.[17] If acute myeloid marrow failure is superimposed in Pseudomonas infection in the burned patient, it would accentuate this deficit in the host defense by further reducing his capacity to respond adequately to infection.

SUMMARY

The hematologic response of thermally injured patients to gram-negative infections has been reproduced in rats with lethal Pseudomonas burn wound infection.

Peripheral granulocytopenia and thrombocytopenia in the infected, burned animal are related to selective suppression of the marrow granulopoiesis and thrombopoiesis which are normally stimulated by burn injury. Peripheral cell destruction is not additionally enhanced by infection.

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Mortensen RF, Eurenius K: Erythrokinetics and ferrokinetics following burn injury. Clin Res 20:44, 1972.

PRESENTATIONS

Eurenius K: Suppression of Granulocyte and Platelet Production in the Infected Burn. Presented at Southern Soc Mtg Amer Fed Clin Res, New Orleans LA, 29 Jan 72.

Newsome TW: Suppression of Granulocyte and Platelet Production in the Infected Burn. Amer Burn Assn, San Francisco CA, 7 Apr 72.

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1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
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3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISP'N INSTR'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	3A061102B71R	01	120
b. CONTRIBUTING				
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

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

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
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	CURRENT 73	0.2	7.0

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21. GENERAL USE
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22. KEYWORDS (Precede EACH with Security Classification Code): (U) Suppurative Thrombophlebitis;(U) Dogs
(U) Thrombophlebitis; (U) Intravenous Catheters; Long-Term Intravenous Therapy

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 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) 71 10 - 72 06 Polyethylene catheters produce marked thrombophlebitis. There is little reaction about the silastic catheters. The effect of bacteremia and A-V fistulas is to be assessed.

*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. 3A061102B71R-01, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: THROMBOPHLEBITIS - ETIOLOGY AND PREVENTION IN BURNED MILITARY PERSONNEL

US ARMY INSTITUTE OF SURGICAL RESEARCH
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1 July 1971 - 30 June 1972

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Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

REPORT TITLE: THROMBOPHLEBITIS - ETIOLOGY AND PREVENTION 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 1971 - 30 June 1972

Investigators: Gary W. Welch, MD, Major, MC
Paul Silverstein, MD, Major, MC
Daniel W. McKeel, Jr, MD, Major, MC

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To examine the role of catheter composition in thrombophlebitis, mongrel dogs have had their external jugular veins catheterized using silastic and polyethylene catheters. The polyethylene catheters produced a marked reaction and thrombophlebitis with thrombosis of the vein, whereas the silastic catheters produced little reaction. After further histologic examination of the catheterized veins, attempts will be made to produce transient bacteremias in catheterized dogs to determine whether or not bacteria will localize in the veins which have become thrombosed. Following this, A-V shunts will be created to determine the effect of flow and catheter composition on thrombophlebitis.

Suppurative thrombophlebitis
Thrombophlebitis
Intravenous catheters
Long-term intravenous therapy

THROMBOPHLEBITIS - ETIOLOGY AND PREVENTION IN BURNED MILITARY PERSONNEL

The development of prolonged intravenous fluid therapy has also created the problem of infusion thrombophlebitis. In most reports, the incidence varies between 26 and 32 % (Andreasen, 1969[1]; Swanson et al., 1969[2]). An additional complication has been that of suppurative thrombophlebitis (Stein et al., 1970[3]). Suggested etiologies of this thrombophlebitis have included (a) pH of the infusate, (b) duration of the infusion, (c) size of the cannula, (d) presence of bacteria, (e) cannula composition, (f) composition of the infusate itself, (g) venous flow characteristics, and (h) skin preparation and technique of venipuncture (Fonkalsrud, 1969[4]; Fonkalsrud et al., 1968[5]; Matzger, 1971[6]; Tse et al., 1971[7]; Nejad et al., 1968[8]).

Initially, attempts were made to evaluate the different catheter compositions in the rabbit ear veins. However, the size of the silastic catheter precluded using rabbits. Therefore, it was decided to use mongrel dogs whose external jugular veins are catheterized, one side with polyethylene and the other with silastic catheters. The catheters are placed in the veins by exposing the veins through a small surgical incision, and then the skin is closed over the catheter. Ten days after introduction of the catheter, the animals are sacrificed and the catheters with the veins are removed en bloc.

Preliminary studies show the polyethylene catheters to produce a great deal of reaction and thrombophlebitis with extensive tissue reaction around the vein, necessitating en bloc dissection of the vein and surrounding muscles. Silastic catheters, on the other hand, produce very little reaction, and the vein can be dissected free. In most cases, the silastic catheterized vein remains patent. After the examination of approximately 6 more dogs for the development of thrombophlebitis, an attempt will be made to produce transient bacteremias in order to seed the thrombophlebitic veins and hopefully, cause a suppurative thrombophlebitis. Next, A-V fistulas will be created and the role of flow along with catheter composition in the development of thrombophlebitis will be examined.

SUMMARY AND CONCLUSIONS

Cannulization of the external jugular veins of dogs with either polyethylene or silastic catheters has resulted in the development of marked thrombophlebitis in the veins catheterized with polyethylene catheters. Those catheterized with silastic have evidenced little reaction. Because these catheters are buried subcutaneously

and there is no infusate, it would appear that catheter material plays a significant role in the development of thrombophlebitis. Further plans include the examination of the role of transient bacteremia in development of suppurative thrombophlebitis, and the role of flow in the development of thrombophlebitis will be examined by the creation of A-V fistulas.

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PRESENTATIONS AND/OR PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
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3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISP'N INSTR'N	9A. SPECIFIC DATA CONTRACTOR ACCESS	9. LEVEL OF SUM
	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	3A061102B71R	01	081
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code) (U) Gastrin Levels Following Thermal Injuries 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
70 09	71 12	DA	C. In-House

17. CONTRACT/GRANT: Not Applicable
a. DATES/EFFECTIVE: EXPIRATION:
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18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 72	0.5	14.0
	CURRENT 73	0	0

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22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Gastrin; (U) Gastric Secretions; (U) Curling's Ulcer

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

23. (U) Measurement of serum gastrin levels serially in patients with thermal injuries.

24. (U) Twenty-two patients with burns of greater than 30% of the total body surface were included. Following an overnight fast blood was drawn at intervals 1, 2, 4, and 6 weeks post injury. The sera were frozen, transported to Dr J.E. McGuigan at the J Hillis Miller Medical Center, Gainesville, Florida, for radioimmunoassay of gastrin levels.

25. (U) 70 09 - 71 12 At completion of the study, data revealed serum gastrin values falling into the lower portion of the control range. No correlation to burn size, interval postburn, sepsis, or gastrointestinal bleeding was noted. The data are being submitted for publication.

*Available to contractors upon originator's approval

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

FINAL REPORT

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

REPORT TITLE: GASTRIN LEVELS FOLLOWING THERMAL INJURIES OF MILITARY PERSONNEL

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

1 July 1971 - 30 June 1972

Investigators:

Thomas W. Newsome, MD, Major, MC
Morris J. Asch, MD, Lieutenant Colonel, MC
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Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

REPORT TITLE: GASTRIN LEVELS FOLLOWING THERMAL INJURIES OF MILITARY PERSONNEL

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

Period covered in this report: 1 July 1971 - 30 June 1972

Investigators: Thomas W. Newsome, MD, Major, MC
Morris J. Asch, MD, Lieutenant Colonel, MC
James E. McGuigan, MD*
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Reports Control Symbol MEDDH-288(R1)

Curling's ulcerations of the gastroduodenal mucosa remain a serious complication of thermal injury. Previous studies from this Institute have demonstrated normal levels of gastric acid secretion in a burn population and suggested that deficits in gastric mucous production may create a relative hyperacidity. This would assume greater significance if the gastric mucosa has been damaged by postburn ischemia.

Recent radioimmunoassay techniques have allowed sensitive measurements of serum gastrin, the hormonal stimulus for gastric acid secretion. Since levels had not been previously determined in stress situations, they were measured serially in a population of patients with extensive thermal injuries.

Serum gastrin levels in these patients fell into the lower portion of a range previously documented in presumably normal control patients. There was no apparent relation to burn size, the interval postburn, sepsis, or gastrointestinal bleeding.

Gastrin
Gastric secretions
Curling's ulcer

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GASTRIN LEVELS FOLLOWING THERMAL INJURIES OF MILITARY PERSONNEL

Although acute gastroduodenal ulceration in burn patients has been correlated with burn size and sepsis, the precise etiology remains obscure.[1-5] Altered secretion of gastric acid does not distinguish a burn population in general or patients with Curling's ulcer within that population.[6] O'Neill, however, has demonstrated a quantitative depression of mucous production in burned dogs[7] and many investigators have proposed that the gastric mucosa is damaged by early postburn ischemia.[1,8,9] In this milieu normal acid levels could represent a relative but significant hyperacidity.

Definition of the hormonal stimulus for gastric acid secretion[10-14] has been rapid following the isolation of gastrin from antral mucosa in 1962 and its laboratory synthesis in 1964.[15] The development of a sensitive radioimmunoassay[16] has permitted accurate quantitation of serum gastrin levels in a number of ulcerogenic states including the Zollinger-Ellison syndrome,[17] hypercalcemia,[18-19] pernicious anemia,[20] and peptic ulcer disease.[19,21] Since serum gastrin levels in stress situations have not been reported, they have been measured serially in a population of patients with extensive thermal injuries and form the basis of this communication.

METHODS

Twenty-two patients admitted to the US Army Institute of Surgical Research between November 1970 and September 1971 were included in this study. Each was admitted to the Institute in the first week postburn and extent of burn ranged from 30 to 90% of the total body surface. Patient ages ranged from 2 to 68 years but a homogeneous group was formed by 16 patients of ages 18 to 36 years. The presence of only 4 females in the group reflected the composition of the military population served.

All patients were resuscitated using the Brooke formula[22] as a guide to fluid administration and their burns were treated topically with mafenide acetate cream. Nasogastric suction was employed until normal gastrointestinal function returned and was used to relieve any subsequent gastric dilatation. Active gastrointestinal bleeding was treated by iced saline lavage and sedation. In no patient was hypercarbia documented during the course of the study and no patient received intravenous hyperalimentation.

Blood specimens were drawn after an overnight fast from each patient at postburn intervals of one, 2, 4 and 6 weeks. The sera were collected by centrifugation and immediately frozen. At a later

date they were thawed and gastrin levels determined by the radioimmunoassay technic of McGuigan.[16]

Due to the difficulty inherent in manipulating these severely burned patients for gastric collections, the secretory studies performed previously in this Institute[6,7] were not repeated.

RESULTS

Serum gastrin levels are tabulated with the clinical data in the Table. The representative nature of this burn group is indicated by the relatively high incidence of sepsis (9/22) and concurrent massive upper gastrointestinal bleeding (3/22). All 3 patients with significant gastrointestinal bleeding died. At autopsy 2 had gastric ulcerations and the other a large duodenal ulcer. Two other patients died of sepsis later in their hospital courses.

All serum gastrin levels fell within a range previously recorded in a control population[21] but tended to reside at the lower end of this range. This may be explained by the younger age of the burn study group, for gastrin levels appear to be age related.[21]

In the gastrin data there were no patterns discernibly related to magnitude of burn injury, postburn interval, sepsis, or gastrointestinal bleeding. The levels associated with clinical hemorrhage were 235 pg/ml (Patient No. 3), 65 pg/ml (Patient No. 19), and 110 pg/ml (Patient No. 22).

DISCUSSION

Normal serum gastrin levels in patients with extensive thermal injuries are consistent with O'Neill's demonstration of unaltered acid secretion in this population.[6] He, also, noted no significant difference in patients with actual ulceration.

These findings strengthen the suggestion that Curling's ulcer, like other trauma-related stress ulcers and steroid ulcers, reflects a deficiency in the gastroduodenal mucosal barrier.[23,24] An association with postburn serum corticosteroid elevations[25-27] has been suggested by the similarity between mucosa deficits after burn injury[7] and those in steroid-treated rats.[28] On the other hand, adrenalectomized animals are not protected from stress ulceration.[29,30]

Another hypothesis is that stress ulceration is related to the reflux of intestinal chyme into the stomach accompanying shock. This has been shown to induce histologic mucous deficits and subsequent mucosal ulceration.[31,32] Indeed, ion flux studies of acid

Serum Gastrin Levels in Patients with Extensive Thermal Injury

Burn Size %	Patient No.	Sex	Age	Gastrin Levels (pg/ml)[a] 1	2	4	6	GI Bleed	Comments
30 - 39	1	M	26	< 20	60	60	< 20	No	Uncomplicated hospital course
	2	M	27	< 20	< 20	< 20	< 20	No	Febrile,septic 2 weeks postburn.uneventful recovery
	3	M	53	400	210	360	235	No	Uncomplicated course
	4[b]	M	68	< 20		< 20		No	Died acute pneumonia 6 weeks postburn
	5	F	19	< 20		< 20		No	Uncomplicated course
	6	F	49	< 20	< 20	< 20		No	Fungal infection of burn wound 1 week postburn,excised;subsequently uncomplicated course
	7[b]	F	2		235			Yes	Fungal burn wound infection;bled from large duodenal ulcers;died 2 weeks postburn from sepsis
	8	M	24	< 20	< 20	< 20	< 20	No	Deep electrical injuries,intermittent sepsis;multiple amputations,ultimately satisfactory healing
	9	M	23	60	< 20	< 20	< 20	No	Uncomplicated course
	10	M	34	100	105	110	130	No	Febrile course for 2 weeks postburn. healed satisfactorily

Table - continued

Burn Size %	Patient No.	Sex	Age	Gastrin Levels (pg/ml)[f] 1	2	4	6	GI Bleed	Comments
40 - 49	11	M	27	< 20	< 20	< 20	< 20	No	4+ Guiac positive stool 1 week postburn but stable Hct; uncomplicated course
	12	M	28	< 20		< 20	< 20	No	Deep burns; uncomplicated course
	13	M	41	< 20	60		< 20	No	Preburn history of epigastric pain. first 2 weeks postburn marked by pneumonia,sepsis,prepyloric erythema at gastroscopy,normal gastric acid secretion,no bleeding
	14	M	51	60	< 20	< 20		No	Uncomplicated course
	15	M	36	< 20		75	< 20	No	Acute thrombophlebitis 1 week postburn, heparinized; uncomplicated healing
	16*	F	18	< 20	< 20	< 20	60	No	Protracted,fatal staphylococcal septicemia
50+	17	M	33	< 20	< 20	< 20	< 20	No	Pneumonia 1st week postburn; subsequently uncomplicated course
	18	M	23	< 20	< 20	< 20	< 20	No	Multiple fractures requiring bilateral amputations of lower extremities;intermittently septic,ultimately healed
	19*	M	21	65	< 20			Yes	Septic with bilateral Providencia pneumonia;bled intermittently from duodenal ulcer;died 2 weeks postburn

43-4

Table - continued

Burn Size %	Patient No.	Sex	Age	Gastrin Levels (pg/ml)/ 1	2	4	6	GI Bleed	Comments
	20	M	21	80	< 20	< 20	< 20	No	Preburn history of epigastric pain; uncomplicated course
	21	M	26	< 20	< 20		< 20	No	Uncomplicated course
	22•	M	30	110				Yes	Bled 1 week postburn from gastric ulcer, died with burn wound infection, pneumonia

/ Range in a control population: 0 - 200 pg/ml

• Died

bathed gastric mucosa have demonstrated decreased mucosal resistance to hydrogen ion back diffusion from lumen to mucosa in animals after contact with bile salts.[33,34] A similar change in mucosal permeability has occurred after exposure to serotonin,[35] a compound which produces, in the experimental animal, lesions similar to stress ulcers.[35,36]

By demonstrating that the hormonal stimulus for gastric acid secretion, as well as the actual secretion itself, is unaltered by burn injury, our study reemphasizes the apparently secondary role of gastric acid in Curling's ulcerogenesis.

SUMMARY

Serum gastrin levels have been measured in a group of patients with extensive thermal injuries. They are unaltered by burn injury nor do they correlate with burn size, sepsis, or development of gastroduodenal ulceration.

This is consistent with previous acid secretory studies and suggests that Curling's ulcer diathesis is determined primarily by deficits in the barrier function of the gastric mucosa rather than by abnormal acid production.

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PRESENTATIONS AND/OR PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
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3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. 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	3A061102B71R	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	Cont	DA	C. In-House

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18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 72	0.3	8.4
	CURRENT 73	0.1	3.0

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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) 71 10 - 72 06 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 disociated 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.

*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. 3A061102B71R-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

1 July 1971 - 30 June 1972

Investigators:

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James V. Wessels, MD, Captain, MC*
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Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A061102B71R-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 1971 - 30 June 1972

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 for 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 2 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.

Etoxadrol
CL1848C
Burn anesthesia

From the Department of Anesthesiology, Brooke General Hospital, Brooke Army Medical Center, Fort Sam Houston, Texas 78234.

CLINICAL EVALUATION OF ETOXADROL (CL1848C) FOR USE IN BURNED MILITARY PERSONNEL

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 patients.

A pilot study of 10 patients has been undertaken to observe the anesthetic properties, the physiologic effects, and the post-operative psychological manifestations of CL1848C when used for elective operations during the course of burn management.

Progress: Two patients have thus far received CL1848C, and their case reports follow:

Case No. 1. The patient was a 29 year old caucasian male who had received 3% total body surface electrical burns on 5 Oct 1971 to the left foot, left lateral thigh and buttocks, all third degree. At the time of his accident, he sustained a fall and a mild concussion, from which he was completely recovered. He was scheduled for operative debridement of his burn wounds on 12 October 1971. He was otherwise in excellent health.

The patient was premedicated only with atropine, 0.8 mg IV, 10 minutes prior to the induction of anesthesia. At 0845, he received 60 mg (0.75 mg/kg) of etoxadrol which was given over a period of one minute. Blinking and flickering of the eyelids began approximately one minute later and at the same time the patient became unresponsive to commands. Mild to moderate spontaneous random movements of the head and all extremities also began at this time and continued throughout the anesthetic. Lip smacking and extension of the tongue occurred frequently and sighing, clearing of the throat, and vocalization only occasionally. Withdrawal from the surgical stimuli was not abolished by 2 additional doses of 20 mg etoxadrol each at 0910 and 0920, but was relieved somewhat by the addition of N_2O (6 L) and O_2 (3 L) by mask at 0925. The surgical procedure was concluded at 0948.

The airway was well maintained until nitrous oxide was added, at which time the tongue became flaccid and the patient required a nasal pharyngeal airway. The mouth had become rigidly closed at this time. Ventilation was well maintained throughout the anesthetic, and tidal volumes varied from 700 to 1400 cc. However, the pattern of ventilation, particularly after the operation was begun, was uneven. Arterial blood gases drawn at 0907 revealed a pO_2 of 98, Pco_2 34, and pH 7.44.

A mild hypertension and tachycardia were noted shortly after induction, but the highest pressure recorded was only 160/90 mm Hg. The ECG revealed a sinus rhythm at all times.

A rectal temperature probe revealed a temperature of 98.8° F on induction, which rose to 99.2 at the end of the procedure. Although a circulating water blanket was used at a temperature of 98, the patient was, for the most part, undraped and the ambient temperature of the operating room was 64° F. Under similar circumstances, a patient anesthetized with halothane in our operating theater would be expected to decrease his temperature 2 to 5 degrees.

At the conclusion of the procedure, the patient was taken to our recovery area which is for the most part quiet and private. He became arousable and was able to move extremities on command at 1010 hours. He could give his name at 1125 and name the hospital by 1225. During this early recovery phase, he could remember dreams of a pleasant nature.

Complete control of eye movement and speech was not regained until much later. Nystagmus was still present, and the patient complained of blurred vision. When he opened his mouth to speak, often lip movement without sound was produced. At 2200 hours that night, there had been no further recovery, and the patient complained of seeing visions of his childhood (from about age 4 on) and of an accident which he had had in Vietnam several months before. He also had at this time a feeling that he was dying or dead. On questioning the patient, it was felt that he was somewhat anxious about his prolonged recovery, and it was elected to sedate him for the remainder of the night. He was given 100 mg pentobarbital IV and later 100 mg IM. The next morning, all that remained of anesthetic residual was blurred vision and a feeling of generalized numbness. However, the patient did not feel that he was completely normal until approximately 40 hours postoperatively.

On direct questioning, the patient did not feel that he would like to have the drug for his next procedure. He also stated that he had been completely free of pain throughout the 2 days of recovery.

The patient was subsequently anesthetized with ketamine, and later with thiopental, N_2O, and O_2, both techniques providing adequate operative anesthesia with recovery periods of normal duration and free of complications.

Case No. 2. The second patient was a 54 year old white female who was in her usual state of good health when her clothes ignited

accidentally and she received a 21% total body surface burn on 5 Oct 1971. She was evacuated to the Institute of Surgical Research on the same day.

Her past medical history was normal except for previous surgical procedures - appendectomy, hysterectomy, and hemorrhoidectomy. Anesthesia in previous procedures was spinal in all cases and without sequelae or complications. She is also under treatment for arthritis and receives aspirin and Premarin.

Her resuscitation in the initial postburn period was uneventful, and her wound remained free of infection. On 8 Nov 1971, 34 days after her injury, she was brought to the operating room for debridement of her burns and heterografting. An intravenous infusion was started, and at 0835 hours, atropine, 0.01 mg/kg, was given intravenously. At 0845, anesthesia was induced with 1 mg/kg of etoxadrol, slowly, over 2 to 3 minutes. The patient appeared to lose consciousness over the next 2 or 3 minutes, but verbalized with a moaning sound in response to being touched during her surgical preparation. Ten minutes after induction, it was decided to give her another 1/2 mg/kg of etoxadrol to see if the moaning could be stopped. It was not successful, and, therefore, 10 minutes later, another 1/2 mg/kg was given. The patient had mild random movements and only once withdrew from her surgical stimulation; however, her moaning in response to painful stimuli continued, and, therefore, 35 minutes after induction of anesthesia, it was decided to add nitrous oxide in a concentration of 60% oxygen to her anesthetic management. Ten minutes later, another 15 mg of etoxadrol was given and repeated again in 10 minutes. Surgery continued uneventfully for a total duration of 80 minutes. Blood gas measurements made during the operation were reported as normal. Her temperature remained at a normal level without elevation or drop during the entire operation, an unusual finding in our operating room where temperatures usually decrease due to exposure of uncovered areas of skin. There was a slight hypertension and tachycardia, which developed immediately after induction, but this was not to a level of any concern. The total dose of etoxadrol was 2 1/2 mg/kg for an 80 minute procedure. The surgeons stated that they felt operating conditions were satisfactory to excellent. However, the moaning was of some concern to both the surgeons and to us, the anesthetists. On the addition of nitrous oxide to the regimen, the patient's airway became somewhat difficult to maintain. The patient was removed to the recovery room 5 minutes after completion of the operation. She was completely unresponsive with minor random motor activity. She required a nasal airway for maintenance of good respiration without obstruction. She first became arousable 15 minutes after arrival in the recovery room, but could not respond to verbal commands, only to painful stimuli. She was

unable to follow any commands for 8 hours postoperatively. During this period, she appeared to be dreaming and frequently in a state of panic, occasionally yelling "help". Random movements increased, and throughout the night the patient hallucinated and screamed wildly. It was necessary several times through the first night to sedate her with Nembutal, 50 mg intramuscularly. Twenty-seven hours postoperatively, the patient finally appeared calm, knew her name, and realized that she was in the postoperative state. She recalled hallucinating at some length, and remembered it as being unpleasant. At this time, she stated she would not like the anesthetic again. According to her estimation, she did not feel entirely normal until approximately three and one-half days postoperatively. From our observations, we concur with this estimation.

The patient subsequently underwent 2 additional anesthetics, one under ketamine and the other under Halothane, both of which were uneventful. The patient responded in a normal fashion to both anesthetics, with none of the effects seen under etoxadrol.

SUMMARY

Intraoperatively, the drug produced anesthesia quite similar to that seen with ketamine. Both patients studied received large doses of CL1848C (1.25 mg/kg and 2.25 mg/kg) according to the normal anesthetizing dose determined at another institution. Yet, neither patient appeared to be fully anesthetized with CL1848C, and both required supplemental agents to complete the intended surgery.

Both patients had prolonged unpleasant recovery periods, 40 hours and 86 hours, respectively.

Based on the work done here and reports of cases done elsewhere, it would appear that CL1848C is indeed very much like ketamine, but much longer acting, and without the potency required to produce sufficient anesthesia in most patients. Continuation of this investigation is awaiting preliminary reports from other investigators regarding the effects of premedication on obtaining adequate surgical anesthesia.

PRESENTATIONS AND/OR PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6973	72 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
71 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	3A061102B71R	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)

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	CURRENT 73	0.5	18.0

19. RESPONSIBLE DOD ORGANIZATION
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NAME: Philip W Rogers, MAJ, MC
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FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code): (U) Aldosterone; (U) Chloride; (U) Potassium; (U) Extracellular volume; (U) Metabolic alkalosis; (U) Respiratory Acidosis; (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) Metabolic alkalosis is a common acid-base disturbance seen in injured or severely ill troops. This study was designed to fully examine the pathophysiology of this disorder.

24. (U) This disturbance of acid-base hemeostasis has been examined by studying the development and correction of hypokalemic metabolic alkalosis and contraction alkalosis in dogs. The effect on acid-base balance of selective aldosterone deficiency was also studied. Since it soon became apparent that aldosterone played a key role in this disorder, its effect on Na transport in the entire nephron was examined. Studies of the renal compensation of chronic respiratory acidosis also have been initiated.

25. (U) 71 07 - 72 06 Results obtained thus far demonstrate that metabolic alkalosis cannot be generated by the kidney in the absence of hyperaldosteronism. Metabolic alkalosis can be maintained by the kidney in the absence of hyperaldosteronism provided a stimulus to proximal reabsorption exists. Potassium deficiency can result in metabolic alkalosis provided hyperaldosteronism exists. No effect of aldosterone on sodium transport at any site of the nephron save the distal exchange site has been demonstrated. The role of the renin-angiotensin-aldosterone system on the generation of bicarbonate is being assessed. Results from these studies are too preliminary to warrant any conclusions at this point.

*Available to contractors upon requestor's approval

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ANNUAL PROGRESS 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

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

1 July 1971 - 30 June 1972

Investigators:

Neil A. Kurtzman, MD, Lieutenant Colonel, MC
Martin G. White, MD, Major, MC
Philip W. Rogers, MD, Major, MC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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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

Period covered in this report: 1 July 1971 - 30 June 1972

Investigators: Neil A. Kurtzman, MD, Lieutenant Colonel, MC
Martin G. White, MD, Major, MC
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Metabolic alkalosis is a common acid-base disturbance seen in injured or severely ill troops. This study was designed to fully examine the pathophysiology of this disorder. The development and correction of hypokalemic metabolic alkalosis and contraction alkalosis has been studied in dogs. The effect on acid-base balance of selective aldosterone deficiency has been also studied. Since it soon became apparent that aldosterone played a key role in this disorder, its effect on Na transport along the entire nephron has been examined. Metabolic alkalosis cannot be generated by the kidney in the absence of hyperaldosteronism. Metabolic alkalosis can be maintained by the kidney, however, provided a stimulus to proximal reabsorption exists. Potassium deficiency can result in metabolic alkalosis provided hyperaldosteronism exists. No effect of aldosterone on sodium transport at any site of the nephron save the distal exchange site has been demonstrated. Aldosterone may exert an effect on the transport of other electrolytes by the kidney.

Aldosterone
Chloride
Potassium
Extracellular volume
Metabolic alkalosis

EFFECT OF CHLORIDE AND EXTRACELLULAR VOLUME ON CORRECTION OF METABOLIC ALKALOSIS--A COMMON PROBLEM IN THE INJURED TROOP

In the last decade our understanding of the pathophysiology of metabolic alkalosis has greatly increased. The expansion of our knowledge in this field has been almost entirely due to the efforts of Schwartz and co-workers.[1-7] While this work is large and varied it can be summarized as follows. With two exceptions (marked potassium depletion and primary aldosteronism) metabolic alkalosis requires chloride deficiency for its genesis and chloride repletion for its correction. These observations have been the subject of an excellent review[8] which should be consulted for the full details of the work mentioned above. Schwartz and his colleagues have interpreted their data as indicating a major role for chloride in the genesis, maintenance and correction of metabolic alkalosis. They have also interpreted their data as indicating a minimal role for potassium in the genesis, maintenance, and correction of metabolic alkalosis.

In the past several years a series of studies from our laboratory[9,10] and those of others[11-14] have presented evidence that requires a reappraisal of the pathophysiology of metabolic alkalosis. This paper summarizes this recent data and presents new data concerning metabolic alkalosis. These data show important roles for extracellular volume, the state of body potassium stores, and mineralocorticoid excess in the genesis and maintenance of metabolic alkalosis.

CONTROL OF RENAL BICARBONATE REABSORPTION

Metabolic alkalosis may be defined as a primary abnormal pathophysiologic event characterized by a gain of bicarbonate ion or a loss of hydrogen ion. Thus, extracellular fluid contains more bicarbonate than under normal circumstances. Persistent metabolic alkalosis is a common clinical occurrence. The fact that it may persist even when no new bicarbonate is added to extracellular fluid is a priori evidence that the kidney is reabsorbing more bicarbonate than under normal circumstances. Were it not doing so metabolic alkalosis would soon correct as a result of renal bicarbonate loss. It is, therefore, imperative to understand the various mechanisms that control bicarbonate reabsorption by the kidney before attempting to make any explanation of the forces called into play during the generation or correction of metabolic alkalosis.

Bicarbonate is believed to be reabsorbed in the proximal and distal convoluted tubules. It is unlikely that large amounts of bicarbonate are reabsorbed in the loop of Henle.[15] Bicarbonate

is reabsorbed by hydrogen ion secretion in exchange for sodium in the proximal tubule (Fig. 1), and hydrogen or potassium secretion in exchange for sodium in the distal tubule. Carbonic anhydrase which catalyzes the hydration of CO_2 to carbonic acid is found in both proximal and distal renal tubular epithelial cells and on the luminal surface of proximal tubular epithelial cells.[16] When hydrogen ion is secreted into the tubular lumen sodium enters the tubular cell. It, along with the bicarbonate ion that resulted from the breakdown of carbonic acid, is returned to the circulation. It is important to remember that sodium may also be reabsorbed as sodium chlodride, in which case electroneutrality is maintained not by cation exchange, but by the passive diffusion of chloride across the tubular epithelium which accompanies the active reabsorption of sodium.

Hydrogen secretion, and therefore bicarbonate reabsorption, will be increased by any event that elevates the intracellular concentration of hydrogen ion. It is for this reason that both potassium deficiency and hypercapnia result in increased bicarbonate reabsorption. Potassium deficiency causes potassium to leave cells in exchange for hydrogen, and hypercapnia results in intracellular acidosis because of increased formation of carbonic acid. Conversely, increased body potassium stores and hypocapnia decrease intracellular hydrogen ion concentration resulting in decreased bicarbonate reabsorption.

Recently, it has been demonstrated that the state of extracellular volume (ECV) exerts a major effect on bicarbonate reabsorption.[9,14] When volume is expanded sodium reabsorption is inhibited which secondarily results in depressed bicarbonate reabsorption. When volume is contracted sodium reabsorption is stimulated and so is that of bicarbonate.

Much evidence has been presented suggesting that chloride deficiency stimulates renal bicarbonate reabsorption.[1-8] Since chloride depletion almost invariably means salt depletion which results in contracted extracellular volume, and since ECV contraction stimulates bicarbonate reabsorption, it is unclear if chloride plays any primary role in bicarbonate reabsorption independent of its effect on volume, Current evidence would indicate that all the effects of chloride on bicarbonate reabsorption are mediated through changes in effective ECV.[17]

When the regulatory role of extracellular volume over bicarbonate reabsorption was elucidated it became necessary to reevaluate the role of potassium as a regulator of bicarbonate reabsorption. Much of the evidence that changes in body potassium stores directly resulted in changes of renal bicarbonate reabsorption rested on evidence obtained from studies in which potassium chloride or potassium bicarbonate were infused into animals also receiving sodium

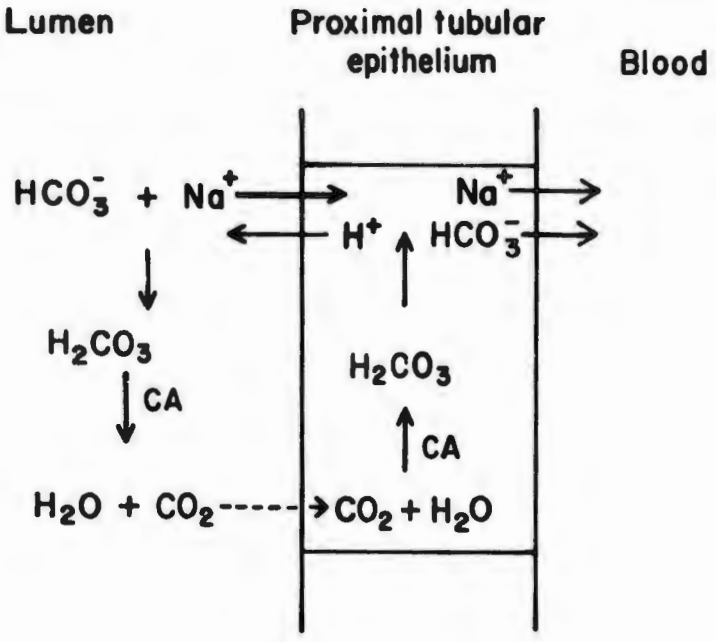

Figure 1. The mechanism of proximal tubular bicarbonate reabsorption.

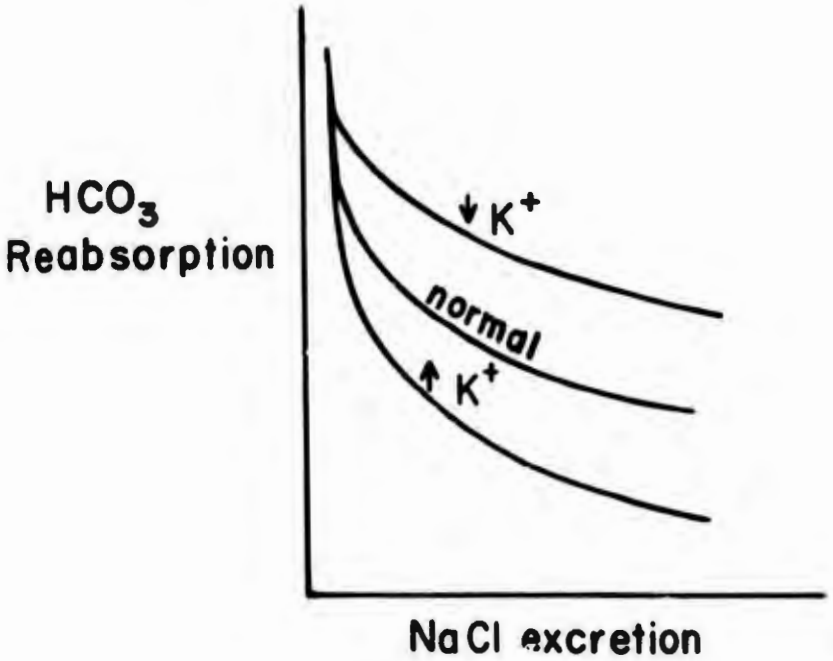

Figure 2. The relationship of bicarbonate reabsorption to NaCl excretion (and inferentially ECV expansion) in potassium loaded, normal, and potassium depleted subjects.

bicarbonate.[18] Such maneuvers resulted in marked depression of bicarbonate reabsorption. It is readily apparent, however, that administration of large amounts of sodium bicarbonate, and potassium chloride or potassium bicarbonate inevitably results in extracellular volume expansion. It was possible, therefore, that potassium loading resulted in depressed bicarbonate reabsorption as a consequence of extracellular volume expansion rather than as a consequence of a direct effect of potassium on proximal tubular epithelial cells. Further doubt was cast concerning the direct regulatory role of potassium on bicarbonate reabsorption by the observation that hypokalemic metabolic alkalosis could be corrected by the administration of sodium chloride.[7] Patients with hypokalemic metabolic alkalosis by definition have high reabsorptive rates for bicarbonate; it has long been assumed that this was at least in part due to the accompanying potassium deficiency. When saline was administered to these patients and metabolic alkalosis corrected it was assumed that the reabsorptive rate of bicarbonate was now normal despite the fact that potassium deficiency had not been corrected. Thus it was tempting to downgrade the role of potassium as a major etiologic agent of metabolic alkalosis.

That potassium exercised a regulatory role over renal bicarbonate reabsorption independent of an effect attributable to volume was demonstrated by us.[19] We measured bicarbonate reabsorption in three groups of dogs at varying levels of salt excretion (Fig. 2). The three groups of animals studied were a normal group, a potassium loaded group, and a potassium depleted group. We showed that at any one level of sodium excretion bicarbonate reabsorption was higher in the potassium depleted animals than in the normal animals and higher in the normal animals than in the potassium loaded dogs. When salt excretion was very low, and inferentially volume markedly contracted, there was no difference in bicarbonate reabsorption between the three groups. From these data we concluded that volume expansion depressed bicarbonate reabsorption to the same degree in potassium loaded, normal, and potassium depleted animals, but that independent of volume the state of body potassium stores exerted an effect on the level at which bicarbonate reabsorption was set. We also concluded that volume contraction was such a major stimulus to increased bicarbonate reabsorption that it could completely overcome the depressive effect of potassium loading on bicarbonate reabsorption. Thus, during volume contraction one would expect the effect of varying levels of body potassium stores on bicarbonate reabsorption to be completely obscured.

These findings suggested that the reason patients with hypokalemic metabolic alkalosis could have their acid-base disturbances corrected by the administration of saline alone was the simple fact that bicarbonate reabsorption in hypokalemic subjects is just as responsive to the depressive effects of volume expansion as it is in normal subjects. It further seemed likely that, while saline administration restored

plasma bicarbonate concentration to normal in patients with hypokalemic metabolic alkalosis, bicarbonate reabsorption was not "normal" in that it would have been lower in "normal" subjects similiarly treated. Another possibility was that these clearance studies did not related to the steady state situation. It was obvious that further balance studies of hypokalemic metabolic alkalosis were indicated.

The relationship of aldosterone to metabolic alkalosis has long been perplexing. Evidence has been presented both for and against its role as a major etiologic factor in metabolic alkalosis. We studied[20] the effects of aldosterone deficiency on renal bicarbonate reabsorption and found that proximal tubular bicarbonate reabsorption occurs independent of any effect of aldosterone. The opposite was found to be true in the case of the distal tubule; aldosterone deficiency was found to markedly impair the ability of the distal tubule to secrete hydrogen ion, and thus to impair its ability to reabsorb or regenerate bicarbonate. With these observations in the background we undertook the following investigations of the pathophysiology of metabolic alkalosis.

GENERATION AND MAINTENANCE OF METABOLIC ALKALOSIS

METHODS

A. The effects of potassium depletion on three groups of dogs were studied. The dogs received the potassium exchange resin sodium polystyrene sulfonate 2 gm/kg/day for seven days and desoxycorticosterone (DOCA) 20 mg/day by intramuscular injection for 14-16 days. They received a "zero" electrolyte diet, identical to that described by Cohen,[13] to which 85 mEq of NaCl were added daily in the case of the first group. The second group had 85 mEq of NaCl and 35 mEq of $NaHCO_3$ added to the diet daily, while the third group had 17 mEq/day of NaCl and 35 mEq/day of $NaHCO_3$ added to their diet. Twelve dogs made up the first group, 7 the second, and 11 dogs comprised the third group. The dogs in this third group were further studied by discontinuing the daily dose of DOCA and then gradually increasing it until metabolic alkalosis redeveloped. At this point 35 mEq/day of $KHCO_3$ was substituted for the 35 mEq/day of $NaHCO_3$ that had been added to these animals' diet.

B. Six dogs were subjected to bilateral adrenalectomy and maintained on 0.75 mg/day of dexamethasone and 0.5 mg/day of DOCA. They were given 2-3 weeks to recover from the effects of surgery and then studied. The daily dose of DOCA was raised to 20 mg; potassium exchange resin was also given for 7 days. When metabolic alkalosis had developed the dose of DOCA was lowered to 0.5 mg/day. When the study was completed all the electrolytes were removed from the dog's diet,. urinary sodium excretion was then monitored as a check

of the adequacy of mineralocorticoid replacement.

C. Six dogs who had recovered from bilateral adrenalectomy were studied. They were placed on a "zero" electrolyte diet and treated with 0.75 mg/day of dexamethasone, 20 mg/day of DOCA, and 20 mg/day, intravenous furosemide. This protocol was followed for six days during which time the observations reported below were made. The animals were then allowed to recover by being placed on the regular kennel diet and maintenance dexamethasone and DOCA. After 2-3 weeks had elapsed the animals were placed on the "zero" electrolyte diet and again treated with 20 mg/day of furosemide. The daily doses of dexamethasone and DOCA were kept at 0.75 mg and 0.5 mg respectively. Measurements were again made over the ensuing six days. The order in which these two protocols were performed was varied from dog to dog.

D. A group of 6 dogs was treated in a fashion identical to that described above in Section A, Group III. When metabolic alkalosis was fully developed all electrolytes were removed from the dog's diet. When the animals elaborated a salt free urine the daily dose of DOCA was reduced to 0.5 mg.

The analytic techniques used to measure Na, K, Cl and HCO_3 were identical to those previously described.[9,10,20]

RESULTS

A. The effects of potassium depletion and high dose DOCA administration for the three groups of animals studied are presented in Figure 3. The mean plasma potassium concentration was 2.3 mEq/L for each of the three groups; the degree of potassium depletion for each group was judged to be about equal. The mean plasma bicarbonate concentration ± SD before and after potassium depletion and DOCA administration is shown for each group. There was a statistically significant increase in bicarbonate concentration in each group. The post-potassium depletion bicarbonate concentrations of 26.1 mEq/L and 29.2 mEq/L in Groups I and II are not significantly different from each other. Both were significantly different from the value of 34.2 mEq/L obtained in Group III.

These data show that the tendency of potassium depletion and mineralocorticoid excess to cause metabolic alkalosis can be significantly modified by variations in salt intake. They are fully in accord with the clearance data, cited above, that show bicarbonate reabsorption in the potassium depleted dog to be depressed by volume expansion with saline.

The results obtained from one of the dogs in Group III are presented in Figure 4. Potassium depletion with exchange resin and DOCA resulted in a marked drop in the plasma potassium concentration and a rapid rise in bicarbonate concentration to well in excess of

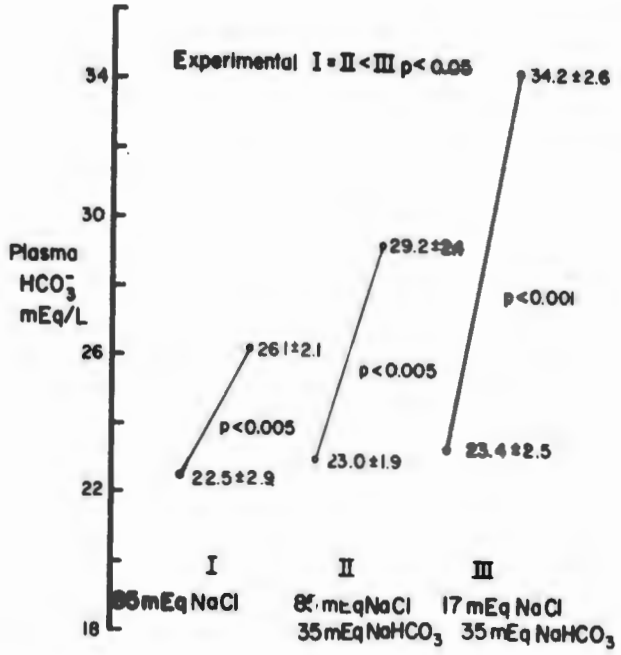

Figure 3. The effect of potassium depletion on plasma bicarbonate concentration in three groups of dogs receiving varying sodium intakes.

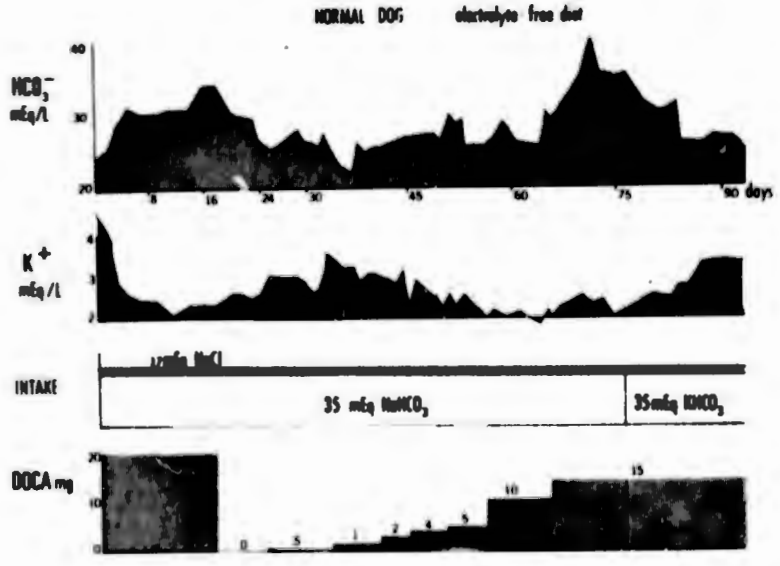

Figure 4. The effect of potassium depletion and repletion, and that of varying doses of DOCA on plasma bicarbonate concentration.

30 mEq/L. When DOCA was withdrawn metabolic alkalosis rapidly corrected. Metabolic alkalosis did not redevelop when the dose of DOCA was gradually increased until a level of 15 mg/day was reached. In none of the dogs studied were we able to induce metabolic alkalosis, using this model, with a dose of DOCA less than 10 mg/day. The changes in plasma potassium concentration noted during the period of increasing DOCA dosage are due to shifts of potassium out of cells as the diet contained no potassium. When metabolic alkalosis had redeveloped the dose of DOCA was kept constant at 15 mg/day and $NaHCO_3$ was removed from the diet with an equal amount of $KHCO_3$ (35 mEq/day) substituted. This resulted in an increase in plasma potassium concentration and a fall in bicarbonate concentration from over 35 mEq/L to control levels. Thus metabolic alkalosis was completely corrected even though there was no change in the anion content of the dog's diet. Similar results were obtained from the other animals in this group. Of great interest is the observation that all of these animals excreted urine that contained no potassium during periods of maximal potassium depletion; this despite the continued administration of large amounts of DOCA and the excretion of large amounts of sodium.

B. The results obtained from one of the adrenalectomized dogs subjected to potassium depletion as described in Group III above is presented in Figure 5. When the potassium depletion and metabolic alkalosis had been generated the dose of DOCA was reduced to 0.5 mg/day. This dose was sufficient to allow all of these dogs to promptly elaborate a sodium free urine when placed on a salt free diet at the conclusion of the study. They elaborated this salt free urine while still markedly potassium depleted thus failing to show any defect in NaCl reabsorption in the presence of potassium depletion as has been demonstrated in the rat.[21] This difference in our study in the dog as contrasted with the earlier one in the rat may lie in the fact that the ability of our potassium depleted animals to conserve salt was always tested on the background of maintenance DOCA treatment. Potassium depletion may result in hypoaldosteronism which in turn results in salt wastage. This group of animals was studied to eliminate the possibility that the correction of metabolic alkalosis seen in Group A following discontinuance of DOCA was due to aldosterone deficiency induced by the effect of potassium deficiency on the zona glomerulosa of the adrenal. As is apparent from the figure this animal (and all the others so studied) corrected its metabolic alkalosis when the dose of DOCA was reduced from the elevated range of 20 mg/day to the maintenance range of 0.5 mg/day. As in the previous group there was no change in the electrolyte composition of the animal's diet nor was there any correction of its potassium deficit.

C. The results obtained from one of the animals in Group C are presented in Figure 6. When this adrenalectomized animal was treated with furosemide, an electrolyte free diet, and 20 mg/day of DOCA it developed metabolic alkalosis. The data points for this protocol are

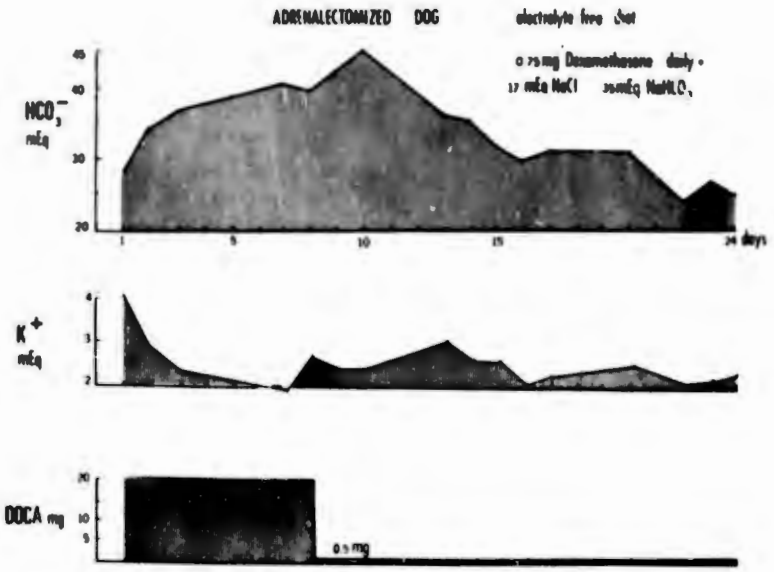

Figure 5. The effect of decreasing the dose of DOCA on plasma bicarbonate concentration in a potassium depleted, adrenalectomized dog.

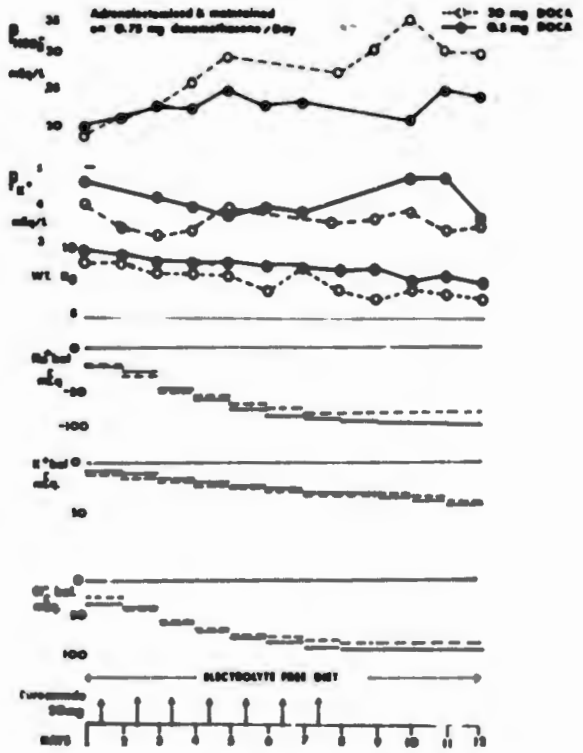

Figure 6. Balance data from an adrenalectomized dog. The open circles and dotted lines plot the data obtained while the dog received 20 mg/day of DOCA, that obtained while the dog received 0.5 mg/day is plotted with closed circles and dotted lines.

plotted using open circles and dashes. When the identical protocol was repeated save that the dose of DOCA was kept at the replacement level of 0.5 mg/day (these data are plotted using closed circles and solid lines) metabolic alkalosis did not develop despite the fact that sodium, potassium, and chloride balances were virtually the same during both protocols. Six animals were studied in the fashion just outlined. Mean plasma bicarbonate at the end of the maintenance DOCA periods was 25.1 ± 2.1(S.D. mEq/L; mean bicarbonate concentration at the end of the high DOCA periods was 30.5 ± 2.4 mEq/L $p < 0.05$. These data show that this form of diuretic induced metabolic alkalosis did not develop in the absence of increased amounts of mineralocorticoid hormone. It did, however, persist, once generated, when the dose of DOCA was lowered from 20 mg/day to 0.5 mg/day.

The observation that metabolic alkalosis will be maintained in the presence of an amount of mineralocorticoid insufficient to generate it in the presence of volume contraction is further documented below.

D. Six dogs were treated in a fashion identical to those described in Section A, Group III. When metabolic alkalosis was fully developed sodium intake was dropped to zero. When urinary sodium excretion was zero the dose of DOCA was dropped to 0.5 mg/day. One such study is presented in Figure 7. As is apparent, dropping the dose of DOCA from 20 to 0.5 mg/day had no effect on plasma bicarbonate concentrations. When the dose of DOCA was similarly dropped in animals in which the diet and urine contained generous amounts of sodium metabolic alkalosis rapidly corrected. This lack of responsiveness of bicarbonate concentration to a drop in DOCA dose in the presence of sodium restriction was seen in all 6 animals so studied.

DISCUSSION

Bicarbonate is reabsorbed in both the proximal and distal tubules. The distal mechanism is at least in part under the control of aldosterone.[20] The proximal mechanism is independent of aldosterone; it is regulated by effective extracellular volume and the level of potassium stores (CO_2 tension also regulates proximal bicarbonate reabsorption,[10] but plays no primary role in metabolic alkalosis). Bicarbonate generation is essentially limited to the distal tubule. At this site in the nephron, when sodium bicarbonate reabsorption is virtually complete, Na^+ for H^+ exchange will result in increased net acid excretion by the formation of titratable acidity and NH_4^+; an increase in net acid excretion results in the return of new bicarbonate to the circulation. Thus, if plasma bicarbonate concentration is to be elevated as a consequence of renal generation of bicarbonate an accelerated distal mechanism is necessary; therefore increased amounts of mineralocorticoid are necessary. Since the distal exchange site exchanges either H^+ or K^+ for Na^+, potassium deficiency is necessary for maximal distal hydrogen ion secretion and thus maximal bicarbonate generation. It is also obvious that adequate amounts of sodium must be

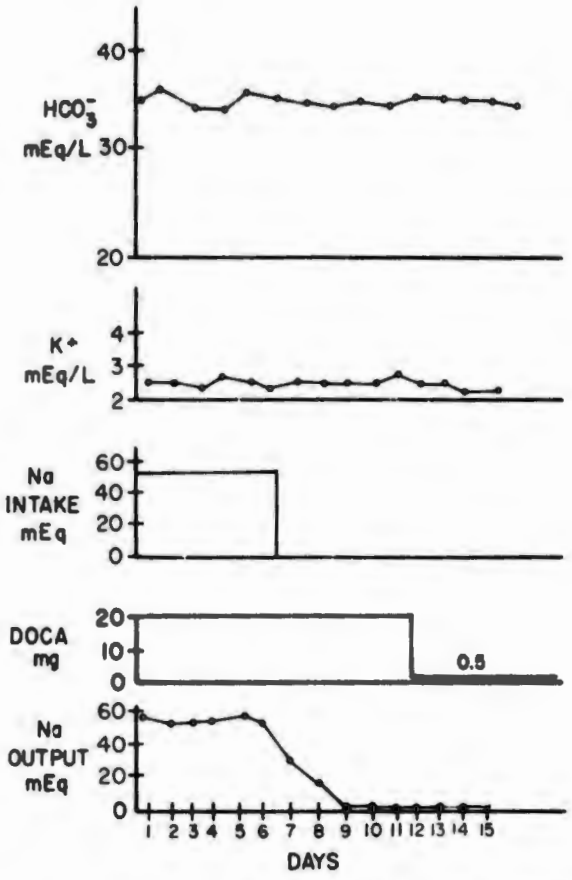

Figure 7. Balance data showing the effect of reduction of sodium and DOCA intake.

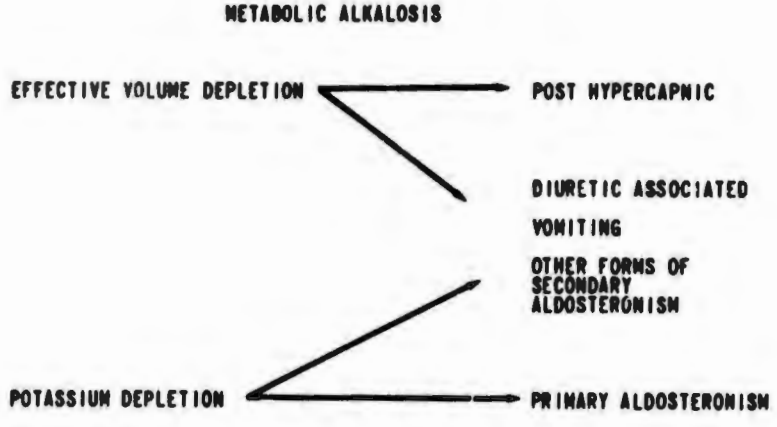

Figure 8. The relationship of ECV and potassium depletion of the maintenance of common types of metabolic alkalosis.

presented to the distal exchange site for maximal renal bicarbonate generation to take place.

These points are strikingly illustrated by the studies presented in Sections A and B. Potassium depletion with high dose DOCA administration results in an increase in plasma bicarbonate concentration (Fig. 3) because of increased distal H^+ secretion. The degree to which bicarbonate concentration rises depends on the salt content of the animals' diet. The more salt ingested the greater the extent of ECV expansion; ECV expansion depresses proximal bicarbonate which results in a lower steady state bicarbonate concentration for any one level of potassium depletion.

The dependence of renal bicarbonate generation on both potassium deficiency and mineralocorticoid excess is demonstrated in Figure 4. Withdrawal of high dose DOCA treatment or correction of potassium deficiency resulted in full correction of metabolic alkalosis. Similarly, metabolic alkalosis did not redevelop until the dose of DOCA had been raised to a high level. Thus, both the generation and maintenance of metabolic alkalosis in this model are the consequence of accelerated distal H^+ secretion. Proximal reabsorption plays little role in maintaining the alkalosis because DOCA induced salt retention and ECV expansion depresses proximal reabsorption countering the stimulatory effect of potassium deficiency.

The proximal tubule plays a role in maintaining metabolic alkalosis when ECV is contracted. The animals studied in Sections C and D were able to maintain metabolic alkalosis, once generated, even when the dose of DOCA was reduced to the low level of 0.5 mg/day.

Thus, those forms of metabolic alkalosis associated with ECV contraction require accelerated distal H^+ secretion to develop, but may be maintained almost entirely as a consequence of enhanced proximal H^+ secretion. Since aldosterone has no regulatory role over proximal bicarbonate reabsorption excess mineralocorticoid activity is not necessary to maintain metabolic alkalosis provided a stimulus to proximal bicarbonate reabsorption (ECV contraction) is present.

The stomach may generate excess bicarbonate via vomiting. Obviously, the generation of metabolic alkalosis through this mechanism will not require hyperaldosteronism. Since vomiting results in ECV contraction proximal bicarbonate reabsorption will be stimulated and metabolic alkalosis maintained. The administration of saline will expand ECV, depress proximal reabsorption, result in bicarbonate diuresis and correction of metabolic alkalosis.

The role of ECV depletion and potassium deficiency in maintaining the common clinical forms of metabolic alkalosis is presented in Figure 8. Post-hypercapnic metabolic alkalosis results from the correction of respiratory acidosis in a patient with ECV

contraction. The kidney generates increased amounts of bicarbonate as a compensatory response to hypercapnia. When the hypercapnia is relieved the kidney is unable to excrete the excess bicarbonate because ECV contraction has stimulated proximal bicarbonate reabsorption. Increasing the amount of salt in the patient's diet or infusing saline corrects this form of metabolic alkalosis by expanding ECV and depressing proximal bicarbonate reabsorption. If the patient has another stimulus to proximal reabsorption such as heart failure or hypoproteinemia this form of metabolic alkalosis will not relent until <u>affective</u> extracellular volume is expanded by treating the primary disorder.

Those types of metabolic alkalosis associated with diuretic therapy are maintained by a combination of volume depletion and potassium deficiency. The metabolic alkalosis of vomiting is maintained primarily because of volume contraction; potassium deficiency, in many patients, plays a minor role in maintaining this type of alkalosis. The relative roles of volume contraction and potassium deficiency in preventing the correction of metabolic alkalosis in other forms of secondary aldosteronism vary depending upon the individual circumstances.

Potassium deficiency, with mineralocorticoid excess, plays the sole role in maintaining the metabolic alkalosis seen in patients with primary aldosteronism (or other diseases in which the primary abnormality is steroid excess). Accelerated distal generation of bicarbonate is responsible for the generation of this type of metabolic alkalosis and accelerated distal bicarbonate reabsorption maintains it. This is the reason that this type of alkalosis fails to respond to volume expansion with saline. Proximal reabsorption is already depressed (considering the degree of potassium depletion present) due to mineralocorticoid induced salt retention and ECV expansion.

When potassium depletion is very severe volume expansion with saline cannot fully correct the associated metabolic alkalosis.[22] Volume expansion depressed proximal bicarbonate reabsorption but it is still set so high that the alkalosis persists. Potassium repletion is required to depress proximal bicarbonate reabsorption in these patients sufficient to allow correction of their alkalosis.

In summary, metabolic alkalosis generated via the kidney requires the presence of hyperaldosteronism and potassium deficiency which together cause increased distal H + for Na + exchange. Hyperaldosteronism is not required for the kidney to maintain metabolic alkalosis once generated if a stimulus for proximal bicarbonate reabsorption is present. Both effective extracellular volume contraction and potassium deficiency stimulate proximal bicarbonate reabsorption.

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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 OC 6954	72 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
71 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	3A061102B71R	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 72	0.5	18.5
FISCAL YEAR CURRENT 73	0.5	15.0

19. RESPONSIBLE DOD ORGANIZATION
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NAME:

21. GENERAL USE: FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code): (U) Bicarbonate Reabsorption; (U) Sodium; (U) Extracellular Volume; (U) Potassium; (U) ADH; (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 hemeostasis are extremely common in injured or ill troops. These disorders are perpetuated, compensated, or corrected by changes in renal bicarbonate reabsorption. This study was undertaken to examine the processes that regulate renal bicarbonate reabsorption.

24. (U) Thus far in this project, the role of effective extracellular volume, acute respiratory acidosis, potassium deficiency, potassium excess and aldosterone deficiency on renal bicarbonate reabsorption have been examined. Work is in progress to examine the role of cylic AMP, parathormone, acetyl-cholene, oxytocin, vasopressin (ADH), prostaglandins on renal bicarbonate reabsorption.

25. (U) 71 07 - 72 06 Thus far we have found that changes in extracellular volume markedly effect the response of renal bicarbonate reabsorption to the other variables mentioned above but when extracellular volume is controlled, acute respiratory acidosis, and potassium deficiency increase bicarbonate reabsorption, while potassium excess depresses bicarbonate reabsorption. ADH in large doses has been shown to be as potent a diuretic agent as ethacrynic acid. It has no effect on bicarbonate reabsorption indicating that its diuretic effect results from inhibition of sodium reabsorption in the ascending limb of the loop of Henle.

*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. 3A061102B71R-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

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

1 July 1971 - 30 June 1972

Investigators:

Neil A. Kurtzman, MD, Lieutenant Colonel, MC
Philip W. Rogers, MD, Major, MC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A061102B71R-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

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

Period covered in this report: 1 July 1971 - 30 June 1972

Investigators: Neil A. Kurtzman, MD, Lieutenant Colonel, MC
Philip W. Rogers, MD, Major, MC

We have previously shown that effective extracellular volume is such a potent regulator of renal bicarbonate reabsorption that the other principle regulators cannot be studied effectively if one does not precisely specify the level of extracellular volume at which one is working. By controlling volume as we have defined the regulatory role of potassium, CO_2 tension, and carbonic anhydrase activity on bicarbonate reabsorption.

The emphasis of work currently being carried out is to define the mechanism by which volume expansion depresses bicarbonate reabsorption. The role of a variety of naturally occurring humoral agents on bicarbonate reabsorption is to be assessed. The first of these, Vasopressin (ADH), in pharmacologic doses is markedly chloriuretic but does not effect bicarbonate reabsorption, indicating that its main site of action in the nephron is distal to the proximal tubule, presumably the ascending limb of the loop of Henle.

Bicarbonate reabsorption
Sodium
Extracellular volume
Potassium
ADH

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
				DA 0D 6978	72 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
71 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	3A061102B71R	01	194
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Procede 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 (Thousands)
FISCAL YEAR	PRECEDING 72	0.3	8.5
	CURRENT 73	0.2	7.0

19. RESPONSIBLE DOD ORGANIZATION
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NAME: R B Lindberg, PhD DA

21. GENERAL USE: FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Procede 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. Procede 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. Clincal 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) 71 07 - 72 06 No significant temperature elevations have been noted in patients draped with this material. Modification of the drape material has improved its draping characteristics and based on use of drapes made of this material in 50 operative cases, it is well accepted by surgeons. Bacteriologic testing of the latest form of sheeting has shown no penetration of staphylococci, Klebsiella, Enterobacter, Providencia or Pseudomonas broth culture fluid during a one hour exposure confirming the excellent bacterial barrier properties of surgical drapes made of this synthetic sheeting material.

*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. 3A061102B71R-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 1971 - 30 June 1972

Investigators:

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Thomas L. Hudson, MD, Colonel, MC *
Robert B. Lindberg, PhD

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Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A061102B71R-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 1971 - 30 June 1972

Investigators: Joseph A. Moylan, Jr., MD, Major, MC
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Thomas L. Hudson, MD, Colonel, MC *
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Reports Control Symbol MEDDH-288(R1)

Asepsis in the operating room is one of the major factors in the prevention of wound infections. An effective bacterial barrier to prevent contamination of the operative site from nonsterile areas has long been needed as cloth drapes have been shown to be permeable to bacterial penetration, especially when wet.

Available disposable operating room drapes are waterproof and do prevent bacterial transgression. However, these materials have poor draping qualities, may produce hyperthermia and allow unimpeded "run-off" of blood and irrigating solutions onto the operating room floor or onto the operating team.

A new sheeting material made of a spun-bonded olefin-cellulosic laminate has been evaluated for its draping characteristics, absorbency, physician acceptance, and bacterial barrier in 50 general surgical cases. Cultures using sterile rodac plates were taken at the margins of the operative field immediately following draping and at the completion of the cases. No wound infections were noted in this series, and nonpathogens in low colony counts were cultured postoperatively from the drape with Staphylococcus albus predominating. The draping qualities were comparable to cloth and no significant temperature elevations were observed. Laboratory testing of the material showed no bacterial penetration of Staphylococcus aureus, Klebsiella, Enterobacter, Providencia, and Pseudomonas after a 4-hour exposure to broth culture fluid containing these organisms, confirming the excellent bacterial barrier properties of the drape.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0D 6384	72 07 01	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. D[illegible]N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
71 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	3A061102B71R	01	312
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) Identification of Presence of Stress in Nursing Personnel Caring for Critically Ill Military Patients (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
70 07	72 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 72	0.9	25.2
	CURRENT 73	0	0

19. RESPONSIBLE DOD ORGANIZATION
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ASSOCIATE INVESTIGATORS
NAME:
NAME:

21. GENERAL USE: FOREIGN INTELLIGENCE NOT CONSIDERED

DA

22. KEYWORDS (Precede EACH with Security Classification Code): (U) Stress; (U) Intensive care; (U) Nursing; (U) Social psychology

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

23. (U) To determine if prolonged exposure to critically Ill individuals, such as patients with burns, in an intensive care situation leads to signs of increased stress in nursing personnel.

24. (U) The approach to this study was to test each subject within the first 48 hours of assignment to a general work area in Brooke General Hospital or Ward 14A. Each subject was retested at the end of three or six months.

25. (U) 71 07 - 72 06 Sixty subjects, 20 ANC and 40 Clinical Specialists were tested in the study. Four officers and 11 Specialists were tested. The final division of subjects was that 10 ANC and 20 Specialists were assigned to Ward 14A, and the same numbers were assigned to the general wards in Brooke General Hospital.

All data were collated, and analyses of variance were performed on the scores of each subject for the two tests used - modified Nowlis and Green Mood Adjective Check List and the Hand-Steadiness Test. No significant differences were found between individuals working in an intensive care unit versus those working on general hospital wards. No differences were noted between the two subgroups retested at the end of 3 months and remaining two subgroups retested at the end of 6 months. Further analyses were carried out on the data comparing ANC and Specialists. No significant differences were found. A multiple regression coefficient will be done at a later time on the eight factors in the MACL.

Unobstrusive measures are being reviewed for their role in making differences between experimental and control groups. Such facets as hostile humor, peaks and valleys of attitudes, mutual support, and general military patterns are under consideration. Planning of nursing personnel off-duty time and rotational work patterns would appear to be an important morale factor.

*Available to contractors upon originator's approval

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FINAL REPORT

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

REPORT TITLE: IDENTIFICATION OF PRESENCE OF STRESS IN NURSING PERSONNEL CARING FOR CRITICALLY ILL MILITARY PATIENTS

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

1 July 1971 - 30 June 1972

Investigator:

Lois A. Johns, Lieutenant Colonel, ANC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

REPORT TITLE: IDENTIFICATION OF PRESENCE OF STRESS IN NURSING PERSONNEL CARING FOR CRITICALLY ILL MILITARY PATIENTS

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

Period covered in this report: 1 July 1971 - 30 June 1972

Investigator: Lois A. Johns, Lieutenant Colonel, ANC

Reports Control Symbol MEDDH-288(R1)

This study was designed to determine if prolonged exposure to critically ill individuals in an intensive care situation, such as patients with burns, led to signs of increased stress in nursing personnel. Sixty subjects (20 Army Nurse Corps officers and 40 clinical specialists) were tested in the study. All data were collated, and analyses of variance were performed on the scores of each subject for the two tests used--modified Nowlis and Green Mood Adjective Check List and the Hand-Steadiness Test. No significant differences were found between individuals working in an intensive care unit versus those working in general hospital wards. No differences were noted between the 2 subgroups retested at the end of 3 months and the remaining 2 subgroups retested at the end of 6 months. Further analyses were carried out on the data comparing Army Nurse Corps officers and that of clinical specialists. No significant differences were found between these groups.

Stress
Intensive care
Nursing
Social psychology

IDENTIFICATION OF PRESENCE OF STRESS IN NURSING PERSONNEL CARING FOR CRITICALLY ILL MILITARY PATIENTS

A study has been completed to determine if prolonged exposure to critically ill individuals in an intensive care situation, such as caring for burned patients, led to signs of increased stress in nursing personnel.

Numerous studies have been done to measure physiological and psychological indicators of stress. Articles in nursing and medical journals have expressed concern about stress placed on nursing personnel. Publications concerning stress and nursing personnel have been philosophical and rarely refer to objective studies. Vreeland and Ellis have made observations of nursing personnel in an intensive care unit but did not carry out a planned study. Cleland, in a field study, showed that a little stress improved nursing performance, but mounting stress made performance drop dangerously.

Stress is some physiological, chemical, or emotional factor to which an individual fails to make a satisfactory adjustment which causes physiologic tensions. In this study, stress was defined as the work experiences of each subject from the time he was first tested until the time of his second testing.

Recommendations have been made that personnel working in intensive care units be relieved from this work area periodically. However, to develop nursing proficiency in a specialty care area, experience is necessary. Time and exposure are essential to acquire experience.

If indicators of stress in an intensive care area can be measured, steps may then be taken to decrease or to divert some of the causes of this stress. Hopefully, this would lead to improved personnel performance and improved nursing care. Guidelines for assignment could lead to an increased performance of nursing personnel and aid in the development of needed educational and staffing program.

Two groups of 30 subjects were used as the study sample. Each group was subdivided into two sections of 15 subjects. Each section was composed of five Army Nurse Corps (ANC) officers and 10 Clinical Specialist (91C) enlisted men. The experimental group was composed of nursing personnel assigned to the intensive care ward of the US Army Institute of Surgical Research (ISR). The members of the control group were assigned to Brooke General Hospital (BGH) in areas other than intensive care or coronary care units.

The 91C subjects had a range of hospital experience as licensed vocational nurses of 0-12 years with a mean experience of two years.

Their ages ranged from 20 to 43 with average age of 29. The average grade was that of Specialist 5th Class with a range from Specialist 4th Class to Sergeant 1st Class. Of interest was the random occurrence, within experimental group 1, of no clinical experience of 91C individuals.

The officer group had male and female subjects who ranged in nursing experience from 0-16 years, with a mean experience of 4 years. The age range was from 22-43 with a mean age of 29. The ranks considered were 2LT to MAJ with the average rank of these subjects being 1Lt.

Each subject was tested immediately after assignment to a work area in Brooke General Hospital or to Ward 14A, ISR. He was given a modified, 40 word, form of the Nowlis and Green Mood Adjective Check List (MACL). Each of the 8 factors in the MACL had 5 words in it. A form of the Hand-steadiness Test was used in which each subject was tested for 3 5-minute periods with a reset period of one minute between trials one and two, and two and three.

Predictions were that individuals assigned to the Intensive Care Ward of the ISR would show significantly higher levels of stress as measured by the 2 testing devices than would the subjects assigned to Brooke General Hospital, and that significant differences would be demonstrated between the 2 groups over time. Multiple regression coefficients were to be used for predictions of potential success or failure within the intensive care area if a computer program was possible to prepare. If lack of significant differences between the groups was found, a review of unobstrusive measures was planned.

All data were submitted to analysis of variance testing. No significant differences were found between the 2 groups, between the ANC officers, nor between the 91C enlisted men for location of assignment or over time (See Tables 1-6, inclusive).

No multiple regression coefficients have been done on the 8 factors of the MACL. This will be done at a future time, and it is predicted that significant negative correlations would be indicative of differences between ICU and non-ICU personnel.

The nonsignificant results of this study indicate that the instruments used were not capable of measuring stress as defined for purposes of the study. Many studies of stress define the term in accord with study hypotheses and quantify the results in terms of the stresses used and/or the measure used.

Cleland in his reports indicated that professional nurses in planned nursing service situations could be tested for levels of stress using a paper and pencil device. The results of his study are

TABLE 1. MOOD ADJECTIVE CHECK LIST

SOURCE	SS	df	MS	F
TIME	476.02	1	476.02	1.550
LOCATION	176.82	1	176.82	0.576
INTERACTION	8.82	1	8.82	
ERROR	17192.93	56	307.02	
TOTAL	17854.58	59	302.62	

TABLE 2. HAND STEADINESS TEST

SOURCE	SS	df	MS	F
TIME	156.82	1	156.82	0.18
LOCATION	.0167	1	.0167	.0002
INTERACTION	1570.82	1	1570.82	
ERROR	48176.93	56	860.30	
TOTAL	49904.58			

TABLE 3. ANC OFFICERS MOOD ADJECTIVE CHECK LIST

SOURCE	SS	df	MS	F
TIME	288.80	1	288.80	1.12
LOCATION	80.00	1	80.00	.309
INTERACTION	259.20	1	259.20	
ERROR	4138.80	16	258.67	
TOTAL	4766.80	19	250.88	

TABLE 4. ANC OFFICERS HAND STEADINESS TEST

SOURCE	SS	df	MS	F
TIME	414.05	1	414.05	.384
LOCATION	2020.05	1	2020.05	.187
INTERACTION	2.45	1	2.45	
ERROR	17266.00	16	1079.125	
TOTAL	19702.55	19	1036.976	

TABLE 5. 91C MOOD ADJECTIVE CHECK LIST

SOURCE	SS	df	MS	F
TIME	216.23	1	216.23	.636
LOCATION	99.23	1	99.23	.292
INTERACTION	60.03	1	60.03	
ERROR	12236.30	36	339.90	
TOTAL	12611.77	39	323.38	

TABLE 6. 91C HAND STEADINESS TEST

SOURCE	SS	df	MS	F
TIME	202.50	1	202.50	.325
LOCATION	270.40	1	270.40	.434
INTERACTION	3960.10	1	3960.10	
ERROR	22430.60	36	623.07	
TOTAL	26863.60	39	688.81	

in question when viewed with the results of this study. Cleland's work was done with specific personnel in specific work situations while the present study attempted to test individuals working in a day-to-day stressful situation.

A disappointing facet of this study was an apparent inability to use the testing instruments as a criterion for selection of personnel more likely to be successful workers in an intensive care unit. A study by Mefferd, et al, would encourage further investigation along these lines for use by the Army Nurse Corps and the Army Medical Department for personnel selection in specialized units.

Evaluation of differences between the two groups studied must be done on the basis of unobstrusive measures. Application of such measures to ICU personnel included noting an extremely low sick-call rate, no instances of absence without leave, and the observation of developing pride in being a member of a special military organization. The latter has been evidenced many times by unelicited comments of nursing service personnel assigned to flight status, by responses of personnel to questions asked them by non-unit personnel, and by parties planned by personnel for all Burn Study Branch personnel regardless of rank or position.

Observation of personnel in the Burn Study Branch has indicated a triangular physician-nurse-corpsman support relationship. The mutual support given by members of the care team is notable for the psychological as well as physical aspects of it.

Nursing personnel have demonstrated ways in which one can determine stress indicators. Personnel request 3 to 4-day weekends off, although scheduling practices are to have these times available in a pattern. Frequent short periods of leave are used as a pattern by civilian personnel. Personnel request areas of assignment at times, e.g., not being assigned into the Intensive Care Cubicle but rather to an open ward, or they have requested to be assigned to the Convalescing Burn Care Ward. Some personnel have had previous records of working well within an ICU situation but have found themselves unable to work with the acutely ill, thermally-injured person. In an effort to cope with the aforementioned situation, a carefully planned orientation and rotational work pattern is carried out under the supervision of the chief nurse so individuals will secure adequate experience in and knowledge of the care of the burned person.

A cyclic pattern of behavior can be noted and observed in the nursing service personnel. The pattern has a direct relationship to the number and type of acutely ill burned persons. A purposeful tenseness is often noted when nursing personnel commit themselves to the care of a given patient together with the physician as the team leader in meeting the patient's physiological and psychological needs. When such a patient dies, the nursing staff noticeably

responds. Hostile humor, as noted by LaGaipa, also has a role in the sense of failure evidenced by the staff's cyclic downswing. A complete, observational study of the cyclic behavioral pattern of the nursing staff would be very useful.

A repetition of this study using different measurement methods of greater sensitivity might well detect evidence of stress differences related to an intensive care situation and be of use in Army Nurse Corps personnel duty assignment actions.

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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 OC 6971	72 07 01	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. SUMM INSTR'N	8a. SPECIFIC DATA CONTRACTOR ACCESS	9. LEVEL OF SUM
71 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	3A061101A91C	00	075
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede 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
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17. CONTRACT/GRANT: Not Applicable
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c. TYPE — d. AMOUNT
e. KIND OF AWARD — f. CUM. AMT.

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
FISCAL YEAR	PRECEDING 72	0.6	9.7
	CURRENT 73	0.6	8.0

19. RESPONSIBLE DOD ORGANIZATION
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SOCIAL SECURITY ACCOUNT NUMBER:

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NAME: Kenneth W Spitzer, CPT, MSC
NAME: DA

21. GENERAL USE: Foreign Intelligence Not Considered

22. KEYWORDS (Precede each with Security Classification Code): (U) Wounded Soldiers; (U) Burns; (U) Lung Mechanics; (U) Pulmonary Diffusion; (U) Ventilation/Perfusion Abnormalities; (U) Blood Gases; (U) Shunt; (U) PV Work

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 natural history of lung function in burned patients to elucidate the pathogenesis of pulmonary complications early postburn.

24. (U) Spirometry; arterial blood gases (room air and 100% oxygen); minute ventilation; tidal volume; respiratory rate; oxygen consumption; respiratory quotient; static lung compliance; dynamic lung compliance; airways resistance; pressure-volume work of breathing; lung volumes (nitrogen washout); lung clearance index; carbon monoxide diffusing capacity were studied serially.

25. (U) 71 07 - 72 06 An additional 24 acutely burned patients have been studied (total 37 patients). Eighteen of the 37 patients studied had a dramatic increase in minute ventilation; 10 patients had no increase in minute ventilation. The indices measured were similar within the two groups with the exception that static lung compliance was more severely depressed in those patients with increased minute ventilation. Hypoxemia, hypercapnea, and acidosis were absent (in 2 patients, the spinal fluid pH and partial carbon dioxide pressure were equally unremarkable). Diffusing capacity for carbon monoxide and lung clearance index were normal. No significant "shunting" was present. Consistent abnormalities were found in lung mechanics with vital capacity either normal or slightly depressed and dynamic lung compliance normally related to the static compliance with no further decrease with increasing respiratory rates. The pressure volume work of breathing was usually high normal or slightly elevated. Lung resistance was normal. Dyspnea was denied. We conclude that this clinical state, previously undescribed, differs from "shock lung," or "trauma lung;" and the driven respiration is due to an extrapulmonary cause and not to a primary derangement of the lung itself.

(*Available to contractors upon originator's approval)

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ANNUAL PROGRESS REPORT

PROJECT NO. 3A061101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: PULMONARY PATHOPHYSIOLOGIC CHANGES FOLLOWING THERMAL INJURY IN BURNED SOLDIERS

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

1 July 1971 - 30 June 1972

Investigators:

Alan H. Morris, MD, Major, MC
Kenneth W. Spitzer, Captain, MSC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A061101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

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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 1971 - 30 June 1972

Investigators: Alan H. Morris, MD, Major, MC
Kenneth W. Spitzer, Captain, MC

Reports Control Symbol MEDDH-288(R1)

Multiple indices of lung function have been serially determined in 35 patients during the early postburn period. Those patients with burns exceeding 40% of the total body surface (and one patient with a 35% total body surface burn) developed a dramatic hyperpnea and tachypnea, usually between the fourth and the eighth postburn days, without dyspnea, and with frequently normal chest x-rays and physical examinations. This preceded by several days the development of pulmonary infiltrates, abnormal physical examinations, and the clinical appearance of obvious intrapulmonary disease. Arterial hypoxemia is absent, arterial Pco_2 values are low, and arterial pH values are usually normal to alkaline, in both groups. Significant right to left shunting is absent, and diffusing capacity for carbon monoxide, as well as the lung clearance index, is normal. In contrast with the well preserved indices reflecting gas transfer, and distribution of inspired air, the mechanical properties of the lung appeared to be almost uniformly deranged. The static lung compliance, and the dynamic lung compliance, were reduced in both groups of patients, more severely in those with burns exceeding 40% of the total body surface. The abnormal compliance, however, did not correlated with the marked change in minute ventilation. These observations have led us to conclude that the observed hyperpnea is due to an extrapulmonary cause. The absence of dyspnea, hypoxemia, right to left shunting, and extensive pulmonary infiltrates, make this clinical picture distinctly different from that of "shock lung", and related syndromes.

Burns
Lung mechanics
Pulmonary diffusion
Ventilation/perfusion abnormalities
Blood gases
Shunt

PULMONARY PATHOPHYSIOLOGIC CHANGES FOLLOWING THERMAL INJURY IN BURNED SOLDIERS

Because of the frequency and severity of pulmonary problems following thermal injury, and because of the paucity of information concerning lung function during this period, we have undertaken an investigation of lung function following severe burns. The first clinical suggestion of pulmonary difficulty has often been the dramatic development of tachypnea and hyperpnea on the third to fifth postburn day, preceding by several days the first appearance of abnormalities of the physical or x-ray examination of the chest, and in the absence of dyspnea.

Of the 35 patients, 18 had a definite and large increase in minute ventilation, and 11 had no significant increase in minute ventilation in the early postburn period. The differences between the levels of ventilation in these 2 groups seemed to be quite large from the third or fourth until approximately the 12th postburn day, but were not significant before or after this period (Fig. 1). It became apparent that not only was this dramatic increase in ventilation in some way related to time postburn but that it was also related to burn size. Of 28 patients for whom adequate information is available, the 17 with burns involving more than 40% of the total body surface all had an increase in ventilation (see table). Eleven of these 17 patients died. Of 12 patients with burns of less than 40% of the total body surface, only one with a burn of 35% of his total body surface had an increase in ventilation, and none died.

% TBS	N	$\uparrow \dot{V}_E$	Died
> 40	17	17	11
< 40	12	1	0

Several indices of gas exchange across the lung were studied, and were unremarkable. Arterial oxygen tensions were normal when the patients breathed room air (Fig. 2). Arterial carbon dioxide tensions were low and the pH was slightly alkaline. Arterial oxygen tensions, studied with the patients breathing 100% oxygen, failed to reveal any significant right to left shunting of blood (Fig. 3). The diffusing capacity for carbon monoxide was within normal limits (Fig. 4). The distribution of inspired air was normal and uniform except in preterminal patients as indicated by the lung clearance index (Fig. 5).

In contrast to the indices of gas exchange, those which reflected mechanical properties of the lung were not normal. The static lung compliance was depressed and was more severely decreased in patients with large burns and a striking increase in minute ventilation (Fig. 6). The dynamic compliance was increased in concert with the decrease in static lung compliance, but did not fall further when respiratory rate increased, suggesting that small airways were not severely deranged (Fig. 7). The lung resistance was normal except in one patient with an inhalation injury, suggesting that large airways were not severely narrowed (Fig. 8). As expected from the decrease in lung compliance, the

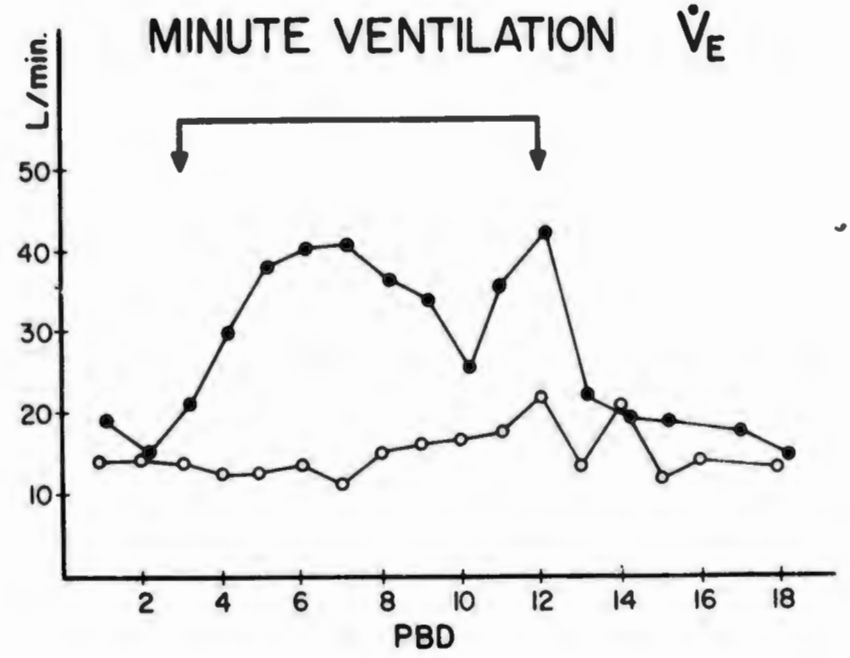

Figure 1

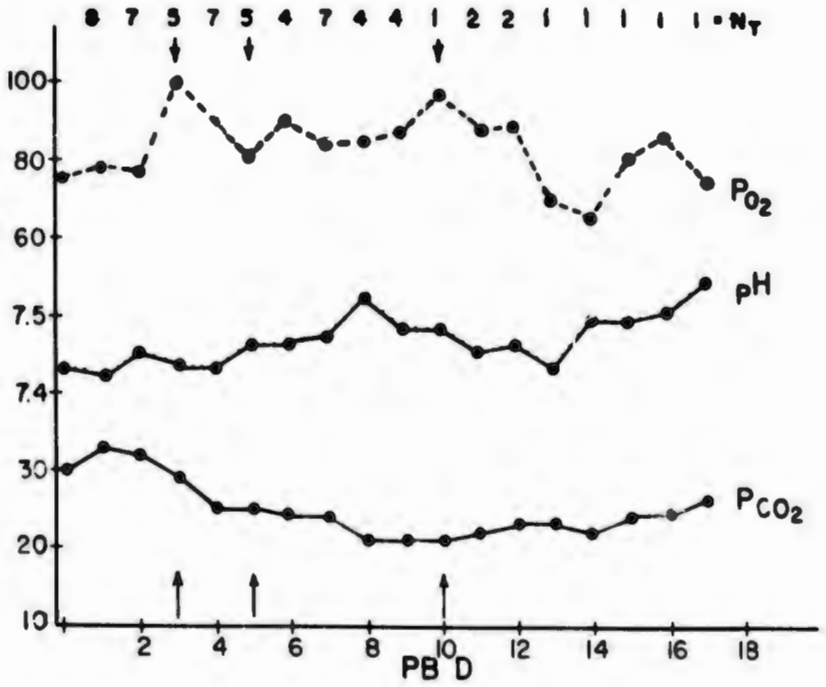

Figure 2

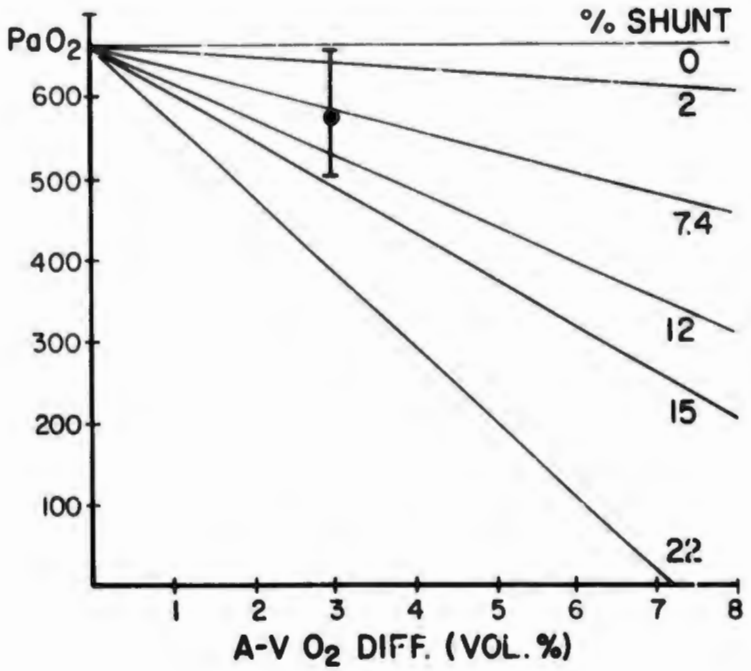

Figure 3

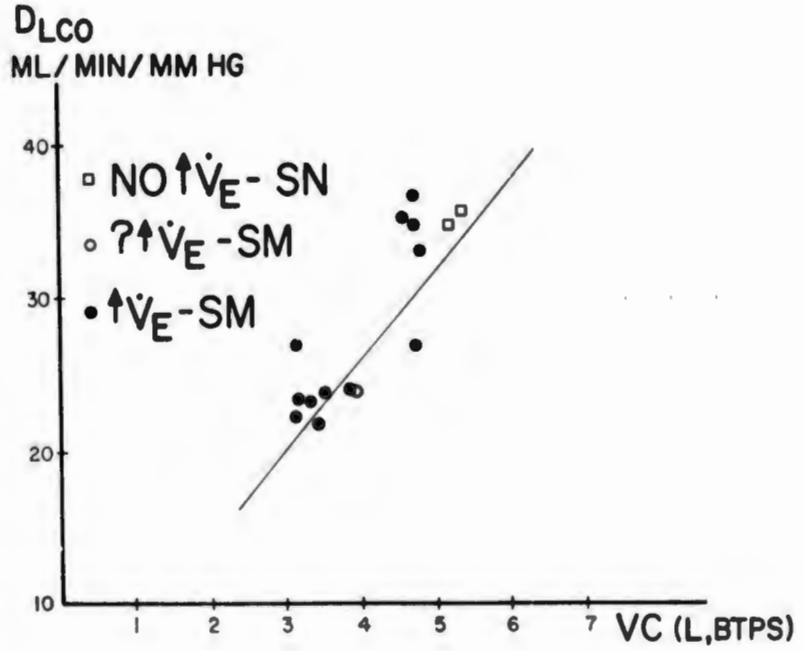

Figure 4

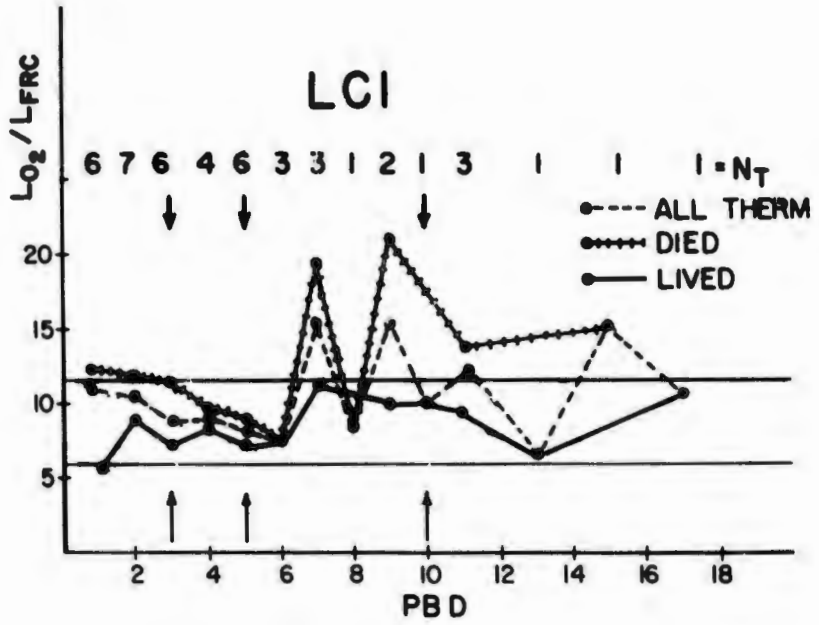

Figure 5

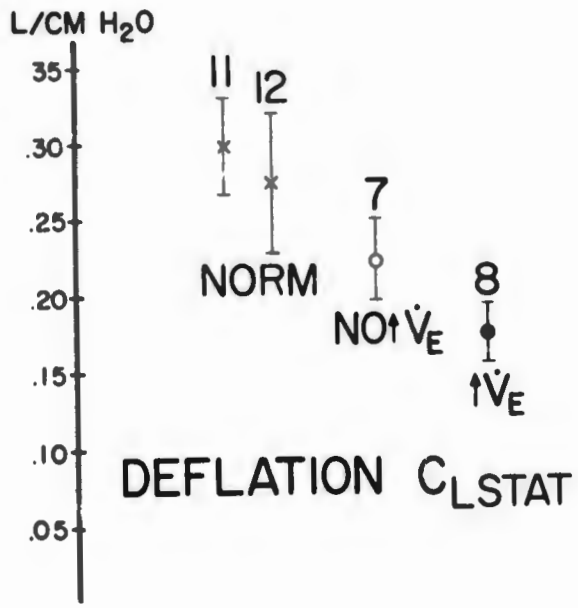

Figure 6

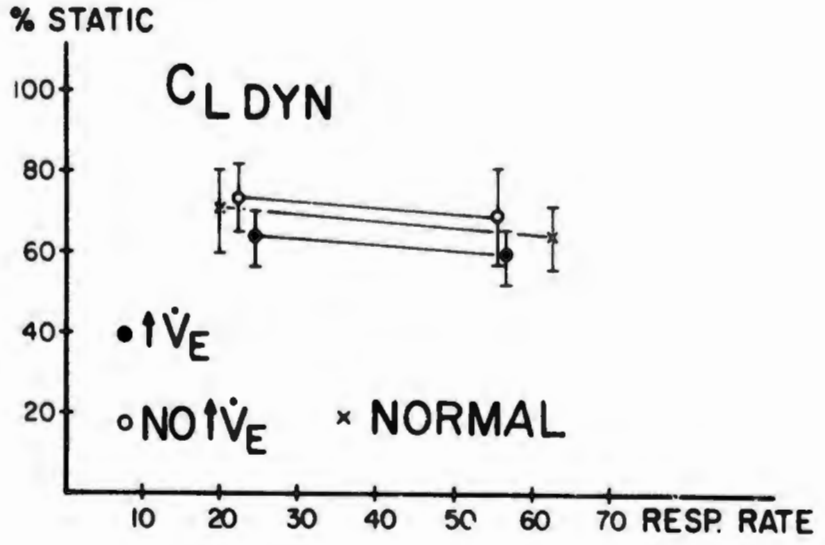

Figure 7

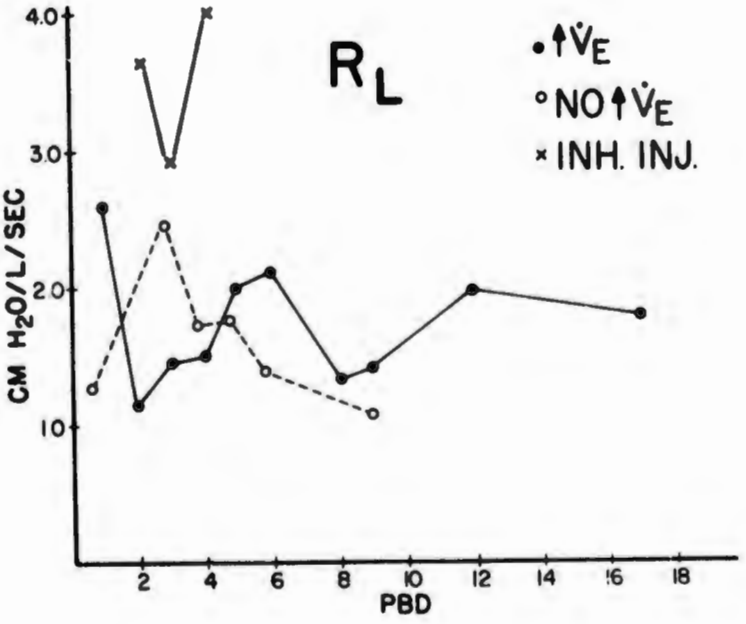

Figure 8

pressure volume work of breathing was increased, but this increase was not dramatic, and approximately half of the measurements in the patients fell within the upper limits of normal for our Laboratory (Fig. 9).

Even though the lung compliance, the only consistently abnormal index of lung function, was frequently decreased, this failed to explain the increase in minute ventilation, as shown by the results obtained in the following 2 characteristic patients. The minute ventilation of a patient's first measurements (as depicted in Fig. 10) with a 60% total body surface burn, rose strikingly and reached a peak of about 50 L/min on the ninth postburn day. During the time when his minute ventilation was strikingly increased, his diffusing capacity for carbon monoxide was normal. His forced vital capacity was approximately 75% of normal, but remained constant. His static lung compliance was reduced, but was not significantly changed throughout this period when the minute ventilation strikingly rose and then fell. The patient was treated with Sulfamylon, which produces, as one of its unwanted effects, carbonic anhydrase inhibition, during the time indicated by the solid bar. Although this is compatible with the conclusion, offered by some, that Sulfamylon is responsible for unusual changes in ventilation in patients with burns, other evidence presented in this report will raise questions about the relationship of Sulfamylon to this increased ventilation and indicate that, whatever role it may play, it is not simple and straightforward.

In the patient whose measurements are depected in Figure 11, with a 46% total body surface,burn, the minute ventilation strikingly increased, reaching a peak of approximately 40 L/min on the eight postburn day. During this entire period, both preceding, during, and after the increase in minute ventilation, the diffusing capacity for carbon monoxide and the force vital capacity were normal. The static lung compliance was slightly depressed initially, and then rose to normal as the minute ventilation increased. This patient was treated with Sulfamylon without interruption throughout the study period. A macroaggregated albumin lung scan, performed on postburn day 7, was within normal limits. In contrast to the observations in this patient, which suggest that Sulfamylon is unrelated to the changes in minute ventilation, are observations (Fig. 12) made in 5 other patients. Minute ventilations are indicated on the vertical axes and postburn days on the horizontal axes. The per cent total body surface is recorded and the periods of Sulfamylon application are indicated by the solid bars. Interruption of Sulfamylon therapy was usually associated with a decrease, and application of Sulfamylon with an increase, in minute ventilation. The patient with a 48% total body surface burn, however, had no change in minute ventilation following the reapplication of Sulfamylon the the 10th postburn day.

Comparison (Fig. 13) of the average minute ventilation of 4 Sulfamylon-treated patients, with burns ranging from 24 to 39% of the body surface, with those of one patient with a 21% burn treated

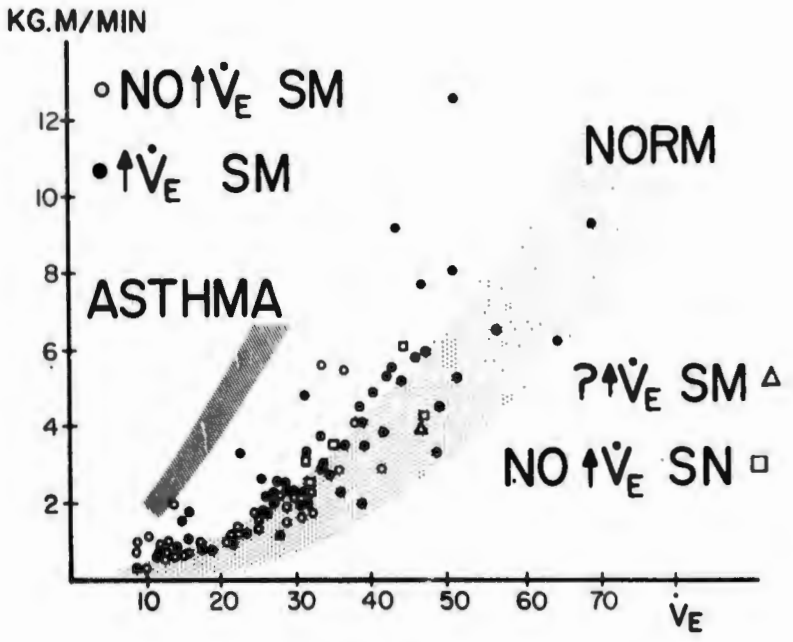

Figure 9

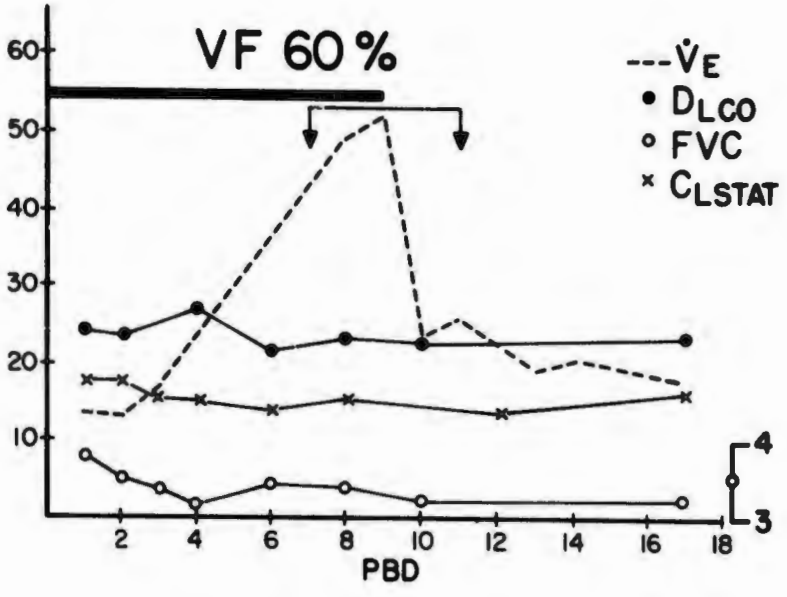

Figure 10

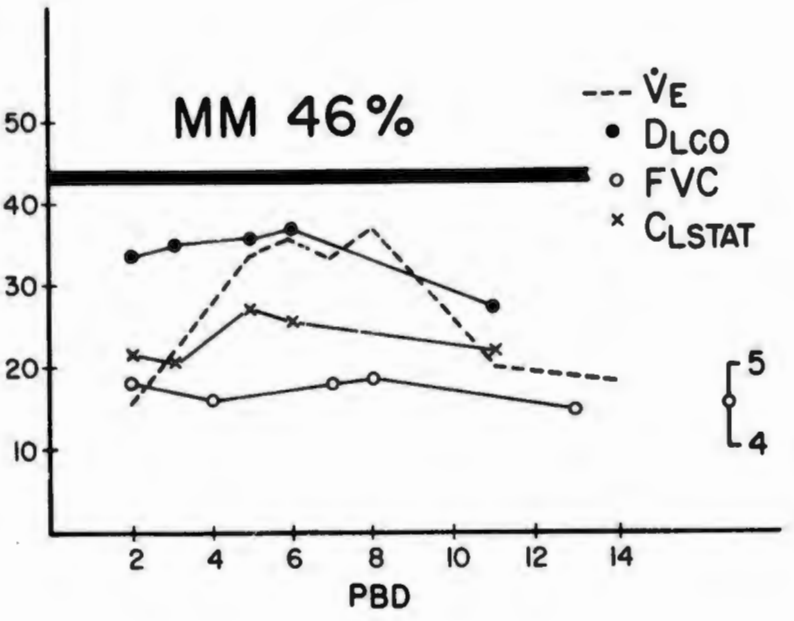

Figure 11

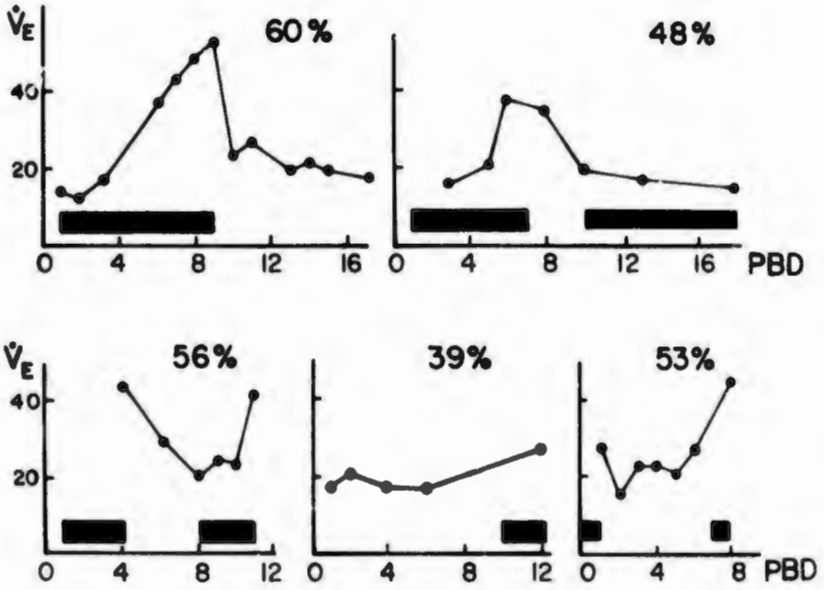

Figure 12

with silver nitrate, and one patient with a 38% burn treated with sulfadiazine, failed to reveal any obvious differences. Neither silver nitrate nor sulfadiazine are carbonic anhydrase inhibitors. Although a correlation exists between the minute ventilation and the combined serum level of Sulfamylon and paracarboxybenzene sulfonamide, its metabolic product (Fig. 14), the scatter of points is large and the minute ventilation cannot be predicted from the serum levels of the drugs. Minute ventilations vary from 10 to 50 liters per minute at serum levels of 8-10 mg%. The contribution of Sulfamylon to the increase in ventilation still remains to be defined.

Since the metabolic rate is increased in patients who suffer thermal injury (Fig. 15), and since this increase is related to burn size, the relationship between minute ventilation and the resting metabolic rate was investigated in order to explore the possibility that the minute ventilation was merely a consequence of the increased metabolic activity of these patients (Fig. 16). There is a distinct difference in the relationship between minute ventilation and metabolic activity in the two groups of patients, indicated by the solid lines, but this is likely a consequence of burn size. Those who did not develop an increase in minute ventilation (burns less than 40% are strikingly similar to normal exercising men (indicated by the dotted line) in terms of the ventilation they generate with respect to their metabolic rates. However, those patients who do develop a large increase in minute ventilation (burns greater than 40%) demonstrate a very different relationship between minute ventilation and metabolic activity.

These observations have led us to two tentative conclusions: (1) that the dramatic increase in minute ventilation is due to some extrapulmonary cause, influenced by both the extent of burn and the time elapsed following injury; (2) that this unusual clinical picture characterized by high minute ventilation, in the face of a normal chest x-ray, frequently normal physical examination, unremarkable blood gases, and in the absence of dyspnea, is distinctly different from "shock lung", "trauma lung", "postperfusion lung", and related syndromes. It is, to our knowledge, previously undescribed.

PRESENTATIONS

Presentations on, "Pulmonary Pathophysiologic Changes Following Thermal Injury", presented to the following:

Univ of Washington Sch of Med, Seattle, Wash, 1 Nov 71.
Univ of Utah Sch of Med, Salt Lake City, Utah, 3 Nov 71.
Washington Univ Sch of Med, St Louis, Mo, 10 Feb 72.
Univ of Colorado Schof Med, Denver, Colo, 15 Feb 72.
Univ of Arizona Sch of Med, Tucson, Arizona, 18 Feb 72.
Univ of New Mexico Sch of Med, Albuquerque, NM, 23 Feb 72.
Univ of Michigan Sch of Med, Ann Arbor, Mich, 27 Mar 72.
Amer Burn Assn, San Francisco, Cal, 8 Apr 72.

PUBLICATIONS

None

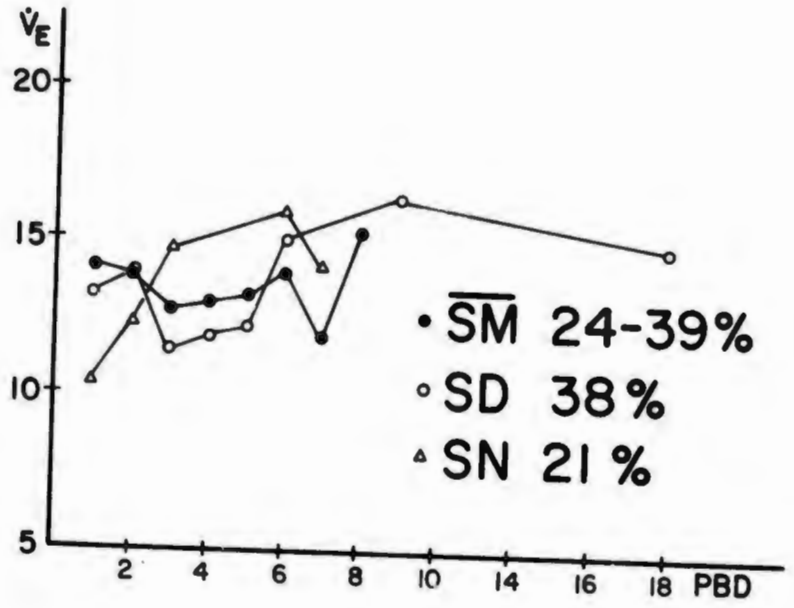

Figure 13

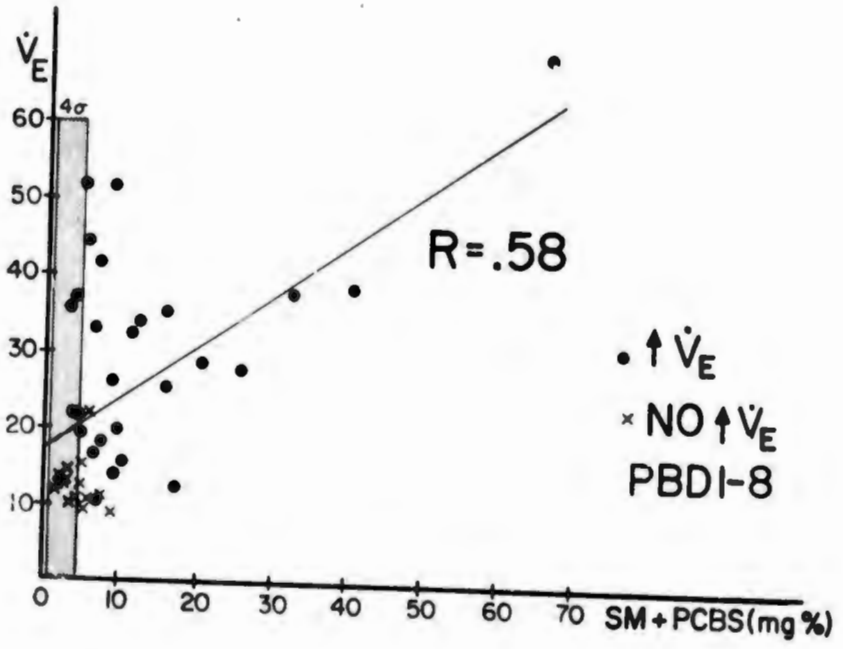

Figure 14

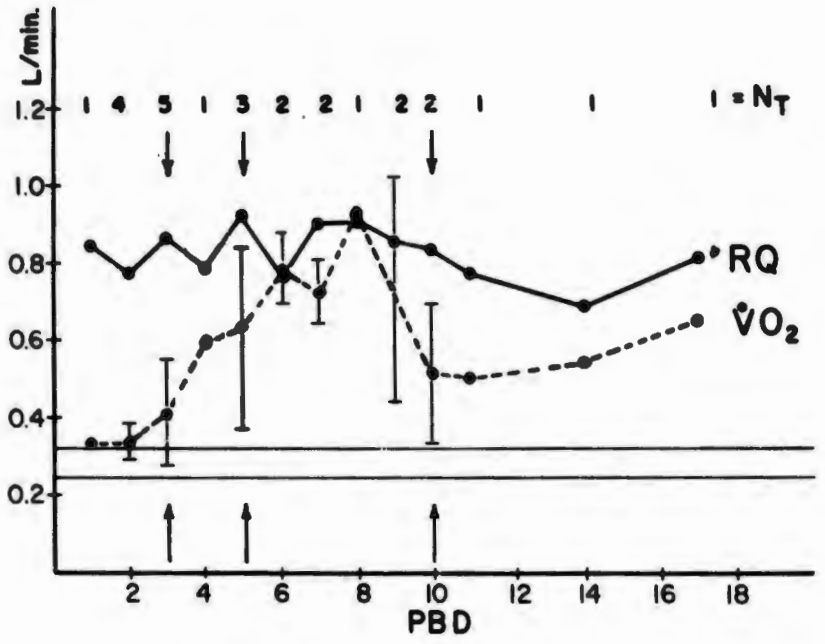

Figure 15

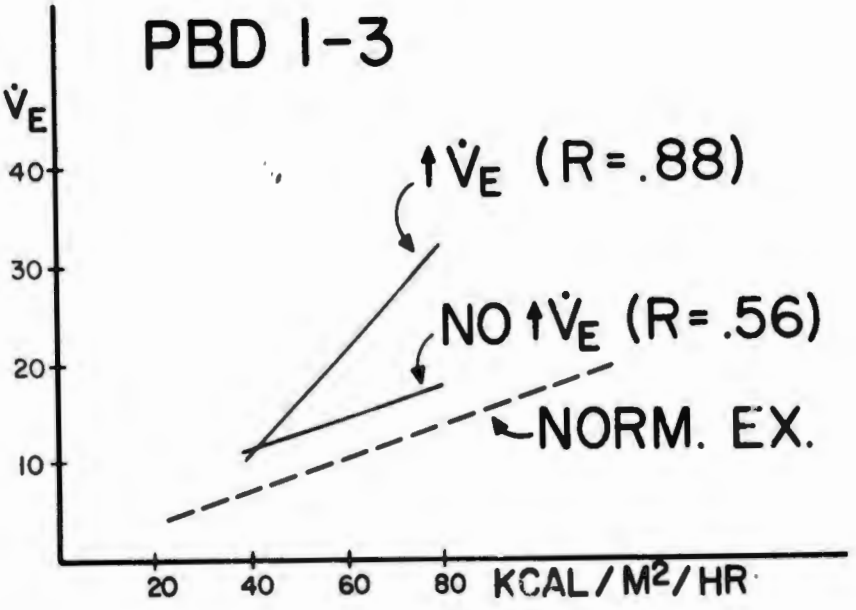

Figure 16

50

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OE 6393	72 07 01	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. DOWNGRADING	7. REGRADING	8. DISP'N INSTR'N	9A. 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	3A061101A91C	00	081
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Proceed 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 72	0.1	13.7
	CURRENT 73	0.2	10.0

19. RESPONSIBLE DOD ORGANIZATION
NAME: US Army Institute of Surgical Research
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20. PERFORMING ORGANIZATION
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SOCIAL SECURITY ACCOUNT NUMBER:
ASSOCIATE INVESTIGATORS
NAME: Glenn D Warden, CPT, MC
NAME: Roger Salisbury, MAJ, MC DA

21. GENERAL USE: FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Provide 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. Proceed 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) A reproducible animal model will be used to evaluate xenograft specimens. Efficacy of the test materials will be judged on the basis of histologic biopsies and duration of adequate wound coverage measured by the day at which 50% epithelial slough of the graft occurs.

25. (U) 71 07 - 72 06 Laboratory evaluation of Lyophilized porcine cutaneous xenograft has been completed. Adequate wound coverage in the animal model was achieved for 10-13 days, as compared to 85 days for commercially supplied fresh porcine xenograft. Histologically, lyophilized skin adheres ultimately to the wound bed without evidence of severe inflammatory, foreign body or immune reaction.

A clinical trial is underway at the present time. Forty patients have been treated sequentially with lyophilized porcine xenograft with no discernible differences from previously used fresh porcine skin.

*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. 3A061101A91C-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 1971 - 30 June 1972

Investigators:

Paul Silverstein, MD, Major, MC
Glenn D. Warden, MD, Major, MC
Roger E. Salisbury, MD, Major, MC
William F. McManus, MD, Major, MC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A061101A91C-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 1971 - 30 June 1972

Investigators: Paul Silverstein, MD, Major, MC
Glenn D. Warden, MD, Major, MC
Roger E. Salisbury, MD, Major, MC
William F. McManus, MD, Major, MC

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. However, due to inadequate supply and limited shelf life, widespread use of cadaver allograft has been restricted. Recent research with biologic dressings has confirmed the efficacy of xenogeneic porcine and canine cutaneous grafts as substitutes for allograft, and fresh and frozen porcine grafts are now commercially available.

Continued investigation with xenografts not requiring freezing or refrigeration has resulted in production of a lyophilized porcine graft sterilized by irradiation and indefinitely shelf-storageable. After reconstitution by soaking in balanced electrolyte solution for 60 to 90 minutes, lyophilized xenograft provides adequate wound coverage in an excised animal model for an average of 10 days. A clinical trial in 40 patients with second-and third-degree burns suggests that it is an adequate substitute for any of the fresh or frozen biologic dressings when used as a temporary wound cover during the period of therapy between time of eschar separation and autografting.

Cutaneous xenograft
Wound cover

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 resulted in an appreciation of the physiologic advantages derived from application of split-thickness skin grafts from allo- and xenogeneic species to granulating surgical and traumatic wounds, especially wounds resulting from thermal injury. The preferred natural dressings are those harvested from human cadaver donors within 24 hours of death and refrigerated, after saturation with penicillin and Streptomycin or other antibiotics.

Fresh porcine xenograft has been similarly produced to alleviate the shortage of human cadaver allograft. However, while fresh porcine skin dressings provide adequate protection to denuded skin surfaces, they suffer from shortcomings of limited viability, possible microbial contamination, and the possibility of eliciting an allergic reaction related to the antibiotic in which they are prepared. Frozen porcine skin was then produced that could be protected from bacterial contamination by irradiation and indefinitely stored in a deep freeze. However, this product had the disadvantages of increased cost and required freezer equipment for storage.

The discovery that nonviable grafts could function as substitutes for fresh skin stimulated production and evaluation of freeze-dried split-thickness skin grafts. Sterilization is achieved by gamma radiation(without the incorporation of antibiotics), and the xenograft is stored in air-free containers. Prior to use, lyophilized skin must be presoaked in balanced salt solution to achieve reconstitution. Cross checks on sterility by culture of commercially purchased spore-strips irradiated with the skin confirm sterility. After reconstitution, any unused portions of skin can be stored for an additional 5-7 days in a standard refrigerator, if handles aseptically and protected from contamination.

LABORATORY INVESTIGATION

Lyophilized split-thickness porcine cutaneous xenografts of varying thickness were applied to clean, excised 20% total body surface (TBS) area wounds on the backs of 200 gm male Sprague-Dawley rats. Grafts were fixed in place with Michel clips. Animals were then returned to their individual cages and observed daily. Adequacy of wound coverage was judged by adherence, purulence, presence of subgraft blebs, and/or serum leakage through the dressings. The endpoint for duration of adequate wound coverage was arbitrarily chosen as that day on which 50% of the graft epithelium had separated and sloughed.

Wound biopsies were evaluated histologically on a periodic basis for documentation of graft adherence and the presence of inflammation, infection, and fibroblastic proliferation at the interface. First and second set applications of grafts were performed at 2-week intervals to evaluate the possible occurrence of accelerated immune rejection.

The results of the above experiments demonstrated that lyophilized porcine xenograft functioned as well as any other xenograft previously tested in the same animal model (Table 1). Adequate duration of coverage in an experimental population of 90 rats was 10-13 days--longer than previously noted with fresh rat allograft (8-10 days) and commercially supplied fresh porcine graft (8.2 days). Optimum thickness of the xenograft prior to reconstitution appeared to be 0.015" - 0.020"-- slightly thicker than that of fresh porcine skin (0.010" - 0.015%). The increased thickness is required because the skin does not fully expand to its pre-lyophilization thickness after reconstitution.

Table 1. Average Duration of Wound Coverage

	Days
Rat allograft	8
Fresh porcine xenograft	8.2
Frozen-irradiated porcine xenograft	7.14
Lyophilized porcine xenograft	10.0

No accelerated rejection was noted with successive applications of porcine xenograft at 2-week intervals. The finding is in agreement with that previously reported for fresh and frozen xenograft. Lack of clinically demonstrable immune response is believed related to failure of revascularization of the grafts. Histologic examination of biopsies taken from xenografted wounds confirmed the clinical observation of excellent wound adherence and demonstrated ingrowth of granulation tissue into the lower dermal collagen network of the graft. No evidence of foreign body multinucleated giant cell or mononuclear immune reactions were noted. Graft integrity was maintained until cellular slough of the epidermis occurred, presumably due to disintegration.

In summary, lyophilized porcine cutaneous xenograft was found to provide excellent wound protection in the ISR standard laboratory rat model for approximately 10 days. It functioned as an adequate substitute for other known biologic dressings and manifested no significant differences in its behavior from previously tested forms of porcine skin. It differed from allograft in its failure to vascularize or show clinical and histological signs of immune rejection. Rat wounds treated with xenograft readily accepted permanent autografts from the animal's abdomen.

CLINICAL INVESTIGATION

On the basis of the animal wound study cited above, lyophilized porcine xenograft was applied to 40 patients with second- and third-degree burns requiring treatment with biologic dressings during the period between separation of eschar and final wound closure with autograft or by spontaneous re-epithelialization. Indications for using xenograft included: (1) protection of granulating wound beds from desiccation,hypertrophy, and infection; (2) as a "test" graft to assess receptiveness of the wound to autograft; (3) as an aid to debridement of small bits of retained devitalized tissue; and (4) to facilitate healing of second-degree burns and alleviate pain. Biopsies and photographs of xenografted wounds were taken periodically.

Lyophilized xenograft was applied from commercially supplied rolls after reconstitution for approximately one hour in sterile, room temperature,physiologic saline solution. Reconstitution was continued until no white, parchment-like areas remained in the skin.

Xenograft was applied to wounds, dermal side down, and either left exposed or dressed in moist saline gauze or stretchable tubular net dressings. Grafts were carefully trimmed to conform to the wound margins with no overlap onto unremoved adjacent eschar or normal skin. Xenografts applied to circumferential extremity burns were routinely protected with dressings from dislocation due to shearing against the bed clothes.

All wounds were inspected at least once daily. Indications for removing and replacing xenograft dressings were: purulence beneath the graft, nonadherence for mechanical reasons, and unexplained temperature elevations. Because of the absence of clinical immune response, xenografts that were well adherent to second-degree wounds were left in place until re-epithelialization was complete and resulted in spontaneous separation of the graft from the wound. Grafts adherent to granulating wounds were left in place an average of 4 days or until autografting was performed.

Graft changes were performed at the bedside or during bathing in

the hydrotherapy tank. No anesthesia or narcotic analgesia was employed. Clinicians graded xenografted wounds on a subjective scale previously employed for evaluation of biologic dressings (Table 2). Results of the clinical trial of xenograft on second- and third-degree burn wounds indicated that lyophilized porcine skin was similar to other biologic dressings. An average of 5 applications was required to achieve a wound grade of 3 or 4 (good adherence with or without granulation bleeding). At this point, wounds were considered to be ready for autografting.

Table 2. Subjective Grading of Xenografted Wounds

1 = no graft adherence with subgraft suppuration
2 = no graft adherence without subgraft suppuration
3 = adherence of graft with no bleeding of granulation tissue when graft removed
4 = adherence of graft with bleeding of granulation tissue when graft removed

While no differences were noted in the clinical behavior of lyophilized skin when compared to fresh or frozen xenograft, there were notable differences in a comparison to fresh cadaver allograft:

Fresh cadaver allograft was observed to adhere better initially to granulating wounds and developed considerably less suppuration at the wound interface than did porcine skin. Therefore, it was usually deemed necessary to change xenograft more frequently during the first week of use. After all devitalized tissue had been removed and a healthy granulating bed developed, porcine skin became well adherent and could be left in place 4 to 5 days.

Less bleeding and a more pale color were observed in the granulating beds treated with porcine xenograft when compared to the rich capillary ooze noted from wounds on which allograft had been applied. These differences are due to the fact that porcine skin does not become vascularized and, therefore, fewer capillaries are disrupted when the grafts are stripped from the wounds. Allograft "takes" to a granulating wound and the neovasculature that develops within it must be ruptured at the time of graft removal. Despite these observations, autograft take has appeared equally good on wounds prepared with either dressing.

The histologic appearance of biopsies from wounds xenografted with lyophilized skin resembled those utilizing fresh or frozen porcine grafts. No significant inflammatory reaction was noted unless excessive suppuration occurred due to incomplete debridement of the wound. No accumulation of foreign body multinucleated giant cells or mononuclear cells was observed at the wound interface or in areas of granulation tissue invasion of the graft. The exact cellular immune relationship between split-thickness cutaneous xenograft and its host is still unclear. No evidence of delayed hypersensitivity, allergy, or accelerated rejection has been elicited by successive applications of xenograft to animal or human wounds.

SUMMARY AND CONCLUSIONS

In laboratory and clinical evaluations, lyophilized porcine cutaneous xenograft appears to provide acceptable wound protection. Because of its ready availability porcine skin is a useful variant of biologic wound dressings, particularly in medical centers previously unable to harvest adequate amounts of cadaver skin.

The 3 forms of porcine graft appear similar provided certain precautions are employed in reconstitution, application, and subsequent wound inspection. The skin must be applied in its natural orientation (epidermal side up). Xenograft changes should not be delayed if subgraft suppuration or an unexplained febrile reaction occurs.

Lyophilized skin provides several advantages over previously developed products--specifically, guaranteed sterility and indefinite shelf storage without freezing or refrigeration. Lyophilized porcine skin is less expensive than the frozen product and comparably priced with the fresh product.

PUBLICATIONS AND/OR PRESENTATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0E 6954	72 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	61101A	3A061101A91C	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	0.1	2.7
	CURRENT 74	0.2	5.0

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)

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TELEPHONE: 512-221-4652

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: John Hunt, MAJ, MC

NAME: DA

21. GENERAL USE

FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Eschar; (U) Excision; (U) Cryosurgery; (U) Liquid Nitrogen; (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 achieve early, safe excision of eschar with minimal blood loss and preservation of viable tissue in burned soldiers.

24. (U) Cryosurgical technics with liquid nitrogen in surgical extirpations permit removal of tissue with minimal blood loss. These techniques will be applied to burn care to determine if delineation between second and third degree burns can be made and safe excision can be undertaken.

25. (U) 71 07 - 72 06 In a pilot study, four pigs with 60% total body surface burns underwent a total excision on the second postburn day using a liquid nitrogen cryosurgical instrument and dermatome excision with minimal blood loss and good differentiation of viable from non-viable tissue. Wounds were autografted after two changes of homograft with excellent take. Further studies are underway.

*Available to contractors upon originator's approval.

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

ANNUAL PROGRESS REPORT

PROJECT NO. 3A061101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: EXCISION OF ESCHAR IN BURNED SOLDIERS

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

1 July 1971 - 30 June 1972

Investigators:

Joseph A. Moylan, Jr., MD, Major, MC
John L. Hunt, MD, Major, MC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A061101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: EXCISION OF ESCHAR 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 1971 - 30 June 1972

Investigators: Joseph A. Moylan, Jr., MD, Major, MC
John L. Hunt, MD, Major, MC

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 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 (Janzekovic Z: Present clinical aspect of burns: A symposium. Derganc M, ed: Maribor, Yugoslavia: CP Mariborski tisk, 1968, pp 99-112, 215-230; Jackson DM: Second thoughts on the burn wound, J Trauma 9:839, 1969) have both reported favorably on 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. Differentiation of viable from nonviable tissue is critical in the process of tangential excision, and such discrimination has been deemed important in minimizing blood loss and surgical stress.

Laboratory evaluation of a cryogenic device using liquid nitrogen to freeze nonviable tissue with subsequent dermatome excision has appeared to permit differentiation of nonviable from viable tissue and to be associated with minimal blood loss. Four Duroc pigs, each of which had been given a 60% full-thickness total body surface burn, 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 load sufficiently rapidly to keep it from freezing.

The frozen tissue was easily differentiated from viable tissue and was readily excised by a dermatome with minimal blood loss. The excised wounds 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.

Application of this technique to 3 selected patients has emphasized the differences between burn patients and laboratory models of burn injury. In the clinical situation, the cryogenic method appears best suited for treatment of burns on relatively smooth, relatively unyielding regular surfaces such as the limbs. Its use in treatment of burns of irregular or "yielding" areas such as the anterior chest, buttocks or abdomen has been associated with less efficient viable-nonviable tissue differentiation and excessive blood loss from such areas. Homograft skin applied to the excisional wounds of these burn patients adhered well and adequacy of removal of nonviable tissue was deemed excellent. Invasive wound sepsis developed in the excised area of one patient despite use of Sulfamylon burn cream on adjacent intact eschar.

In summary, this cryosurgical technique appears to be useful in the treatment of burn wounds of certain anatomic areas of selected patients with extensive total body surface burns. Technological alteration will be needed to improve the general usefulness of this method of excision,especially in terms of better operative hemostasis. Further studies are in progress.

Burn wound excision
Wound care
Cryosurgery
Liquid nitrogen

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0A 6382	72 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
71 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	3A061101A91C	00	080
b. CONTRIBUTING	61102A	3A061102B71R	01	
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 72	0.2	5.3
FISCAL YEAR CURRENT 73	0.3	7.5

19. RESPONSIBLE DOD ORGANIZATION
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NAME: Basil A Pruitt, 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 determine wedge (left atrial) pressure, cardiac output, and other cardiovascular indices of extensively burned patients to evaluate the effect of the injury and resuscitation on pulmonary hemodynamics on burned military personnel.

24. (U) Selected patients with significant burns who exhibit clinical or radiologic evidence of increased pulmonary intra- or extravascular volume are studied by means of pulmonary wedge pressure, cardiac output, urine output, blood pressure and pulse.

25. (U) 72 01 - 72 06 To date, two patients have been partially studied. The first patient's pulmonary artery pressure was increased, presumably due to fluid overload, while the second patient demonstrated increased pulmonary density by x-ray, in the presence of total body hypovolemia and normal pulmonary wedge 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. 3A061101A91C-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 1971 - 30 June 1972

Investigators:

Stephen Slogoff, MD, Major, MC
Gary W. Allen, MD, Major, MC
Basil A. Pruitt, Jr., MD, Colonel, MC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A061101A91C-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 1971 - 30 June 1972

Investigators: Stephen Slogoff, MD, Major, MC
Gary W. Allen, MD, Major, MC
Basil A. Pruitt, Jr., MD, Colonel, MC

Reports Control Symbol MEDDH-288(R1)

Interstitial pulmonary edema is a frequent autopsy finding in patients dying as a result of thermal injury. The determinations of the etiology of this phenomenon in the thermally injured patient is extremely difficult, as frequently the only clinical sign or laboratory evidence of the disease is an abnormal chest x-ray. Clinical findings suggesting cardiogenic pulmonary edema are usually absent in these patients, even though they often respond paradoxically to the usual therapy for pulmonary edema. The disease appears to represent a change in pulmonary-capillary permeability, a phenomenon already documented in other vascular beds in the thermally injured patients.

The purpose of this project is to evaluate the cardiovascular status and pulmonary hemodynamics of these patients in order to establish the etiology of the pulmonary changes that are observed. The techniques involved include measurement of wedge pressure, cardiac output, arterial and central venous blood pressures, and blood volumes. To date, 2 patients have been studied. The first patient demonstrated acute onset of pulmonary edema by x-ray and clinical evidence, and measurements showed that this was probably the result of fluid overload. The patient's central venous, pulmonary artery, and pulmonary wedge pressures were all supranormal and responded appropriately to diuretic therapy. The second patient also showed radiologic evidence of acute pulmonary edema, at which time circulating blood volume was low, central venous pressure and pulmonary artery pressures were high, and wedge pressure was low.

On the basis of these results, this work will be expanded and continued. The findings in the latter patient are illustrative of the physiologic changes of special interest in burn patients.

Burn Cardiovascular hemodynamics
Wedge pressure Resuscitation of fluids

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OD 6976	72 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'D INSTR'N	8B. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
71 07 15	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	3A061101A91C	00	083
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Procede with Security Classification Code): (U) Studies of the Effect of Variations of Temperature and Humidity on Energy Demands of the Burned Soldier in a Controlled Metabolic Room (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
71 07	Cont	DA	C. In-House

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

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 72	0.2	5.5
	CURRENT 73	1.0	25.0

19. RESPONSIBLE DOD ORGANIZATION
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NAME: Arthur D Mason, Jr, MD DA

21. GENERAL USE: FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Provide each with Security Classification Code): (U) Metabolism; (U) Heat loss; (U) Evaporative water loss; (U) Controlled environment; (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 determine the relation of metabolic rate to evaporative water loss in extensively burned patients, to describe the change of metabolic rate with change in ambient temperature and humidity under controlled conditions and to describe the optimum temperature and humidity for treatment areas in terms of energy economy in soldiers.

24. (U) Use of a controlled environment study room to measure metabolic rate at various temperature and humidity levels. Concurrent measurement of water loss, R.Q. and appropriate temperatures in the carefully controlled environment will permit calculation of heat loss components and determination of the critical temperature in those conditions which minimize energy demands in the seriously ill patient.

25. (U) 71 07 - 72 06 An environmental chamber has been designed and installed which has a temperature range of 15 to 40° centigrade, plus or minus 0.5° centigrade, a relative humidity range from 20 to 80 percent, plus or minus 2 percent, limited by a minus 1.1° centigrade and plus 35° centigrade dew point. The room air is introduced through a porous ceiling and flows out through baseboard ventilation ducts in the walls at less than 50 feet per minute. This room has undergone rigorous testing and is equipped with numerous sensors to insure constant environmental conditions for studying. At present time control data on partition of heat loss is being gathered to compare with experiments of severe injured patients. A special feature of the environmental chamber is the control device which can be operated by the patient to establish a comfort level by regulating temperature and humidity changes from a bedside control unit.

* Available to contractors upon contractor's request.

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. 3A061101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: STUDIES OF THE EFFECT OF VARIATIONS OF TEMPERATURE AND HUMIDITY ON ENERGY DEMANDS OF THE BURNED SOLDIER IN A CONTROLLED METABOLIC ROOM

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

1 July 1971 - 30 June 1972

Investigators:

Douglas W. Wilmore, MD, Major, MC
Basil A. Pruitt, Jr., MD, Colonel, MC
Arthur D. Mason, Jr., MD

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A061101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: STUDIES OF THE EFFECT OF VARIATIONS OF TEMPERATURE AND HUMIDITY ON ENERGY DEMANDS OF THE BURNED SOLDIER IN A CONTROLLED METABOLIC ROOM

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

Period covered in this report: 1 July 1971 - 30 June 1972

Investigators: Douglas W. Wilmore, MD, Major, MC
Basil A. Pruitt, Jr., MD, Colonel, MC
Arthur D. Mason, Jr., MD

Reports Control Symbol MEDDH-288(R1)

Nine individuals have been placed in the metabolic room to determine optimal comfort levels in varying temperature and humidity. The environmental chamber, designed to maintain a constant temperature and humidity between the ranges of 15 and 40°C and 20 to 90% relative humidity, was controlled by each individual studied to establish optimal comfort temperature at varying humidities of 40, 60, and 80%. All patients were in the supine position, and wore only underclothing. Optimal comfort levels were achieved after 1 to 4 hours of equilibration by adjustment of room temperature from a bedside control unit. As the humidity increased, the individuals perceived the room warming, and gradually decreased the ambient temperature (see Table). All burn patients set the room at a much warmer level than did the healed convalescing burn patients or control individuals. The healed burn patients, who had lost 10 to 15% of their preburn body weight but had achieved total skin coverage, felt most comfortable in the room slightly warmer than that temperature established by the normal individuals. These responses may reflect changes in the insulation of skin or body fat.

COMFORT TEMPERATURE* SELECTED AT DIFFERENT RELATIVE HUMIDITIES (MEAN TEMPERATURE IN DEGREES CENTIGRADE)

Study Subjects	Number	40	60	80
Normal	3	26.5	26.3	23.8
Healed burn	3	26.7	25.8	25.0
Acute burn	3	35.5	32.0	28.8

* Temperature taken after 1-4 hours of equilibration

Further studies are in progress to fractionate the heat loss from the burn patients and to determine if a warm-dry room will substantially decrease the prodigious metabolic demands in the thermally injured patient.

Metabolism
Heat loss
Evaporative water loss
Controlled environment

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0D 6951	72 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'N INSTR'N	8b. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
71 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	3A061101A91C	00	082
b. CONTRIBUTING	61102A	3A061102B71R	01	
c. CONTRIBUTING				

11. TITLE (Procede 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 72	0.2	6.3
	CURRENT 73	0.3	10.0

19. RESPONSIBLE DOD ORGANIZATION

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ASSOCIATE INVESTIGATORS

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NAME: B A Pruitt, Jr, LTC, MC DA

21. GENERAL USE

FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Procede 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. Procede 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 or above with 30% or greater burns, regardless of sex, who have positive blood cultures or in whom a clinical suspicion of bacteremia or endotoxemia exists, are admitted to the study. Seven cc of heparinized blood are drawn from each patient, the plasma separated by centrifugation, and then tested for the presence of endotoxin using Limulus blood lysate. Inhibitors of the reaction are eliminated by reducing the pH of the plasma to 4 and then raising it to a pH of 6.1 before adding the amebocyte lysate. Blood is drawn daily as long as the patient is suspected of having septicemia or as often as the patient's doctor desires, dictated by the clinical condition of the patient.

25. (U) 71 07 - 72 06 New techniques for extraction of endotoxin from blood are being investigated and an attempt made to develop a simple and reliable test. Thirty-four patient have been admitted to the study to date, with endotoxemia being identified in five. Four had simultaneous positive blood culture, and one developed a positive blood culture one day later. DIFCO E. coli 0111:B4 continues to be our standard. Ample supply of Limulus amebocyte lysate has been obtained.

*Available to contractors upon originator's approval

ANNUAL PROGRESS REPORT

PROJECT NO. 3A061101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

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 1971 - 30 June 1972

Investigators:

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

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A061101A91C -00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: DETECTION OF ENDOTOXIN IN BURNED SOLDIERS WITH SEPSIS

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

Period covered in this report: 1 July 1971 - 30 June 1972

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

Reports Control Symbol MEDDH-288(R1)

The Limulus amoebocyte lysate technic for the assaying of endotoxin is a simple, relatively quick method of detecting the presence of as little as 0.0005 µg of endotoxin/ml of whole blood. "Limulus lysate" was obtained from the blood of horseshoe crabs collected in the vicinity of the Marine Biological Laboratory, Woods Hole, Massachusetts. Twenty-nine liters of amoebocyte lysate were collected. Of these, 19 liters were of a potency that justified testing for suitability in detecting endotoxin in human blood.

One hundred eighty blood samples were tested for the presence of endotoxin, 5% of samples were positive. There was no correlation between a positive test and a positive blood culture. Numbers are too small at present to draw any definite conclusion. Technics of testing and of preparing the amoebocyte lysate have to be further elucidated.

Endotoxin
Sepsis
Assay

DETECTION OF ENDOTOXIN IN BURNED SOLDIERS WITH SEPSIS

There are many technics for the detection of endotoxin, most not well suited for clinical use, measuring different biologic functions and having widely varying sensitivities. Most are biologic assays requiring intact animals and are time consuming. It has been demonstrated that endotoxin in low concentration causes gelation of a protein-containing fluid derived from the blood cell (amoebocyte) of the horseshoe crab, Limulus polyphemus. A method based on this phenomenon is capable of detecting 0.0005 µg of endotoxin per ml of whole blood. This test has proved to be simple, accurate and relatively easy to perform, with results obtained in 4 to 24 hours.

MATERIALS AND METHODS

During July and August 1971, 2 of the investigators prepared Limulus lysate from the amoebocyte of horseshoe crabs harvested at the Marine Biological Laboratory, Woods Hole, Massachusetts.

Technic for this collection was as follows:

Horseshoe crabs, which varied in size from 8 to 12 inches across the widest portion of the carapace, were used. A sterile 13-gauge 3 ½-inch needle was inserted into the junction of the thoracic and abdominal segments on the dorsal surface, after the area had been cleansed with 70% alcohol. All glassware used in preparing the lysate was siliconized and made pyrogen-free by baking in an oven at 180° C for at least 2 hours. The blood was allowed to flow into a 250 cc centrifuge bottle containing 0.125% n-ethyl-malimide solution in 3% sterile, pyrogen-free saline at 40° C, so that when filled there was a 1:1 ratio of blood to NEM solution. After 15 minutes, the mixture was centrifuged at 600 rpm for 6 minutes; the supernate aspirated and the sediment of cells washed in 3% sterile, pyrogen-free saline. The mixture was transferred to a 40cc centrifuge tube, centrifuged again at 600 rpm for 6 minutes, supernate aspirated and cells washed a second time. After this second washing, the cells were then lysed with 35 ml of sterile, pyrogen-free distilled water added to each centrifuge tube. The mixture was held at 4° C and at the end of 24 hours, centrifuged at 2500 rpm for 15 minutes. The supernate (lysate) was removed and tested against a known concentration of Escherichia coli endotoxin (Difco) to ascertain its potency. The test was considered positive when there was the formation of a gel with increase in turbidity.

Once a suitable quantity of high potency lysate had been obtsined clinical use of the test was begun. Burn patients, 5 years old or above with 30% or greater burns, regardless of sex, who had either positive blood cultures or in whom a clinical suspicion of

bacteremia or endotoxemia was entertained, were admitted to the study. From 5-7 cc of heparinized blood was drawn aseptically from each patient into a sterile pyrogen-free plastic syringe and placed in sterile vacuum tubes. The blood was chilled by ice. Plasma was separated from the blood cells by centrifugation and aspirated aseptically. To 1 cc of plasma was added 0.1 cc of 25% glacial acetic acid reducing the pH of the plasma to near 4.0 and precipitating protein. This is mixed well, allowed to set for 3 minutes and then 0.2 cc of 50% phosphate buffer added bringing the pH of the mixture to 6.2 - 6.7 range. The solution is centrifuged at 5000 rpm for 5 minutes and supernate aspirated. To 0.1 cc of the plasma supernate is added 0.1 cc of amoebocyte lysate in 3 mm test tubes and mixed. The tube is placed in a water bath at 37° C for 4 hours and then removed, the test being read for the first time. Twenty-four hours later, it is read for the final time. A positive test is the formation of a clot with increased turbidity.

Blood cultures were prepared at the time of collecting samples for endotoxin study.

From September 1, 1971 to April 11, 1972, 180 blood samples have been tested for the presence of endotoxin with 9 being positive in 6 patients, an incidence of 5%. One patient had 2 plasma samples positive for endotoxin and one other patient 3. The rest were single positive samples. In only one patient was there correlation between a positive lysate test and a blood culture, positive for Pseudomonas and Providencia stuartii, that was obtained the following day. There were no positive blood cultures obtained on the day of or following the positive endotoxin assay in the other patients.

The correlation between positive lysate test for endotoxin and blood cultures was not as high as has been reported by some investigators. However, others have found lack of correlation between blood cultures and endotoxin content of blood. The rate of reactors was lower than has been reported elsewhere. Studies directed to a better means of extraction of the endotoxin from plasma or whole blood are in progress. Attempts are also being made to improve the sensitivity of the lysate.

The number of positive lysate tests for endotoxin are too few at this point to draw definite conclusions. In all of the patients there were clinical signs of sepsis or endotoxemia at the time that a positive lysate test for endotoxin was obtained. With improvement in sensitivity of lysate and method of extraction, a higher rate of positive reactions is to be expected.

PUBLICATIONS AND/OR PRESENTATIONS

None

ANNUAL PROGRESS REPORT

PROJECT NO. 3A061101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: DETECTION OF ENDOTOXIN IN BURNED SOLDIERS WITH SEPSIS PART II. NATURAL VARIATION IN SENSITIVITY OF LIMULUS AMOEBOCYTE LYSATE TO ENDOTOXIN

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

1 July 1971 - 30 June 1972

Investigators:

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Basil A. Pruitt, Jr., MD, Colonel, MC
Arthur D. Mason, Jr., MD

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A061101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: DETECTION OF ENDOTOXIN IN BURNED SOLDIERS WITH SEPSIS PART II. NATURAL VARIATION IN SENSITIVITY OF LIMULUS AMOEBOCYTE LYSATE TO ENDOTOXIN

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

Period covered in this report: 1 July 1971 - 30 June 1972

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

Reports Control Symbol MEDDH-288(R1)

The exquisite sensitivity of lysate from amoebocyte of Limulus polyphemus to endotoxin offers a new approach to the study of endotoxemia and endotoxin shock. However, reactivity of lysate varies with individual animals. Results with 950 crabs bled on Cape Cod in July-August 1971 were assessed for such variation. The availability of a standard reacting substance is vital to potential clinical application of this reaction. Eight hundred seventy-three samples, each from 120 ml of Limulus blood, were tested, as were pools of comparable lysates. Intensity of reaction was graded from 1+ to 4+. The objective was detection of 0.001 µg/ml of endotoxin, since this level of sensitivity is needed if the reaction is to be useful clinically. 0.4% of all samples reacted at 4+ with 0.001 µg ET/ml, 11% at 3+ or 4+, and 52% at 2+. Marked temporal variation occurred in lysate harvested from 7 July to 12 August. No 4+ reactors were seen. Four of 19 pools, with 4% of total lysate collected reacted at 3+ or 4+ with 0.001 µg ET/ml. Detailed cross-comparison of lysates is needed to make this reaction potentially useful.

Endotoxin Shock
Limulus Sepsis

DETECTION OF ENDOTOXIN IN BURNED SOLDIERS WITH SEPSIS PART II. NATURAL VARIATION IN SENSITIVITY OF *LIMULUS* AMOEBOCYTE LYSATE TO ENDOTOXIN

The possible role of endotoxin in the etiology of septic shock has prompted 5 decades of research directed toward clarifying this phenomenon. Assay of circulating and tissue-fixed endotoxin has been difficult and equivocal because of the technically cumbersome and insensitive technics which were available. The discovery that the clotting mechanism of *Limulus polyphemus*, the horseshoe crab, could be triggered by trace amounts of endotoxin[1,2] has led to development of an essentially new, simple and quick test for detecting endotoxin at the nanogram level. This is the clotting, or gelation of the protein extractable from the amoebocytes, or blood cells. Levin[3] and Reinhold[4] have reported detection of endotoxin in the blood of septic patients. This is a potentially very important tool for study of burn patients, and a program was set up to obtain a supply of potent amoebocyte lysate to be used in studying endotoxemia and endotoxin shock in severely burned patients. Collection of horseshoe crabs was carried out on Cape Cod from July 11 through August 25, 1971. Little is known about seasonal variations in reactivity of the coagulating protein, but temporal variation has been noted, and when it became apparent that this was occurring, detailed records of the differences were made.

Horseshoe crabs are most readily collected in shallow, sandy estuarine areas, and the bays on Cape Cod have long provided an optimal source. The crabs are not in shallow water in cold weather and collecting can be made only from late spring to early fall. The animals were transported by truck, as soon as collected, to holding tanks at the Marine Biological Laboratory at Woods Hole. They were bled within 3 to 4 days, but never on the day of capture. They were bled from the dorsal haemocele, through the hinge tissue of the carapace, using a pyrogen-free, siliconized No. 13 needle. The bleeding, incidentally, need not be lethal, and the animals were routinely returned to Vineyard Sound. 120 ml of anticoagulant, which consisted of 0.125% H-ethyl-maleimide in 3% sodium chloride, buffered at pH 7.2, was placed in a 250 ml centrifuge bottle and an equal volume of blood was collected in this solution. On average, 100 ml of blood per crab was obtained. This highly potent anticoagulant is required to prevent activation of the *Limulus* clotting system. If amoebocyte rupture occurs in the presence of *Limulus* plasma, prompt, complete clotting occurs. Only siliconized, pyrogen-free glass and nonwettable plastic can come in contact with *Limulus* blood without causing clotting.

Cells were gently sedimented and washed twice in sterile, pyrogen-

free, 3% sodium chloride, which is isotonic for Limulus blood. The washed amoebocytes were suspended in pyrogen-free distilled water, in which the reactive protein is eluted. After 24 hours in the cold, the cells were removed by centrifugation. The supernatant fluid was the amoebocyte lysate. The cell mass from 120 ml of blood was washed and eluted in 35 to 40 ml of 3% saline, then suspended in distilled water. The lysate tubes were tested, pooled according to reactivity and stored at 4° or -70° C.

Since man is one of the mammals most sensitive to the effect of endotoxin, with lethal doses being expressed in nanogram or less per kilogram, a sensitivity level of 0.001 µg per ml, or 1 nanogram per ml, must be achieved by a test substance if it is to be useful. The Limulus reaction has not yet been reduced to a precise quantitative end point. It is expressed in terms of clot and of turbidity. A dense, firm, very turbid clot, which forms within one hour after endotoxin and lysate have been mixed, is designated as a 4+ reaction. A 3+ reaction shows a softer clot, which still remains in place when the tube is slanted, with dense turbidity. A 2+ reaction is a clot that flows when inverted, and a dispersed turbidity with some flakes of coagulum. One-plus reactions are still clotted, but the clot is softer still, with slight turbidity and particles of coagulum settled in the tube.

The reactivity of individual tubes from 873 samples, each tube representing the lysate from 120 ml of Limulus blood are summarized in Table 1.

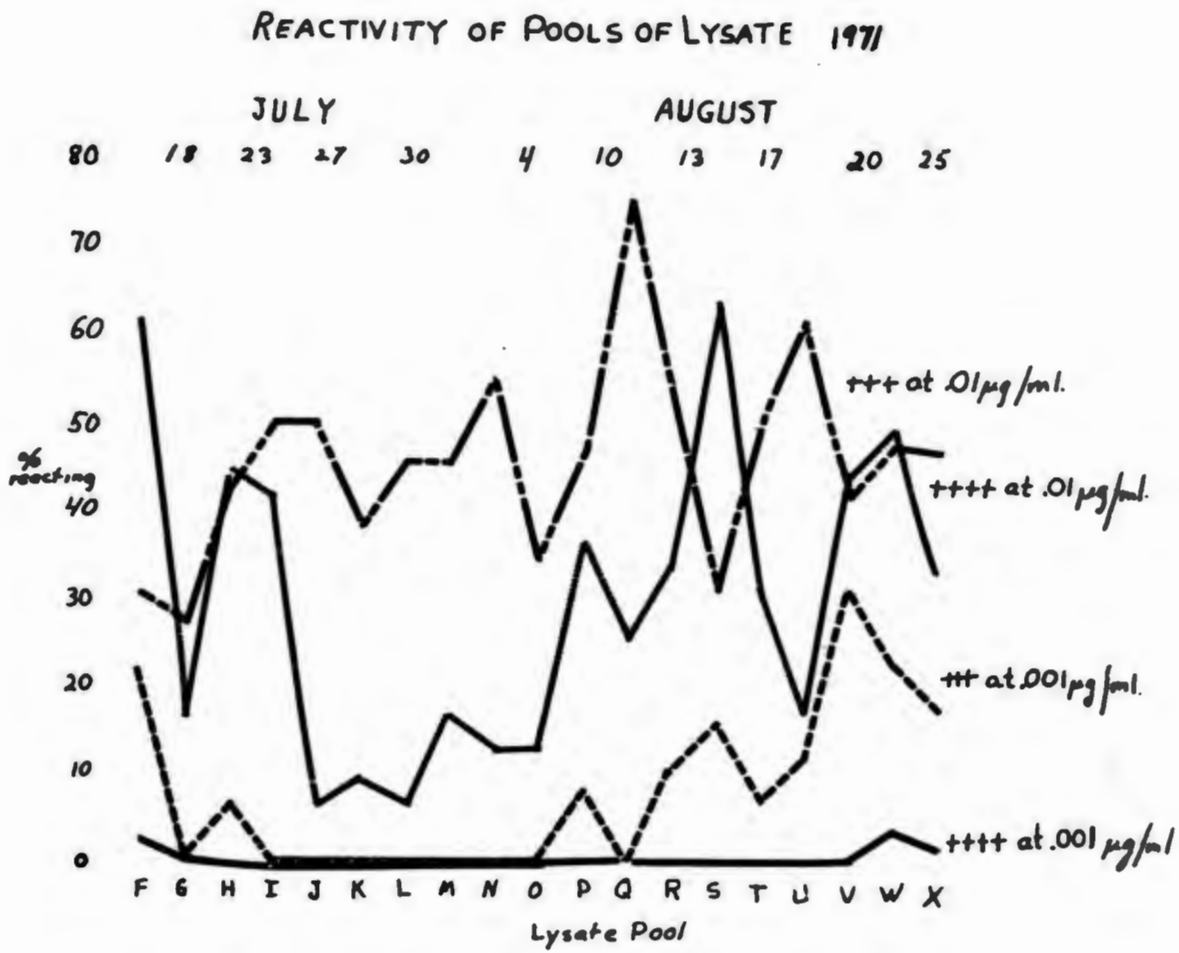
Although meaningful end points of reaction must detect at least 0.001 µg per ml if they are to be useful in clinical studies, all lysates were tested at 2 levels, with 0.01 and 0.001 µg per ml of endotoxin. Five pools, A through E, were tested before those listed, but at that time the lowest test dilution was 0.01 µg per ml. Since these were the first pools collected, and since technics of collection were being standardized, they were not included in this comparison. On the 18th of July, one tube of those collected reacted at 4+ with 0.001 µg per ml of endotoxin, and 22% of all tubes reacted at 3+. From that time, for 14 successive collections, until 24 August, no tubes of lysate reacted with 0.001 µg of endotoxin at the 4+ level. Even at the 3+ level, only once prior to 10 August were there tubes reacting with 0.001 µg. From 10 August onward, the percentage of strong reacting samples slowly increased, and at the end, from 20 to 25 August, a larger proportion of samples showed a high level of sensitivity. The temporal changes in Limulus lysate sensitivity are shown graphically in the figure.

There was little drop in the proportion of strains reacting with

Table 1
REACTION OF LIMULUS AMOEBOCYTE LYSATE
WITH E. COLI ETs JULY-AUGUST 1971

POOL	NO. TUBES	DATE	% REACTING WITH 0.01 µg/ml					% REACTING WITH 0.001 µg/ml				
			4+	3+	2+	1+	Neg.	4+	3+	2+	1+	Neg.
F	36	18/7	61	30	6	3	0	3	22	44	19	11
G	48	21/7	17	27	42	12	2	0	0	0	31	69
H	45	23/7	44	42	13	0	0	0	7	58	31	4
I	24	25/7	42	50	4	4	0	0	0	79	17	4
K	55	29/7	9	38	34	9	9	0	0	44	24	33
L	48	30/7	6	46	42	4	2	0	0	56	35	8
M	48	2/8	17	46	33	2	2	0	0	14	67	19
N	31	4/8	13	55	32	0	0	0	0	29	55	16
O	32	6/8	12	34	50	3	0	0	0	19	59	22
P	60	10/8	37	47	15	2	0	0	8	53	29	10
R	48	13/8	33	54	12	0	0	0	10	69	18	2
S	19	16/8	63	31	5	0	0	0	16	37	42	5
T	63	17/8	32	51	17	0	0	0	6	46	44	0
U	72	19/8	17	61	21	0	0	0	11	43	36	10
V	68	20/8	44	41	15	0	0	0	31	47	20	1
W	63	24/8	49	48	3	0	0	3	22	46	28	0
X	64	25/8	33	47	17	3	0	1	17	48	19	14

55-4


REACTIVITY OF POOLS OF LYSATE 1971
JULY
AUGUST
80
18
23
27
30
4
10
13
17
20
25
70
60
50
% reacting
40
30
20
10
0
+++ at .01μg/ml.
++++ at .01μg/ml.
+++ at .001μg/ml.
++++ at .001μg/ml
F G H I J K L M N O P Q R S T U V W X
Lysate Pool

0.01 µg of endotoxin at the 3+ level, but 4+ reactions with 0.01 µg per ml dropped to a low level from mid July to mid August. These lots were those which showed a 3+ reaction with 0.001 µg per ml. A rising incidence of samples with 3+ reactions at 0.001 µg per ml occurred during the last half of August. The optimal lysate was that reacting with 0.001 µg per ml at the 4+ level. The minimal amount of this material, even at summer's end, was evident.

The totals of individual tubes of lysate and the reacting levels obtained are summarized in Table 2. Reaction at 3+ and 4+ levels with 0.01 µg per ml of endotoxin occurred in 77% of the tubes collected, but at the critical level of reaction with 0.001 µg per ml of endotoxin, only 11% reacted. The number of 4+ reactors with 0.001 µg per ml was negligible. Reacting tubes were pooled according to degree of activity, at the time each collection was tested. Since the amounts of 4+ reacting substance at 0.001 µg per ml were low, 3+ and 4+ reactors were pooled. Weaker reactors were pooled in matching strengths, so that maximum usefulness of each tube would be retained. The pooled lysates were tested in detail to determine exact titers, within limitation of the method. At the same time, a careful assessment of the valid end point was made, so that, with appropriate controls, the minimal amount of endotoxin in a clinical sample could be assayed. Table 3 summarizes these values for the 2+ and 1+ end points. There were 14 out of 37 pools in which well-defined (but less than solid) gel, and legible turbidity, could be demonstrated with 1.25 nanograms of endotoxin per ml. Three out of these 14 pools, totalling 1000 ml of lysate, would detect 1 nanogram of endotoxin or less at the 3+ to 4+ level.

The remaining lysate pools could detect 2 to 5 nanograms of endotoxin per ml. While this was not sensitive enough for clinical trial, it served adequately for experimental assay of endotoxin in animal experiments, and for assaying endotoxin in tissues at autopsy.

The technic for collecting Limulus amoebocyte lysate is obviously still subject to improvement. Recent reports[3] have described a modified collection technic which yielded a much more sensitive lysate, and a more effective technic for extracting endotoxin from plasma for assay. However, the seasonal variations which we observed would still affect the relative potency of different lots of lysate.

This series of observations indicates that a marked seasonal fluctuation in reactivity of amoebocyte lysate occurs. There is much still unknown about the life cycle of the horseshoe crab, which may be responsible for this variation. The amoebocyte count of the bloods varied markedly; in general, cell "rich" bloods yielded more potent lysate, but there were numerous exceptions in each direction. The individual amoebocytes contain refractile granules from which the

Table 2.

TOTAL BOTTLES OF LYSATE AND REACTING TITERS

WITH E. COLI ET

INTENSITY OF REACTION	REACTION WITH 0.01 µg ET:NO. OF BOTTLES		REACTION WITH 0.001 µg ET:NO. OF BOTTLES	
	TOTAL	%	TOTAL	%
4+	276	31.6	4	0.4
3+	398	45.6	92	10.5
2+	189	21.6	367	42.0
1+	26	2.9	283	32.4
PLUS MINUS OR NEGATIVE	9	1.0	118	13.5

EACH BOTTLE = 20 ml OF LYSATE, FROM 120 ml OF DISTILLED H_2O EXTRACT FROM (AVE) 1.25 LIMULUS.

Table 3.

1971:

LYSATE POOL TITERS IN ASCENDING ORDER

TOTAL POOLS	ET AND REACTION ENDPOINT (µg/ml)	
	2+	+
5	0.005	0.0025
2	0.005	0.00125
16	0.0025	0.00125
11	0.00125	0.0006
1	0.0006	0.0003
2	0.0003	< 0.0003
37		

active protein is eluted, and granule-poor amoebocytes certainly yield lysate less reactive than that from granule-rich cells. This finding parallels the reactivity results.

The results of this collection of lysate from over 900 crabs, in July and August 1971, may not be typical of all years, but the fact that such prolonged, low-level results can occur call for awareness on the part of those collecting this reagent. It would appear best that collections be made in late June or early July, and again in late August and early September, if the more potent reacting substance is to be obtained. The supply of horseshoe crabs is not infinite, and if this ancient life form is to persist, it should be harvested with maximum opportunity for acquisition of effective material. Study of the gelation reaction at both extremes of reaction is needed to obtain a better understanding of this potentially useful phenomenon.

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PRESENTATION

Lindberg RB: Natural Variation in Sensitivity of Limulus amoebocyte lysate to endotoxin. Fed. Soc. Exper. Biol. Atlantic City, NJ, 12 Apr 1972.

PUBLICATION

Lindberg RB, Inge WW,Jr, Mason AD,Jr, Pruitt BA,Jr: Variation in sensitivity of Limulus amoebocyte lysate to endotoxin. Fed Proc 31: 791, 1972.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0E 6383	72 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	61101A	3A061101A91C	00	078
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) Isolation and Characterization of Mycotoxins from Phycomycetes Recovered from 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 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 72	0.3	2.5
	CURRENT 73	0.3	5.0

19. RESPONSIBLE DOD ORGANIZATION
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NAME: Robert B Lindberg, PhD
NAME: George M Helmkamp, Jr, CPT, MSC DA

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Mycotoxins; (U) Phycomycetes; (U) Rhizopus; (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 isolate toxic products from Rhizopus rhizopodiformis, a representative Phycomycete, and to determine their toxic effects in rats to improve therapy of burned troops.

24. (U) Live and heat-killed fungal spores, hyphal fragments, soluble cytoplasm, and broth culture filtrate will be inoculated intravenously, subcutaneously and intracerebrally into healthy male rats.

25. (U) 72 02 - 72 06 Intravenous injection of live fungal spores produces 100% mortality within 48-54 hours. Fungal lesions involved the gastrointestinal tract, kidneys, lungs, and brain of all animals, and less commonly the heart, skin, and adrenals. The liver and spllen were rarely involved. Subcutaneous injections of live spores were not fatal, but led to the production of encapsulated abscesses around groups of spores. Intracerebral inoculation produced weakness and lethargy clinically. Histologic involvement included intracerebral, meningeal, ependymal and choroid plexus lesions. The character of the lesions suggests soluble toxic products may be involved in their pathogenesis.

*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. 3A061101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: ISOLATION AND CHARACTERIZATION OF MYCOTOXINS FROM PHYCOMYCETES RECOVERED FROM BURNED SOLDIERS

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

1 July 1971 - 30 June 1972

Investigators:

Daniel W. McKeel, Jr., MD, Major, MC
F.D. Foley, MD
Malcolm N. Goodwin, Jr., MD, Major, MC
George M. Helmkamp, Jr, Captain, MSC
Robert B. Lindberg, PhD

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A061101A91C-00 , IN-HOUSE LABORATORY INDEPENDENT RESEARCH

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Period covered in this report: 1 July 1971 - 30 June 1972.

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Rats infected hematogenously with Rhizopus rhizopodiformis spores develop visceral lesions that mimic human cerebral, pulmonary, gastrointestinal and renal phycomycosis. Such rats also provide a model to study phycomycotic vascular thrombosis and host sensitization to fungal antigen. The histologic morphology and absence of fungi in some types of visceral lesions suggests these fungi may liberate soluble toxic factors. Future efforts will be to isolate these toxins and to study their mode of action and intracellular site of origin.

Mycotoxins
Phycomycetes

ISOLATION AND CHARACTERIZATION OF MYCOTOXINS FROM PHYCOMYCETES RECOVERED FROM BURNED SOLDIERS

Fungal burn wound infections (FBWI) have increased markedly in ISR patients since the institution of Sulfamylon topical antibacterial therapy in 1964.[1] A recent serial biopsy study of 70 ISR patients demonstrated that 58.8% of third degree burns became colonized with yeasts and fungi by the third postburn week.[2] Data obtained during 1970-1971 suggests that the trend is continuing.

Phycomycetes, saprophytic fungi which are of low pathogenicity in healthy humans, can produce lethal disseminated infections in patients with severe burns,[1,3-5] diabetic acidosis,[6] and other debilitating diseases.[7] Complications of phycomycotic infections are often said to stem from the propensity of the fungus to invade blood vessels and disseminate. Phycomycetes can also produce local destructive effects. Fat saponification, collagen lysis, cartilage penetration, vascular wall destruction, focal necrosis, neural invasion and destruction, and penetration through coagulated dermal collagen are often observed in fungal burn wound infection by Phycomycetes. The possibility that these locally destructive effects could delay wound healing and provide an entry point for bacterial infection deserves consideration. Although some of the above effects may be mediated through lytic enzymes produced by the fungus, non-enzymatic toxins may also be involved. As early as 1914, Gortner and Blakeslee[8] described a lethal toxin from a Phycomycete, Rhizopus nigricans, which produced spreading necrosis and edema following subcutaneous injection. Since that time mycotoxins have been purified from many fungi,[9] especially Aspergillus species,[10] while the Phycomycetes have been neglected. From Aspergillus have been isolated fibrinolysin (aspergillin 0), hemolysin, carcinogen (aflatoxin), edema producing toxin, neurotoxin, endotoxin (primarily a nephrotoxin) and assorted toxic metabolites.[10-16] The production of similar toxins by Phycomycetes, should this be established, would have obvious implications regarding the pathogenesis and treatment of potentially life-threatening infection caused by these fungi in burned and other susceptible patients.

MATERIALS

The fungus selected for study was Rhizopus rhizopodiformis (Pr), a Phycomycete known to be a human pathogen,[17] which had been originally isolated on 28 January 1970, from a burned human extremity amputated for deeply invasive fungal burn wound infection (FBWI). It has proved stable during many subcultures on Sabouraud's dextrose agar medium. Previous experimental studies at the Institute

(Foley FD, Bruck HM, Warden GD, Hunt JL, Lindberg RB, McKeel DW Jr: Unpublished observations) had shown this fungus to be capable of producing lethal, disseminated FBWI in Holtzman rats made diabetic with either alloxan or streptozotocin and seeded with 10^6 Rr spores applied to a recent, dorsal 10-30% scald burn. Similar application of Rr spores to nondiabetic Holtzman rats with 20% dorsal scald burns was not lethal; however fungal proliferation was demonstrable histologically in viable tissue beneath the burn eschar.

A. SPORE PREPARATION

Methods. Rr spores were harvested from mature brown-black cultures grown on solid Sabouraud's medium by scraping with a sterile rod in sterile Ringer's lactate (RI) medium. Large hyphal and sporangial fragments were removed by passing the spore suspension through gauze or cotton. The concentration of spores was corrected in a white blood cell hemocytometer. Adjustment of spore concentration and serial tenfold dilutions were done using RL.

B. SPORE INJECTIONS

1. Intravenous injections. Intravenous injections were performed by injecting spore suspensions (10^2-10^7 spores in one ml RL) into the tail veins of 100-200 gm healthy, male Holtzman rats. Rats were either autopsied 2-8 hours after death or observed for mortality purposes only. Pairs or single rats given 10^7 spores intravenously were sacrificed by bleeding at 8,17,31, and 34 hours postinoculation to observe sequential morphologic changes occurring in infected animals. Control animals were similarly studied after being given one ml RL via the tail vein.

2. Subcutaneous injections. Subcutaneous injections employed 10^7 Rr spores in one ml RL injected subcutaneously between the scapulae of 100-200 gm healthy male Holtzman rats. Animals were sacrificed by intraperitoneal barbiturate overdose at 10 days postinoculation. Control rats received similar injections of one ml RL.

3. Intracerebral injections. Intravenous injections were done by injecting 0.1 ml RL containing 10^7 Rr spores through a one mm burr hole into the right cerebral cortex of 100 gm, healthy, male Holtzman rats. Control injections of 0.1 ml RL were done in the opposite cerebral hemisphere. Rats were sacrificed at 10 days postinoculation by intraperitoneal injection of an overdose of barbiturate.

C. HISTOLOGIC STUDIES

All autopsied rats were carefully examined by one of us (DWM) for gross lesions, some of which were photographed. Selected blocks of brain, pituitary, lungs, liver, spleen, pancreas, adrenals,

kidneys, esophagus, stomach, intestine, bone marrow, spinal cord and skeletal muscle were fixed in 10% neutral buffered formalin and processed for light microscopy or were fixed in 2.5% buffered glutaraldehyde or 4% paraformaldehyde and processed for electron microscopy. Light microscopy sections were often stained with a newly developed PAS-Giemsa stain which simultaneously demonstrates bacteria and fungi (McNeel DW Jr, Worley BL, USAISR Anl Res Rpt, June 1972). Ultra thin sections were double stained with uranyl acetate and lead citrate and were examined with an RCA EMU4 electron microscope.

RESULTS

A. MORTALITY

Subcutaneous spore injections were not lethal, nor were any symptoms noted in these rats. Intracerebral injections also were not fatal except rarely when acute hydrocephalus developed or when a large dural sinus was inadvertently entered during inoculation. Some surviving animals developed lethargy, limb weakness and ataxia during the second week after intracerebral spore inoculation.

Tenfold serial dilutions of Rr spores in RL were given intravenously to 132 healthy, male Holtzman rats with the results shown in Table 1. Spore doses of 10^5-10^7 were uniformly lethal, the largest dose producing death in 1.8 days. Approximately 60% of rats given 10^4 spores died, while only slight or no mortality was noted with lower doses.

B. VISCERAL LESIONS

The distribution of visceral lesions in the infected rats which died are listed in Table 2. Although to date relatively small numbers of rats have been examined histologically, especially those given 10^2-10^5 spores, the data indicates that kidney and brain lesions occur commonly in all groups, whereas lung, gastrointestinal, liver, spleen and heart lesions occur commonly only in rats given 10^6 or 10^7 spores.

C. HISTOPATHOLOGY OF VISCERAL LESIONS

The PAS-Giemsa proved invaluable in detecting hyphae in areas such as heart, spleen and liver. Since only a few animals have been sacrificed sequentially to study the pathogenesis of lesions, observations mostly pertain to rats autopsied shortly after death occurred spontaneously.

1. Heart. The most extensive lesions occurred in the 10^7 group. Broad areas of myocardial necrosis involved the right more than the left ventricles. Although hyphae could be demonstrated

Table 1. Mortality in Healthy, Male Rats Following Intravenous Inoculation of Rhizopus rhizopodiformis Spores

No. Rats	Dose (Spores in 1 ml RL)	Mean Day of Death	Mortality %
20	10^7	1.8	100
25	$10^6 \pm$	4.1	100
24	$10^5 \pm$	6.5	100
23	$10^4 \pm$	8.3	60
20	$10^3 \pm$	9.3	10
20	$10^2 \pm$	--	0

Holtzman strain male rats weighing 100-200 grams were inoculated via the tail vein with the indicated dose of fungal spores suspended in one ml sterile RL. The date and hour of death were recorded. Data represents mean of 3 groups of animals given separate spore preparations at each dose level.

Table 2. Visceral Lesions in Healthy Rats Given Intravenous Injections of Rhizopus rhizopodiformis Spores

	Spore Dose			
	10^7	10^6	10^5	10^4
Brain	3	3	2	2
Pituitary	2	1	0	0
Lung	3	3	2	0
Liver	3	3	0	0
Spleen	3	3	0	0
Pancreas	3	2	0	0
Kidney	3	3	3	3
Adrenal	1	0	0	0
Gastrointestinal	3	3	0	0
Spinal cord	1	0	0	0
Skeletal muscle	2	0	0	0
Heart	3	3	0	0

Code: 0 = never seen 1 = rarely seen
2 = sometimes seen 3 = commonly seen

Experimental conditions were as described in the text and Table 1.

in some lesions, many necrotic areas had no visible hyphae even in PAS-Giemsa stained sections.

2. Lungs. Rats given 10^7 spores developed diffuse lesions throughout the lungs. Light and electron microscopy revealed germinating enlarged spores lodged in septal blood vessels and surrounded by polymorphonuclear leukocytes (PMN) as early as 8 hours after injection. Animals examined at later intervals showed occasional vascular thrombi surrounding fungal hyphae, progressively increasing interstitial hypercellularity, necrosis of type II granular pneumocytes and accumulation of large amounts of lamellar and lattice-like forms of surfactant in the alveoli. After 17 hours, germinating spores were also surrounded by macrophages, histiocytes, and giant cells. Electron microscopy revealed broad zones of finely fibrillar material surrounding some spores and hyphae which could be an antigen-antibody reaction forming at the fungal cell wall-host tissue interface. Another observation which may indicate how host cells become sensitized to fungal antigen was that peeled off layers of fungal cell walls often appeared to be enclosed by portions of host cell cytoplasm; coated vesicles, which are thought to play a primary role in protein uptake by a variety of cells, often enclosed portions of the delaminated cell wall material within the interior of these host cells.

3. Liver. Lesions appeared as microscopic foci of parenchymal necrosis. Early lesions contained clear cells with swollen vesicular nuclei; hyphae were only rarely demonstrable. Later lesions showed occasional giant cells, plus many mononuclear cells, lymphocytes and eosinophils.

4. Spleen. Fungal hyphae penetrated the capsule and parenchyma and produced minute foci of necrosis. The predominant cellular response was neutrophilic.

5. Gastrointestinal System. Rats given 10^6 and 10^7 spores always had numerous lesions throughout the gastrointestinal tract including the lower esophagus. Earliest lesions involved the submucosal microvasculature, many of which were thrombosed. Neutrophils and eosinophils infiltrated the submucosa. Later lesions showed myriads of proliferating hyphae throughout the serosa, muscularis, and mucosa. Light and electron microscopy showed that hyphae in some areas penetrated mucosal glandular cells without producing necrosis, but in other areas were associated with intense cellular destruction. Rats given 10^6 spores uniformly developed intense, hemorrhagic, necrotizing colitis and bloody diarrhea. Vascular thrombosis was particularly prominent in these animals. Gastrointestinal lesions in animals that survived more than 4 days were sometimes granulomatous and usually contained giant cells.

6. Kidneys. All animals that died, irrespective of dose, had kidney lesions. Enlargement progressed with time post-

inoculation, thus was most marked in rats given lower fatal doses, i.e., 10^4 and 10^5 spores. Lesions included acute pyelonephritis, renal vein thrombosis with hemorrhagic infarction (10^6 only), granulomas with giant cells (10^4, 10^5), and, occasionally, obstruction secondary to fungal proliferation. The predominant initial cellular response was neutrophilic. Periarterial eosinophil accumulation was noted in animals given 10^4 and 10^5 spores dying between 6-9 days.

7. Brain. Intravenous injection of 10^4 - 10^7 spores produced widespread lesions in the cerebrum, cerebellum and brainstem but only rarely in the spinal cord. The lesions were perivascular foci of necrosis and edema with early accumulation of neutrophils and later of macrophages and giant cells. Fibrillar material and cell wall phagocytosis similar to that seen in lung lesions were noted around proliferating spores and hyphae by electron microscopy. Intracerebral spore injections led to intense necrosis at the inoculation site much greater than in control injection sites. More distant areas of the central nervous system showed acute and granulomatous mycotic meningitis, ependymitis, choroid plexitis, and cerebritis. The parenchymal lesions associated with proliferating hyphae were similar to hematogenous lesions produced by intravenous inoculation. Vascular thrombosis and perivascular edema were common components of both types of lesions.

8. Subcutaneous Lesions. Rarely, metastatic fungal lesions developed in skin and adjacent skeletal muscle in rats injected intravenously with 10^7 spores. Subcutaneous injection of 10^7 spores led to the production in 10 days of an encapsulated abscess with myriads of nongerminated spores in the center lying among necrotic cellular debris.

SUMMARY

Rats infected hematogenously with Rhizopus rhizopodiformis spores develop visceral lesions that mimic human cerebral, pulmonary, gastrointestinal and renal phycomycosis. Such rats also provide a model to study phycomycotic vascular thrombosis and host sensitization to fungal antigen. The histologic morphology and absence of fungi in some types of visceral lesions suggest these fungi may liberate soluble toxic factors. Future efforts will be to isolate these toxins and to study their mode of action and intracellular site of origin.

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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 6388	72 07 01	DD-DR&E

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
	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	3A061102B71P	05	023
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) Definitive Identification of Burn Wound Fungi In Fatally Burned Military Personnel by Culture and by Histopathology (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
71 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 72	0.4	13.7
	CURRENT 73	0.1	4.0

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21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Burns; (U) Fungi; (U) Autopsy; (Military burn fatalities

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

23. (U)(a)To determine mycotic genera and/or species pathogenic in burn wounds; (b) to determine whether or not histologic identification of fungi can be improved; (c) to determine if earlier, i.e., histologic, identification would assist selection of treatment of burned patients with fungus infections.

24. (U) (a) Culture burn wound and internal organs for fungi at autopsy; (b) obtain genera and species identification of infecting organisms; (c) make detailed morphologic descriptions of fungi in sections taken adjacent to cultured tissue; (d) correlate results of b and c.

25. (U) 71 12 - 72 06 Autopsy cases selected for study due to expected high yield of fungus are being collected as they occur. Eight cases have been collected in the past three months. High yields have been obtained in these cases. Genera have been determined. Reference labs are being consulted for species identification. Special stains on histologic sections are in preparation.

*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. 3A061102B71P-05, BASIC RESEARCH IN SUPPORT OF MILITARY MEDICINE

REPORT TITLE: DEFINITIVE IDENTIFICATION OF BURN WOUND FUNGI IN FATALLY BURNED MILITARY PERSONNEL BY CULTURE AND BY HISTOPATHOLOGY

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

1 July 1971 - 30 June 1972

Investigators:

Daniel W. McKeel, Jr., MD, Major, MC
Malcolm N. Goodwin, Jr., MD, Major, MC
Beverly L. Worley, BS
Robert B. Lindberg, PhD

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A061102B71P-05, BASIC RESEARCH IN SUPPORT OF MILITARY MEDICINE

REPORT TITLE: DEFINITIVE IDENTIFICATION OF BURN WOUND FUNGI IN FATALLY BURNED MILITARY PERSONNEL BY CULTURE AND BY HISTOPATHOLOGY

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

Period covered in this report: 1 July 1971 - 30 June 1972

Investigators: Daniel W. McKeel, Jr., MD, Major, MC
Malcolm N. Goodwin, Jr., MD, Major, MC
Beverly L. Worley, BS
Robert B. Lindberg, PhD

Reports Control Symbol MEDDH-288(R1)

This study was undertaken to provide more definitive data concerning the histopathologic and cultural identification of fungi affecting fatally burned patients at the US Army Institute of Surgical Research. A review of the pathologic findings and mycology cultures of 23 patients who died during the latter half of 1971 revealed a continued high incidence of fungal burn wound colonization (87%) and deep invasive infection (61%). Fusarium, Candida, Mucor and Aspergillus species were most frequently isolated. Positive visceral cultures of fungi occurred in 48%; Fusarium represented 62.5% of visceral isolates. The emergence of Fusarium as a dominant burn wound pathogen and the clinical significance of frequent positive visceral cultures of this organism warrant further study.

The growing importance of Fusarium as a pathogen in burned humans was further documented by a collaborative study with Dr. Norman Conant, a recognized expert in medical mycology at Duke University. Fungal growth was obtained from 88.7% of the specimens of burn wound submitted; Fusarium represented 65.4% of these isolates.

A new histologic stain (modified buffered Giemsa--periodic acid Schiff) has been developed which simultaneously demonstrates bacteria and fungi in the same tissue section. This excellent cytologic stain should greatly facilitate future studies of fungal morphology and histopathology.

Fungi
Burns

DEFINITIVE IDENTIFICATION OF BURN WOUND FUNGI IN FATALLY BURNED MILITARY PERSONNEL BY CULTURE AND BY HISTOPATHOLOGY

Previous reports from this Institute[1,7] have documented the increasing clinical importance and occurrence of mycotic infection of the burn wound and its complications. Candida, Phycomycetes and Aspergillus were the commonest burn wound pathogens in these studies, although many other types of fungi were less frequently isolated. The importance of Candida as a potentially lethal pathogen in burned patients has been stressed in several recent reports.[8,9]

Recent experience has indicated that mycotic burn wound infection is increasingly prevalent in patients autopsied at this Institute. This trend was first documented [1] when review of the histologic burn wound sections from all ISR patients dying in the pretopical chemotherapy era (1961-63) and posttopical chemotherapy era (1964-69) showed a tenfold increase in wound infections due to Phycomycetes and Aspergillus species during the latter period. The present study was initiated to better define the genera and species of mycotic organisms causing burn wound infection, and to determine whether histologic definition of fungal burn wound infection can be improved. This report summarizes initial progress toward these goals.

A. MORPHOLOGIC AND CULTURAL IDENTIFICATION OF MYCOTIC GENERA IN POSTMORTEM BURN WOUND SECTIONS

Data from 23 burn patients autopsied by one of us (DWM) at this Institute during the latter half of 1971 were reviewed to assess the current incidence of mycotic burn wound infection in patients who succumb. The patient population had a mean age of 26.5 years (15 males, 8 females). Burns were predominantly flame or scald injuries and involved an average of 61.1% of the total body surface (range 22.5-94.5%). Average survival postburn was 12 days (range 3-29 days). Virtually all the patients were treated with topical mafenide acetate (Sulfamylon burn cream) application to the burn wound, and a majority received systemic antibiotics as well. The primary cause of death in all but two patients was attributed to causes other than the fungal infection, the one exception being a 48-year-old diabetic physician who received a 34.5% scald burn and died 27 days later with florid ketoacidosis and oculorhinocerebral phycomycosis which led to blindness, cavernous sinus and carotid artery thrombosis, and multifocal mycotic abscesses throughout the cerebrum.

An average of 8.2 histologic slides per case were examined from the 23 cases to assess the incidence of fungi in the burn wound. These findings were correlated with postmortem mycology

cultures with the following results:

1. Fungi were seen in the burn wound in 87% (20/23) of the patients.

2. Histologically diagnosed invasive fungal burn wound infection (FBWI) occurred in 70% (14/20) of the patients with fungi. In spite of this high incidence, visceral mycotic lesions were rarely seen histologically, although fungi were isolated from viscera or venous thrombi in 11 of the 20 patients (55%).

3. Fungi were seen histologically in 53.3% (81/152) of all burn wound areas which, when cultured, yielded growth from 38.2% (see Table 1). Considering all histologic sections of burn wound (including those not cultured) from these 23 patients, 54.5% (103/189) had one or more genera of fungi in the burn wound.

4. Fifty-eight portions of burn wound yielded 65 mycotic isolates representing 13 genera in addition to sporadic mycotic isolates from viscera and venous thrombi. The distribution of mycotic genera cultured from the burn wound and viscera/thrombi are listed in Table 1. These data show that 38.2% of portions of burn wound cultured had one or more fungi, among which Fusarium (23.3%), Candida, Mucor and Aspergillus were commonest. Fusarium isolates represented 62.5% of all positive viscera/thrombus cultures. Fusarium was seen histologically in the lung in bronchiolar mucous, suggesting an airborne mode of transmission to that organ.

The above results indicate an extremely high incidence of fungal burn wound infection in ISR patients who died during the latter part of 1971. The overall incidence is higher than that reported by Nash et al.[1] for the period 1964-1969, although they examined data from a much larger number of autopsied patients. They found that approximately 65% of patients had burn wound sections which contained one or more fungi, and noted a marked increase in deep infections by broad fungi such as Phycomycetes and Aspergillus. The emergence of Fusarium and the decline of Phycomycetes as predominant burn wound pathogens has apparently occurred during 1970-71, since the studies reported by Nash et al.[1] and a prospective biopsy study of fungal burn wound colonization in 70 consecutive ISR burn patients seen from 15 April to 15 August 1969, reported by Bruck et al.[2] The latter study implicated Candida albicans as the most frequent burn wound colonizer (70% of biopsies) followed by Phycomycetes and Aspergillus. Review of the original mycology culture data from the latter study revealed that of 34 positive cultures from 29 biopsies, only 3% were Fusarium (one positive culture).

B. DEFINITIVE IDENTIFICATION OF BURN WOUND FUNGI

Because of possible species differences in virulence,

Table 1. Mycotic Genera Cultured from Twenty Burn Patients at Autopsy, USAISR, August - December 1971.

Genus	Burn Wound	Viscera/Thrombus
Fusarium	15	15 (11 in lung, 1 each in liver, spleen, adrenal, bladder
Candida	9	4 (2 in thrombi, 2 in lung)
Mucor	8	0
Aspergillus	7	2 (both in lung)
Cephalosporium	7	0
Scopulariopsus	5	1 (lung)
Alternaria	4	0
Absidia	3	0
Helminthosporium	2	0
Mycelia sterilis	2	0
Rhizopus	1	0
Penicillium	1	1 (lung)
Sephedonium	1	1 (lung)
	65	24

elaboration of soluble toxic products and response to therapy, and for epidemiologic purposes, it would be desirable to accurately define not only the genera but also the species of fungi which infect the burn wound. Such species differences are well known in bacterial infections and may determine the clinical course, survival and treatment of an affected individual. An illustration pertinent to burn patients would be the different clinical illnesses associated with infection by Pseudomonas aeruginosa and Pseudomonas pseudomallei. Dr. Norman Conant, an internationally recognized expert in clinical medical mycology, kindly offered to assist in the identification of fungi. Results have been reported from 7 patients autopsied at ISR between October 1971 and February 1972. We submitted 62 portions of burn wound which were suspected of harboring fungi by their gross appearance at the autopsy table; these were alcohol flamed and implanted immediately on slants of Sabouraud's dextrose agar and shipped to Dr. Conant. No mycotic growth was obtained from 11.3% (7/62); the remaining 88.7% (55/62) yielded 36 Fusarium, 14 yeasts (Candida), 9 Aspergillus, and one Phycomycete. Thus, Fusarium represented 65.4% of the fungi isolated from these specimens. Reference laboratories are being consulted to obtain final species identifications of these isolates. Additional specimens have been submitted, but results are not yet available.

C. IMPROVEMENT OF HISTOLOGIC IDENTIFICATION OF FUNGI.

A combined histologic stain which simultaneously demonstrates gram positive and negative bacteria plus fungal yeast forms and hyphae has been developed. The stain uses a modified buffered Giemsa method developed at this Institute by Teplitz and Davis [10] as an excellent general cytologic and bacterial stain, combined with the periodic acid-Schiff (PAS) reaction to stain fungal cell walls. The method, which is detailed in Table 2, was developed by Major McKeel and Mrs. Beverly Worley and has been evaluated by the other ISR staff pathologists. The excellent cytologic, bacterial and mast cell staining of the original modified Giemsa method is preserved and enhanced by the addition of PAS staining. Fungal cell walls are often deep blue or magenta, whereas cytoplasmic elements are often rose, pink or pale blue. Candida stains more intensely than with either modified Giemsa or PAS alone. This stain should provide savings in slide preparation time and costs, as well as eliminating the need for using separate histologic stains to demonstrate bacteria and fungi. The method should find wide application in other military and civilian histology laboratories. A manuscript is being prepared for publication of the method and its applications.

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Table 2. Procedure for PAS-Giemsa Combined Stain for the Simultaneous Histologic Demonstration of Bacteria and Fungi

Reagents:

0.5% Periodic Acid -
0.5 gm Periodic Acid
100 ml Dist. H_2O

Schiff Reagent -
Harleco Schiff Reagent #2818 or make Coleman-Feulgen Reagent

Stock 2.6 pH Buffer
24.2 ml .2N HCl
50 ml .2M Glycine
Qs to 200 ml with distilled H_2O. Check and adjust pH to 2.6. Store in refrigerator.

Stock Giemsa Stain
1 gm Giemsa Stain (Allied Chemical)
66 ml Glycerine
Heat overnight in 60°C. oven. Cool to room temp., then add
66 ml Methanol
Filter and store in refrigerator.

Working Giemsa (Use only one day)
3 ml Stock buffer
3.5 ml Stock giemsa
45 ml Dist. H_2O
Heat to 60°C. in oven prior to use. pH 3.3 to 3.4

Procedure:

1. Deparaffinize and hydrate through alcohols to dist. H_2O.
2. Place in 0.5% periodic acid for 5 min.
3. Rinse in dist. H_2O.
4. Place in Schiff's Reagent for 15 min.
5. Rinse in running tap water for 5 min.
*6. Stain in Harris' Hematoxylin for 1 min.
*7. Rinse in tap water.
*8. Differentiate quickly in acid alcohol and blue in tap water.
9. Stain in heated working giemsa in 60°C. oven for 40 min.
10. Rinse in dist. H_2O.
11. Differentiate in Abs. Acetone.
12. Clear in equal parts acetone-xylene, then 2 changes xylene.
13. Mount in permount.

*Steps 6,7, and 8 may be omitted for routine use, if desired. Staining with Hematoxylin gives more intensive nuclear staining, useful for photographing.

Results: Bacteria stain a bright blue. Fungi stain magenta.

Pruitt BA Jr: Fungal burn wound infection. JAMA 215:1664-1666, 1971.

2. Bruck HM, Nash G, Stein JM, Lindberg RB: Studies on the occurrence and significance of yeasts and fungi in the burn wound. Ann Surg, in press.

3. Bruck HM, Nash G, Foley FD, Pruitt BA Jr: Opportunistic fungal infection of the burn wound with Phycomycetes and Aspergillus. Arch Surg 102:476-482, 1971.

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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 OC 6985	72 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
71 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	3A061102B71P	08	067
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) Lymphocyte Corticosteroid Binding in the Rat after Thermal Injury - A Model of Changes Observed 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 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 72	0.4	16.4
FISCAL YEAR CURRENT 73	0	0

19. RESPONSIBLE DOD ORGANIZATION
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NAME: A A Johnson, BS DA

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Corticosteroids; (U) Steroid Binding; (U) Transcortin; (U) Lymphocytes

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

23. (U) (a) To measure postburn injury serum corticosteroid levels in a laboratory rat model and military burn patients. (b) To measure serum transcortin (corticosteroid binding globulin) binding activity in these subjects after a burn injury.

24. (U) Fluorisine technique is used for measuring serum corticosteroids and tritiated corticosteroid absorption binding technique is used to measure transcortin binding capacity.

25. (U) 71 07 - 72 06 (a) Postburn serum corticosteroid levels are elevated in relation to burn size in both burn patients and experimental scald burned rats. (b) Serum transcortin levels are increased only in burned rats, theoretically protecting them from hypercorticism.. No parallel use in steroid binding globulin was seen in the patient population.

*Available to contractors upon originator's approval.

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FINAL REPORT

PROJECT NO. 3A061102B71P-08, BASIC RESEARCH IN SUPPORT OF MILITARY MEDICINE

REPORT TITLE: LYMPHOCYTE CORTICOSTEROID BINDING IN THE RAT AFTER THERMAL INJURY - A MODEL OF CHANGES OBSERVED IN BURNED SOLDIERS

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

1 July 1971 - 30 June 1972

Investigators:

Karl Eurenius, MD, Major, MC
Richard F. Mortensen, MS, SP 5
Avery A. Johnson, BS

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A061102B71P-08, BASIC RESEARCH IN SUPPORT OF MILITARY MEDICINE

REPORT TITLE: LYMPHOCYTE CORTICOSTEROID BINDING IN THE RAT AFTER THERMAL INJURY - A MODEL OF CHANGES OBSERVED 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 1971 - 30 June 1972

Investigators: Karl Eurenius, MD, Major, MC
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Reports Control Symbol MEDDH-288(R1)

An adsorption technique has been adapted to measure both the total corticosteroid-binding capacity (CBC) and corticosteroid-binding globulin (CBG) fraction of rat serum following a standardized third-degree scald burn over 30% of their body surface area. A maximum increase of 20 µg cortisol bound/100 ml sera (CBC) over unburned controls was seen 4 days following injury. This increase in CBC was entirely accounted for by a twofold increase in the CBG fraction. Changes in corticosterone levels paralleled alterations in the CBG capacity. Increased CBG capacity was still indicated when CBG fraction was corrected for changes in serum globulin levels following a burn. Albumin binding capacity (ABC) was elevated the first day postburn only.

In contrast to the rat, no significant alteration in binding capacities occurred in 33 human burn patients studied. The extent of the burn injury ranged from 12-69% of body surface area. As a result, the level of unbound cortisol was as much as 5 times normal in the early postburn period and remained elevated up to 40 days after injury. The presence of large amounts of unbound cortisol in these sera implies either unprotected hypercorticism or binding of excess cortisol to other serum components. It is concluded that the human, unlike the rat, does not respond with an increase in the synthesis of CBG following a severe burn.

Corticosteroids
Steroid binding
Transcortin
Lymphocytes

LYMPHOCYTE CORTICOSTEROID BINDING IN THE RAT AFTER THERMAL INJURY - A MODEL OF CHANGES OBSERVED IN BURNED SOLDIERS

A high plasma concentration of 17-hydroxycorticosteroids is associated with burns in both laboratory animals and man. Since only the unbound or albumin-bound corticosteroid is thought to be biologically active, an increase in the level of corticosteroid-binding globulin (CBG), an alpha-globulin, might protect thermally injured laboratory animals or man from the effects of a high plasma corticosteroid concentration. By measuring the binding activity of serum proteins, an assessment of the relative amounts of bound and unbound corticosteroids can be made. A simple adsorption method was employed to measure both the total corticosteroid-binding capacity (CBC) and the fraction of the total binding capacity due to CBG. Albuminin-binding capacity was not measured since it is not saturable under these experimental conditions. Changes in the binding capacity are directly related to the availability of the 2 serum proteins for binding: albumin, which has a high capacity, but low affinity for corticosteroids; or CBG, which has a low capacity, but high affinity with an association constant 10^5 greater than that of albumin.

MATERIAL AND METHODS

Sprague-Dawley rats were burned by immersion in water at 100° C for 10 seconds in a fiberglass mold to allow a dorsal burn of 30% of their body surface (Walker, et al).[1] Sera from these rats, and from 33 adult human male burn patients (10-70%) total body surface burn were assessed for corticosteroid and cortisol levels with a fluorometric technique and transcortin and albumin binding by selective adsorption with dextran-coated florisil using labeled cortisol-1,2-^{3}H before and after heat denaturation of transcortin.

RESULTS

Rats. Albumin binding of cortisol was directly related to steroid concentration and exhibited no saturation point. Transcortin binding could be saturated, rose after injury to one and one-half times normal by the fourth day postburn, and returned toward normal by the 10th day. This curve reflected a similar rise in serum corticosteroid levels during the postburn period.

Humans. Unlike the rat, human transcortin binding was not increased after injury, and, in fact, fell slightly despite almost two-fold increase in circulating cortisol levels. The depression of transcortin binding was greater with larger burn size.

SUMMARY

An adsorption technique was adapted to measure transcortin binding of rat serum following thermal injury. A maximum increase in the rat serum transcortin was seen 4 days following a third-degree burn. Changes in corticosterone levels paralleled alterations in the transcortin. Increased levels were still indicated when transcortin was corrected for changes in serum globulin levels following a burn. The binding by albumin remained relatively constant postburn.

In contrast to the rat, no significant alteration in transcortin occurred in human burn patient serum. As a result, the level of cortisol in excess of binding was as much as 5 times normal in the early postburn period and remained elevated up to 40 days after injury. It is concluded that the human, unlike the rat, does not respond with an increase in serum corticosteroid-binding activity following the stress of a severe burn.

REFERENCE

1. Walker HL, Mason AD Jr: J Trauma 8:1049, 1968.

PUBLICATIONS

1. Mortensen RF, Johnson AA, Eurenius K: Serum corticosteroid binding following thermal injury. Clin Res 19:31, 1971.

2. Mortensen RF, Johnson AA, Eurenius K: Serum corticosteroid binding following thermal injury. Proc Soc Exptl Biol Med 139: 877, 1972.

PRESENTATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6983	72 07 01	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. SMRY'S HISTRY	9A. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
71 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	3A061102B71P	08	068
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Procede with Security Classification Code): (U) Platelet and Megakaryocyte Kinetics in Burned Rat - Laboratory Study of Hematologic Changes Occurring 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 11	72 05	DA	C. In-House

17. CONTRACT/GRANT: Not Applicable
a. DATES/EFFECTIVE: EXPIRATION:
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18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 72	0.3	14.8
FISCAL YEAR CURRENT 73	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) Thrombocytosis; (U) Platelet Survival; (U) Burn Thrombokinetics; (U) Megakaryocytes

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

23. (U) Document postburn thrombocytosis, platelet survival, and platelet and megakaryocyte kinetics.

24. (U) Platelet counts, chromium 51 tagged survival, bone marrow examination, label distribution and collection.

25. (U) 71 07 - 72 05 Thrombocytosis in burned animals, preceded by transient thrombocytopenia; shortened platelet survival; selective early increase in bone marrow megakaryocytopoiesis have been documented. This information has been published in Journal Laboratory Clinical Medicine, volume 79:247, 1972, and the project is completed.

* Available to contractors upon originator's approval.

DD, FORM 1 MAR 66 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65 AND 1498-1, 1 MAR 66 (FOR ARMY USE) ARE OBSOLETE

FINAL REPORT

PROJECT NO. 3A061102B71P-08, BASIC RESEARCH IN SUPPORT OF MILITARY MEDICINE

REPORT TITLE: PLATELET AND MEGAKARYOCYTE KINETICS IN THE BURNED RAT--LABORATORY STUDY OF HEMATOLOGIC CHANGES OCCURRING IN BURNED SOLDIERS

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

1 July 1971 - 30 June 1972

Investigators:

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Peter M. Meserol, MS, Sp5
P. William Curreri, Lieutenant Colonel, MC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A061102B71P-08, BASIC RESEARCH IN SUPPORT OF MILITARY MEDICINE

REPORT TITLE: PLATELET AND MEGAKARYOCYTE KINETICS IN THE BURNED RAT--LABORATORY STUDY OF HEMATOLOGIC CHANGES OCCURRING 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 1971 - 30 June 1972

Investigators: Karl Eurenius, MD, Major, MC
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Reports Control Symbol MEDDH-288(R1)

Thrombocytosis is a common observation after burn injury. The present studies were performed to evaluate platelet and megakaryocyte kinetics in laboratory rats inflicted with a 30%, third-degree, scald burn. Studies included labeled platelet (^{51}Chromium) survival and distribution, and femoral bone marrow megakaryocyte concentration and morphology. Twenty-four hours after burning, thrombocytopenia was observed as a result of decreased platelet survival and burn wound sequestration. This was followed immediately by a sustained thrombocytosis resulting from both a synchronous response in bone marrow megakaryocyte production and a return of platelet survival to normal. This sustained response may be identical to that observed in other forms of tissue trauma and inflammation; and suggests that in addition to thrombocytopenia other stimuli to platelet production exist.

Thrombocytosis
Platelet survival
Burn thrombokinetics
Megakaryocytes

PLATELET AND MEGAKARYOCYTE KINETICS IN THE BURNED RAT--LABORATORY STUDY OF HEMATOLOGIC CHANGES OCCURRING IN BURNED SOLDIERS

Thrombocytosis is a common occurrence after thermal injury, and is associated with elevated fibrinogen concentration and factor V and VIII activity.[1] Thrombocyptopenia has also been observed in some burned patients, and this is thought to reflect platelet consumption.[1] The dynamics of platelet production and survival are not well understood in burns; therefore these studies were undertaken in a burned animal model.

Sprague-Dawley rats were inflicted with a 30% third-degree scald burn.[1] Following injury, ^{51}Cr blood volume, platelet count, ^{51}Cr-labeled platelet survival, bone marrow megakaryocyte content, size, and nuclear maturation were determined.

Results indicate a significant depression in platelet concentration after burn injury followed by a sustained thrombocytosis.

^{51}Cr-labeled platelet survival indicated that platelet survival was shortened during the early postburn period, and that this was an extrinsic effect, since acute postburn platelets demonstrated a normal survival curve in normal animals. Labeled platelet sequestration was identical in burned animals one hour, 5 days and 30 days after injury, and control animals with regard to liver, spleen, lung and kidney accumulation, but there was a 50-fold increase in label in burned skin as compared to an equal area of control or unburned skin.

Bone marrow megakaryocyte content increased to 4-fold normal levels by 48 hours, and remained elevated throughout the 35-day observation period. Mean megakaryocyte volume fell initially and then rose to supernormal values, suggesting the appearance of a group of young cells which were synchronously mature. This was confirmed by nuclear maturation studies which indicated an abrupt shift to binucleated megakaryocytes which in 5 days grew to 8, 16, and 37 lobed cells.

The bone marrow megakaryocyte and platelet responsetto burn injury is immediate and effective. It may be identical to that response seen in other forms of tissue trauma and inflammation. The elevated platelet counts noted in polycythemia vera, essential thrombocythemia, and inflammatory conditions reflect increased platelet production in the presence of normal platelet survival. In the early stages, thrombocytopenia and increased platelet

destruction may play an important role in the resultant thrombocytosis of burned rats. However, in the later stages of recovery enhanced megakaryocyte activity persists in the presence of both normal platelet survival and thrombocytosis. This suggests that another mechanism for platelet stimulation may exist.

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1. Walker HL, Mason AD Jr: A standard animal burn. J Trauma 8:1049, 1968.

PUBLICATION

Eurenius K, Mortensen RF, Meserol PM, Curreri PW: Platelet and megakaryocyte kinetics following thermal injury. J Lab Clin Med 79:247-257, 1972

PRESENTATIONS

None

60

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OE 6389	72 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	3A061102B71P	08	070
b. CONTRIBUTING				
c. CONTRIBUTING				

11. 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)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
71 09	Cont	DA	C. In-House

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

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 72	0.3	9.4
	CURRENT 73	0.3	10.0

19. RESPONSIBLE DOD ORGANIZATION
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ASSOCIATE INVESTIGATORS
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NAME: E D Foley, MD DA

21. GENERAL USE: FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Artificial Tendons; (U) Flexor Tendon Injuries; (U) Homografts; (U) Chickens

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

23. (U) To study the effects of 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 media ink injections performed. Biopsies of all tendon sheaths were repeated.

25. (U) 71 09 - 72 06 Neo-tendon sheaths appear to form about the implanted prostheses and histologic evaluation of this tissue is underway. Improved prostheses are being fabricated. Functional results are being assayed and will be analyzed later.

* 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. 3A061102B71P-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

1 July 1971 - 30 June 1972

Investigators:

Roger E. Salisbury, MD, Major, MC
Basil A. Pruitt, Jr., MD, Colonel, MC
Arthur D. Mason, Jr., MD
F.D. Foley, MD

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A061102B71P-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 covered in this report: 1 July 1971 - 30 June 1972

Investigators: Roger E. Salisbury, MD, Major, MC
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Reports Control Symbol MEDDH-288(R1)

Severe burns and trauma of the hands produce fibrosis, diminution of soft tissue and fixation of normal gliding planes. Conventional tendon grafting is highly unsuccessful because of uncontrollable adhesions between grafts and surrounding tissues. Experimental and clinical work with artificial tendons has clearly demonstrated superior results in the severely damaged hand. A 2-stage operation is performed that converts undifferentiated connective tissue into a new tendon sheath, preparing a suitable bed for a tendon graft.

The purpose of these investigations will be to study the effects of artificial tendons on undifferentiated connective tissue beds, to study the blood supply of the newly formed sheath and tendon autografts and homografts, and to compare end function between an autograft in a sheath and stored bank homograft tendon. In the first part of this study 10 chickens, approximately 12 weeks old, had the flexor tendons removed from the third toe of the right foot and replaced with an artificial tendon. Artificial tendons were also placed in the soft tissue of the back. Re-exploration performed up to 32 days postoperatively revealed newly formed sheaths around the artificial tendons, both in the leg and in the back, which differed from ordinary flexor tendon sheath and from each other.

Artificial tendons
Flexor tendon injuries

LABORATORY EVALUATION OF ARTIFICIAL TENDONS AND HOMOGRAFTS FOR USE IN MILITARY PERSONNEL WITH SEVERE FLEXOR TENDON INJURY

Consistently good results in flexor tendon repair after severe burns and hand trauma has not been a reality. Far too often the repaired tendon becomes densely adherent to surrounding connective tissue by scar resulting in absence of gliding. The use of artificial tendons in flexor tendon injuries of the hand has produced results superior both clinically and experimentally to any other technique tried thus far. The technique necessitates a two-stage operation and a supply of tendon autograft. Unfortunately, in the severely traumatized patient, autograft is not always available. Therefore, cadaver tendon homograft, successfully used clinically by some investigators on a small scale, presents an interesting possibility in the patient with massive trauma or in instances of mass casualties.

The purpose of these studies is to sequentially investigate (1) the effect of artificial tendons on undifferentiated connective tissue beds and study the nature of newly formed sheaths histologically by electron microscopy and to compare them with normal tendon sheaths, (2) to study the blood supply and metabolism of sheaths and tendon autograft and homograft, and (3) to compare the function between autograft in a sheath and homograft in a sheath.

METHOD

In this experiment, 10 chickens of approximately 12 weeks of age and 2 kg body weight were used as the experimental animal. In all cases the chicken was anesthetized with Penthrane and the right foot prepared with a five-minute scrub with pHisoHex solution and alcohol. Following sterile draping, a lateral incision was made in the long toe, and the flexor tendon system identified. The flexor profundus and sublimis tendons were removed as well as most of the sheath, leaving intact two pulleys. An artificial tendon of length equal to the segment of profundus tendon removed was then inserted under the pulleys and anastomosed distally and proximally with interrupted sutures of 4-0 nylon. The foot was cast in flexion, the animal turned on his side, and following sterile preparation and draping, a 2-inch segment of artificial tendon was then inserted in the soft tissue of the back. All casts were removed within three weeks and unsacrificed animals were then allowed to ambulate ad lib. Animals were serially sacrificed from 7 to 34 days postoperatively. Specimens of normal flexor tendon sheaths were taken for controls as well as artificial neo-sheaths from the back and from the toes which had been operated on.

RESULTS

In all cases undifferentiated connective tissue had formed

sheaths around artificial tendons placed in the soft tissue of the back as well as in the injured toe. Grossly, the sheath in the back was filmy, clear and moist on the surface next to the tendon. Sheaths that had formed around the artificial tendons placed in the toes were much thicker than the sheaths that had formed in the back or those on the control normal flexor tendons. Microscopic examination revealed that normal tendon sheath consisted of two layers. The layer adjacent to the gliding tendon prosthesis varied from single to several cells thick and was incomplete in some areas where uncovered collagen fibers were exposed. Underneath was a collagen layer that varied in thickness depending on the part of the sheath that was removed. Specimens from the back revealed formation of a "neo-sheath" as early as 7 days postimplantation that likewise consisted of two layers. At 7 days, an incomplete monocellular layer was adjacent to the artificial tendon. Underneath was a fine collagenous layer that was loosely packed. Specimens taken as late as 28 days revealed the 2 layers to be better defined. Biopsies of the specimens of neo-sheaths in the foot and the injured toe as well as normal tendon sheaths in uninjured toes were both taken from "no man's land." The "neo-sheath" in the foot was composed of dense collagen with a surface layer of collagen fibers partially covered by fibroblasts. The underlying collagenous layer was markedly thicker than normal flexor tendon sheath or the sheath that had formed in the back.

CONCLUSION

Artificial tendons implanted in the toes and soft tissue of the back were successful in causing undifferentiated connective tissue to form a "neo-sheath" about the implanted material. Significantly, the sheaths did differ from each other. Perhaps unlimited motion and the mechanical trauma of walking elicited a connective tissue response that formed a thicker sheath of more dense collagen in the toe. The tendon implanted in the soft tissue of the back was essentially immobile and a lesser connective tissue response was elicited. Significantly, both neo-sheaths seemed to possess a fibrous collagen component with an incomplete cellular lining similar to normal tendon sheath. Therefore, their differences are in degree rather than in kind. Electronmicroscopy studies and scanning electronmicroscopy studies are in progress which may elucidate ultrastructural differences as well.

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2. Hunter JM, Salisbury RE: Flexor tendon reconstruction in severely damaged hands. J Bone and Joint Surg 53A, 829, 1971.

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PUBLICATIONS AND/OR PRESENTATIONS

None

61

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0E 6396	72 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
	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	3A061102B71P	08	069
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) Biologic Dressings for Skin Graft Donor Sites 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
71 08	72 03	DA	C. In-House

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

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In $ thousands)
FISCAL YEAR	PRECEDING 72	0.4	9.2
	CURRENT 73	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) Biologic Dressings; (U) Skin Graft Donor Sites

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

23. (U) To compare rates of healing of donor sites covered with biologic dressings as opposed to conventional treatment.

24. (U) Twenty-one burned patients ready for skin grafting were studied. Using the left or right thigh for a donor site, a split thickness graft 10/1000 inch thick was taken with the Brown dermatome. The donor site was divided into thirds and in a random order covered with porcine heterograft, fine mesh gauze or left open with only a blood coagulant cover. All donor site coverings were allowed to separate spontaneously. Rate of healing was evaluated usually and by biopsy of each donor site at 10 days postoperatively and 30 days after discharge from the hospital.

25. (U) 71 08 - 72 03 Histologically documented incorporation of porcine collagen in healing donor sites with marked edema, inflammatory change and delayed healing indicate that physiologic dressings do not exert beneficial effects on split thickness skin graft donor site.

*Available to contractors upon originator's approval.

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FINAL REPORT

PROJECT NO. 3A061102B71P-08,BASIC RESEARCH IN SUPPORT OF MILITARY MEDICINE

REPORT TITLE: BIOLOGIC DRESSINGS FOR SKIN GRAFT DONOR SITES IN BURNED TROOPS

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
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1 July 1971 - 30 June 1972

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Douglas W. Wilmore, MD, Major,MC
Paul Silverstein, MD, Major,MC
F. D. Foley, MD

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A061102B71P-08, BASIC RESEARCH IN SUPPORT OF MILITARY MEDICINE

REPORT TITLE: BIOLOGIC DRESSINGS FOR SKIN GRAFT DONOR SITES 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 1971 - 30 June 1972

Investigators: Roger E. Salisbury, MD, Major,MC
Douglas W. Wilmore, MD, Major,MC
Paul Siverstein, MD, Major,MC
F.D. Foley, MD

Reports Control Symbol MEDDH-288(R1)

Biologic dressings aid healing of second degree burns, promote granulation of denuded surfaces, decrease bacterial proliferation on open wounds and increase rate of epithelialization. This study was designed to determine if application of a biologic dressing would accelerate the healing of a split thickness skin graft donor site.

Split thickness skin at 10/1000th inch was harvested from the anterior portion of the thigh in 17 patients requiring grafting. The donor site was divided into thirds and each area randomly assigned 1 of 3 methods of treatment, which included porcine xenograft, saline soaked fine mesh gauze or no dressing. Surface covering on all wounds was allowed to separate spontaneously and time of complete healing of each respective donor area determined by daily inspection. Photographs and biopsies were taken at 10 and, in selective cases, at 30 days postoperatively. Biopsy specimens were coded and evaluated for healing by light microscopy.

Rate of donor site healing and character of the healed skin were comparable by visual and biopsy examination when comparing 3 methods of donor site treatment in 11 patients. However, in 6 patients incorporation of all or part of the xenograft in the split thickness donor site areas occurred, resulting in erythematous, raised wounds with delayed healing. Histologic evaluation revealed disorganized healing with host fibroblast invasion around or through portions of the porcine graft and incorporation of porcine dermis between host epidermis and deep dermal structures.

Application of porcine cutaneous xenograft does not improve split thickness skin graft donor site healing and may even promote disorganized healing and formation of foreign body granuloma.

Biologic dressings
Skin graft donor sites

BIOLOGIC DRESSINGS FOR SKIN GRAFT DONOR SITES IN BURNED TROOPS

In the seriously burned patient donor sites may be quite limited. Though recipient sites may be ready for grafting, a recently utilized donor area may not be healed enough to "recrop". Techniques that would hasten healing and retard infection of donor sites would be valuable. It has been found that homografts and heterografts applied to second degree burns lead to early epithelization, less bacterial contamination, more organized histologic healing and better cosmesis than similar burns left uncovered.

The purpose of this study was to evaluate a technique that might hasten healing of split thickness skin graft donor sites.

METHOD

In 17 patients requiring skin grafting, the left or right anterior thigh was chosen as a donor site. All donor sites were prepared with a 5 minute Betadine scrub and then draped with sterile sheets. Split thickness skin, 10/1000th inch thick, was taken with the Brown dermatome. Pressure was applied to the donor site with 4 x 4s soaked in dilute epinephrine solution, 1:100,000 concentration, to achieve hemostasis. Once hemostasis was achieved, the donor site was divided into thirds and randomly treated with porcine xenograft, saline soaked fine mesh gauze or left open for blood coagulum. Xenografts were observed daily and changed if subgraft suppuration developed. Fine mesh gauze and blood coagulum were allowed to separate spontaneously. Rate of healing was evaluated visually and by biopsies of the donor sites taken at 10 days and in selected cases, 30 days postoperatively. Specimens were evaluated by light microscopy for epithelialization, differentiation of epidermal layers and changes in dermis. Donor sites were evaluated visually each day for differences in color or texture. If obvious differences were noted, color pictures were taken. If any donor site became purulent cultures were taken and the frequency of infection tabulated.

RESULTS

In 11 patients visual and biopsy evaluation of donor sites revealed no difference in the rate of healing or the character of the healed wound. Though the fine mesh gauze, blood coagulum and xenograft separated on different days, there was no difference in the rate of healing. However, 6 patients demonstrated incorporation of the porcine xenograft in the healing donor site. These donor sites appeared erythematous and raised. Histologic evaluation revealed incorporation of dermal collagen, disorderly epidermal

maturation and formation of milia at the graft-recipient junction. Visual re-evaluation at 30 days did not reveal any improvement. Significantly there were no infections of any donor sites nor did any xenografts have to be removed.

CONCLUSIONS

Coverage of split thickness donor site with a biologic dressing of porcine xenograft does not accelerate donor site healing. In fact, it caused cosmetic and histologic complications in a significant number of patients in this series. No instances of xenograft incorporation have been documented as yet with second degree burns. It is obvious that a split thickness skin defect created by the dermatome behaves differently than a partial thickness burn in its response to porcine biologic dressings. Therefore, while pigskin may be useful in covering healing second degree burns, it is inadviseable to use it for coverage of donor sites.

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PRESENTATION

Foley FD: Pathology of the xenograft covered donor site. Amer Burn Assoc, San Francisco, April 1972.

PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OD 6397	72 07 01	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DOD'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
71 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	3A061102B71P	08	066
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) Volume Control of Renal Glucose Reabsorption - Laboratory Study of Changes in Sick and Injured Troops (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 72	0.5	11.5
	CURRENT 73	0.5	15.0

19. RESPONSIBLE DOD ORGANIZATION
NAME: US Army Institute of Surgical Research
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SOCIAL SECURITY ACCOUNT NUMBER:

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

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code): (U) Combat wounded soldier
(U) Glucose; (U) Extracellular Volume; (U) Sodium; (U) Glomerular Filtration Rate

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

23. (U) Disorders of glucose metabolism characterized by over production of glucose and under utilization of glucose are extremely common in troops subject to trauma or medical illness. These disorders of glucose metabolism would not result in symptomatic hyperglycemia if the kidney excreted the excess glucose. Since the kidney commonly does not excrete such excess amounts of glucose, this study was undertaken to determine if glucose is reabsorbed by the kidney by an independent rate limited transport mechanism or if glucose reabsorption is linked to that of sodium as has recently shown to be true for uric acid, calcium, phosphate, bicarbonate, etc. In addition, the effect of glomerular filtration rate on glucose reabsorption was examined.

24. (U) Since sodium reabsorption is controlled by extracellular volume, glucose reabsorption was measured in dogs with normal, expanded, and contracted extracellular volumes.

25. (U) 71 07 - 72 06 Results thus far obtained have shown that glomerular tubular balance for glucose exists and that this glomerular tubular balance may be disrupted when sodium reabsorption is changed. The therapeutic implication from these studies is that disorders of glucose metabolism which may result in hyperglycemia, can be ameliorated if proper attention to salt and water balance is paid and if extracellular volume contraction is avoided. Future studies will examine the effect of cylic AMP, prostaglandins, acetylcholine, parathormone, and oxytocin on renal glucose reabsorption. The effect of vasopressin on glucose and sodium reabsorption has been examined. ADH depresses sodium reabsorption without affecting glucose transport indicating that ADH exerts its inhibitory effect distal to the proximal tubule presumably in the ascending limb of the loop of Henle.

*Available to contractors upon originator's approval

FINAL REPORT

PROJECT NO. 3A061102B71P-08, BASIC RESEARCH IN SUPPORT OF MILITARY MEDICINE

REPORT TITLE: VOLUME CONTROL OF RENAL GLUCOSE REABSORPTION--LABORATORY STUDY OF CHANGES IN SICK AND INJURED TROOPS

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

1 July 1971 - 30 June 1972

Investigators:

Neil A. Kurtzman, MD, Lieutenant Colonel, MC
Philip W. Rogers, MD, Major, MC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A061102B71P-08, BASIC RESEARCH IN SUPPORT OF MILITARY MEDICINE

REPORT TITLE: VOLUME CONTROL OF RENAL GLUCOSE REABSORPTION--LABORATORY STUDY OF CHANGES IN SICK AND INJURED TROOPS

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

Period covered in this report: 1 July 1971 - 30 June 1972

Investigators: Neil A. Kurtzman, MD, Lieutenant Colonel, MC
Philip W. Rogers, MD, Major, MC

Reports Control Symbol MEDDH-288(R1)

This study was undertaken to determine if glucose reabsorption by the kidney was linked to that of sodium as has recently been shown to be true for uric acid, calcium, phosphate and bicarbonate. Since sodium reabsorption is controlled by extracellular volume, glucose reabsorption was measured in dogs with normal, expanded, and contracted extracellular volumes. Also, the effect of glomerular filtration rate on glucose reabsorption was examined.

Sodium
Glucose
Extracellular volume
Glomerular filtration rate

VOLUME CONTROL OF RENAL GLUCOSE REABSORPTION--LABORATORY STUDY OF CHANGES IN SICK AND INJURED TROOPS

Disorders of glucose metabolism characterized by overproduction of glucose and underutilization are common in troops subject to trauma or medical illness. These disorders of glucose metabolism would not result in symptomatic hyperglycemia if the kidney excreted this excess glucose. Since the kidney commonly does not excrete this excess glucose, this study was undertaken to determine if glucose is reabsorbed by the kidney by an independent rate limited transport mechanism or if glucose reabsorption is linked to that of sodium. Since sodium reabsorption is controlled by extracellular volume, glucose reabsorption was measured in dogs with normal, expanded, and contracted extracellular volumes. Our data show that glomerular tubular balance for glucose exists and that this balance is disrupted when sodium reabsorption is changed. Furthermore, changes in sodium reabsorption are directly paralleled by changes in glucose reabsorption. The relationship of sodium reabsorption to that of glucose is demonstrated in Figure 1. The data demonstrating glomerular tubular balance for glucose is presented in Figure 2.

Glucose reabsorption has been used as a marker of proximal tubular reabsorption. ADH, which is markedly chloriuretic and natriuretic, does not affect glucose reabsorption indicating that ADH exerts its effect on sodium transport distal to the proximal tubule.

PUBLICATIONS

Kurtzman, NA, White, MG, Rogers, PW, Flynn JJ III: The relationship of sodium reabsorption and glomerular filtration rate to renal glucose reabsorption. Proc Amer Soc Nephrology 5:42, 1971.

Kurtzman, NA, White MG, Rogers PW, Flynn JJ III: The relationship of sodium reabsorption and glomerular filtration rate to renal glucose reabsorption. J Clin Invest 51:127, 1972.

PRESENTATION

Kurtzman NA: The Relationship of Sodium Reabsorption and Glomerular Filtration Rate to Renal Glucose Reabsorption. Amer Soc of Nephrology meeting, 22 Nov 71, Wash DC.

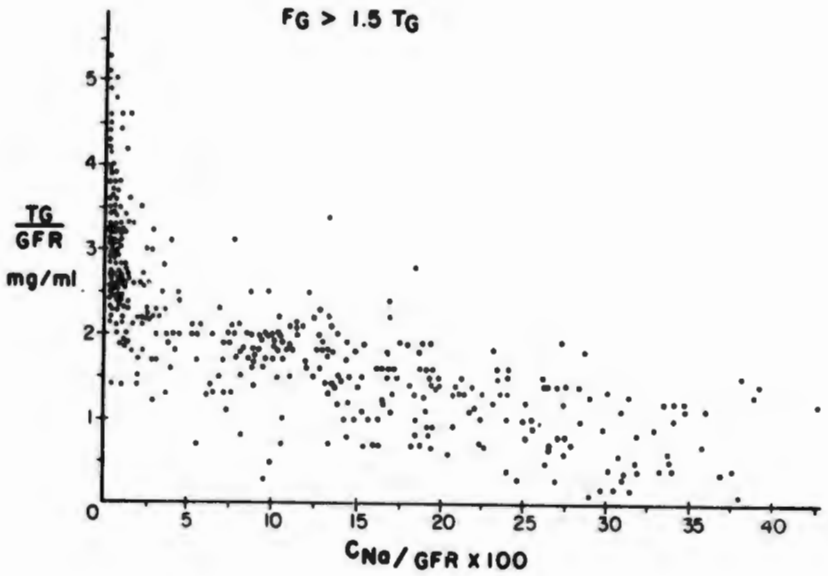

Figure 1

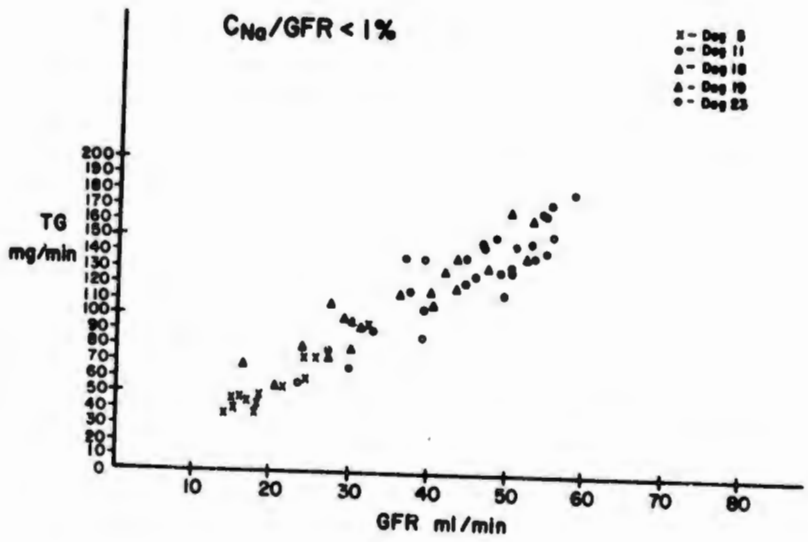

Figure 2

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OD 6398	72 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
71 07 01	K.COMPLETION	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62110A	3A062110A821	00	111
b. CONTRIBUTING	61102A	3A061102B71R	01	
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)

(U) Platelet Function 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
70 06	72 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 72	0.3	12.8
	CURRENT 73	0	0

19. RESPONSIBLE DOD ORGANIZATION

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20. PERFORMING ORGANIZATION

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ASSOCIATE INVESTIGATORS

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NAME: P W Curreri, LTC, MC DA

21. GENERAL USE

FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Burn; (U) Coagulation; (U) Platelets

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

23. (U) Platelet function with respect to coagulation studies, including disseminated intravascular coagulation in thermally injured rats and selected burn patients.

24. (U) Assessment of ADP platelet aggregation in burn patients and a laboratory animal model.

25. (U) 71 07 - 72 06 Rats: Immediate postburn suppression and subsequent overshoot of ADP induced platelet aggregation. Plasma-serum factor in acute postburn period capable of suppressing normal platelet aggregation.

Humans: ADP induced platelet aggregation is depressed after burn injury and returns only very slowly to normal, in relation to burn size.

*Available to contractors upon originator's approval.

DD FORM 1 MAR 66 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65 AND 1498-1, 1 MAR 66 (FOR ARMY USE) ARE OBSOLETE.

FINAL REPORT

PROJECT NO. 3A062110A821-00, COMBAT SURGERY

REPORT TITLE: PLATELET FUNCTION IN THE BURNED SOLDIER

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

1 July 1971 - 30 June 1972

Investigators:

Karl Eurenius, MD, Major, MC
Gerald Rothenberg, MD, Major, MC*

* From the Department of Pathology, Brooke Army Medical Center, Fort Sam Houston, Texas 78234

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A062110A821-00, COMBAT SURGERY

REPORT TITLE: PLATELET FUNCTION IN THE BURNED SOLDIER

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

Period covered in this report: 1 July 1971 - 30 June 1972

Investigators: Karl Eurenius, MD, Major, MC
Gerald Rothenberg, MD, Major, MC*

Reports Control Symbol MEDDH-288(R1)

Platelet aggregation is suppressed in the rat immediately after burn injury. This suppression appears to be mediated by a plasma factor. By 24 hours after burning, platelet aggregation is supernormal and then falls to normal. Enhanced aggregation could not be related to an extrinsic mechanism, but may be related to the appearance of a cluster of new young platelets in response to injury. These changes are discussed with relation to coagulation.

Burn Coagulation Platelets

* From the Department of Pathology, Brooke Army Medical Center, Fort Sam Houston, Texas 78234.

PLATELET FUNCTION IN THE BURNED SOLDIER

In burned patients we have frequently observed alterations in hemostasis, resulting in life-threatening hemorrhage or thrombosis. Survey studies indicate significant elevations in fibrinogen and platelet concentration and factor V and VIII activity, although some patients fail to support this response and bleed. A recent examination of platelet and megakaryocyte kinetics in scald burned rats demonstrated early thrombocytopenia due to burn wound sequestration, followed by sustained thrombocytosis (Eurenius, et al).[1] One *in vitro* test of *in vivo* platelet function is platelet aggregation, the agglutination of platelets in the presence of adenosine diphosphate (ADP) or substances which mediate the release of endogenous ADP. We have assessed aggregation in rats after burn injury.

MATERIALS AND METHODS

Sprague-Dawley, male rats (150-220 gm) were inflicted with a 30%, third-degree, scald burn under pentobarbital anesthesia (Walker, et al).[2] At one and 6 hours, and 1, 2, 4, 6, 15, 20, and 30 days postburn, these rats were bled by cardiac puncture using plastic syringes, and a 9 volume blood: 1 volume, 3.2% trisodium citrate anticoagulation mixture prepared. Ten animals were examined at each period. Platelet rich plasma (PRP) was collected as the supernatant material after centrifugation at 350 xg, 15 min RT, and platelet poor plasma (PPP) was collected as the supernatant material after centrifugation of the remaining blood at 1100 xg for 10 min RT. Platelet counts were determined with phase microscopy in counting chambers after dilution of the plasma with 1% ammonium oxalate.

Aggregation was recorded and measured using a recording densitometer equipped with magnetic stirrer (1100 RPM) and constant temperature block (37° C). To each 1.2 ml sample of pre-warmed, pre-recorded PRP, 0.05 ml of ADP was added. Aggregation curves were recorded, traced, weighed and converted to integrated area per 10^6 platelets per mm^3 PRP, since earlier studies demonstrated a linear relationship between PRP platelet concentration and the area described by the aggregation curve.

A group of rats similarly anesthetized and burned was assessed for calcium and urea concentration, thrombin and partial thromboplastin time, factor VIII activity (activated PTT assay), plasma fibrinogen and blood platelet count.

RESULTS

A prompt reduction in aggregation was observed immediately after burn injury, followed at 24-48 hours by a brief but significant "overshoot" and a gradual return to normal. Mean curves of control, 1 hour, 48 hours and 6 day tracings are presented in figure 1, and all aggregation data are presented in Figure 2. Associative coagulation data from the same observation periods are presented in the table.

Aggregation of control, 1 and 48 hour postburn PRP, after preincubation (15 min, 37° C) with equal volumes of PPP from each of the 3 groups was next studied. Results, figure 3, indicate that there is no significant change in the reaction when each PRP is preincubated with its own plasma (B, D), or when control PRP is preincubated with 48 hour postburn plasma (C), but a significant suppression of normal PRP aggregation occurred in the presence of PPP from 1 hour postburn rats, indicating the presence of an extrinsic inhibitor.

DISCUSSION

In vitro platelet aggregation may be elicited by various substances, including ADP, soluble enzymes (thrombin), collagen, particulate matter, saturated fatty acids and antiplatelet serum. Catecholamines, heat denatured plasma, and lecithin have also been reported to be effectors. The reaction is mediated in most cases through endogenous ADP. The magnitude of this reaction is dependent upon platelet and ADP concentration, temperature, turbulence, calcium, fibrinogen and pH.

We noted both significant depression and augmentation of the aggregation response in burned rats. At the time that suppression was noted (1 hour), the thrombin time was significantly prolonged in the absence of heparin and in the presence of increased fibrinogen concentration. This suggested the presence of fibrin degradation products, further confirmed by the subsequent decrease in platelet count, fibrinogen and factor VIII (see Table). Fibrin split products have been associated with increased and decreased platelet aggregation. Decreased platelet aggregation has been reported in cirrhotics with fibrinolysis as measured by prolonged thrombin times. Although depressed platelet adhesion has been observed in uremia, we were unable to demonstrate renal failure throughout the postburn period. Failure to aggregate as a result of hypercorticosteronism is unlikely since steroid levels are elevated not only initially but throughout the convalescent period (Mortensen, et al).[3] Although intrinsic platelet damage may explain this early inhibition of aggregation, we have also demonstrated a potent extrinsic inhibitor. It is unlikely that the factor is ADP, released from red cells during the hemolysis making the platelets refractory to further ADP challenge, since mixing normal

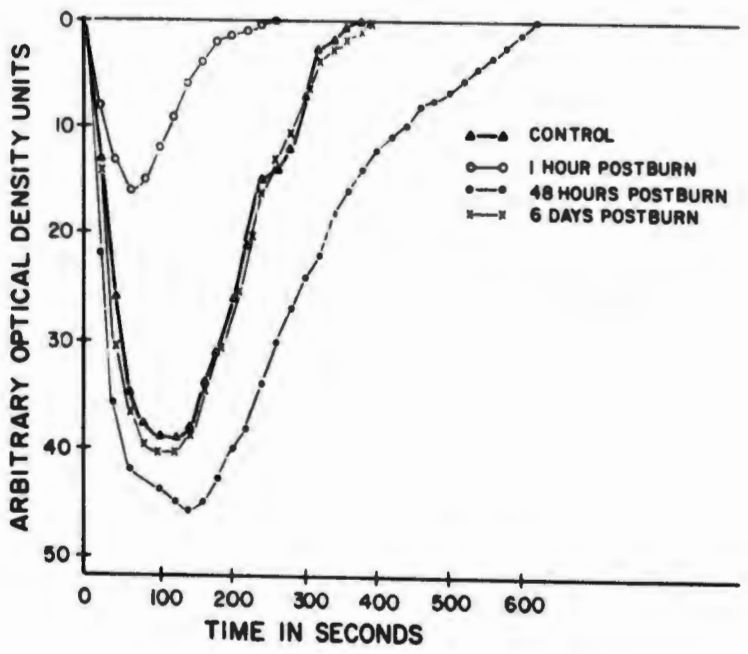

Figure 1. Mean aggregation curves for platelets from control, 1 hour and 2 and 6 days postburn animals. Results are expressed as optical density change per million platelets in response to 8.5 x 10^{-6} M ADP, and plotted as a function of time in seconds.

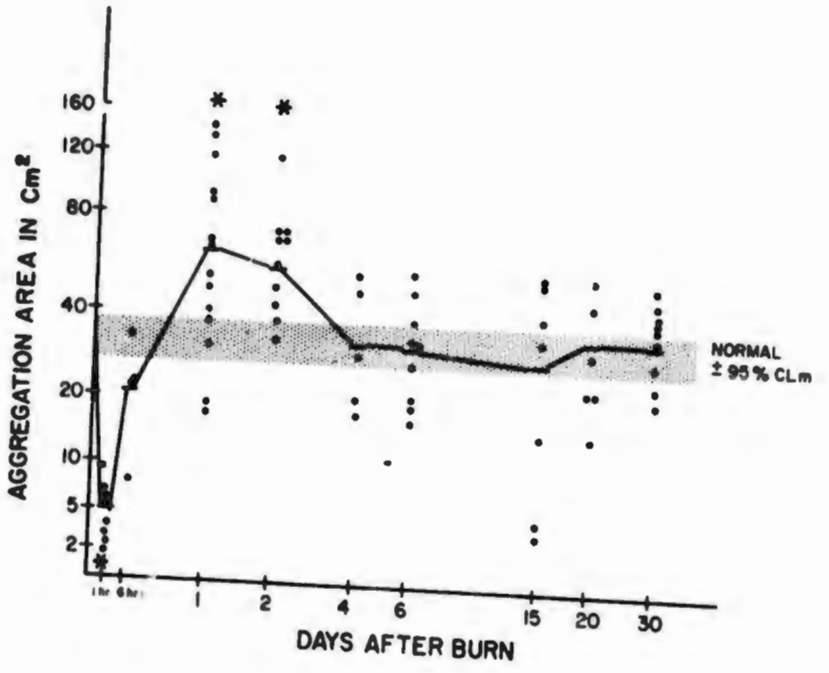

Figure 2. Change in platelet aggregation, measured by curve integration in cm^2 as a function of time postburn. Mean values at 1 hour and 1 and 2 days postburn differ significantly from control $p < 0.005$.

Factors Affecting Platelet Function

	Ca++ (mg %)	BUN (mg %)	P.T. (sec.)	P.T.T. (sec.)	Factor VIII (% of Control)	T.T. (sec.)	Fibrinogen (mg %)	Platelet Count
Control	5.5	18	13.4	39.2	100	15.5	155	1.16 x $10^6/mm^3$
1 Hour	4.9	23	15.2	63.9	125	24.6	181	1.15
6 Hour	-	-	14.2	74.6	80	14.7	99	0.91
1 Day	7.1	24	12.1	82.6	160	11.4	724	0.62
2 Day	-	-	13.1	98.2	180	12.5	580	1.10
3 Day	6.9	19	13.8	58.9	150	25.2	354	1.48
4 Day	6.2	20	13.1	57.0	110	14.8	209	1.61
6 Day	5.4	16	12.9	71.8	110	14.5	239	1.92
10 Day	6.5	22	13.0	72.0	200	20.1	230	1.72

Ca++ - Total serum calcium in mg per cent
BUN - Blood urea nitrogen in mg per cent
P.T. - Prothrombin time in seconds
P.T.T.- Partial thromboplastin time in seconds (one stage)
VIII - Factor VIII activity as per cent of control
T.T. - Thrombin time in seconds
Fibrinogen - Fibrinogen concentration in mg per cent
Platelet Count - Platelets x $10^{-6}/mm^3$ whole blood

63-5

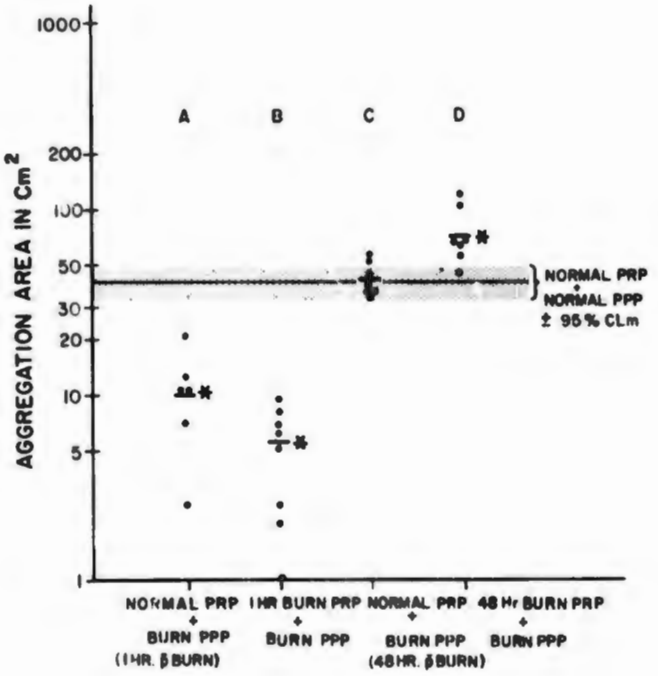

Figure 3. Platelet aggregation, measured by curve integration of normal platelets incubated with either 1-hour postburn plasma (A), or 48-hour postburn plasma (C). Compared with 1 hour (B) and 48 hour (D) postburn platelets incubated in their own plasma. Significantly different mean from that of control indicated by *.

platelets with 1 hour postburn PPP did not cause aggregation before the addition of ADP.

The enhanced aggregation observed in rats 24 and 48 hours after burn is of some interest. Endotoxin, fibrin split products, and the postoperative state have been associated with augmented aggregation. Postoperative hyperaggregation has been attributed to the release of young platelets with an increased metabolic requirement. Young human platelets aggregate better with collagen than old platelets, but this has not been confirmed with ADP. We have previously described the appearance of a synchronous burst of new platelets after burn injury beginning at 24 hours (Eurenius, et al).[1]

If in vitro aggregation reflects an important aspect of platelet function in hemostasis, factors affecting this mechanism including fibrin degradation products, may be closely related to the hemorrhage and thrombosis seen in burned patients.

REFERENCES

1. Eurenius K, Mortensen RF, Meserol PM, Curreri PW: Platelet and megakaryocyte kinetics following thermal burn. J Lab Clin Med 79:247-257, 1972.

2. Walker HL, Mason AD Jr: A standard animal burn. J Trauma 8:1049-1051, 1968.

3. Mortensen RF, Johnson AA, Eurenius K: Serum corticosteroid binding following thermal injury. Proc Soc Expt Med Biol 139:877, 1972.

PRESENTATIONS AND/OR PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6976	72 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
71 07 01	K, COMPLETION	U	U	NA	NL	☑ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62110A	3A062110A821	00	106
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)

(U) Circulation in the Extremities of Burned Troops (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
69 07	72 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 72	0.3	10.4
	CURRENT 73	0	0

19. RESPONSIBLE DOD ORGANIZATION

NAME: US Army Institute of Surgical Research

ADDRESS: Ft Sam Houston, Tx 78234

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20. PERFORMING ORGANIZATION

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Burn Study Branch

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SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Roger Salisbury, Maj, MC

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

21. GENERAL USE

FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Circulation; (U) Extremity; (U) Thermal Injury; (U) Escharotomy

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

23. (U) To assess circulatory changes in patients with extremity burns and to evaluate monitoring effectiveness of flowmeter in low flow states and venous disease.

24. (U) All patients with extremity burns are studied with an ultrasonic flow meter to evaluate presence of distal extremity flow and need for escharotomy. The flow meter also was employed to monitor blood pressure of patients in shock and to document venous obstruct in patients with suspected thrombophlebitis.

25. (U) 71 07 - 72 06 The Doppler flowmeter is clinically useful in determining peripheral blood flow in patients with circumferential limb burns and its use has decreased the performance of escharotomy by 50%. A systolic blood pressure unobtainable by conventional means was recorded in 33 patients either in shock with massive limb edema, or in the pediatric age group and was valuable in guiding therapy. Thrombophlebitis was documented in 8 patients in whom a venogram was difficult to obtain because of the burn injury. Clinical use of the instrument continues, but the study is completed.

(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.

FINAL REPORT

PROJECT NO. 3A062110A821-00, COMBAT SURGERY

REPORT TITLE: CIRCULATION IN THE EXTREMITIES OF BURNED TROOPS

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

1 July 1971 - 30 June 1972

Investigators:

Joseph A. Moylan, Jr, MD, Major, MC
Roger E. Salisbury, MD, Major, MC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A062110A821-00, COMBAT SURGERY

REPORT TITLE: CIRCULATION IN THE EXTREMITIES OF BURNED TROOPS

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

Period covered in this report: 1 July 1971 - 30 June 1972

Investigators: Joseph A. Moylan, Jr, MD, Major, MC
Roger E. Salisbury, MD, Major, MC

Reports Control Symbol MEDDH-288(R1)

The Doppler flowmeter is clinically useful in determining peripheral blood flow in patients with circumferential limb burns and its use has decreased the performance of escharotomy by 50%. A systolic blood pressure, unobtainable by conventional means, was recorded in 33 patients, either in shock with massive limb edema or in the pediatric age group, and was valuable in guiding therapy. Venous occlusion was documented in 8 patients in whom a venogram was difficult to obtain because of the burn injury. Clinical use of the instrument continues, but the study is completed.

Circulation
Extremity
Thermal injury
Escharotomy

CIRCULATION IN THE EXTREMITIES OF BURNED TROOPS

In a controlled prospective study (Moylan, et al),[1] the Doppler ultrasonic flowmeter has been found to be a sensitive instrument in evaluating distal extremity blood flow following circumferential third degree limb burns. Continued usage of the flowmeter for this test and newer uses are the subject of this report.

During the year 1971, 47 patients with limb burns averaging 85% of the extremity surfaces were admitted to the US Army Institute of Surgical Research. A total of 116 limbs had some third degree involvement. From our prior experience, over 80% of these limbs would necessitate escharotomies, based on clinical judgment. However, using the Doppler flowmeter, only 18% of this group required an escharotomy. The ultrasonic flowmeter permitted a limited escharotomy in 4 upper limbs when return of flow to terminal digits was noted following a decompression limited to the wrist. The Doppler flowmeter allowed repeated monitoring of each limb to confirm the adequacy of flow over the next 48 hours.

The Doppler flowmeter was also used to monitor blood pressure in both adults and children, in whom it would have been otherwise unobtainable because of severe upper limb edema, the presence of burn per se, or absence of upper limbs. During this period, 33 patients were in this category, many being children. Measurement of blood pressure in these circumstances was a valuable adjunct to therapy as all required vasopressors to maintain an adequate blood pressure.

Many patients with lower limb burns had persistant edema in their legs following the diuretic period. The etiology of the persistent edema was not clear. In 8 individuals in whom a venogram was difficult to obtain because of the burn injury, venous occlusion was diagnosed and the course of therapy was evaluated by repeated Doppler flowmeter examinations. In other patients, the patency of their major venous tributaries was established in the presence of limb edema and no anticoagulant therapy instituted. There were no pulmonary emboli, either clinically or at autopsy, in those patients who expired in this group.

Clinical use of the ultrasonic flowmeter continues but the study has been completed.

REFERENCE

1. Moylan JA,Jr, Inge WW,Jr, Pruitt BA,Jr: Circulatory changes following circumferential extremity burns evaluated by ultrasonic

flowmeter: An analysis of 60 thermally injured limbs. J Trauma 11: 763, 1971.

PRESENTATIONS AND/OR PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6981	72 07 01	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. ENTER'D INSTR	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
71 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	62110A	3A062110A821	00	113
b. CONTRIBUTING	61102A	3A061102B71R	01	
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)

(U) Urinary Tract Infections in Burned Military Patients (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
69 05	72 06	DA	C. In-House

17. CONTRACT/GRANT: Not Applicable

a. DATE/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 72	0.3	8.4
	CURRENT 73	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) Bacteriuria; (U) Urinary tract infection

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 effect of the presence and duration of an indwelling urethral catheter on the incidence of urinary tract infections. To assess the relation, if any, between organisms in the urinary tract and in the blood stream.

24. (U) Blood and urine cultures will be obtained at time of catheterization, 48 hours, one, two, four and eight weeks postburn.

25. (U) 71 07 - 72 06 Probit analysis revealed that after 11 days of continuous catheterization 50% of patients had significant urinary tract bacterial counts (greater than 10 to 5 colonies per cc of urine). Only a few urinary tract infections failed to clear following catheter removal and required antibiotic therapy. The causative organisms were common inhabitants of the burn wound.

*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. 3A062110A821-00, COMBAT SURGERY

REPORT TITLE: URINARY TRACT INFECTIONS IN BURNED MILITARY PATIENTS

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

1 July 1971 - 30 June 1972

Investigators:

Jon M. Reckler, MD, Major, MC
Andrew M. Munster, MD, Lieutenant Colonel, MC
Robert B. Lindberg, PhD
Arthur D. Mason, Jr., MD

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

65-11

ABSTRACT

PROJECT NO. 3A062110A821-00, COMBAT SURGERY

REPORT TITLE: URINARY TRACT INFECTIONS IN BURNED MILITARY PATIENTS

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

Period covered in this report: ! July 1971 - 30 June 1972

Investigators: Jon M. Reckler, MD, Major, MC
Andrew M. Munster, MD, Lieutenant Colonel, MC
Robert B. Lindberg, PhD
Arthur D. Mason, Jr., MD

Reports Control Symbol MEDDH-288(R1)

Urethral catheterization is essential to evaluate the adequacy of resuscitation in the patient with extensive burns by monitoring the urinary output. Urinary tract infections related to catheterization are among the most important complications attendant to their use and appear to be directly related to the duration of catheter residence within the bladder. This prospective study was designed to determine the incidence of urinary tract infections and the organisms involved in catheterized burn patients, to establish the incidence of persistent infection at the time of discharge from the hospital, and to describe the pathogenesis of such infections.

Thirty-four male patients between the ages of 15 and 45 years, with burns of from 5 to 76% of the total body surface (mean burn size 40%), admitted to our burn center within 48 hours of injury and requiring placement of an indwelling urethral catheter were included in the study. Quantitative urine cultures were obtained serially until discharge. Depth and location of burn, duration of catheterization and the clinical course of each patient were documented.

Probit analysis of the data revealed that after 11 days of continuous catheterization 50% of patients had significant urinary tract bacterial counts, i.e., greater than 10^5 colonies per cc of urine. Only a few urinary tract infections failed to clear following catheter removal and required antibiotic therapy. The causative organisms were common inhabitants of the burn wound.

Bacteriuria
Urinary tract infection

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OE 6951	72 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
	K. COMPLETION	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62110A	3A062110A821	00	108
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) Cecal Wound Healing in Burned and Non-Burned Rats - Laboratory Model Simulating 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 02	71 09	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 72	0.5	10.0
FISCAL YEAR CURRENT 73	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) Wound Healing; (U) Burned Rats; (U) Cecum

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

23. (U) To compare the healing in the incised cecum of burned and non-burned Sprgague-Dawley rats.

24. (U) Four groups of animals were studied at 3, 5 and 7 days post surgery, bursting pressures of the cecum being measured. Four groups contained 10 animals each and were: (1) 40% burned rat, no operation; (2) 40% burned rat with abdominal and cecal incision; (3) non-burned rat with abdominal incision only and (4) non-burned rat with abdominal and cecal incisions.

25. (U) 71 02 - 71 09 After excluding those who died prematurely or for various technical reasons were unsuitable, a total of 186 rats were studied. At 3 days postoperatively the bursting pressures of the incised cecums of both non-burned and burned rats were equal compared to non-incised cecums which were twice as strong. At 5 days postoperatively the incised cecums of burned rats were weaker than the incised cecum in non-burned rats showing delayed healing, probably a result of the burn. However at 7 days postoperatively the incised cecums of the burned animals were almost as strong as non-incised cecums of the non-burned rats.

*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 66 AND 1498-1, 1 MAR 66 (FOR ARMY USE) ARE OBSOLETE.

FINAL REPORT

PROJECT NO. 3A062110A821-00, COMBAT SURGERY

REPORT TITLE: CECAL WOUND HEALING IN BURNED AND NONBURNED RATS - LABORATORY MODEL SIMULATING BURNED SOLDIERS

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

1 July 1971 - 30 June 1972

Investigators:

Wellford W. Inge, Jr., MD, Lieutenant Colonel MC
Arthur D. Mason, Jr., MD
Harrel L. Walker, MS
Andrew M. Munster, MD, Lieutenant Colonel, MC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A062110A821-00, COMBAT SURGERY

REPORT TITLE: CECAL WOUND HEALING IN BURNED AND NONBURNED RATS - LABORATORY MODEL SIMULATING BURNED SOLDIERS

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

Period covered in this report: 1 July 1971 - 30 June 1972

Investigators: Wellford W. Inge, Jr., MD, Lieutenant Colonel, MC
Arthur D. Mason, Jr., MD
Harrel L. Walker, MS
Andrew M. Munster, MD, Lieutenant Colonel, MC

Reports Control Symbol MEDDH-288(R1)

It has been a clinical impression that intestinal anastomoses, as well as abdominal incisions heal more slowly in burn patients as compared to comparable surgical incisions and procedures in nonburned patients. In an attempt to study healing in intestinal incisions, a surgical incision was made in the antimesenteric border of the cecum of Sprague-Dawley rats. Four groups of animals were studied: (1) burned, no cecal incision; (2) burned, abdominal and cecal incisions; (3) nonburned, abdominal incision only; and (4) nonburned, abdominal and cecal incision. Healing of the cecal incision was tested by measuring the bursting pressure of the cecum. At 3 days postoperatively the incised cecums of both the nonburned and burned rats were equally weak as compared to non-incised cecums which were twice as strong. At 5 days postoperatively the incised cecums of the burned rats were weaker than the incised cecums of the nonburned rats which were as strong as the non-incised cecums of both groups. However, at 7 days postoperatively the incised cecums of both burned and nonburned animals were almost as strong as non-incised cecums. One can conclude from this study that there is a lag period in healing in the incised cecum of the burned rat at 5 days postoperatively but by one week postoperatively healing as measured by recording the bursting pressures of the cecum has returned almost to that of the nonburned animal.

Wound healing
Burned rats
Cecum

CECAL WOUND HEALING IN BURNED AND NONBURNED RATS - LABORATORY MODEL SIMULATING BURNED SOLDIERS

To prove or disprove the premise in the laboratory that intestinal anastomoses healed more slowly in the burned patients as compared to nonburned patients, cecal incisions were made in burned and nonburned Sprague-Dawley rats and the bursting pressures of the cecum recorded at 3, 5, and 7 days postoperatively.

METHOD

Sprague-Dawley rats weighing from 180-200 gm were divided into 4 groups, 10 rats in each group.

1. 40% burn with no operation.
2. 40% third degree burn with abdominal and cecal incisions.
3. Nonburned rats with abdominal incisions only.
4. Nonburned rats with abdominal and cecal incisions.

All rats, whether burned or not, were anesthetized with sodium pentobarbital 20-25 mg/kg and were given 10 cc intraperitoneal saline for resuscitation. At 3 days postburning, all of the animals were anesthetized as before and operations were performed as indicated by the assigned group. Abdominal incisions were made in the midline and were 2½ cm in length. Cecal incisions were made 1½ cm in length on the antimesenteric border and closed with a single layer of simple running 3-0 chromic catgut. Abdominal incisions were closed in 2 layers with muscle and fascia approximated with a running 3-0 chromic catgut suture and skin closed with a running 3-0 silk. Then at 3, 5, or 7 days postoperatively all animals were sacrificed. The cecum was dissected out, excised and bursting pressure of the cecum measured by inserting a cannula into the ascending colon, submerging the cecum in a saline filled beaker, inflating the cecum with a Harvard pump containing 50 cc syringe filled with air and recording the pressure curve on a Sanborn recorder (Gray, 1967;[1] Ravitch, 1967[2]). Animals dying before time to be sacrificed were excluded from the study. Technical problems with dissection and preparing the cecum for bursting excluded others.

Pressure setting on the recorder was from 0 - 200 mm Hg and speed of the paper at 2.5 mm/sec. Air was delivered to the cecum at 37.2 ml/min.

RESULTS

Bursting pressures of animals at 3, 5, and 7 days postoperatively

are listed for each group in Tables I, II, and III. Sixty-five animals were studied at 3 days postoperatively with average bursting pressure of the incised cecum being 38 mm of Hg for burned animals and 41 mm Hg for nonburned animals. The average bursting pressure of non-incised cecums of burned and nonburned animals was 102 and 103 mm Hg respectively. Sixty-seven animals were studied 5 days after operation with mean bursting pressures of 61 mm Hg for incised cecums of burned animals, 92 mm Hg for incised cecums of nonburned animals with 93 and 95 mm Hg respectively for non-incised cecums in burned and nonburned animals. At 7 days postoperatively, 64 animals were studied. Average bursting pressure for the incised cecum of burned animals was 90 mm Hg and the incised cecum of nonburned animals 103 mm Hg. Average pressures of 100 and 110 were obtained for the non-incised cecums of burned and nonburned animals. The mean pressures are shown in the figure for all groups at 3, 5, and 7 days postoperatively.

DISCUSSION

The literature describes studies in epidermal regeneration, (Scapicchio, 1968)[3] the effect of radiation and thermal burns on the intestinal mucosa, (Baker, 1968)[4] and impaired healing resulting from infection, (Smith, 1967)[5] as well as the effect of shock on wound healing (Schmidt, 1967).[6] There is no literature on intestinal wound healing in burns. The clinical impression is that surgical incisions in the burn patient heal more slowly than those in nonburned patients. This study shows that in the rat at 3 days post surgery there is little difference in the strength of incised rat cecum whether the animal is burned or not. Weakness demonstrated by measuring the bursting pressures of the cecum is related to the incision itself. Healing at this point is the same in both animals. The 95% confidence limits of the bursting pressure for burned incised animals are 26 - 49 mm Hg and for incised nonburned animals 33 - 49 mm Hg. At 5 days postoperatively the healing in the incised cecum of the burned animal lags behind that of the nonburned animal. Cecal bursting strengths of the burned animal with no incision in the cecum and nonburned animal with no incised cecum are essentially the same. The 95% confidence limits of bursting pressure for incised cecums on the 5th postoperative day in the burned animals is 50 -73 mm Hg as compared to 79 - 106 mm Hg for the incised cecums of nonburned animals. At 7 days postoperatively healing of the incised cecum of burned animals has progressed so that the 95% confidence limits of cecal bursting pressure are 67 - 113 mm Hg as compared to 88 - 118 mm Hg for unburned animals with cecal incisions.

All of the animals studied received the same diet, were not treated with antibiotics or other drugs nor were they infected. Generally, the burned animals appeared to gain less weight and were not as active as the nonburned animals.

TABLE I. THREE DAYS POSTOPERATIVELY

Burned		Non Burned	
Incised cecum	Not Incised Cecum	Incised cecum	Abdominal Incision
70	85	35	110
50	80	30	115
15	115	40	115
20	90	20	120
45	80	35	95
65	85	55	110
20	105	45	85
15	105	35	110
60	130	55	90
15	100	75	95
50	130	40	125
15	100	35	75
50	120	35	100
	105	15	100
	105	15	90
		35	90
		75	125
		65	120
			105
Average 38	102	41	103
95% CL* 26-49	94-111	33-49	97-110

*Confidence Limits

TABLE II. FIVE DAYS POSTOPERATIVELY

Burned		Non Burned	
Incised cecum	Not Incised Cecum	Incised cecum	Abdominal Incision
40	110	75	85
20	100	150	85
80	95	120	95
80	95	70	85
75	70	80	95
20	95	110	140
35	102	45	85
75	105	80	85
45	95	105	85
75	85	80	85
70	90	105	90
65	100	100	105
75	85	115	105
50	110	50	110
85	75	100	105
	90		95
	85		115
			85
			95
			85
Average 61	93	92	95
95% CL* 50-73	88-99	79-106	90-102

*Confidence Limits

TABLE III. SEVEN DAYS POSTOPERATIVELY

	Burned		Non Burned	
	Incised cecum	Not Incised Cecum	Incised cecum	Abdominal Incision
	135	125	125	100
	130	100	125	110
	20	70	120	120
	15	135	20	95
	135	105	100	120
	105	105	120	90
	130	105	110	85
	135	120	150	100
	95	90	125	115
	100	105	105	110
	15	95	95	100
	95	95	85	130
	60	95	100	110
	105	125	100	130
	25	85	70	110
	145	95		130
		80		
		75		
Average	90	100	103	110
95% CL*	67-113	92-108	88-118	103-117

*Confidence Limits

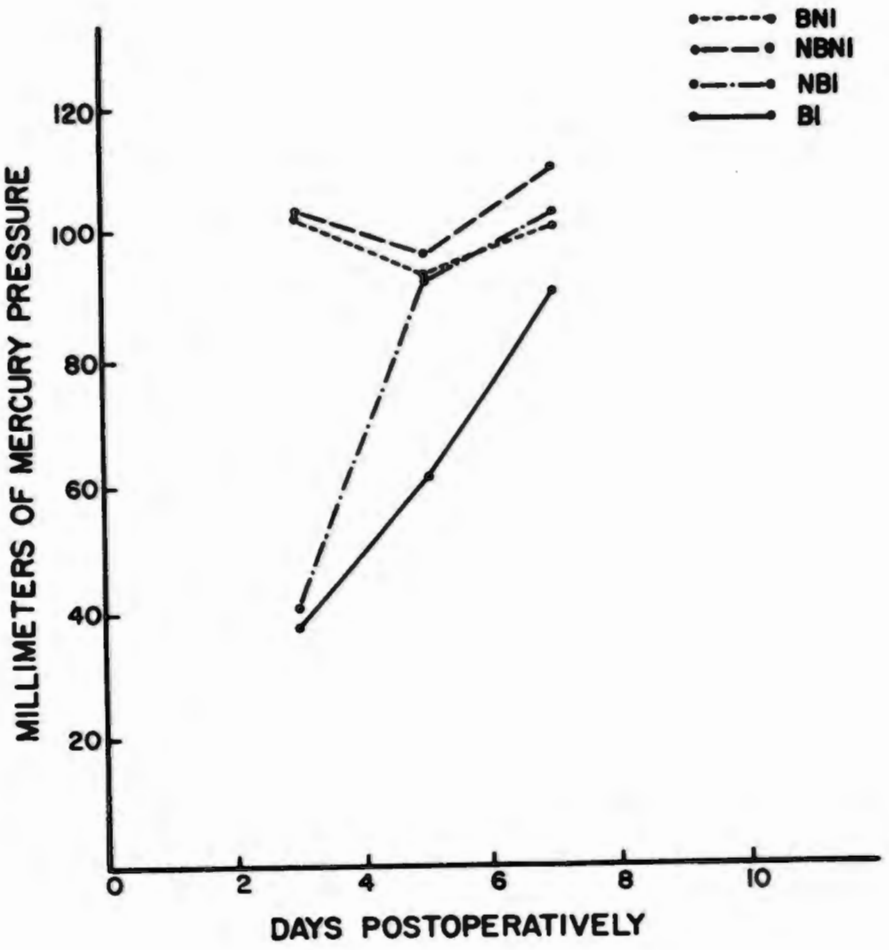
LEGEND FOR FIGURE

Average cecal bursting pressure of burned and unburned rats with and without cecal incisions.

B - burned

N - non

I - incised


BNI
NBNI
NBI
BI
MILLIMETERS OF MERCURY PRESSURE
120
100
80
60
40
20
0
2
4
6
8
10
DAYS POSTOPERATIVELY

66-7

A technical problem encountered was in dissection of the incised cecum from the abdominal cavity. Frequently the cecum would be adherent to the small intestine and to the parietal peritoneum. In dissecting the cecum free, the suture line was disrupted in some animals. In order to prevent this a portion of the parietal peritoneum was left intact on the incision in some specimens which may have variably increased bursting strength. This was not a problem at 3 days postoperatively since adhesions were easily lysed. At 5 days postoperatively this was more of a problem but was not as much of one as at 7 days postoperatively. No difference in formation of adhesions could be seen in burned as compared to nonburned animals. They seemed to be less severe in the burned animal at 5 days post surgery than the nonburned animal which would also coincide with the differences in healing noted in this study.

One should realize in attempting to extrapolate this animal data to the human that healing in the rat proceeds at a much faster rate than in the human. This model might be useful in the study of the influence of infection, growth hormone and other drugs on healing.

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6. Schmidt GH: Effect of hemorrhagic shock on wound healing in rats and guinea pigs. J Surg Res 7:513, 1967.

PRESENTATIONS AND/OR PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
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3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA CONTRACTOR ACCESS	9. LEVEL OF SUM
	K. COMPLETION	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62110A	3A062110A821	00	109
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) Peripheral Neuropathy in a Thermally Injured Military Population (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
71 01	72 02	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 72	0.4	11.2
	CURRENT 73	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) Physical Therapy; (U) Peripheral Neuropathy

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

23. (U) Document the extent of idiopathic peripheral neuropathies in burn patients and the effects of therapy on their ultimate prognosis.

24. (U) 150 consecutively admitted patients with thermal injures were studied with reference to sensory and motor function of the extremities.

25. (U) 72 01 - 72 02 Study was comp'eted and revealed the majority of peripheral nerve injuries to be related to either the initial injury or to post injury trauma to the involved nerve. In a small number of patients no cause for the nerve deficit was identified and a truly idiopathic neuropathy existed.

*Available to contractors upon originator's approval.

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FINAL REPORT

PROJECT NO. 3A062110A821-00, COMBAT SURGERY

REPORT TITLE: PERIPHERAL NEUROPATHY IN A THERMALLY INJURED MILITARY POPULATION

US ARMY INSTITUTE OF SURGICAL RESEARCH
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1 July 1971 - 30 June 1972

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Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A061110A821-00, COMBAT SURGERY

REPORT TITLE: PERIPHERAL NEUROPATHY IN A THERMALLY INJURED MILITARY POPULATION

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

Period covered in this report: 1 July 1971 - 30 June 1972

Investigators: William J. O'Brien, III, First Lieutenant, AMSC
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Donald H. See, MD, Major, MC*
Leah Palm, Captain, AMSC

Reports Control Symbol MEDDH-288(R1)

Of 150 consecutively admitted patients with thermal injuries, 11 (7%) were found to have peripheral neuropathies when studied with reference to sensory and motor function of the extremities. Five of these neuropathies could be directly related to thermal necrosis or discrete trauma, but the etiology of the remainder was obscure and involved areas remote from the burn wound. Possible contributing factors were ascertained by history, when possible, with special attention paid to splinting and positioning at the time of injury.

Patients with clinically apparent neurologic injury were evaluated by electromyography (EMG) for accurate documentation of defects. Sensory mapping and manual muscle testing were obtained initially and repeated in conjunction with the EMG every 6 weeks. Treatment of the study patients included splinting as required, active and passive range of motion exercises and intensive physical therapy, in an attempt to minimize atrophy of the involved muscle groups.

Serial EMG's as long as 6 months postinjury confirmed mild residual weakness not interfering with functional activity in 8 of 11 patients (73%). Evidence of reinnervation in process was manifest in the tracings of 7 patients (64%). Three patients demonstrated no evidence of regeneration of the involved nerve(s), and the last case was suggestive of a myopathic process.

Physical therapy
Peripheral neuropathy

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PERIPHERAL NEUROPATHY IN A THERMALLY INJURED MILITARY POPULATION

One hundred and fifty consecutive patients with thermal injuries were studied with reference to sensory and motor function of the extremities. Eleven (7%) were found to have previously undiagnosed peripheral neuropathies. Since the average age of the patients admitted to the Institute of Surgical Research was under 30, an incidence of 7% seemed rather high. Five of the 11 neuropathies could be directly related to thermal necrosis or sharp trauma but the etiology of the remainder was obscure and involved areas remote from the burn wound. Possible contributing factors were ascertained by history, when possible, with special attention paid to splinting and positioning at the time of injury. The purpose of this study was to document the actual extent of injury and the effects of therapy on ultimate results.

MATERIALS AND METHODS

Patients included in this study were examined clinically for sensory and motor dysfunction of the extremities. In addition to a careful history, the defects discovered were evaluated by electromyography and nerve conduction studies. Manual muscle and sensory tests were employed in conjunction with the EMGs to further elucidate the defect. The muscles tested were graded by standard voluntary manual muscle testing techniques.

The sensory testing employed the patient's ability to feel a pinprick over the cutaneous distribution of the peripheral nerve thought to be involved. The muscle and sensory tests were repeated weekly, while the EMGs were repeated at 6-week intervals.

The patients with circumferential burns of the extremities were tested for adequacy of peripheral perfusion by the Doppler Flowmeter. Ischemia of the extremities due to severe postburn edema was treated by elevation, active and assisted exercise or escharotomy to minimize tissue damage. This served to eliminate inadequate perfusion as one possible cause of peripheral neuropathy.

Treatment on a daily basis incorporated splinting to maintain functional position and programs of therapeutic active and assisted exercise to minimize loss of function.

The study was discontinued at the time of each patient's discharge from the hospital. Follow-up was maintained for up to 6 months postinjury to allow adequate assessment of treatment regimens.

RESULTS

There were 6 nerve injuries of obscure etiology that occurred in extremities distant from the burn and unaccompanied by obvious physical trauma. Three involved the ulnar nerve causing decreased or lost sensation to pinprick over the C8 dermatome. The dorsal and volar interosseus muscles were weak or not functioning, causing the patient inability to abduct and adduct the digits.

Three of the neuropathies of uncertain etiology could be attributed by history to positioning during prolonged evacuation from the scene of the accident. These cases were probaly due to compromise of the ulnar nerve due to pressure on the nerve as it traverses the medial epicondyle of the humerus.

These ulnar nerve injuries presented with similar histories typical of positional insult to the nerve. Fortunately, the follow-up EMGs indicated reinnervation in progress in 2 of the 3 patients with some return of active intrinsic muscle function. All 3 of these patients were discharged from the study with functional hands after an intensive program of physical and occupational therapy.

The remaining 3 neuropathy cases involved the brachial plexus, and the etiologies of these injuries were not easily identified.

The first case presented with a bilateral brachial plexus syndrome with more involvement on the left than the right. The patient recalled being lifted by the arms.

The second plexus injury presented with bilateral complete brachial plexus involvement. The history was unremarkable.

The last plexus injury seen in the study occurred in a female injured in an auto accident. By history she was pulled from the car by the right arm. Three days after admission she awoke with a flaccid right upper extremity--typical of an upper trunk injury.

These cases were treated over an 8-week period with active assisted, active and resistive exercise programs. Follow-up EMG and voluntary manual muscle tests revealed resolution of the weakness in all 3 cases to the point of functional strength. They were discharged from the study with functional upper extremities, with a prognosis for full recovery.

In the acute treatment of the thermally injured patient, attention is focused, as it should be, on the critical problems of treatment and coverage of the burn wound. In addition to the burn wound, incidental but injury related problems, such as peripheral nerve injuries may be diagnosed. These neuropathies can be attributed to direct thermal necrosis, sharp trauma, pressure and

some have questionable etiology. Of the nerve injuries not caused by thermal necrosis or sharp trauma, one-third might have been prevented by attention to careful positioning during evacuation.

The ulnar nerve seems to be most prone to injury during patient transfer, due to its anatomical course over the medial epicondyle of the humerus. In this study the prognosis for recovery from this type injury was good if active physical therapy is utilized to prevent atrophy of the involved muscle groups.

The brachial plexus injuries found in this study do not lend themselves well to a specific etiologic category. While 2 of the 3 patients were moved by traction on their upper extremities, their injuries were not easily explained on the basis of brachial plexus stretch, since the plexus involvement was too generalized to be explained by a simple axillary strain injury. One patient did not have onset of weakness until 3 days postinjury and then it was quite rapid. After extensive neurologic consultation these enigmas were attributed to brachial neuropathy of questionable etiology. The third patient was not lifted or pulled by the arms to his knowledge, yet he presented with a bilateral brachial plexus injury with involvement of all of the peripheral nerves in both extremities; no explanation is proposed for his injury.

DISCUSSION

The prognosis for these plexus injuries also seems favorable since slight residual weakness is the only sequela noted. A point of interest is the fact that the nerve conduction velocities of the involved nerves were within the normal range each time they were studied in patients with injuries not due to thermal necrosis or sharp trauma. This indicates that the value of sequential nerve conduction studies in these injuries is questionable.

SUMMARY

One hundred and fifty consecutive patients with thermal injuries were studied in reference to sensory and motor function of the extremities. Eleven were found to have sensory and/or motor dysfunction. Five of these neuropathies could be directly attributed to thermal necrosis or discrete trauma. Of the remaining 6 cases, 3 could be attributed to inappropriate positioning during lengthy evacuation.

Eight of 11 patients showed resolution of their weakness with EMG evidence of reinnervation in progress. Three patients with nerve injuries due to direct trauma or thermal necrosis showed no return of function.

PUBLICATIONS AND/OR PRESENTATIONS

None

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1 July 1971 - 30 June 1972

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