REPORT MEDDH-288(R1) AD_____

ANNUAL RESEARCH PROGRESS REPORT

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

1 July 1974 - 30 June 1975

Prepared for:
US ARMY MEDICAL RESEARCH & DEVELOPMENT COMMAND
WASHINGTON, D.C. 20314

DESTROY THIS REPORT WHEN IT IS NO LONGER NEEDED,
DO NOT RETURN IT TO THE ORIGINATOR.

APPROVED FOR PUBLIC RELEASE. DISTRIBUTION UNLIMITED

FINDINGS IN THIS REPORT ARE NOT TO BE CONSTRUED
AS AN OFFICIAL DEPARTMENT OF THE ARMY POSITION,
UNLESS SO DESIGNATED BY OTHER AUTHORIZED DOCUMENTS.

IN CONDUCTING THE RESEARCH DESCRIBED IN THIS REPORT
THE INVESTIGATORS ADHERED TO THE "GUIDE FOR LABORATORY
ANIMAL FACILITIES AND CARE", AS PROMULGATED BY THE
COMMITTEE ON THE GUIDE FOR LABORATORY ANIMAL FACILITIES
AND CARE OF THE INSTITUTE OF LABORATORY ANIMAL
RESOURCES, NATIONAL ACADEMY OF SCIENCES NATIONAL
RESEARCH COUNCIL.

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE

READ INSTRUCTIONS BEFORE COMPLETING FORM

1 REPORT NUMBER | 2 GOVT ACCESSION NO | 3 RECIPIENT'S CATALOG NUMBER

4 TITLE (and Subtitle)
Annual Research Progress Report

5 TYPE OF REPORT & PERIOD COVERED
1 July 1974 - 30 June 1975

6 PERFORMING ORG. REPORT NUMBER

7 AUTHOR(s)

8 CONTRACT OR GRANT NUMBER(s)

9 PERFORMING ORGANIZATION NAME AND ADDRESS
U.S. Army Institute of Surgical Research
Fort Sam Houston, Texas 78234

10 PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS
3A161102B71R 01
3A161101A91C
(cont'd)

11 CONTROLLING OFFICE NAME AND ADDRESS
US Army Medical Research & Development Command
Washington, DC 20314

12 REPORT DATE
30 June 1975

13 NUMBER OF PAGES
520

14 MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)

15 SECURITY CLASS (of this report)

15a DECLASSIFICATION/DOWNGRADING SCHEDULE

16 DISTRIBUTION STATEMENT (of this Report)
Approved for public release. Distribution unlimited.

17 DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18 SUPPLEMENTARY NOTES

19 KEY WORDS (Continue on reverse side if necessary and identify by block number)

20 ABSTRACT (Continue on reverse side if necessary and identify by block number)

This report documents the clinical and laboratory activities of the US Army Institute of Surgical Research during fiscal year 1975. 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 initial management of burned patients and on studies related to prevention and treatment of burn wound infection.

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

3A161102B71P-02
3A162110A821-00

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

SGRD-US

SUBJECT: Annual Research Report FY 1975

TO: See Distribution

Annual report(s) of the US Army Institute of Surgical Research for FY 1975 are forwarded under provisions of the OTSG Regulation 70-31, dated 2 April 1969. Qualified requesters may obtain copies of this report from Defense Documentation Center, Cameron Station, Alexandria, Virginia 22314.

Basil A. Pruitt, Jr.

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

FOREWORD

Over 100,000 burn patients require hospital care in the United States annually. The admission of over 2,000 burn patients to the US Army Burn Facility in Japan in 1967-1969, including over 1,100 patients in CY-68 alone, gives indication of the magnitude and seriousness of thermal injury as a military medical problem. A severe burn adversely affects every organ system and presents, usually in exaggerated form, all the pathophysiologic changes that occur in patients with lesser forms of trauma. The study of burn injury in man or laboratory animals, therefore, would seem to require no further justification, since the information generated has not only military relevance by definition but can be and, in fact, has been directly applied to all forms of trauma as a contribution to national health care. Certainly the best example of the economic and medical effectiveness of regional care is this burn center, established by the US Army in 1947.

A recurring preoccupation with what are called "key words" and statements of relevance would only dilute the above facts, since an extensive burn is widely recognized as the most severe injury to which man is liable. In fact, recent inquiries suggesting that computer modeling can substitute for human or animal studies provide an excellent example of how "perception" can be dulled by unwarranted use of modish words and phrases by those with vested interests and no familiarity with the actual problems. Such in vitro modeling, as should be obvious to all, is only possible when the precise interactions of all variables are known with certainty--a situation which does not exist today. Animal studies are not carried out for their own sake but for what can be applied to burned man to reduce death and disability. To those of us who care for burn patients, the goal-directed research and clinical care activities reported in this volume appear not only of unquestioned military relevance but of potential benefit to all trauma patients.

Basil A. Pruitt, Jr.

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

TABLE OF CONTENTS

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OA 6380	75 07 01	DD DR&E(A)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
74 07 01	D. CHANGE	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO. CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A161102B71R	01	115
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)

(U) Clinical Operation, Center For 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
50 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 75	49.9	607
	CURRENT 76	50	637

19. RESPONSIBLE DOD ORGANIZATION

NAME: US Army Institute of Surgical Research

ADDRESS: Fort Sam Houston, Texas 78234

RESPONSIBLE INDIVIDUAL

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

TELEPHONE: 512-221-2720

20. PERFORMING ORGANIZATION

NAME: US Army Institute of Surgical Research
Clinical Division

ADDRESS: Fort Sam Houston, Texas 78234

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

NAME: Hugh D. Peterson, COL, MC

TELEPHONE: 512-221-3301

SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS

NAME:

NAME: DA

21. GENERAL USE

FOREIGN INTELLIGENCE NOT CONSIDERED

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

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

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

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

25. (U) 74 01 - 74 12 During 1974, 244 patients were admitted to the Institute of Surgical Research. Specific attention to the early diagnosis and treatment of inhalation injury involved the use of fiberoptic bronchoscopy and xenon scan for diagnosis and the ongoing evaluation of steroids for treatment. Early fluid resuscitation and the incidence of acute stress ulceration as determined by fiberoptic gastroscopy are currently being assessed. As in previous years pulmonary infection with gram negative organisms continued to be a frequently observed complication of thermal injury and intensive investigation of methods to prevent and more adequately treat these complications continues. Several cases of acute bacterial endocarditis have been encountered within the past year and intensive effort is being devoted to prevention and treatment of this entity. Continuing evaluation of topical therapeutic agents is proceeding with an attempt to delineate the true role of topical therapy in care of the large thermal injury. General principles of management previously developed at this unit remain unchanged. Several new clinical approaches to the treatment of the extensive thermal injury and its complications are presently being evaluated.

*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. 3A61102B71R-01, RESEARCH IN BIOMEDICAL SCIENCES

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

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

1 January - 31 December 1974

Investigators:

Hugh D. Peterson, DDS, MD, Colonel, MC
Robert N. Agee, MD, Major, MC
Willard A. Andes, MD, Major, MC
Daryl R. Erickson, MD, Major, MC
Norman S. Levine, MD, Major, MC
James M. Long III, MD, Major, MC
Joseph C. McAlhany, Jr., MD, Major, MC
Richard H. Merrill, MD, Major, MC
James W. Taylor, MD, Major, MC
Douglas W. Wilmore, MD
Dorothy M. Berry, Lieutenant Colonel, ANC
Marbeth G. Michael, Lieutenant Colonel, ANC
Wilma F. Hall, Lieutenant Colonel, AMSC
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: CLINICAL OPERATION, CENTER FOR TREATMENT OF BURNED SOLDIERS

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

Period covered in this report: 1 January - 31 December 1974

Investigators: Hugh D. Peterson, DDS, MD, Colonel, MC
Robert N. Agee, MD, Major, MC
Willard A. Andes, MD, Major, MC
Daryl R. Erickson, MD, Major, MC
Norman S. Levine, MD, Major, MC
James M. Long III, MD, Major, MC
Joseph C. McAlhany, Jr., MD, Major, MC
Richard H. Merrill, MD, Major, MC
James W. Taylor, MD, Major, MC
Douglas W. Wilmore, MC
Dorothy M. Berry, Lieutenant Colonel, ANC
Marbeth G. Michael, Lieutenant Colonel, ANC
Wilma F. Hall, Lieutenant Colonel, AMSC
Basil A. Pruitt, Jr., MD, Colonel, MC

Reports Control Symbol MEDDH-288(R1)

Two hundred and fourty-four patients with thermal injury were admitted to the Clinical Division of the United States Army Institute of Surgical Research during the calendar year of 1974. The Institute of Surgical Research's main emphasis continues to be providing optimal care to military personnel and civilians with major thermal injuries. Clinical investigation has continued into the physiology, biochemical and bacteriologic aspects of thermal injury. In addition a new facet has been added to our care of the thermally injured patient in that early definitive plastic reconstruction has been undertaken in healed patients prior to their discharge, and a small group of patients have been selected for long term rehabilitation and reconstruction. During the calendar year of 1974 23 separate patients were admitted electively for reconstructive procedures. The personnel of this unit have also participated in many education programs both military and civilian. This report summarizes the activity of the Clinical Division of the United States Army Institute of Surgical Research in 1974 and cites the recognizable complications which have contributed to mortality and morbidity in burn patients. Clinical evaluations have been carried out of post-burn pulmonary changes, the metabolic response of the patient to his injury, biologic dressings, synthetic dressings, electrolyte changes in the post-burn period, high

voltage injury, post burn protein metabolism, Laser excision of burns, gastroesophageal endoscopy, enzymatic debridement; and a comparison made of sulfamylon verses silver sulfadiazine as a topical agent. In 1974 144 patients were air evacuated by the ISR burn team or 61% of all admissions.

Thermal injury	Heterograft
Topical therapy	Resuscitation
Autograft	Air evacuation
Homograft	Mortality

CLINICAL OPERATION, CENTER FOR TREATMENT OF BURNED SOLDIERS

The Clinical Division of the United States Army Institute of Surgical Research continued through the year of 1974 to have as its primary objective the provision of clinical care for thermally injured soldiers. The number of admissions declined from 261 in 1973 to 244 patients in 1974.

In 1974 there were a total of 113 aeromedical evacuation flights, 109 of these were CONUS flights (within the Continental United States) with 144 patients evacuated. All patients within a radius of 200 miles of Brooke Army Medical Center requiring air evacuation were transported by helicopter. There were 27 flights for that purpose. There were 4 flights outside of the Continental United States all to Alaska.

CLINICAL MANAGEMENT

In depth description of the management of patients with thermal injury as practiced by this Institute are found in previous Annual Reports and in numerous scientific publications. Therefore the following remarks will be limited to new and current methods of clinical therapy.

The most significant change in burn therapy at the Institute of Surgical Research during the calendar year of 1974 was a result of a comparison study of sulfamylon versus silver sulfadiazine as the initial topical antimicrobial agent. The initial study consisted of 84 patients managed by burn size and age and randomized in pairs. One member of each pair receiving sulfamylon the other receiving silver sulfadiazine. The LA 50 was 20% better in the 15 to 40 age group in those patients treated with silver sulfadiazine than the sulfamylon treated patients. It was therefore elected in July of 1974 to use silver sulfadiazine as the initial topical antimicrobial agent. Through the remainder of 1974 silver sulfadiazine was used exclusively and at the end of the year the LA 50 was still in excess of 63.5% in the 15 to 40 age group with the inclusion of all inhalation injuries.

The pulmonary complications which have continued to cause morbidity and mortality early in the course of the thermally injured patient are less frequently seen in the silver sulfadiazine treated patient. Silver sulfadiazine offers poor bacteriologic control of the burn wound so that patients with extensive burns (those over 65 to 70% with a large third degree component) pursue a septic course starting two to three weeks post burn. It is our opinion at present that although sulfamylon offers better bacteriologic control of the burn wound it accentuates hyperventilation in the early burn period especially immediately after resuscitation in those with burns over 30%. This appears to result from either carbonic anhydrase inhibition or an as yet unidentified activity and suggests that sulfamylon should be employed cautiously if at all in the early post-burn period.

The ^{133}Xenon lung scan continues to be a useful diagnostic modality for acute inhalation injuries. Although a small number of false positive and false negative scans have been identified, these patients can usually be separated on clinical grounds and bronchoscopic findings. Recently the morphologic changes in the tracheobronchial tree resulting from inhalation injury have been evaluated by fiberoptic bronchoscopy. Large airway chemical tracheobronchitis has been identified as a variant of inhalation injury in the absence of ^{133}Xenon scintiphotographic parenchymal changes resulting from the inhalation of products of incomplete combustion. With the two modalities the inhalation injury can be divided anatomically into a large airway injury and parenchymal injury. At present an investigation is underway to assess the efficacy of systemic steroids administered in high doses within the first 72 hours post inhalation injury. Routine chest roentgenography, fiberoptic bronchoscopy, ^{133}Xenon lung scan and pulmonary function studies are being used to evaluate diagnostic accuracy and the effect of treatment on inhalation injury.

An evaluation of Sutilain enzymatic debridement of burned hands has been completed. The basic findings were that there was rapid dissolution of the surface of the eschar in the enzyme treated hand, however, a graftable wound bed was achieved no more rapidly than if saline soaks had been used since the enzyme appears to be ineffective in removing deep dermal elements and necrotic fat. The only use of enzymatic debridement for burned hands is for softening the eschar more rapidly. Such a use may be an alternative to digital escharotomy.

Glucose kinetics have been determined in 9 patients following thermal injury using a computerized one pool model to analyze data generated from intravenous and oral glucose tolerance curves. Glucose production is increased to 3 to 4 times normal between the seventh and fifteenth day post injury and then decreases to normal with closure of the burn wound. The hyperglycemia seen during the post injury period is related to increased glucose production not decreased glucose disappearance. This is in marked contrast to the glucose kinetics during the resuscitation period or septic episodes which are characterized by prolonged disappearance of the glucose from the glucose space and minimally elevated glucose production.

During 1974 research has continued in an effort to modify the central nervous system adrenergic response following thermal injury. No decrease in hypermetabolism has been seen with the use of topical anesthesia, regional deinnervation of injured extremities, salicilate administration, calcium infusion, or ingestion of L-Dopa. The hypermetabolic response of normal man to cold exposure was decreased by inhaling 70% helium and 21% oxygen when compared with studies using room air alone. Evaluation of the role of the inert gases on central nervous system adrenergic function is now being pursued in both normal subjects and patients.

Again in 1974 the two major fluid electrolyte disturbances seen at the Institute of Surgical Research in the thermally injured patients

were hypernatremia and hyponatremia. Hyponatremia reflecting a water deficit was found to be the most common electrolyte abnormality in burn patients as a whole. Hyponatremia is the most common electrolyte change in the burned child who has seizures and it is caused by either excessive administration or too rapid administration of electrolyte free fluids or the "leaching" effect of silver nitrate dressings. This finding calls for caution in the administration of non-electrolyte fluids in small children and a careful monitoring of all patients in silver nitrate dressings.

Interest was continued during 1974 in primary excision of third degree burns. Final evaluation has determined that the carbon dioxide laser in its present state is of advantage to the cutting current of the electrocautery, but that both modalities are superior to the scalpel excision. Excisions have fallen into two categories, massive burns excised and covered with allograft or xenograft which have uniformly done poorly, and localized third degree burns in areas amenable to excision in patients with moderate size burns (40 to 65%). The latter have virtually always done well.

Tangential excision has also been evaluated during 1974. The tangential excisions fall into two groups. The first being tangential excisions of burns of the hands with early autografting. When the hand to be tangentially excised had deep second degree burns the results were uniformly excellent, when the injury was full thickness the results were hard to distinguish from those observed by allowing the eschar to separate spontaneously. Tangential excision was also employed in patients having full thickness wounds with tenacious eschar. The eschar was tangentially excised until freely bleeding yellow fat was encountered. When small test areas of graft were placed on the yellow fat no take was accomplished. The routine treatment therefore was to wrap the tangentially excised extremity in sulfamylon solution soaks and allow granulation tissue to form. Wound maturation usually takes place in 7 to 14 days following which the wound can be closed with "meshed" grafts with excellent results.

Nebulized gentamycin was evaluated as a deterent to pulmonary infection during 1974. Patients that developed a roentgenologically evident infiltrate post-burn were treated in a double blind study with either nebulized gentamicin or nebulized saline. Twenty patients have been studied to date. Seven patients in each group were fully treated and evaluated. Six of seven patients treated with gentamicin cleared their infiltrates whereas only two of seven given normal saline cleared their infiltrates. None of six patients in the gentamicin treated group died of bronchopneumonia, whereas three of six in the saline group died of bronchopneumonia. The study is continuing to determine the reliability and significance of these findings.

Gastroesophageal endoscopy with the fiberoptic endoscope has continuted to be a valuable diagnostic modality through 1974. It has been found to be a reliable and sensitive method of diagnosing upper GI

disease. Colonoscopy has been undertaken on six patients within 72 hours post burn. Although three of these patients showed evidence of gastroduodenal disease on gastroduodenoscopy, all of the patients had normal colonic mucosa. Before the occurrence of microvascular ischemia with mucosal damage of the colon can be documented, additional patients must be studied with upper and lower tract endoscopy within the early post-burn period.

EDUCATION

During the period of this report, 3 surgical residents from Brooke Army Medical Center, 1 from Fitzsimons, 1 from David Grant USAF Medical Center, Travis AFB, California and 1 civilian physician from Bexar County Hospital participated as active members of the medical staff for periods of 1 to 3 months as part of their surgical training. One physician from the Army of Germany and one from the Army of Venezuela spent three months training with the unit. Two Navy and five Army reserve officers completed their tours of active duty training with our unit for periods of 2 to 6 weeks. Two interns from the Department of Surgery at Brooke each served one month of duty at the unit as did one general medical officer from the Out Patient Department and one general surgeon from the Army Hospital in Okinawa. One civilian physician from Syracuse N.Y. spent two weeks training and one senior medical student from the University of Arizona spent a month in observer training. Thirty-nine reserve officers and 25 National Guard officers and paramedical personnel were given tours and briefings. Twenty-one foreign visitors from the following countries: Belgium, Pakistan, Thailand, Paraguay, Jordan, Israel, Australia, India, England, Mexico, West Germany and Sweden received briefings on the care of the thermally injured patient and on the overall mission of the Institute of Surgical Research. Approximately 103 civilian and military physicians and 192 nurses, students and paramedical personnel visited and were briefed during 1974.

Numerous scientific presentations concerning various aspects of thermal injury werymade by members of the Clinical Division at local, state, regional and national meetings as listed at the end of this section.

STATISTICAL RESUME

During the year 1974, 244 thermally injured patients were admitted to the Institute of Surgical Research. As in 1973 no patients were air evacuated by the Institute of Surgical Research Burn Team from the Far East. There were 226 dispositions during 1974 and the subsequent data will be based on those dispositions. The patients ranged in age from 5 months to 83 years with 180 males and 46 females. The average age of the patient was 28.5 years with an average burn size of 41.5%, and a 19.8% average third degree component. The average burn index was 30.5%. Out of 226 dispositions, 178 had third degree burns (78.8%). Forty-five patients were less than 15 years of age with an average age of 6.3 years.

The average total burn in the pediatric age group was 33% with 18.5% being third degree. The burn index in children was 26%. Of the 45 pediatric patients admitted, 35 had some third degree burn (77.8%).

The mortality in the pediatric age group was 35.5%. In the group of pediatric burn patients who died, the average age was 6.3 years and the average burn size was 60% with 38.6% being third degree. The overall mortality for the year 1974 was 42.9% or 97 patients out of the 226 expired, of which 76 were male and 21 were female. The average age of the patient who died was 32 years and in this group the average total burn was 60.8% with 35% being third degree for a burn index of 48%. The increase in mortality compared to previous years is partly related to the fact that no patients from Southeast Asia have been air evacuated and the number of acute admissions has increased markedly as has the average total per cent burn increased in the patients admitted to the Institute of Surgical Research. Of the 97 patients who expired 85 or 87.6 had some third degree burn. Autopsies were performed in 79 patients (81.4%) of all the deaths. The average post burn day of death was 21.4 which is a marked increase from the 11.8 of 1973 and may represent the effect of the change in topical agent used in the early post burn period.

Table 1 identifies the source of admission of patients during the calendar year 1974. The majority of the burns were from the Continental United States. Table 2 summarizes the burn etiology in 1974. Table 3 summarizes the effect of age and total body surface burn on mortality.

Table 4 lists the mortality rates in increments of 10% total body surface burn from the years 1971 through 1974. Table 5 presents the survival and mortality rate of patients with greater than 30% body surface burns in the calendar years 1955-1974. It should be noted that there are no marked changes.

Table 6 shows a comparison of burn mortality in the pre-topical antimicrobial years 1962-1963 with the cummulative index since 1965 when Sulfamylon and later silver sulfadiazine have been used. As previously reported the improvement is primarily in that group of burn patients whose injury is 30-60% with little if any change in those patients with greater than 60% injury.

The average hospital stay in 1974 was 42.3 days. When convalescent leave for active duty military personnel was excluded the average hospital stay was 39.6 days. The average post burn admission day to the Institute of Surgical Research was 2.8. This figure reflects a decrease in the average post burn day of admission from 11.2 days in 1970 to 9 days in 1971 and 7 days in 1972 and 5 days in 1973. The decrease in the average admission time is because the patient population originates in the Continental United States and also reflects the rapid aeromedical

Table 1. Source of Admission, 1974

Area	A	AD	AF	AFD	N	ND	VAB	Other	TOTAL
1st Army	3	1	0	0	1	0	4	6	15
3rd Army	3	3	0	3	2	3	8	15	37
5th Army	12	9	10	13	2	3	18	64	131
6th Army	2	1	8	2	0	0	3	9	24
MDW	2	0	0	0	0	0	0	0	2
Alaska	1	0	1	0	0	0	1	2	5
Germany	2	2	2	0	0	0	0	0	6
England	0	0	0	1	0	0	0	0	1
Spain	0	0	0	0	0	1	0	0	1
Mexico	0	0	0	0	0	0	0	1	1
San Salvador	0	0	0	0	0	0	0	1	1
Okinawa	0	0	0	1	0	0	0	0	1
Thailand	0	0	0	0	0	0	0	1	1
	25	16	21	20	5	7	34	98	226

A - Army
AF - Air Force
D - Dependent
N - Navy, Marine Corps & US Coast Guard
VAB - Veterans Administration Beneficiary
Other: Civilian Emergency (95)
US Public Health Service Beneficiary (2)
Bureau of Employees' Compensation Beneficiary (1)

Table 2. Burn Etiology, 1974 - 226 Dispositions

Causes	Number of Patients	% Disposition	Deaths	% Mortality
Gasoline & Kerosene	54	23.9%	23	42.6%
Structural Fires	21	9.3%	14	66.7%
Motor Vehicle Accidents	16	7.1%	9	56.3%
Aircraft Accidents	8	3.5%	6	75.0%
Open Flames	18	8.0%	7	38.9%
Electrical	17	7.5%	5	29.4%
Hot Liquid	17	7.5%	3	17.6%
Chemical	2	0.9%	1	50.0%
Others	26	11.5%	11	42.3%
Butane, Propane or Natural Gas Exp.	44	19.5%	17	38.6%
Welding Accidents	3	1.3%	1	33.3%
TOTAL	226		97	

Table 3. Age, Body Surface Involvement & Mortality, 1974

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	2	1	1	0	0	1(1)	0	0	0	0	5	1	20.0
1-2	2	1	1	0	0	0	0	0	0	0	4	0	0
2-3	1	1	0	0	1(1)	1(1)	0	1(1)	0	0	5	3	60.0
3-4	2	0	0	0	3(2)	0	0	0	0	0	5	2	40.0
4-5	0	0	2	1	0	0	0	0	0	0	3	0	0
5-10	1		0	0	2(2)	1(1)	2(2)	0	1(1)	1(1)	9	7	77.8
10-15	4		1	2(1)	2	1	0	1(1)	1(1)	0	14	3	21.4
15-20	3	2	5(1)	3	1	3(2)	3(3)	1(1)	1	3(3)	25	10	40.0
20-30	7	6	5	8(3)	15(6)	9(5)	8(7)	1	5(5)	1(1)	65	27	41.5
30-40	4	5	1(1)	6	2	5(2)	1(1)	3(3)	5(5)	1(1)	33	13	39.4
40-50	0	2	4	8(3)	4(3)	5(3)	3(2)	1(1)	0	3(3)	30	15	50.0
50-60	0	1	6(1)	2(1)	4(2)	1(1)	0	1(1)	1(1)	0	16	7	43.8
60-70	0	2	2(1)	1(1)	1(1)	0	1(1)	0	0	0	7	4	52.2
70-80	0	0	0	0	1(1)	1(1)	0	1(1)	0	0	3	3	100.0
80-90	0	0	0	1(1)	0	0	1(1)	0	0	0	2	2	100.0
Total	26	24	28	32	36	28	19	10	14	9	226		
Deaths	0	0	4	10	18	17	17	9	13	9		97	
% Mortality	0	0	14.3	31.2	50.0	60.7	89.5	90.0	92.9	100			42.9

Note: Deaths shown in parentheses.

Table 4 Per Cent Body Surface Involvement and Mortality, 1971 - 1974

% Burn	0-10	10-20	20-30	30-40	40-50	50-60	60-70	70-80	80-90	90-100	Total
					(1971)						
No. Burned	50	65	57	39	34	20	12	8	10	6	301
Deaths	0	0	2	7	14	12	11	7	9	6	68
% Mortality	0	0	3.5	17.9	41.2	60	91.7	87.5	90	100	22.6
					(1972)						
No. Burned	47	56	43	42	36	23	22	16	11	5	301
Deaths	1	2	7	13	15	13	21	15	11	5	103
% Mortality	2.1	3.6	16.3	31	41.7	56.5	95.4	93.8	100	100	34.2
					(1973)						
No. Burned	39	35	46	26	38	32	20	16	12	9	273
Deaths	0	0	7	9	21	22	17	16	12	9	113
% Mortality	0	0	15.2	34.6	55.3	68.8	85	100	100	100	41.4
					(1974)						
No. Burned	26	24	28	32	36	28	19	10	14	9	226
Deaths	0	0	4	10	18	17	17	9	13	9	97
% Mortality	0	0	14.3	31.2	50	60.7	89.5	90	92.9	100	42.9

Table 5. Per Cent Burn Versus Survival, 1955-1974

Year	Survivors (burns over 30%)			Deaths		
	No. Cases	Average % Burn		No. Cases	Average % Burn	
		Total	3°		Total	3°
1955	20	39.5	20.3	21	55.6	38.1
1956	22	41.0	17.3	20	57.8	37.8
1957	19	38.4	24.1	17	57.1	38.8
1958	15	42.3	21.6	23	56.5	35.3
1959	29	43.1	20.6	24	63.1	38.1
1960	17	44.2	20.1	30	57.8	37.3
1961	18	44.2	25.0	31	58.0	39.7
1962	18	42.7	21.4	54	59.1	46.2
1963	28	45.8	19.6	57	69.0	41.0
1964	40	41.8	14.8	37	65.0	42.4
1965	47	43.8	21.0	33	66.0	33.4
1966	68	41.5	14.9	59	59.9	31.3
1967	103	42.7	13.3	51	59.9	32.3
1968	143	44.2	12.6	38	54.6	24.6
1969	113	43.2	11.1	70	58.7	26.4
1970	92	39.4	10.7	70	51.9	32.6
1971	63	41.9	14.0	68	60.8	38.0
1972	62	42.0	17.2	103	56.7	35.9
1973	47	43.7	19.6	113	60.3	36.2
1974	55	43.9	12.2	97	60.8	35.9

Table 6. Comparison of Burn Mortality Rates, 1962-1963 and 1964-1974

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
1964-74	1603	45	2.8	463	80	17.3	395	126	31.9	247	123	49.8	423	365	86.3

evacuation carried out by the Institute of Surgical Research.

During the year 1974 1,094 operations were performed on 195 patients, an average of 5 operations per patient. Three hundred and ninety-two anesthetics were performed on 176 patients or 3 anesthetics per patient. A total of 670 ward procedures were performed. Two hundred and fifty-eight autografting procedures were carried out on 104 patients or approximately 2 1/2 procedures per patient. Thirty-eight patients had 92 applications of allograft or an average of approximately three applications per patient.

Porcine cutaneous xenograft was applied to 28 patients 103 times or an average of 4 times per patient. There was a marked decrease in the use of porcine cutaneous xenograft during 1974. Evaluation of this material on wounds ready to accept autograft and on wounds not clean enough to autograft revealed that it was least desirable of any of the materials tested. Cadaver allograft was aseptically harvested from 33 donors, a drop from 56 in 1973. Escharotomies were performed on 63 patients or 27.9% of all dispositions. Twenty-eight patients required amputations, 15 of these were major.

Tracheostomy was performed in 37 patients or 16.4% of all dispositions. The specific indications for tracheostomy were the need for prolonged ventilatory support, upper airway obstruction, and inhalation injury.

One hundred and fifty-two patients or 62% of all dispositions had at least one blood culture drawn during their hospitalization and 100 patients had a positive blood culture. For further information on bacteriologic data the reader is referred to the succeeding chapters. Suppurative thrombophlebitis occurred in 11 patients or 5% of all dispositions. In keeping with the high index of suspicion of suppurative thrombophlebitis, 31 cutdowns were explored. An important aspect of burn therapy is monitoring the burn wound and this is done with frequent wound biopsies. Eighty-four patients or 37% of all dispositions in 1974 had at least 1 wound biopsy performed.

A total of 623,450 cc of blood were administered to 140 patients or 62% of all dispositions. An average of 4,531 cc of blood were given to each of the 140 patients.

Seventy-nine patients or 35% of the dispositions had some type of associated injury upon admission. Five per cent of the patients had a major fracture. Other significiant orthopedic problems which developed during the hospital stay included exposed tendon and bones of all four extremities, 3 open knee joints, 1 open shoulder joint and many open small joints of the hand. There were 3 cases of significant osteomyelitis. There were frequent ophthalmologic injuries in the 226 dispositions. They included corneal burns, corneal abrasion, corneal laceration and thermal injury of the lids. Significant long term sequelae of

eyelid burns occurred in the form of all degrees of ectropion. Of the surviving patients in 1974 there was only one with permanent visual impairment, that occurring secondary to a corneal scar resulting from a deep corneal burn.

Another significant problem of the thermally injured patient is chondritis. Twelve patients or 5% of all dispositions developed auricular chondritis during 1974 and chondrectomy was performed in 17 ears. Several patients lost a portion of their ears due to direct thermal trauma.

Gastrointestinal complications again were quite prominent in our burn population. Gastrointestinal bleeding of some type occurred in 42 patients or 18.6% of all dispositions. Gastrointestinal ulcers diagnosed either roentgenographically or endoscopically occurred in 48 patients or 21.2% of all dispositions. Duodenal or gastric ulcers were the cause of bleeding in 23 patients. Five patients developed perforated gastroduodenal ulcers and 12 patients had exploratory laparotomy for complications secondary to ulcer disease. Superior mesenteric artery syndrome occurred in three patients, two requiring surgery, both with an unfavorable termination, a third managed conservatively did well. Other major gastrointestinal complications detected by fiberoptic endoscopy included gastritis in 35 patients, duodenitis in 12 patients, esophagitis in 29 patients and esophageal ulcerations in 10 patients. Acute acalculous cholecystitis was diagnosed at post mortem examination in three patients but in no patient was it the cause of death. Pancreatitis usually of the interstitial type and of mild degree was diagnosed in 10 patients, a fourfold decrease from 1973 probably related to changing pathologic criteria. Major renal complications in our burn population include some degree of renal failure usually as a terminal event in 54 patients. Hemodialysis was carried out on nine patients and peritoneal dialysis on 1 patient.

Cardiac complications continued to play a significant role in patient morbidity and mortality. Nine patients sustained an acute myocardial infarction and all succumbed to the insult. During 1974 there were several established cases of acute bacterial endocarditis. Staphylococcus aureus coagulase positive was the predominant organism. Five cases were diagnosed prior to death, four of these were resistant to intensive antibiotic therapy and had a fatal termination. A fifth case had his mitral valve replaced with subsequent clearing of his blood stream infection, only to succumb 32 days later to a gram negative mediastinitis. At post mortem examination the prosthetic valve was well seated and free of bacterial involvement. The remainder of the myocardial infectious problems were those related to terminal sepsis.

Pulmonary problems continued to be a significant cause of morbidity and mortality in 1974 as they had in the past. However, with the discontinuance of early post-burn Sulfamylon burn cream, topical therapy, early pulmonary problems were reduced and those problems related to long term sepsis in the debilitated patient were of relatively greater consequence.

With the patients surviving longer yet dying of later septic complications there was an increase in both hemotogenous pneumonia and bronchopneumonia. Inhalation injuries diagnosed either by bronchoscopy or 133Xenon scan were noted in 51 patients or 22.6% of the dispositions. The other significant pulmonary problem was pneumothorax occurring in 15 patients either associated with use of a mechanical ventilator or insertion of a subclavian venous cannula. Pulmonary emboli were diagnosed in 24 patients during 1974 either by clinical findings and lung scan or at autopsy examination.

SUMMARY

During 1974 226 patients were admitted to the U.S. Army Institute of Surgical Research. The basic change in the therapeutic approach to the burn patient is the discontinuance of Sulfamylon burn cream in the early post burn period. There were fewer significant early pulmonary complications, a much more benign early post burn course, an increase of the LA50 over recent past years but with the prolonged survival we have seen a relative increase in septic deaths in patients with large burns. Infection continues to be the most frequent cause of mortality in the thermally injured patient. Clinical research efforts are oriented towards evaluation of control of the septic process with ongoing research on the efficacy of various topical agents, the role of hydrotherapy in the management of the burn wound, the use of systemic antibiotics, immunosuppression with excision and allografting in massive burns, and other efforts to improve the survival of the burned soldier.

Table 7. Causes of Death, 1974

Patient	Age	Sex	% Burn Total	% Burn 3°	PBD Death	Cause of Death
1	18	F	100	100	2	Cardiovascular collapse; inhalation injury severe
2	43	M	97	97	1	Cardiovascular collapse; inhalation injury severe
3	19	M	96	74	14	Gram-negative sepsis (Proteus, Enterobacter and E. coli)
4	19	M	95	95	2	Severe inhalation injury
5	46	M	95	86.5	14	Septicemia (Klebsiella)
6	9	M	92.5	92.5	1	*Cardiovascular collapse; cerebral edema
7	29	M	90.5	90.5	4	Severe inhalation injury
8	39	M	90.5	90.5	2	Cardiovascular collapse; severe inhalation injury
9	41	M	90	76	15	Bronchopneumonia; systemic sepsis (Providencia)
10	7	F	89	87	22	Burn wound sepsis; hematogenous pneumonia (Pseudomonas)
11	12	M	88	6	1	Cardiovascular collapse; acute renal failure, pulmonary edema & congestion
12	31	M	87.5	83	5	Pulmonary edema, severe
13	20	F	86	30	18	Burn wound invasion with vasculitis, bacterial (cocci) and mycotic (septate hyphae)
14	39	M	85	74	5	Right lower lobe pneumonitis; renal failure
15	38	M	84	57	30	Burn wound sepsis; hematogenous pneumonia, multiple renal abscesses organism Enterobacter
16	26	M	84	0	28	Diffuse intra-alveolar and interstitial hemorrhage with infarction both lungs; hemorrhage and necrosis adrenal glands bilaterally
17	23	F	83	57	8	*Cardiorespiratory arrest, etiology undetermined

* Autopsy not performed

Table 7. Causes of Death, 1974

Patient	Age	Sex	% Burn Total	% Burn 3°	PBD Death	Cause of Death
18	21	M	83	43.5	17	Sepsis (Klebsiella and Enterobacter)
19	34	M	82	71	20	Sepsis (Enterobacter)
20	59	M	82	40	6	Cardiovascular collapse secondary to severe arteriosclerotic cardiovascula disease
21	32	M	81.5	38	17	Bilateral adrenal hemorrhage and necrosis; pulmonary edema
22	24	M	80	12	3	Severe inhalation injury with superimposed bilateral bronchopneumonia
23	31	M	75	49.5	41	Systemic sepsis (Klebsiella and staphylococcus)
24	14	M	75	5	23	*Gram-negative sepsis (Pseudomonas)
25	73	M	75	2	3	Cardiovascular collapse; inhalation injury
26	50	F	75	0	31	Gram-negative sepsis (Klebsiella, Providencia stuartii)
27	16	M	74.5	74.5	0	*Severe inhalation injury
28	2	F	74	74	1	*Severe inhalation injury; bilateral pneumonitis
29	43	M	71.5	54	9	Severe inhalation injury; Serratia pneumonitis and septicemia
30	39	M	71	24.5	20	Bilateral bronchopneumonia (Pseudomonas, Klebsiella); marked adrenal hemorrhage and necrosis
31	32	M	71	22	16	Staphylococcal burn wound invasion and staphylococcal sepsis
32	19	F	69	59	15	Burn wound sepsis (Providencia stuartii); acute bacterial endocarditis (Providencia stuartii)
33	26	M	69	47.5	50	Sepsis (Pseudomonas)
34	42	F	69	39	20	*Septicemia (E. coli)

* Autopsy not performed

Table 7. Causes of Death, 1974

Patient	Age	Sex	% Burn Total	% Burn 3°	PBD Death	Cause of Death
35	28	M	69	23	5	Severe inhalation injury; bilateral pulmonary embolus, massive on right
36	28	M	66	60	5	Inhalation injury; pneumonitis and hyperkalemia
37	5	M	65	65	7	*Septicemia (Enterobacter)
38	27	M	65	46.5	44	*Gram-positive and negative septicemia (Staphylococcus, E coli and Providencia)
39	35	M	64	52.5	23	Gram-negative sepsis (Enterobacter)
40	18	M	64	45.5	12	Sepsis (Klebsiella); tension pneumothorax secondary to pulmonary abscess; acute peritonitis secondary to perforated gastric ulcer
41	18	M	63	3	49	Acute bacterial endocarditis with secondary embolic phenomenon to celiac axis; infarction to small bowel and peritonitis (Staphylococcus aureus)
42	8	M	62	62	2	Cardiovascular collapse; acute renal failure
43	24	M	62	47	26	Hemorrhagic necrotizing bronchopneumonia; perforated duodenal ulcer
44	26	M	61.5	5.5	15	Severe inhalation injury with secondary bronchopneumonia
45	25	M	61	56	10	Septicemia (E. coli, Providencia)
46	83	M	61	51	14	Sepsis (E. coli)
47	67	M	61	29	1	Severe inhalation injury
48	48	M	60.5	0	32	Sepsis (Pseudomonas); hematogenous pneumonia (Pseudomonas)
49	27	M	59.5	45	15	Inhalation injury; bilateral pneumonia
50	23	M	58.5	41	8	Inhalation injury; bilateral pneumonia

* Autopsy not performed

Table 7. Causes of Death, 1974

Patient	Age	Sex	% Burn Total	% Burn 3°	PBD Death	Cause of Death
51	18	M	57.5	41.5	75	Sequelae of inhalation injury, sepsis (Enterobacter)
52	25	M	57.5	20.5	11	Sepsis (staphylococcal); pneumonia
53	56	M	57	32	28	Sepsis (Klebsiella)
54	24	M	56.5	33	19	Fungal burn wound invasion septate hyphae and yeast forms; bilateral bronchopneumonia
55	19	M	56	54	15	Septicemia (Pseudomonas); severe inhalation injury
56	25	M	56	40	22	Pseudomonas burn wound sepsis
57	34	F	55.5	29	46	Aspiration pneumonia
58	11/12	F	55	40	31	Gram-negative sepsis (Enterobacter, Providencia)
59	45	M	55	19.5	70	Bilateral bronchopneumonia secondary to inhalation injury, burn wound sepsis (Pseudomonas)
60	7	M	54.5	24	86	Suppurative thrombophlebitis; bilateral pulmonary embolism; systemic sepsis (Staphylococcus aureus coagulase positive)
61	30	M	54	52	9	Severe inhalation injury with bilateral bronchopneumonia
62	76	M	53	49	20	*Septicemia (Enterobacter)
63	47	F	53	3	11	Acute bacterial endocarditis (Staphylococcus aureus) valves involved tricuspid and pulmonary
64	45	M	51	0	28	Bilateral severe bronchopneumonia complicated by severe arteriosclerotic heart disease
65	2	F	50.5	33.5	9	*Cardiac arrest, etiology unknown

* Autopsy not performed

Table 7. Causes of Death, 1974

Patient	Age	Sex	% Burn Total	% Burn 3°	PBD Death	Cause of Death
66	25	M	49.5	0	7	Severe inhalation injury
67	52	F	49	20.5	6	Sepsis (Enterobacter)
68	55	M	47	5	24	*Staphylococcal septicemia; bilateral staphylococcal pneumonia
69	26	F	47	0	70	Acute bacterial endocarditis (Staphylococcus) valve involved tricuspid; bilateral hematogenous pneumonia
70	8	F	46.5	42.5	47	Acute hemorrhagic pancreatitis; acute myocardial infarction
71	46	M	46.5	20.5	22	Adult hyaline membrane disease, lungs, bilateral
72	70	M	46	14.5	10	*Bilateral pneumonia (Pseudomonas)
73	2	M	46	0	6	Bacterial burn wound invasion and systemic sepsis (Staphylococcus)
74	25	M	45.5	11	38	Acute bacterial endocarditis (Staphylococcus aureus coagulase positive); bilateral septic pulmorary emboli
75	27	M	45	35	50	*Cardiac arrest, etiology undetermined
76	62	M	44.5	1	7	Severe inhalation injury
77	25	M	43	29	89	Suppurative pericarditis secondary to surgery for bacterial endocarditis (Enterobacter)
78	48	M	43	25	95	Gram negative sepsis (E. coli, Klebsiella)
79	43	M	43	16	55	Sepsis (Klebsiella); mucus plug in trachea
80	5	F	41.5	24.5	17	Sepsis (Pseudomonas)
81	26	M	41	41	57	*Cardiac arrest, etiology undetermined

* Autopsy not performed

Table 7. Causes of Death, 1974

Patient	Age	Sex	% Burn Total	% Burn 3°	PBD Death	Cause of Death
82	3	F	41	0	4	Pulmonary and cerebral edema
83	3	F	40.5	38.5	6	Inhalation injury; cerebral edema
84	40	M	39.5	26.5	9	Acute pulmonary edema and cerebral edema
85	13	F	39	23	9	Pseudomonas burn wound sepsis
86	27	M	36.5	22.5	7	*Cerebral contusion and cerebral edema
87	45	M	36	3	11	*Cerebral vascular accident secondary to severe hypertension
88	48	M	35	0	19	Inhalation injury; sepsis secondary to mural thrombus right atrium (Staphylococcus)
89	26	F	34	33	36	Septicemia (Providencia stuartii)
90	83	M	34	0	5	Myocardial infarction
91	20	M	32	16.5	16	Inhalation injury with secondary pneumonitis
92	63	M	30.5	27.5	23	Acute myocardial infarction
93	54	M	30	8	40	Extensive bilateral pneumonia (Pseudomonas)
94	38	F	26	26	26	Severe bilateral bronchopneumonia
95	50	M	25	0	53	Sepsis secondary to bronchopneumonia (Klebsiella)
96	19	M	23.5	0	3	*Severe inhalation injury
97	60	M	23	18	18	*Diabetes mellitus complicating burn; seizure disorder cerebral vascular insufficiency; aspiration pneumonitis

* Autopsy not performed

PRESENTATIONS

Wilmore DW: Metabolic Aspects of Burn Care. Univ of Kan Trauma Conf, Kansas City, Kan 7 Jan 74.

Wilmore DW: Treatment of Burns. Grand Rounds, Department of Surgery, Vanderbilt University, Nashville, Tenn 12 Jan 74.

Pruitt BA Jr: 1) Current Methods of Burn Care; 2) Acid-base Disturbances in Injured Man. ACS Trauma Course, New Orleans, LA 14-17 Jan 74.

Salisbury RE: Evaluation of Digital Escharotomy in Thermally Injured Hands.

Pruitt BA Jr: Newer Opportunistic Infections in Burns.

Levine NS: A Comparison of Coarse Mesh Gauze Versus Biologic Dressings. Seventh Anl Symp. of Military Plastic Surgery, Wash DC 23 Jan 74.

Wilmore DW: Energy Metabolism Following Injury. Grand Rounds, Dept of Surgery, Univ of Maryland, Baltimore, MD 25 Jan 74.

McGranahan BG: Nursing Care in Burns. Sch of Aerospace Med, Brooks AFB TX 25 Jan 74.

McAlbany JC Jr: Treatment of Burns. Off Basic Course, Academy of Health Sciences, Fort Sam Houston, TX 25 Jan 74.

Long JM III: Serum and Liver Vitamin A Levels in Thermally Injured Patients. Technical Conf on Parenteral Vitamins, A.M.A., F.D.A., Wash DC 28 Jan 74

Wilmore DW: Metabolism Following Injury. Dept of Surgery, Univ of Ohio, Toledo, OH. 5 Feb 74.

Long JM III: Total Nutrition by Vein. Southwest Foundation Forum, San Antonio, TX. 6 Feb 74.

Salisbury RE: Artificial Prostheses for Tendon Injuries: Mechanical Problems and Cellular Response. Interdisciplinary Conf, Biomedical Research Problems in a Changing World. Institute for Molecular Physics, Univ of Maryland. 6 Feb 74.

Orcutt TW and Hayward CD: The Burn Insult. Nursing students, San Antonio College, San Antonio, TX 11 Feb 74.

McGranahan BG: Nursing Care of Burns. AORN Convention, New Orleans, LA 12 Feb 74.

Hunt JL: The Treatment of Burns. Medical Aspects of Advanced Warfare Course, USAF Sch of Aerospace Med, Brooks AFB, TX. 12 Feb 74.

Pruitt, BA Jr: Discussant of paper on water holding skin lipid. Society of University Surgeons Meeting, St. Louis, MO. 14-16 Feb 74.

Taylor JW: Burn Care. Univ of Tex Med Sch at San Antonio, San Antonio, TX 21 Feb 74.

McGranahan BG: Burn Treatment. R.N. Club, Randolph AFB, TX 25 Feb 74.

Wilmore DW: Energy Balance in Acute Illness. Tex State Nutritional Council, Univ of Tex at San Antonio, San Antonio, TX 28 Feb 74.

Wilmore DW: Resuscitation Following Burn Injury. Intl Med Assembly of Southwest Tex, San Antonio, TX 1 Mar 74.

Agee RN: Classification of Burns. Students Intensive Care Nursing Course, Brooke Army Medical Center, Fort Sam Houston, TX 4 Mar 74.

The following presentations were given to the Brooke Army Medical Center-Univ of Texas Medical School at San Antonio Symp on Surgical & Orthopaedic Aspects of Trauma, San Antonio, TX 5 Mar 74:

Pruitt BA Jr: Fluid Resuscitation and Early Care of the Burn Patient

Agee RN: Topical Therapy in Burn Wound Care

Orcutt TW: Coverage of the Burn Wound

Levine NS: *Excision of the Burn Wound*

Salisbury RE: Special Considerations in Treatment of Hand Burns

Petroff PA: Inhalation Injury and Other Pulmonary Complications

Long JM III: Gastrointestinal Complications.

Hunt JL: Diagnosis and Treatment of Newer Opportunistic Infections

Wilmore DW: Nutritional and Metabolic Considerations in the Treatment of Burn Patients

Peterson HD: Reconstructive Surgery of the Burn Patient and the Treatment of Scars.

Orcutt TW: Burns. Allied Medical Officers, Sch of Aerospace Medicine, Brooks AFB, TX 8 Mar 74.

Taylor JW: Burn Wound Therapy. Students Intensive Care Nursing Course, Brooke Army Medical Center, Fort Sam Houston, TX 11 Mar 74.

Taylor JW, McGranahan BG, Hall WF, Diaz HM: Team Approach to Care of Burn Patients. Physical Therapy School, Academy Health Sciences, Fort Sam Houston, TX 11 Mar 74.

Pruitt BA Jr: Early Complications of Burns. Hahnenann Continuing Education Program, Crozer-Chester Med Ctr, Philadelphia, PA 12 Mar 74.

Long JM III: Hyperalimentation. Students Intensive Care Nursing Course, Brooke Army Medical Center, Fort Sam Houston, TX 15 Mar 74.

Orcutt TW: Treatment of Burns. Officers Basic Course, Academy of Health Sciences, Fort Sam Houston, TX 15 Mar 74.

McGranahan BG: Nursing Care and Treatment of Burns. Flight Nurses and Technicians, Sch of Aerospace Med, Brooks AFB TX 15 Mar 74.

Blumle ML: Role of the Research Nurse. Incarnate Word Sch of Nursing San Antonio, TX 18 Mar 74.

Salisbury RE: Pseudosheaths Found by Gliding Tendon Prostheses. Thomas Jefferson Univ, Philadelphia, Pa. 15 Mar 74.

The following presentations were given at the Methodist Hospital Burn Seminar, Gary, Indiana on 22-23 Mar 74:

Orcutt TW: 1) The Burn Insult; 2) Initial Management; 3) Contemporary Burn Wound Management; 4) Complications in Burns

Curtis NA: 1) Intensive Care Nursing Assessment and Intervention; 2) Psychological Problems in Burn Patients

Townsend JC: Physical Therapy Management of Burn Patients

Shaw AL: Occupational Therapy and Splint Management

Hunt JL: Burn Victim. Continuing Medical Education Course Evaluation of Trauma University of Tex Med Sch at San Antonio, San Antonio, TX 23 Mar 74

Long JM III: Clinical Aspects of I.V. Hyperalimentation. Seminar on Intravenous Feeding Techniques for Nurses, Pharmacists and Physicians, N. California Soc of Hospital Pharmacists. Oakland, CA 23 Mar 74.

Wilmore DW: Fluid Electrolyte Balance. Course lecturer, American College of Surgeons, Houston, TX 25-26 Mar 74.

Wilmore DW: Hormone Regulation of Metabolism. American College of Surgeons, Houston, TX 27 Mar 74.

Long JM III: Potential Complications of Intravenous Hyperalimentation. Grand rounds, Wilford Hall USAF Hospital, Lackland AFB, TX 30 Mar 74.

Czaja AJ: Acute Gastroduodenal Disease after Thermal Injury: An Endoscopic Evaluation of Incidence and Natural History. Amer College of Physicians, New York, NY 4 Apr 74.

The following presentations were made at the American Burn Association Anl mtg in Cincinnati, Ohio 4-6 Apr 74:

Podgornoff WC: Parenteral Nutrition in Burned Patients: Nursing Considerations

Salisbury RE: Evaluation of Extended Digital Escharotomy in Thermally Injured Hands

Taylor JW: Thermal Injury During Pregnancy

Andes WA: Myocardial Infarction in the Thermally Injured Patient

Levine NS: A Comparison of Coarse Mesh Gauze vs 'Biological Dressings' on Granulating Wounds

McAlhany JC Jr: Histochemical Study of Gastric Mucosubstances After Thermal Injury

Long JM III: Emergence of Fusarium and Cephalosporium as a Cause of Invasive Burn Wound Infection

Andes WA: Acute Hematologic Changes in the Severely Burned Patient

Warden GD: Evaluation of Leukocyte Chemotaxis in Thermally Injured Patients

Wilmore DW: Effects of Human Growth Hormone and High Caloric Feeding in the Post-Traumatic Metabolic Response Following Thermal Injury

Long JM III: Burn Injury: Diagnosis, Treatment and Prevention. San Antonio Chapter, American Soc of Mechanical Engrs mtg 18 Apr 74

Hunt JL: What's New in Burns. Weber County Medical Society mtg, Hill Air Force Base, Utah 18 Apr 74

Long JM III: What's New in the Metabolic and Nutritional Support of Burn Patients; 2) Current Therapy for Burn Injuries. Seminar - Medical Research and Consultant Staff, McGaw Laboratory, Glendale, CA 26 Apr 74

Long JM III: Intravenous Hyperalimentation. Seminar - California State Society of Intravenous Therapists, Los Angeles, CA 27 Apr 74

Pruitt BA Jr: Nutrition for the Hypermetabolic Burn Patient. Case discussion of two burn patients. Symp on the Treatment of Burns. Beverwijk, Holland 26-27 Apr 74.

Long JM III: Clinical Aspects of IV Hyperalimentation. Kane County Medical Society, Geneva, IL 1 May 74

Long JM III: Intravenous Hyperalimentation. Seminar for Interns, Residents and Nursing Staff, Rush-Presbyterian St Luke's Medical Center, Chicago, IL 1 May 74

Pruitt BA Jr: Discussant of paper on Enzymatic Debridement of Burns. American Surgical Assn mtg, Colorado Springs, CO 1-3 May 74.

Long JM III: Intravenous Hyperalimentation. Staff Dept of Surgery Univ of Chicago Med Sch, Chicago, IL 2 May 74

Long JM III: Principles of Intravenous Hyperalimentation. Evanston Hospital, Evanston, IL 2 May 74

Long JM III: The Current Management of Burn Injury. Texas Medical Assn on Trauma, Dallas, TX 3 May 74

Hunt JL: Life Saving Measures for the Critically Injured. Cincinnati, OH 8 May 74

Pruitt BA Jr: Early and Late Problems in Management of Major Burns. Western New York Chapter ACS mtg, Wanakah, NY 9-10 May 74.

Pruitt BA Jr: Organization of County Units. Anl mtg of American Trauma Society, Chicago, IL 12 May 74.

Czaja AJ: Acute Duodenal Disease after Thermal Injury: Assessment by Early and Serial Endoscopy. Amer Soc for Gastrointestinal Endoscopy. San Francisco, CA 22 May 74.

Long JM III: Current Treatment of Burns. Seminar for Military Pharmacists, San Antonio, TX 24 May 74

Wilmore DW: Total Intravenous Nutrition. Seminar for Military Pharmacists, San Antonio, TX 24 May 74

The following presentations were made at a Team Symposium on Burns at the Midwest Health Congress, Kansas City, MO 10-12 Jun 74:
Agee RN: Management of Thermal Injuries
Curtis NA: Nursing Care of the Burn Patient
Townsend JC: Physical Therapy for the Burn Patient
Shaw AL: Occupational Therapy in the Burn Unit

Pruitt BA Jr: 1)Discussant of paper by J. Kohn of London, entitled, "The Role and Value of Antiseptics in a Burn Unit." 2) Discussant of paper by R. Zellner of Wudwigshafen, W. Germany, entitled "Problems of Vaccination Against Pseudomonas Infection" and 3) "From the Clinic to the Laboratory and Back--The Effectiveness of Interdisciplinary Burn Research at the US Army Institute of Surgical Research" Inauguration Symp on Burns, Univ Med Ctr, Ljubljana, Yugoslavia. 7-8 Jun 74.

Curtis NA: Nursing Care of the Burn Patient. Flight nurses and technicians, Sch of Aerospace Med, Brooks AFB, TX 25 Jun 74

Czaja AJ: Acute Gastroduodenal Disease Following Thermal Injury. Univ of Tex Health Science Center, San Antonio, TX 12 Jul 74.

Long JM III: IV Hyperalimentation. Nurses and Pharmacists, St. Mary's Hospital; 2) Practical Aspects of IV Hyperalimentation. Medical staff mtg, St. Mary's Hospital; 3) Consultant visit, Burn Unit Washoe County Medical Center, Reno, NV 15 Jul 74.

Long JM III: Intravenous Hyperalimentation. Florida Society of Hospital Pharmacists and Nurses of the Miami area, Hollywood, FL 19 Jul 74

Peterson HD: Management of Thermal Injuries. Officers Basic Course,

Academy of Health Sciences, Fort Sam Houston, TX 19 Jul 74

Berry DM: Nursing Research. Students Intensive Care Nursing Course, Brooke Army Medical Center, Fort Sam Houston, TX 22 Jul 74

Peterson HD: 1) Acute Management of Burns. Arkansas Trauma Research Society Physicians; 2) Nurses Role in the Management of the Acute Burn. Arkansas Trauma Research Society Nurses, Arkadelphia AR 2 Aug 74

Long JM III: Practical Aspects of Intravenous Hyperalimentation. Univ of TX Med Sch Surgical Conf, Medical students, Interns, Residents and visiting staff. San Antonio, TX 9 Aug 74

Agee RN: Treatment of Burns. Officers Basic Course, Academy of Health Sciences, Fort Sam Houston, TX 12 Aug 74

Agee RN: Treatment of Burns. Officers Basic Course, Academy of Health Sciences, Fort Sam Houston, TX 16 Aug 74

Long JM III: Prevention and Treatment of Electrical Injuries. USA Communications Command Regional Conf, San Antonio, TX 20 Aug 74

Peterson HD: Treatment of Burns. Officers Basic Course, Academy of Health Sciences, Fort Sam Houston, TX 30 Aug 74

Agee RN: Classification of Burns - Initial Care. Students Intensive Care Nursing Course, Brooke Army Medical Center, Fort Sam Houston, TX 4 Sep 74

Taylor JW: Burn Wound Therapy. Students Intensive Care Nursing Course, Brooke Army Medical Center, Fort Sam Houston, TX 9 Sep 74

Czaja AJ: Acute Gastroduodenal Disease after Burns. Mayo Clinic Gastroenterology Unit, Rochester, Minn 13 Sep 74.

Long JM III: 1) Hypermetabolism and Nutritional Support After Major Thermal Injury; 2) The Interrelationship of Fat and Carbyhydrate as Caloric Sources for Total Intravenous Nutrition. International Congress of Parenteral Nutrition, Montpellier, France 12-14 Sep 74

Erickson DR: Complications, Infection, Inhalation Injuries. Students Intensive Care Nursing Course, BAMC, Fort Sam Houston, TX 16 Sep 74

Pruitt BA Jr: 1) The Use of Topical Chemotherapy and Tissue Biopsies for the Control and Monitoring of Burn Wound Infection: Results in over 2900 Burn Patients; 2) Workshop moderator and plenary session panelist for plenary session on "Infection and Sepsis". Fourth International Congress on Burn Injuries, Buenos Aires, Argentina. 15-21 Sep 74.

Hunt JL: Life Saving Measures for the Critically Injured. Cincinnati, OH 8 May 74

Pruitt BA Jr: Early and Late Problems in Management of Major Burns. Western New York Chapter ACS mtg, Wanakah, NY 9-10 May 74.

Pruitt BA Jr: Organization of County Units. Anl mtg of American Trauma Society, Chicago, IL 12 May 74.

Czaja AJ: Acute Duodenal Disease after Thermal Injury: Assessment by Early and Serial Endoscopy. Amer Soc for Gastrointestinal Endoscopy. San Francisco, CA 22 May 74.

Long JM III: Current Treatment of Burns. Seminar for Military Pharmacists, San Antonio, TX 24 May 74

Wilmore DW: Total Intravenous Nutrition. Seminar for Military Pharmacists, San Antonio, TX 24 May 74

The following presentations were made at a Team Symposium on Burns at the Midwest Health Congress, Kansas City, MO 10-12 Jun 74:
Agee RN: Management of Thermal Injuries
Curtis NA: Nursing Care of the Burn Patient
Townsend JC: Physical Therapy for the Burn Patient
Shaw AL: Occupational Therapy in the Burn Unit

Pruitt BA Jr: 1)Discussant of paper by J. Kohn of London, entitled, "The Role and Value of Antiseptics in a Burn Unit." 2) Discussant of paper by R. Zellner of Wudwigshafen, W. Germany, entitled "Problems of Vaccination Against Pseudomonas Infection" and 3) "From the Clinic to the Laboratory and Back--The Effectiveness of Interdisciplinary Burn Research at the US Army Institute of Surgical Research" Inauguration Symp on Burns, Univ Med Ctr, Ljubljana, Yugoslavia. 7-8 Jun 74.

Curtis NA: Nursing Care of the Burn Patient. Flight nurses and technicians, Sch of Aerospace Med, Brooks AFB, TX 25 Jun 74

Czaja AJ: Acute Gastroduodenal Disease Following Thermal Injury. Univ of Tex Health Science Center, San Antonio, TX 12 Jul 74.

Long JM III: IV Hyperalimentation. Nurses and Pharmacists, St. Mary's Hospital; 2) Practical Aspects of IV Hyperalimentation. Medical staff mtg, St. Mary's Hospital; 3) Consultant visit, Burn Unit Washoe County Medical Center, Reno, NV 15 Jul 74.

Long JM III: Intravenous Hyperalimentation. Florida Society of Hospital Pharmacists and Nurses of the Miami area, Hollywood, FL 19 Jul 74

Peterson HD: Management of Thermal Injuries. Officers Basic Course,

Erickson DR. Acute Problems in Respiratory Care. Christmas Seal League, Pittsburgh, PA 20 Sep 74.

Peterson HD: Current Concepts in the Care of the Acute Burn. CENTO Group mtg, Fort Sam Houston, TX 23 Sep 74

Long JM III: Nutritional Support of Thermally Injured Patients. Univ of Zurich, Kantonspital, Zurich, Switzerland 26-28 Sep 74

Pruitt BA Jr: United Nations Conference of Government Experts on the Use of Certain Conventional Weapons, Lucerne, Switzerland. 29 Sep - 1 Oct 74.

Long JM III: Practical Aspects of Intravenous Hyperalimentation. New Mexico Society of Hospital Pharmacists, Albuquerque, N. M. 2 Oct 74

Wilmore DW: Criteria for Evaluation of Protein Metabolism. FDA, Washington, D.C. 8 Oct 74

Long JM III: Practical Aspects of Intravenous Hyperalimentation. Georgia State Society of Hospital Pharmacists, Atlanta GA 9 Oct 74

Hall WF: Recent Changes in Physical Therapy Treatment of Burn Patients. Chief Physical Therapists of the Health Services Command, Joint Services Seminar, San Antonio, TX 10 Oct 74

Long JM III: Practical Aspects of Intravenous Hyperalimentation. N. Florida Society of Hospital Pharmacists, Orlando, FL 11 Oct 74

Long JM III: New Developments in Intravenous Hyperalimentation. Central Texas Society of Hospital Pharmacists, San Antonio, TX 15 Oct 74

Wilmore DW: Parenteral Nutrition. Johnson City Medical Society, Johnson City, TN 15 Oct 74

Michael MG and Berry DM: Orientation to the Nursing Care and Research Mission at the Institute of Surgical Research. Defense Advisory Committee of Women in Service. 16 Oct 74

Michael MG, Berry DM and Podgornoff WC: Institute of Surgical Research Nursing Seminar. Academy of Health Sciences, Fort Sam Houston, TX 16 Oct 74

Wilmore DW: Hypothalamic Function Following Thermal Injury. American Assn for the Surgery of Trauma. Hot Springs, VA. 17 Oct 74

The following presentations were made and meetings attended by Pruitt BA Jr in connection with the ACS Mtg Miami Beach, FL 20-25 Oct 74:

1) Meeting, ACS Board of Governors; 2) Recorder for ACS Discussion Group; 3) Meeting, ACS Pre- and Postoperative Care Committee; 4) Meeting, ACS Committee on Trauma; 5) Meeting, National Burn Information Exchange; 6) N. American Chapter Internatl Society of Surgery Meeting; 7) Symposium on Stress Ulcers

Levine NS: Laser Excision of Third Degree Burns. American Assn for the Surgery of Trauma. Hot Springs, VA 18 Oct 74

McAlhany JC Jr: Status of the Gastric Mucosal Barrier in Thermally Injured Patients: Correlation with Gastroduodenal Endoscopy. Surgical Forum American College of Surgeons, Miami Beach, FL 22 Oct 74

Long JM III: Fat Carbohydrate Interaction: Nitrogen Sparing Effect of Varying Caloric Sources for Total Intravenous Feeding. Surgical Forum American College of Surgeons, Miami Beach, FL 22 Oct 74

Berry DM: Status of Mandatory Continuing Education in the U.S. ANC Officers, Academy of Health Sciences, Fort Sam Houston, TX 22 Oct 74

Peterson HD: Bacteriology of the Burn Wound. American Society of Plastic and Reconstructive Surgeons, Houston, TX 29 Oct 74

Levine NS: A Comparison of Laser, Scalpel, and the Electrocautery in Burn Wound Excisions. American Society of Plastic and Reconstructive Surgeons, Houston, TX 30 Oct 74

Wilmore DW: Criteria for Evaluation of Protein Metabolism. AMA Nutritional Advisory Group, Chicago, IL 1 Nov 74.

Michael MG: Orientation to Burn Nursing. Stu Nurses and staff of Univ of Tex Student Health Center at Austin, BAMC Fort Sam Houston, TX 6 Nov 74

Wilmore DW: Modification of Catecholamines During Endotoxemia. Assn of Academic Surgeons, Los Angeles, CA 8 Nov 74

The following presentations were made and meetings attended by Pruitt BA Jr at the Burn Toxin Conference, London, England 5-6 Nov 74: 1) Chairman, Morning Scientific Session; 2) Presentation: Shock, Solutions and Sepsis, the Causes of Death in Burn Patients.
Burn Symposium and Inauguration Ceremonies of the Burn Treatment Center, IMTR Hospital, Loverval, Belgium; and 2) Presentation "Opportunistic Infections in Burn Patients--Diagnosis and Treatment"; and 3) Concluding remarks at Inauguration Sumposium. 7-9 Nov 74

Wilmore DW: Food-Fuel Interaction. Univ of Tex Med Sch at San Antonio, San Antonio, TX 11 Nov 74

[illegible] MC: Orientation to Burn Nursing. Stu Nurses, St Phillips [illegible], BAMC FSH, TX 11 Nov 74

Long JM III: 1) Hormonal Regulation of Metabolism; 2) Relative Nitrogen Sparing Effects of Intravenous Carbohydrate and Fat Emulsion. Physicians and Basic Scientists, Conference on Intravenous Caloric Sources and Amino Acids, Palm Springs, CA 11 Nov 74

[illegible] JB: Orientation to Procedures and Practice of Burn Nursing. [illegible] USAF Sch of Aerospace Med, Brooks AFB, TX 12 Nov 74

Long JM III: Intravenous Amino Acids. Univ of Tex Med Sch at [illegible], Houston, TX 12 Nov 74

[illegible] DW: Care of the Critically Injured Patient. Symposium, [illegible] Univ of Texas Med Sch at Dallas, Dallas, TX 13 Nov 74

Pruitt BA Jr: Burn Seminar for Nurses, Univ of Texas Health Sciences Center, Dallas, Texas. Presentations: 1) Shock; 2) Metabolism Following Thermal Injury. 13 Nov 74

Pruitt BA Jr: Symposium on Principles and Practices of Antibiotic Therapy, Univ of Cincinnati Medical Center, Cincinnati, Ohio. Presentation: Susceptibilities in Burn Patients. 14 Nov 74

Peterson MD: Plastic Surgery in the Military. Senior Army Chaplains BAMC Chaplains Course, FSH TX 14 Nov 74

Erickson DR: Panel Member, Medical Advisory Committee of the Christian and Missionary Alliance Foreign Department, New York, NY 14 Nov 74

Long JM III: Review and Update of Intravenous Hyperalimentation. Salt Lake City, UT 16 Nov 74

Agee RN: The Treatment of Burns. Sch of Aerospace Med, Brooks AFB TX 19 Nov 74

Pruitt BA Jr: Treatment of Acute Stress Ulcers; and 2) Discussant of paper presented at the Western Surg Assn Mtg, San Francisco, CA 21-23 Nov 74

Czaja AJ: Acute Gastric Disease After Cutaneous Thermal Injury. 82nd Annual Session of the Western Surgical Assn, San Francisco CA 22 Nov 74

The following presentations were made at the Workshop for Texas Nursing Student Assn, San Antonio College, San Antonio, TX 23 Nov 74:
Taylor JM: The Treatment of Burns
Pedegonaff ME: Nursing Care of the Burn Patient

Long JM III: Priorities for Care of Acute Thermal Injury. Academy of Health Sciences Physician's Assistant Program students, USA ISR, BAMC, Fort Sam Houston, TX 26 Nov 74

Erickson DR: The Emergent Care of the Burn Patient. S. Dak Health Dept, Dept of Public Safety, Annl Emergency Med Tech Refresher Course, Pierre, SD 30 Nov 74

Pruitt BA Jr: Symposium on Burns, Medical Service of Comision Federal de Electricidad, Mexico City, Mexico. Presentations: 1) Sepsis in Burn Patients and its Treatment; 2) Homologous and Heterologous Grafting in Burn Patients. 5-7 Dec 74.

Long JM III: Priorities for Care of Acute Thermal Injury. Academy of Health Sciences Physician's Assistant Program students, USA ISR, BAMC, Fort Sam Houston, TX 3 Dec 74

Long JM III: Priorities for Care of Acute Thermal Injury. Academy of Health Sciences Physician's Assistant Program students, USA ISR, BAMC Fort Sam Houston, TX 10 Dec 74

Long JM III: Priorities for Care of Acute Thermal Injury. Academy of Health Sciences Physician's Assistant Program students, USA ISR, BAMC, Fort Sam Houston, TX 17 Dec 74

PUBLICATIONS

Pruitt BA Jr: Open and closed treatment of burns with povidone-iodine by NG Georgiade and WA Harris (Commentary) Plast Reconstr Surg 53:82-83, 1974

Pruitt BA Jr: Complications of thermal injury. Clinics in Plast Surg 1:667-691, 1974.

Wilmore DW, Lindsey CA, Moylan JA, Fallona GR, Pruitt BA Jr., Unger RH: Hypergluconemia after burns. Lancet 1:73-75, 1974.

Welch GW, McKeel DW Jr., Silverstein P and Walker HL: The role of catheter composition in the development of thrombophlebitis. SG&O 138: 421-424, Mar 1974

McManus WF, Hunt JL, Pruitt BA Jr: Postburn convulsive disorders in children. J Trauma 14:396-401, May 1974

Slogoff S, Allen GW, Wessels JV, Cheney DH: Clinical experience with subanesthetic ketamine. Anesthesia & Analgesia 53:354-358, 1974

Wilmore DW: Evaluation of the patient. In Total Parenteral Nutrition, White PL (Ed), Acton, Mass., Publishing Sciences Group, Inc., Publishers, 1974, pp. 11-21.

Long JM, Wilmore DW, Mason AD Jr, Pruitt BA Jr: Fat-carbohydrate interaction: Nitrogen sparing effect of varying caloric sources for total intravenous feeding. Surg Forum 25:61-63, 1974.

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

Hunt JL, McManus WF, Haney WP and Pruitt BA Jr: Vascular lesions in acute electric injuries. J Trauma 14:461-473, 1974

Wilmore DW, Moylan JA Jr, Bristow BF, Mason AD Jr and Pruitt BA Jr: Anabolic effects of human growth hormone and high caloric feedings following thermal injury. SG&O 138:875-884, 1974

Salisbury RE, Mason AD Jr, Levine NS, Pruitt BA Jr and Wade CWR: Artificial tendons: Design, application and results. J Trauma 14:580-586, 1974

Slogoff S, Allen GW: The Role of Baroreceptors in the Cardiovascular response to ketamine. Anes & Analgesia, Current Researches 53:704-707, 1974

Warden GD, Mason AD Jr, Pruitt BA Jr: Evaluation of leukocyte chemo-

taxis in vitro in thermally injured patients. J of Clin Invest 54:1001-1004, 1974

Wilmore DW, Long JM, Mason AD Jr, Skreen RW and Pruitt BA Jr: Catecholamines: Mediator of the hypermetabolic response to thermal injury. Ann Surg 180:653-669, 1974

Lindberg RB and Latta RL: Phage typing of Pseudomonas aeruginosa: Clinical and epidemiologic considerations. 130:S33-S42, Nov 74

Wilmore DW: Nutrition and metabolism following thermal injury. In Clinics in Plastic Surgery 1, Moncrief JA (Ed), Philadelphia, W.B. Saunders, Publishers, 1974, pp. 603-619.

Czaja AJ, McAlhany JC Jr, Pruitt BA Jr: Acute gastroduodenal disease after thermal injury. New Engl J of Med 231:925-929, Oct 31, 74.

Salisbury RE, McKeel DW Jr, Pruitt BA Jr and Mason AD Jr: Morphologic observations of neosheath development of undifferentiated connective tissue development around artificial tendons. J Biomed Mater Res Symp No 5 (Part 1), 175-184, 1974.

Salisbury RE, McKeel DW, Mason AD Jr: Ischemic necrosis of the intrinsic muscles of the hand after thermal injuries. J of Bone and Joint Surg 56-A: 1701-1707, Dec 74.

Pruitt BA Jr: Infections caused by Pseudomonas species in patients with burns and in other surgical patients. J of Infectious Diseases 130, Supplement S8-S14, Nov 74.

Salisbury RE, Silverstein P and Goodwin MN Jr: Upper extremity fungal invasions secondary to large burns. Plast Reconstr Surg 54:654-659, Dec 1974.

McAlhany HC Jr, Czaja AJ, Villarreal Y, et al: The gastric mucosal barrier in thermally injured patients: correlation with gastroduodenal endoscopy. Surg Forum 25:414-416, 1974.

Czaja AJ, McAlhany JC Jr, Pruitt BA Jr: Acute duodenal disease after thermal injury: assessment by early and serial endoscopy. Gastrointest Endoscopy 20:178, 1974.

EXHIBITS

The following exhibits were displayed during the year 1974:

"Serial Endoscopic Assessment of Acute Gastroduodenal Disease Following Thermal Injury"
1) American College of Physicians Anl Conv, New York NY 1-4 Apr 1974

2) American Gastroenterological Assn Anl Conv, San Francisco CA 22-24 May 1974
3) American College of Surgeons Anl Conv, Miami Beach, FL 21-25 Oct 1974

"Fiberoptic Bronchoscopy in Evaluation of Inhalation Injury"
1) American College of Surgeons Anl Conv, Miami Beach, Fl 21-25 Oct 1974.

"Reconstruction of the Thermally Injured Upper Limb"
1) American Academy of Orthopedic Surgeons, Dallas, TX 17-22 Jan 1974.
2) American College of Surgeons 60th Anl Clinical Congress, Miami Beach, FL 21-25 Oct 1974

MOTION PICTURES

The following motion pictures were shown during the year 1974:

"Energy Metabolism and Energy Support Following Thermal Injury"
"Physical Therapy in the Treatment of Burns of the Hand"
"Heterotopic Calcification About the Elbow"
"Laboratory and Clinical Evaluation of Porcine Xenograft as a Temporary Burn Wound Cover"
"Management of Upper Extremity Burns"
Exhibited at the Fourth International Congress on Burn Injuries, Buenos Aires, Argentina during the week of 16-21 September 1974.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION* DA OA 6983	2. DATE OF SUMMARY* 75 07 01	REPORT CONTROL SYMBOL DD-DR&E(AR)636
3. DATE PREV SUM'RY 74 07 01	4. KIND OF SUMMARY D. CHANGE	5. SUMMARY SCTY* U	6. WORK SECURITY* U	7. REGRADING* NA	8. DISP'N INSTR'N NL	8b. SPECIFIC DATA-CONTRACTOR ACCESS ☒ YES ☐ NO	9. LEVEL OF SUM A. WORK UNIT

10. NO. CODES.*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A161102B71R	01	168
b. CONTRIBUTING				
c. CONTRIBUTING				

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

12. SCIENTIFIC AND TECHNOLOGICAL AREAS* 003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
62 02	Cont	DA	C. In-House

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

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
PRECEDING FISCAL YEAR 75	1.3	75
CURRENT FISCAL YEAR 76	1.5	78

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

20. PERFORMING ORGANIZATION
NAME:* US Army Institute of Surgical Research
Surgical Study Branch
ADDRESS:* Fort Sam Houston, Texas 78234
PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME:* Douglas W. Wilmore, MD
TELEPHONE: 512-221-5712
SOCIAL SECURITY ACCOUNT NUMBER
ASSOCIATE INVESTIGATORS
NAME: Albert J. Czaja, MAJ, MC
NAME: Basil A. Pruitt, Jr., COL, MC DA

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

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

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

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

24. (U) Planned clinical and laboratory studies relating to acute and chronic effects of injury. Studies conducted in this branch have included both clinical investigation involving patients and normal individuals and laboratory studies involving animal models.

25. (U) 74 07 - 75 06 The Surgical Study Branch continued investigations of the metabolic response to thermal injury and the effects of physical trauma on wound healing, susceptability to infection, and gastrointestinal and liver function. The effects of nutritional support on post-traumatic metabolic response and organ system alteration is also being evaluated. The metabolic kitchen provides support for these investigations, in addition to preparation of specialized diets for patients with feeding problems, and the provision of diets containing known constituents to be used in a wide variety of metabolic balance studies. In addition, members of the branch provide nutritional and gastrointestinal consultation for all Institute patients and other individuals outside the Institute with complex gastrointestinal, metabolic-nutritional problems.

*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. 3A161102B71R-01, RESEARCH IN BIOMEDICAL SCIENCES

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

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

1 July 1974 - 30 June 1975

Investigators:

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

Reports Control Symbol MEDDH-288(R1)

Unclassified

ABSTRACT

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

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

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

Period covered in this report: 1 January 1974 - 31 December 1974

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

Reports Control Symbol MEDDH-288(R1)

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

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

Research projects include the definition of the post-traumatic metabolic response, the neuroendocrine mediators for this response, and the relationship between energy metabolism and ambient conditions. In addition, stress ulcers have been studied extensively by endoscopy, biopsy of the gastric mucosa, measurement of acid secretion and back diffusion from the stomachs of seriously injured soldiers. Liver function and bilirubin conjugation studies have been determined to reflect hepatic alteration following trauma.

Trauma
Post-traumatic metabolism
Combat casualty
Gastrointestinal function
Liver function

CLINICAL OPERATION, SURGICAL STUDY BRANCH FOR TREATMENT OF INJURED SOLDIERS

The three major activities of the Surgical Study Branch are: 1) delivery of medical and surgical care to the thermally injured soldier admitted to this institute; 2) clinical and laboratory research in problems related to care and rehabilitation of burn patients, and application of this knowledge to other critically ill patients; 3) the education of medical and paramedical personnel in the care of the seriously injured.

Members of this branch round daily to evaluate the patient problems in the Clinical Division of the US Army Institute of Surgical Research. The branch chief serves as coordinator of all clinical research, and all branch members provide consultative service and care in the areas of post-traumatic metabolism and nutrition, energy balance, gastrointestinal function, and hepatic dysfunction. The expertise of the branch members has been utilized by members of the Brooke Army Hospital to see patients in consultation on the General Surgical, General Medical, Gastroenterologic, Pediatric, and Dermatologic Services of this medical center. Techniques and modalities developed in this unit are currently applied to the care delivered to seriously injured patients who remain hospitalized until all wounds are healed.

Clinical and laboratory research in this division may be placed in the following categories: 1) temperature regulation; 2) post-traumatic metabolism; 3) energy balance; 4) nutritional support of critically ill soldiers; 5) description of the evolution and etiology of stress ulcers of the gastric mucosa of burn patients; 6) description of hepatic dysfunction which occurs following injury, with specific emphasis of the interrelation between hepatic glucose production and energy production of the burn patient.

Branch members participate actively in teaching activities of this unit, the Brooke Army Medical Center, and are affiliated on the staff of the Medical School of the University of Texas at San Antonio. In addition, branch members have actively participated in local, national, and international meetings to present and discuss their research findings and increase the scientific interchange in these areas of study.

PUBLICATIONS AND/OR PRESENTATIONS:

See report of Clinical Division, USAISR

ANNUAL PROGRESS REPORT

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

REPORT TITLE: ANESTHESIOLOGY

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

1 January 1974 - 31 December 1974

Investigators:

Allister K. Morris, MD, Major, MC
Gary W. Welch, MD, Major, MC
Stephen Slogoff, MD, Major, MC

Reports Control Symbol MEDDH-288(R1)

Unclassified

ABSTRACT

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

REPORT TITLE: ANESTHESIOLOGY

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

Period covered in this report: 1 January 1974 - 31 December 1974

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

Reports Control Symbol MEDDH-288(R1)

In 1974, 140 of 226 patients whose dispositions were completed at the United States Army Institute of Surgical Research were given 433 anesthetics at this institute. This figure includes some 17 patients who had reconstructive plastic procedures alone. These 17 patients underwent a total of 53 procedures. In the tables to follow, the plastic procedures are excluded, and 123 patients who had 380 anesthetics are used for computation of the statistics. Of the anesthetics given, 37.37% were ketamine, 29.47% were nitrous oxide-oxygen and supplementation, 18.95% were halothane, 9.47% were ethrane, 1.84% were regional anesthetics, and 2.9% were of other than those agents listed above. Of those patients receiving anesthesia at the Institute of Surgical Research, the mean number of anesthetics per patient was 3.76. Four intraoperative complications occurred during the year, and will be discussed in detail in the text. No intraoperative deaths occurred. During this year, subanesthetic ketamine for debridement or dressing change in the Hubbard tank was rarely used, and most debridements were carried out as major procedures in the operating room.

Anesthesia

ANESTHESIOLOGY

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

PREOPERATIVE PREPARATION

Patients for elective surgery are held NPO after midnight. This usually involves a fasting period of some 8 to 14 hours. Infants and children through age four are permitted clear liquids until 0200 hours. Using this regimen, we have had one instance of vomiting and aspiration on induction in patients for elective surgery. Seriously ill or dehydrated patients are given intravenous fluid preoperatively, including Ringer's lactate and 5% dextrose in Ringer's lactate or saline solution. Solutions of essentially the same nature are used for pediatric cases.

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 utilizing an anesthesia bag, suitable connectors, and a 100% oxygen source. Patients requiring oxygen but no ventilatory assistance are transported with the oxygen source being delivered to the patient by mask or nasal oxygen prongs. Once in the operating room, patients requiring ventilatory assistance may be ventilated manually or with an Air-Shield anesthetic ventilator. Circulatory status is assessed by hematocrit, serum electrolytes and osmolality, and urine output, in addition to direct or indirect measurements of blood pressure. Central venous pressures are measured relatively frequently and, on occasion, a Swan-Ganz catheter is placed for measurement of pulmonary artery and pulmonary wedge pressures.

PREMEDICATION

In general, a small amount of narcotic plus either hydroxyzine or diazepam is given to adults preoperatively. Atropine may be given in combination with these drugs or is given intravenously prior to the induction of general anesthesia. Pediatric patients generally receive a very similar regimen, in reduced dosage, however. Premedications are eliminated altogether in the extremely ill patient or in the child who is particularly afraid of injections.

TYPES OF ANESTHESIA

A. General Anesthesia

1. Ketamine

Ketamine is an intravenous dissociative general anesthetic which has been available for clinical use for approximately six years. Approximately 37% of our anesthetics in the operating room are now administered with this agent. Since cardiovascular reflexes and tone are well preserved, and a patent airway with good ventilation is usually maintained, even in the lateral and prone positions, this anesthetic has permitted numerous operations to be carried out without the use of an artificial airway. These factors plus the tremendous versatility of the agent for somatic procedures have significantly improved anesthetic management for the thermally injured patient.

2. Nitrous oxide supplement

This technique is used in approximately 30% of our procedures. Its versatility and relative lack of cardiovascular depression make it the second most frequently employed anesthetic agent at this time. When relaxants are added to this regimen, the trachea is intubated and respiration is controlled. Although pancuronium, d-tubocurarine, and gallamine are all nondepolarizing relaxants which are available, pancuronium has been the only one of the three to be used with any frequency this year. 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.

3. Halothane with or without nitrous oxide in oxygen

Approximately 19% of the anesthetics in our institution are performed with this combination of agents. It is relatively easy to administer, is nonflammable, and, with proper dosage adjustment, shows minimal cardiovascular depression. We have to date observed no cases of halothane hepatotoxicity. Due to the extreme difficulty of monitoring blood pressure in many of our patients, the use of ketamine and nitrous oxide with supplementation has resulted in a decrease in the general use of this agent.

4. Enflurane (Ethrane^{R}) with nitrous oxide in oxygen

Ethrane is a newly available, nonflammable, inhalational anesthetic which has the chemical structure of a halogenated ethyl-methyl ether. The action of this agent is very similar to halothane in uptake, onset of action, and emergence time. In extensive clinical

trials prior to release, and six months of clinical usage, the drug has demonstrated no propensity for hepatic toxicity. The tendency to produce twitching or involuntary motor activity, associated with seizure activity on electroencephalography at very deep levels of anesthesia, has not been seen at this institution. During the year 1974, approximately 10% of the patients at the Institute of Surgical Research received enflurane with or without nitrous oxide for their anesthesia.

5. Low-dose ketamine - "subanesthetic ketamine"

Although this agent was used extensively during the year 1973, it is difficult to maintain that patients who were amnesic and analgesic were not anesthetized. It has also been demonstrated that, although rare in occurrence, these patients can lose their patent airway unexpectedly and the presence of an anesthesiologist is always necessary. Currently, this technique is being used for very occasional debridements, suture removal, and dressing changes in small children.

B. REGIONAL ANESTHESIA

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

3. Blood pressure cuff of the usual type or of the Infrasonde trademark type, or direct intra-arterial monitoring.

4. Central venous pressure assessment.

5. EKG.

6. Sponge weighing.

7. Measurement of urine output during surgery.

B. RESPIRATION

1. Counting of respiratory rate.

2. Observance of chest and rebreathing bag.

3. Auscultation of chest.

4. Periodic assessment of blood gases intraoperatively when indicated.

C. TEMPERATURE

1. Rectal or esophageal thermistor probe.

It should be noted that the k-thermia heating-cooling blanket has proved to be of significant value in maintaining body temperature when large areas of the body are exposed. In addition, it can help to lower body temperature rapidly and safely when a febrile episode occurs intraoperatively. Ambient temperature in the operating room is maintained at 75° F., and this has been shown to be of benefit in minimizing heat loss.

COMPLICATIONS

Case No. 1

Significant bradycardia and hypotension, probably secondary to hypoxia

This 21 year old white male was injured in an oil rig explosion, in which he sustained a 32% total body surface burn. The face, upper extremities, anterior chest, and back were involved, and carbonaceous sputum was found on bronchoscopy. Approximately one week prior to the anesthetic, the patient spiked temperatures to 103, and blood cultures at that time were positive for Klebsiella. The patient was started on intravenous gentamycin, and a subsequent blood culture (two days later) revealed no growth. The patient remained alert and cooperative; however, in the subsequent three days, the patient became progressively tachypneic with blood gases being reported as normal. Two days prior to operation, the patient had an increase in respiratory rate to 60 per minute, and the chest x-ray revealed diffuse interstitial edema. Blood gases at this time confirmed the decreased ventilation, with a rise in pCO_2 from approximately 30 mmHg to 44 mmHg. The patient was felt to

be in acute respiratory distress, and was intubated with a nasotracheal tube. Sedation and paralysis were necessary intermittently to maintain respirator control. Blood gases at this time on the ventilator were: pO_2 129, pCO_2 44.5, pH 7.30, on an inspired oxygen concentration of 50%. The patient experienced a drop in hematocrit, and the next day bright red blood was noted in the nasogastric tube. Four units of blood had been given in the previous 24 hours, and lavage had failed to control the upper GI hemorrhage. The patient was taken to the operating room, where anesthesia was induced with Valium, 5 mg, and ventilation was controlled with increments of pancuronium. Another 5 mg increment of Valium and 50% nitrous oxide were administered for the performance of a tracheostomy. The nitrous oxide was discontinued for the insertion of the tracheostomy tube, and, after the tracheostomy was complete, the 50% nitrous oxide was reinstituted and another 5 mg increment of Valium was given. Whole blood and fresh-frozen plasma were given as the abdominal operation commenced. The pulse strength was felt to diminish some 15 minutes into the abdominal portion of the operation, and this was noted to be concomitant with a decrease in the urine output. The patient was felt to be volume depleted. A second unit of whole blood was administered, along with a second unit of fresh-frozen plasma, and 200 mg of Lasix was given IV because of the decreased urine output. Twenty-five milligrams of ephedrine were given IV as a temporizing measure while the second unit of whole blood and fresh-frozen plasma were being administered, with a noted increase in the pulse strength. The pulse strength was noted to decrease as the effects of the ephedrine began to wear off, and isuprel was begun. The patient was placed on 100% oxygen, and the CO_2 cannister, which had been depleted, was replaced. The patient became bradycardiac during the change of the CO_2 cannister, but responded to .8 mg of atropine IV. One ampule of sodium bicarbonate was given at this time. Throughout the rest of the procedure, the patient was maintained on 100% oxygen, and 250 mg increments of ketamine were given to insure that the patient was amnesic. A third unit of whole blood and two more units of sodium bicarbonate were given during the remainder of the procedure. As the skin was being closed, the patient was noted to again be bradycardiac; 1.6 mg of atropine was given IV and closed chest massage was begun; .5 mg of epinephrine was given intravenously with return of pulse rate and pressure. Postoperatively, the patient was placed back on the ventilator, with adequate blood gases on 50% oxygen. The next day, the patient was alert and reactive with respirator support. One day later, the patient was noted to be having respiratory difficulty, and the problem appeared to be a tracheostomy tube obstruction. His blood gases were consistent with a respiratory acidosis and hypoventilation. The patient developed a significant bradycardia while his tracheostomy tube was being changed, and subsequently developed a cardiac arrest with unsuccessful resuscitation. Postmortem examination showed a large right pulmonary artery embolus, which was possibly the terminal event.

The postmortem examination also showed a pneumonia, bacterial in nature, which was hemorrhagic, necrotizing, cavitating, bilateral, and severe. The organisms responsible for this pneumonitis were Providencia stuarti, E. coli, and Staphylococcus. There was also interstitial pneumonitis and edema with hyaline membranes.

Comment

This patient's decrease in pulse pressure was at first felt to be volume related, but, when the pulse pressure did not increase with what seemed to be an adequate volume replacement, attempt was made to augment perfusion with an isuprel drip. When this appeared to be of minimal value in augmenting the patient's pressure, the possibility was entertained that the exhausted CO_2 cannister might be contributing to the patient's hypotensive state. CO_2 is a potent vasodilator in the absence of an adequate catecholamine response. It is possible, however, that secondary to this patient's severely diminished pulmonary capacity that he became hypoxic even on 100% oxygen during the short period of time necessary to change the CO_2 cannister. The second episode of bradycardia, however, appeared while the patient was on 100% oxygen and being continuously ventilated. This tendency of poor response to volume loading and vasopressors has been demonstrated on numerous occasions in burn patients who have been septic or are septic and require anesthesia. A somewhat better response has been obtained in some of these patients when Dopamine has been the vasopressor employed. Dopamine was not yet available at the time of this patient's anesthetic.

Case No. 2

Aspiration of gastric contents on induction of anesthesia

This 50 year old white male, heavy equipment operator, sustained a 27.5% total body surface electrical burn when his equipment came in contact with a high-voltage wire. This patient had undergone two previous general anesthetics without difficulty. On induction of anesthesia with 500 mg of thiopental at the beginning of his third anesthetic, the patient vomited and aspirated a moderate amount of gastric contents. Blood gases on 100% oxygen showed a shunt consistent with aspiration and continued to deteriorate over the next 30 minutes. The patient was given solumedrol, 125 mg IV. Three subsequent doses of solumedrol, 125 mg each, were given at six-hour intervals postoperatively. The patient was placed on an MA-1 ventilator postoperatively with 10 cm of water, positive end-expiratory pressure being applied. Steady decreases in the inspired oxygen concentration were possible, and, over the next two days, the patient had progressed to the point that he was extubated and placed on mask oxygen. His ability to oxygenate continued to improve, and, one week later, his PO_2 on room air was 70 mmHg.

Comment

This patient's history was significant in that he had complained of occasional epigastric distress, especially after a large meal. Subsequent endoscopy and upper GI series failed to reveal any abnormalities. The patient had two other general anesthetics without difficulty prior to his discharge.

Case No. 3

Right mainstem bronchial intubation with equal breath sounds noted bilaterally

This 31 year old white male was admitted for elective reconstruction of his left hand, which had been burned in a gasoline fire. He had undergone a 39% total body surface burn with healing resulting in hypertrophic scar requiring release. Anesthesia in this patient was induced with thiopental, and succinylcholine was given to facilitate intubation. The patient was intubated without difficulty. Cords appeared normal, and breath sounds were equal to auscultation bilaterally. Immediately postintubation, the patient was noted to have a decreased compliance. The endotracheal tube was noted to be free from obstruction. Spontaneous respiration returned, and the patient was begun on halothane with the nitrous oxide-narcotic technique being abandoned. This brought about some increase in the patient's compliance; however, arterial blood gases on 100% oxygen showed a PO_2 of 184, a pCO_2 of 75, with a pH of 7.23. A decision was made at this time to cancel the case since there was the possibility of aspiration, although this was considered unlikely. Breath sounds were rechecked and found to be equal bilaterally, and the chest appeared to move equally bilaterally with ventilation. At this point, a fiberoptic bronchoscope was introduced down the endotracheal tube to rule out some mechanical obstruction, and it was found at this time that the endotracheal tube was slightly down the right mainstem bronchus. The tube was pulled back with immediate increase in the PO_2 on 100% oxygen to 220 mmHg. A subsequent chest x-ray was within normal limits, and the patient was placed on a ventilator with 10 cm of PEEP because of the length of time required to make the diagnosis of right mainstem intubation. The patient was ventilated overnight, with restoration of normal pulmonary function. Extubation was accomplished at this time.

Comment

It has recently been pointed out that equal breath sounds may be a misleading factor in determining right mainstem bronchial intubation. It is recommended that, wherever possible, the endotracheal tube cuff be located by palpation with the simultaneous injection of a sufficient

amount of air to allow this palpation. In this patient, this procedure may have allowed a more rapid diagnosis of the right mainstem intubation.

Case No. 4

Hypotension under anesthesia secondary to septic shock

This 14 year old white male sustained a 75% total body surface burn when his clothing was ignited while flying a model airplane which made contact with a power line. There was no electrical injury. The patient did well during resuscitation, and continued to do well despite positive blood cultures requiring intravenous antibiotics. After two weeks of doing relatively well, the patient became lethargic, confused, and sustained a blood pressure drop to a systolic of 60 mmHg. The diagnosis of septic shock was made, and the patient was taken to the operating room to explore his old IV sites for the source of infection. The left antecubital fossa showed a rather large area of tissue necrosis beneath viable skin, as well as destruction of the vein. This had a greenish culture, and cultures subsequently grew Pseudomonas. This area was extensively debrided and left open. During the anesthetic, which consisted of small amounts of ketamine and 50% nitrous oxide, the patient progressively became hypotensive. An attempt was made to start a central venous line prior to administration of vasopressors. The patient became progressively more hypotensive and bradycardiac, which necessitated the administration of atropine, epinephrine, and Dopamine through a peripheral IV. There was good return of pulse and blood pressure, and an adequate urine output toward the end of the case. The patient was returned to the ward and placed on an MA-1 respirator. His inspired oxygen concentration was quickly reduced to 40% with good blood gases. Because of the persistent gram negative blood cultures, and because of the uncertainty of the site of his sepsis, the patient's topical therapy was changed from Silvadene to Sulfamylon. He was continued on Dopamine and progressively deteriorated over the next 48 hours. He became more and more hypotensive and required more and more Dopamine to maintain his blood pressure above 80 systolic. The patient required larger and larger doses of Dopamine and was resuscitated from two cardiac arrests, but subsequently became more and more hypotensive and expired.

Comment

This case illustrates the relative effectiveness of Dopamine to control hypotension during septic shock, but its relative ineffectiveness when it must be continued and the source of sepsis cannot be eliminated.

TABLE 1. OVERALL PATIENT DATA, USAISR (1965-1974)

	A No. of Patients	B No. Patients Anesthetized (ISR Only)	C B/A X 100	D Total Anesthetics (ISR Only)	E D/A	F D/B	G Average Per Cent Burn
1965	174	107	61.5	495	2.84	4.63	33
1966	311	181	58.2	713	2.29	3.94	30
1967	389	239	61.4	670	1.72	2.80	28
1968	389	259	66.6	794	2.04	3.07	30
1969	294	189	64.3	601	2.04	3.18	36
1970	321	198	61.7	497	1.55	2.51	30
1971	301	179	59.5	475	1.58	2.65	31
1972	301	183	60.8	575	1.91	3.14	34
1973	273	141	51.6	377	1.38	2.67	38.5
1974	226	123 (17)*	54.4	380 (53)*	1.68	3.09	41.57

*Numbers in parentheses represent plastic and reconstructive procedures not counted in statistics.

TABLE 2. NATURE OF SURGERY, USAISR

	1971		1972		1973		1974	
Procedure	No.	%	No.	%	No.	%	No.	%
Debridement and/or homograft	74	15.5	113	19.7	81	21.5	86	22.60
Autograft	252	52.9	295	51.3	198	52.6	216	56.9
Orthopedics	62	13.0	51	8.9	30	8.0	31	8.1
Ear (chondrectomy)	19	4.0	18	3.1	10	2.6	6	1.60
Eye and lid	18	3.8	4	0.7	7	1.8	6	1.60
Intra-abdominal	8	1.7	45	7.8	8	2.1	14	3.70
Tracheostomy & bronchoscopy	22	4.6	38	6.6	25	6.6	7	1.80
Other	21	4.4	11	1.9	18	4.8	14	3.70
Total	476		575		377		380	

TABLE 3. EMPLOYMENT OF ANESTHETIC AGENTS AT ISR 1964-1974

(IN PERCENT OF TOTAL FOR THE YEAR)

	64	65	66	67	68	69	70	71	72	73	74
Ketamine	0	0	0	0	0	4.8	18.7	27.3	44.0	33.5	37.37
N_2O-supplement	0.6	3.5	1.3	0	.3	4.7	8.4	18.7	13.0	18	29.47
Halothane	87.0	68.3	92.9	97.0	99.4	86.9	66.8	47.3	40.9	40.3	18.95
Enflurane	0	0	0	0	0	0	0	0	0	2.6	9.47
Regional block	6.0	8.0	1.2	0	.3	1.8	5.2	5.7	1.9	.8	1.84
Methoxyflurane	0	20	0	0	.1	.8	.4	.2	0	0	0
Cyclopropane	4.8	.6	.7	0	0	0	0	0	0	0	0
Other	1.6	0	3.9	3.0	0	1.0	.4	.6	.2	4.8	2.90
Total number of anesthetics	332	495	713	670	794	601	497	476	575	377	380 (53)*

*Plastic procedures not included in anesthetic statistics for total of 433 anesthetics.

TABLE 4. GENERAL ANESTHESIA INDUCTION AGENTS, USAISR - 1974

Agent	Number of Inductions	Percent of Total
IV barbiturate	167	46.1
IV ketamine	144	39.8
IM ketamine	22	6.1
IV other	11	3.0
Inhalation	18	5.00
Total	362	100

TABLE 5. TYPE OF AIRWAY DURING GENERAL ANESTHESIA, USAISR - 1974

	Number of Anesthetics	Percent of Total Number of General Anesthetics
Mask	128	33.7
Endotracheal tube (oral and nasal)	102	26.8
Tracheotomy	28	7.4
Natural airway	122	32.1
Total	380	100

TABLE 6. USE OF MUSCLE RELAXANTS, USAISR - 1974

Total General Anesthetics	Number of Anesthetics Where Muscle Relaxants Used	Pancuronium	dT-Curarine	Gallamine	Succinylcholine
373	73	73	0	0	0 (2)*
Percent of total general anesthetics	19.57	19.57	0	0	0

	Number of Anesthetics	Percent of Total General Anesthetics
Muscle relaxant used	73	19.57
Used for intubation	60	16.08
Used for relaxation	14	3.75

*Used on two plastic cases.

PUBLICATIONS AND/OR PRESENTATIONS:

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA OA 6956	75 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
75 07 01	D. CHANGE	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A161102B71R	01	141
b. CONTRIBUTING				
c. CONTRIBUTING				

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

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*
003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
52 07	Cont	DA	C. In-House

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

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 75	1.1	28
FISCAL YEAR CURRENT 76	1.0	29

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

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

20. PERFORMING ORGANIZATION
NAME:* US Army Institute of Surgical Research
Metabolic Branch
ADDRESS:* Fort Sam Houston, Texas 78234

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME:* William D. Myers, LTC, MC
TELEPHONE: 512-221-6532
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: Richard H. Merrill, LTC, MC
NAME: DA

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

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

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

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

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

25. (U) 74 01 - 74 12 Sixteen patients were hemodialyzed by the ISR Renal Section for a total of 58 patient treatments. One peritoneal dialysis was carried out. Of the 17 patients, only one survived. The rapid bedside clotting test adapted by the section earlier continues to be most useful in patient management during hemodialysis. Femoral vein catheterization with Unipuncture hemodialysis has markedly improved acute hemodialysis access. A video tape has been made of the technique and presented at professional meetings where much interest has been generated. Other areas of clinical investigation are also underway, with particular emphasis on pathophysiology of acute renal failure.

*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. 3A161102B71R-01, RESEARCH IN BIOMEDICAL SCIENCES

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

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

1 January 1974 - 31 December 1974

Investigators:

William D. Myers, MD, Lieutenant Colonel, MC
Richard H. Merrill, MD, Major, MC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

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

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

Period covered in this report: 1 January 1974 - 31 December 1974

Investigators: William D. Myers, MD, Lieutenant Colonel, MC
Richard H. Merrill, MD, Major, MC

Reports Control Symbol MEDDH-288(R1)

Sixteen patients were hemodialyzed by the ISR Renal Section for a total of 58 patient treatments. One peritoneal dialysis was supervised. Of the 17 patients, only one survived. The rapid bedside clotting test adapted by the section earlier continues to be most useful in patient management during hemodialysis. Femoral vein catheterization with Unipuncture hemodialysis has markedly improved acute hemodialysis access. A video tape has been made of this technique and presented at professional meetings where much interest has been generated. Other areas of clinical investigation are also underway, with particular emphasis on pathophysiology of acute renal failure.

Renal failure
Hemodialysis
Soldiers
Peritoneal dialysis

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

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

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

In addition to the dialysis support provided to the hospital and the unit, several pilot studies have been initiated. A new technique introduced into the Hemodialysis Unit for controlling blood anticoagulation during dialysis has proven most beneficial. The results of this innovation were reported at the Southeastern Dialysis and Transplant meeting and have been published. In addition, a videotape has been produced showing our technique of unipuncture dialysis via femoral catheter and has been submitted as a display as well as presented at scientific meetings. Many visiting physicians have expressed interest in the femoral unipuncture technique of acute hemodialysis and requested literature. Other projects underway include measurement of the residual blood volume in coils, evaluation of urinary sediment in thermally injured patients, calcium metabolism in the thermally injured patient, and postburn renal histology. Plans for the future include investigation of intrarenal blood flow in the thermally injured patient to better define the pathophysiology of azotemia and renal failure.

PRESENTATIONS

Merrill, RH. Acute venous dialysis utilizing the unipuncture apparatus. Southeastern Dialysis & Transplantation Association Meeting, Charleston, South Carolina, August 16, 1974.

PUBLICATIONS

Merrill RA. Reduced calcium absorption after nephrectomy in uremic patients. New Eng J Med 291:458-460, 1974.

Merrill RA. Onycholysis, fungal versus drug induced. Southern Med J 67:667-678, 1974.

Merrill RA. Reduced calcium absorption after nephrectomy in uremic patients. New Eng J Med 291:458-460, 1974.

Merrill RA. Positive mono-spot test in histocytic medullary reticulosis. In press, Am J Clin Path.

Merrill RA. Kidney transplantation in the active duty soldier. In press, Military Medicine.

EXHIBITS

Merrill RH. Acute venous unipuncture dialysis, American College of Surgeons Meeting, Miami Beach, Florida, October, 1974.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA OD 6951	75 07 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISP'N INSTR'N	8b. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
74 07 01	D. CHANGE	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A161102B71R	01	084
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)*

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

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
71 03	Cont	DA	C. In-House

17. CONTRACT/GRANT: Not Applicable

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:*

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 75	.6	16
FISCAL YEAR CURRENT 76	.4	11

19. RESPONSIBLE DOD ORGANIZATION

NAME:* US Army Institute of Surgical Research

ADDRESS:* Fort Sam Houston, Texas 78234

RESPONSIBLE INDIVIDUAL

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

TELEPHONE: 512-221-2720

20. PERFORMING ORGANIZATION

NAME:* US Army Institute of Surgical Research
Clinical Division

ADDRESS:* Fort Sam Houston, Texas 78234

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

NAME:* Robert B. Lindberg, PhD

TELEPHONE: 512-221-2018

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Virginia C. English, MS

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

21. GENERAL USE

FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Endotoxin; (U) Sepsis; (U) Assay; (U) Burns; (U) Humans

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

23. (U) To evaluate Limulus lysate coagulation as a test for endotoxin in body tissues and fluids as an aid in characterizing endotoxic shock and sepsis in burned soldiers

24. (U) Plasma is extracted with glacial acetic acid. Liver and other tissues are triturated and extracted in a comparable manner, then assayed for endotoxin.

25. (U) 74 07 - 75 06 Livers of 60 patients dying as a result of severe burns were assayed for endotoxin content. 90% of patients showed endotoxin in liver in amounts ranging from .001 ug/gm to 64 ug/gm. Mean bacterial content of 10^3 colonies per gm was not related to endotoxin content. The correlation of endotoxin with lethal sepsis suggests that the buildup of endotoxin may represent an intact sequestering mechanism but a breakdown of detoxification.

*Available to contractors upon originator's approval

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

ANNUAL PROGRESS REPORT

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

REPORT TITLE: DETECTION OF ENDOTOXIN IN BURNED SOLDIERS WITH SEPSIS

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

1 July 1974 - 30 June 1975

Investigators:

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

Report Control Symbol MEDDH-288 (R1)

UNCLASSIFIED

ABSTRACT

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

REPORT TITLE: DETECTION OF ENDOTOXIN IN BURNED SOLDIERS WITH SEPSIS

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

Period covered in this report: 1 July 1974 - 30 June 1975

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

Report Control Symbol MEDDH-288(R1)

Endotoxin is demonstrated in plasma, serum or tissues by extracting with acetic acid or with chloroform. Livers of 60 patients dying with severe burns were examined by extracting and measuring for the presence of endotoxin. Ninety per cent of the liver samples were positive for endotoxin, in amounts ranging from 0.64 ug/gm to 0.001 ug/gm, with a median value of 0.06 ug/gm. Mean values of 10^3 bacteria per gram of tissue were found, but analysis of assay results in non-septicemic patients, and of endotoxin-negative livers validate the finding in liver samples, and indicate a potential causal relationship between sepsis and endotoxin build-up in liver.

Endotoxin
Sepsis
Assay
Burns
Humans

DETECTION OF ENDOTOXIN IN BURNED SOLDIERS WITH SEPSIS

The detection of nanogram and even picogram amounts of endotoxin by use of the Limulus amoebocyte lysate gelation reaction has occasioned much renewed interest in the role of endotoxin in causing septic shock. Repeated attempts to detect endotoxin in the serum or plasma of septic patients have been made (1,2). For reasons not understood, it has not been possible consistently to detect endotoxin in such samples. In this laboratory, approximately one-third to one-half of patients whose clinical condition, including gram negative septicemia, would be expected to lead to a positive test for endotoxemia, have indeed shown a positive Limulus coagulation reaction. The rate of positive reactions has not been such that the reaction has received wide acceptance as an aid in diagnosis of endotoxins, nor has it appeared to be of prognostic significance. The experience of this laboratory parallels that of other investigators. There are, however, many other facets of the endotoxin problem that can be assessed with this sensitive reaction. One of these is the detection and assay of endotoxin in liver tissue at autopsy. Endotoxin has been shown, in experimental models, to be removed rapidly by the liver, most probably on a basis similar to that in which foreign bodies such as serum molecular aggregates can be detected in liver macrophages, after introduction into the blood. Study of liver tissue at autopsy was initiated in 1973, and a preliminary report was submitted (2). Endotoxin could, indeed, be detected in blocks of liver tissue collected at autopsy. One-gram amounts were collected, homogenized in Ten Broeck grinders, the homogenate taken up in pyrogen-free water, and assayed in a manner paralleling the trichloracetic acid method for plasma endotoxin. Sixty patients had liver samples collected at autopsy. Since the possibility that endotoxin recovered might reflect bacterial contamination rather than sequestered endotoxin, quantitative counts were made on each sample as soon as it was received.

Although 1000 coliform bacteria per ml from a broth culture will give a positive Limulus gelation reaction, it does not follow that a count of 10^3 bacteria per gram of tissue will of necessity give a positive reaction. Even in grossly contaminated specimens the amount of endotoxin present may far exceed that accounted for by the bacteria present. Negative Limulus gelation reactions can occur with tissues containing 10^3 or 10^4 organisms per gram. In cultures, the amount of endotoxin demonstrable from Enterobacteriaceae is on the order of 0.001 ug or less when 1000 organisms per ml are present. The larger amounts of endotoxin demonstrable in liver samples are interpreted as pre-formed endotoxin taken up by liver cells.

---

1. Lindberg RB, English VC, Pruitt BA, Jr, Mason AD, Jr: Detection of endotoxin in burned soldiers with sepsis. USA Institute Surg Res Ann Res Prog Rpt FY 1973, BAMC, Ft Sam Houston, Texas, Section 6.

2. Lindberg RB, English VC, Mason AD, Jr, Pruitt BA, Jr: Detection of endotoxin in burned soldiers with sepsis. USA Institute Surg Res Ann Res Prog Rpt FY 1974, BAMC, Ft Sam Houston, Texas, Section 5.

Endotoxin levels in the livers of 60 patients, 91% of whom had positive blood cultures during life, are shown in Table 1. Among reacting samples, 43 out of 54 exhibited endotoxin at 0.01 ug/gm or higher, and over half of the reactors had from 0.02 to 0.08 ug/gm present. These are significant amounts above the level that bacterial content of liver samples would account for. The mean counts of bacteria in liver samples at the various levels of endotoxin content are shown in the third column. The bacterial counts, in these groups large enough to have a meaningful mean value, were essentially constant over the whole range of endotoxin values. The constancy of the bacterial counts reinforces the conclusion that the endotoxin values found in liver of these fatal burns are not a function of the bacterial content of the tissue, but are independent of them. This distinction is critical if the presence of endotoxin in the liver is to be assessed in relation to the problem of sepsis in the burned patient. The possibility that it represents a sequestering mechanism which is incomplete, in that it fails to detoxify the endotoxin after it is fixed in macrophages or histiocytes, is an attractive one.

Table 1. Endotoxin Levels in Liver Tissues from Autopsies, ISR, 1974

Endotoxin ug/gm	No. Patients	Average Bacterial Count/gm
$>0.64^{*}$	4	-
0.64	1	-
0.32	1	10^{4}
0.16	3	$10^{4.5}$
0.08	11	$10^{3.3}$
0.04	10	$10^{3.3}$
0.02	8	$10^{3.0}$
0.01	5	$10^{3.5}$
0.005	2	$10^{3.0}$
0.002	5	$10^{2.5}$
0.001	4	$10^{3.0}$
Negative (<0.0001)	6	
Total tested	60	
Median level: 0.06 ug/gm		

All patients with endotoxin in the liver did not exhibit bacteremia. In Table 2, 6 patients are shown, in none of whom a positive antemortem blood culture was obtained. The levels of endotoxin were, on average, lower than the overall total. The gram negative species recovered from the liver were typical of those found in the entire population examined. The tissue counts in these liver samples gave a median value of $10^{3.1}$, i.e., the numbers were comparable to the majority of patients who had a diagnosis of septicemia.

In 6 other patients, no endotoxin was recovered from patients who had unequivocal septicemia. These patients are summarized in Table 3. Four of the 6 had prolonged episodes of septicemia, with multiple positive blood cultures with a variety of gram negative bacilli, as well as staphylococci and group D streptococci. The bacterial counts in the liver samples had a median value of $10^{3.1}$, exactly as did those patients with negative blood cultures but with positive endotoxin in the liver. The distinctive attribute of these patients with sepsis but no endotoxin in the liver was, in 4 of them, the very prolonged series of septicemic episodes.

The species of organisms found in the liver specimens reflect the blood stream content of bacteria in this group of patients. The liver and spleen were found by Teplitz and Lindberg to offer a more precise reflection of the blood stream content than could be obtained by postmortem heart blood culture. The number of strains of the various species recovered are shown in Table 4. The gram positive species included a relatively high proportion of group D streptococci. Among gram negative species, the proportion of Escherichia coli and Klebsiella pneumoniae was much higher than would have been expected in view of the distribution of these species recovered in blood culture. In contrast, only one strain of Pseudomonas aeruginosa was recovered; again, this low incidence was surprising in view of the frequency with which Pseudomonas is recovered from blood cultures.

STABILITY OF LIMULUS AMOEBOCYTE LYSATE

The validity of the ongoing study of endotoxin requires a reliable source of amoebocyte lysate. In 1973, a report on reactivity of lysate stored at 4° C and at -70°C was compiled; the success of the -70°C storage was indicated. However, further use of aliquots of the lysate pools collected in 1971 occasioned continued control testing of samples immediately upon thawing and at intervals during storage at 4°C. Table 5 presents the results of these assays. It was readily within the capability of all the lysate samples to generate a usable reaction at 0.00125 ug/ml of endotoxin, but the lysate held at -70°C until the time of use was more potent than the same lysate thawed and held at 4°C. There are circumstances in which amounts of endotoxin smaller than 0.001 ug/ml are to be assayed, but in the system thus far employed, this degree of sensitivity is redundant. The stock of lysate collected in 1971 still includes a usable volume of sensitive and specific reacting material.

DISCUSSION

Further evidence for the appearance of endotoxin in the liver of severely

Table 2. Endotoxin in Liver of Patients with Negative Antemortem Blood Cultures

Autopsy No.	No. of Blood Cultures	Liver		
		Endotoxin Level ug/gm	Bacterial Count Per Gram	Predominant Organisms in Liver
A-10	4	0.038	7.6×10^3	E coli, Serratia marcessens
A-30	1	0.019	7.5×10^1	Staph aureus, Entero aerogenes
A-33	3	0.01	4.0×10^2	E coli, Ps aeruginosa
A-46	6	0.01	4.6×10^8	Entero cloacae
A-64	3	0.0012	5.1×10^3	Alcaligenes odorans var. viridans
A-69	2	0.08	4×10^2	Ps aeruginosa

Table 3. Blood Culture and Postmortem Liver Cultures in Patients with Negative Endotoxin in Liver

Autopsy No.	Antemortem Blood Culture		Postmortem Liver	
	No. of Cultures	Species Recovered	Quantitative Count Per Gram	Species Recovered
A-5	4	Prov stuartii Group D strep Candida	5×10^5	Strep group D
A-12	4	Ps aeruginosa *Entero cloacae* *Serratia* sp	2.3×10^1	Entero cloacae Strep group D Serratia sp
A-37	17 (6 positive)	Staph aureus Ps aeruginosa Prov stuartii	2×10^4	Proteus mirabilis E coli Prov stuartii
A-58	51 (11 positive)	Entero cloacae Klebsiella sp Strep group D Ps aeruginosa Prot mirabilis Prov stuartii	7×10^3	Ps maltophilia Klebsiella sp Entero cloacae
A-67	65 (12 positive)	Staph epidermidis Staph aureus Klebsiella sp Strep group D E coli	1.2×10^3	E coli Prov stuartii
A-75	15 (14 positive)	Bacillus sp Klebsiella sp Entero cloacae Strep group D Prot mirabilis	3.8×10^3	*Prot mirabilis* Klebsiella sp Staph aureus E coli Entero cloacae

Table 4. Species of Bacteria Found in Autopsy Liver Specimens from which Endotoxin was Recovered

Species Rcovered	No. of Cultures
Strep non hemolytic group D	12
Staph aureus	15
Staph epidermidis	5
Corynebacterium sp	2
Bacillus sp	1
Candida sp	4
Enterobacter cloacae	16
Enterobacter aerogenes	8
Serratia marcessens	7
Klebsiella pneumoniae	22
Escherichia coli	28
Proteus mirabilis	11
rettgeri	1
vulgaris	1
Providencia stuartii	21
Pseudomonas aeruginosa	1
No. of cultures gram positive only	0
No. of cultures gram negative only	28
No. of cultures gram positive and negative	29

Table 5 The Integrity of Limulus Lysate Stock

Lysate	Month	Degree of Reaction at ET Concentration in ug						
		0.01	0.005	0.0025	0.00125	0.0006	0.0003	0.00015
X-1	1-14*	4	4	4	3	2	2	1
	3-11**	4	4	4	4	3	2	1
	4-2*	4	4	3	2	2	1	1
	4-17**	4	4	4	4	3	2	2
	4-30*	4	4	4	3	2	±	±
	5-8*	4	4	4	3	2	1	1
U-2a#1	8-5**	4	4	4	3	3	2	2
	11-11*	4	3	3	2	2	1	0
	11-13*	4		3	1	1	1	0
	11-14*	4	3	2	1	1	1	
	11-16*	4	3	2	1	1	1	
	11-18**	4	3	3	3	3	2	
	12-1*	4	3	2	1	1	1	
	12-9*	4	3	2	2	1	1	
	12-16*	4	3	2	2	1	1	
V-1	8-14*	4	4	4	3	3	3	
	12-21*	4	4	4	3	2	1	
T2a#2	5-17*	4	4	3	3	3	2	1
	7-8*	4	4	3	3	3	2	1

* Stored in refrigerator at 4°C.
** Stored in Revco freezer at -70°C and thawed before use
0 = No reaction, 1 = Positive but weak, unstable clot, 2 = Slightly more stable clot than 1+
3 = Tight clot, but slightly disrupted on tipping tube, 4 = Firm clot forms in < 1 hour, does no disrupt on tipping tube

All lysates assayed by using E. coli 0111 B4 lipopolysaccharide (Difco) as endotoxin source

burned patients with sepsis has been shown by the operations described here. The significance of bacteria in the liver as potential sources of the endotoxin reaction has been scrutinized; it appears very unlikely that the bacteria present contribute to the reactions observed. Ninety per cent of the liver samples exhibited endotoxin, in concentrations ranging from 0.64 ug/gm to 0.001 ug/gm. This finding may represent a basic mechanism contributing to demise of the septic patient. Cells within the liver clearly contain the endotoxin, and it may well disrupt essential lysate function or contribute to the phenomenon of septic shock.

PUBLICATIONS AND/OR PRESENTATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA OA 6397	75 07 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
74 07 01	D. CHANGE	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A161102B71R	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

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 75	.6	17
	CURRENT 76	.6	17

19. RESPONSIBLE DOD ORGANIZATION

NAME:* US Army Institute of Surgical Research
ADDRESS:* Fort Sam Houston, Texas 78234

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

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

20. PERFORMING ORGANIZATION

NAME:* US Army Institute of Surgical Research
Microbiology Branch
ADDRESS:* Fort Sam Houston, Texas 78234

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME:* Robert B. Lindberg, PhD
TELEPHONE: 512-221-2018
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: Anthony A. Contreras, MS
NAME: DA

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Antibiotic sensitivity; (U) Pseudomonas; (U) Burns; (U) Chemotherapy; (U) Providencia

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

23. (U) Monitoring of bacterial sensitivity to antibiotics is needed to assure correctness of prompt presumptive antibiotic therapy in patients with sepsis. New antibiotics are sought to circumvent the recurrent problem of antimicrobial resistance as it occurs in burned soldiers.

24. (U) MIC by tube-dilution technic is applied to appropriate isolates, including all septicemic strains.

25. (U) 74 07 - 75 06 Ninety-six percent of all strains tested were from blood cultures. Former Methicillin resistance gave way to an unprecedented high level of sensitivity in Staph aureus. Pseudomonas aeruginosa was affected only by Gentamicin, Colymicin, and Carbenicillin. Klebsiella and Enterobacter cloacae were sensitive only to Minocin and Colymicin. Amikacin was highly effective against Enterobacter and Klebsiella and 60% of strains of Providencia were sensitive.

*Available to contractors upon originator's approval

DD FORM 1 MAR 88 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, NOV 88 AND 1498-1, MAR 88, FOR ARMY USE, ARE OBSOLETE.

ANNUAL PROGRESS REPORT

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

REPORT TITLE: ANTIBIOTIC SENSITIVITY OF CURRENT MILITARY BURN PATIENT FLORA

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

1 July 1974 - 30 June 1975

Investigators:

Robert B. Lindberg, PhD
Anthony A. Contreras, MS
Harvey O.D. Smith, Jr, SP6
Edward C. Plowey, SP5
Daniel T. Zamora, SP6
Larry B. Hensley, SP5

Report Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

REPORT TITLE: ANTIBIOTIC SENSITIVITY OF CURRENT MILITARY BURN PATIENT FLORA

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

Period covered in this report: 1 July 1974 - 30 June 1975

Investigators: Robert B. Lindberg, PhD
Anthony A. Contreras, MS
Harvey O.D. Smith, Jr,SP6
Edward C. Plowey, SP5
Daniel T. Zamora, SP6
Larry B. Hensley, SP5

Report Control Symbol MEDDH-288(R1)

The incidence of sepsis in burn patients, as indicated by number of strains of staphylococci and of gram negative bacteria recovered from the blood stream, was higher in 1974 than in any recent year. Staphylococcus aureus, primarily a monotype epidemic, was more sensitive to the methicillin group of antibiotics, and to aminoglycosides, cephalothins and tetracyclines than in previous years, although these drugs frequently failed to effect clinical cure even with in vitro sensitive strains present. Enterobacter cloacae appeared on an epidemic scale for the first time, with high tissue levels of organisms connoting high invasive potential. It was sensitive mainly to minocin and colymycin, as was the case with Klebsiella pneumoniae, although the latter species was less widespread as a cause of sepsis. Pseudomonas aeruginosa, still a major cause of sepsis, was sensitive to colymycin, gentamycin and carbenicillin, although response of Pseudomonas septicemia to these antibiotics was often inadequate. Further testing of two new antibiotics, Amikacin and Ticarcillin, showed the former to hold great potential for Enterobacter, Klebsiella and, to a lesser degree, Providencia. The latter was extremely active with Pseudomonas, and if it is pharmacologically sound, its clinical trial in Pseudomonas sepsis is indicated.

Burns
Antibiotic sensitivity
Chemotherapy
Pseudomonas
Providencia

ANTIBIOTIC SENSITIVITY OF CURRENT MILITARY BURN PATIENT FLORA

Antibiotic treatment is not administered as an all-protecting shield against burn infection, but when sepsis supervenes, systemic antibiotic is the principal therapeutic weapon available. The level of antibiotic sensitivity of major microbial species in the burn ward thus becomes of immediate and pressing concern in treatment of burn patients. The population of bacteria to which such patients are exposed is largely the endemic flora of the burn ward at any given time. Specific strains of various species populate the burn ward, and over a period of time, such strains have optimal conditions for derivation of resistant forms. Study of antibiotic sensitivity summarized at suitable intervals, offers the best available guide lines for antibiotic use, and also can indicate those antibiotics which, having become ineffective by increase in resistant strains, should be considered for suspension from extensive use, in the hope that sensitive strains may re-emerge.

TECHNIC AND SOURCES OF STRAINS

Minimum Inhibition Concentration (MIC) technic, as described in this Institute's Annual Research Progress Report for 1973, is used routinely for assessing antibiotic sensitivity (1). The test dilution ranges are 25 ug/ml down to 0.078 ug/ml. For carbenicillin, the range is 2500 ug/ml down to 19 ug/ml. Constant monitoring is applied to inoculum size, which is a critical factor in reliability and reproducibility of results. End points of inhibition are assessed on the basis of no visible growth for MIC.

The use of an MIC technic has the advantage of expressing the sensitivity as an actual concentration of antibiotic under precisely controlled conditions. Its disadvantage is the time consuming nature of the procedure, which limits the number of tests that can be done. As a result, most of the MIC procedures are done on blood stream isolates. This is consistent with a policy of restrained use of systemic antibiotics; they are not used to prevent proliferation of bacteria on the burn wound, nor in any other sense as a prophylactic measure.

Sources of strains tested are shown in Table 1. There were 649 strains tested, of which 623 were blood stream isolates. There were small numbers of strains from sputum, wound and other sources tested, but the sensitivity data are essentially that of strains recovered from blood cultures from septic patients. This is, of course, a form of selection in itself, and is heavily weighted in that it tests only those organisms that have shown some degree of invasive capability. This may well be a distinct advantage in the circumstances under which these data are used as a guideline: the clinician is not, after all, concerned with the overall microbial flora of the burn patient but with the control of sepsis.

---

1. Lindberg RB, English VC, Pruitt BA, Jr, Mason AD, Jr: Detection of endotoxin in burned soldiers with sepsis. USA Inst. Surg. Res. Ann. Res. Progress Rpt FY 1973, BAMC, Fort Sam Houston, Texas. Section 6.

Table 1. Sources and Species of Bacteria Tested for Antibiotic MIC, 1974

Species	Total No. Tested		Sources of Strains			
	Patients	Strains	Blood	Lung	Wound	Other
Staph aureus	45	222	214	2	3	3
Staph epidermidis	16	16	16	0	0	0
Strep sp (alpha & non-hemolytic)	9	17	16	1	0	0
Ps aeruginosa	35	76	70	4	1	1
Klebsiella pneumoniae	29	73	73	0	0	0
Entero.cloacae	47	119	116	2	0	1
E coli	12	30	29	0	0	1
Prot mirabilis	9	22	22	0	0	0
Prov stuartii	29	57	53	4	0	0
Serratia marcessens	10	17	14	2	0	1
Totals		649	623	15	4	7

No. of patients with at least one sensitivity test run: 104

The number of strains of each species tested reflects the relative invasiveness and the importance of this species on the overall burn ward infection problem. Staphylococcus aureus, Enterobacter cloacae, Pseudomonas aeruginosa, and Klebsiella pneumoniae were the strains of greatest import. Providencia stuartii, for the first time in 5 years, was, while still numerically important, of far less import than the 4 major species.

The number of patients who contributed strains is of great significance in making clear the nature of the microbial population sampled. Clearly, if one prolonged septicemia contributed 5% to 10% of strains sampled, then any consistent sensitivity pattern of such a strain could bias the overall susceptibility pattern of this population. There were 104 patients from whom strains tested for sensitivity were acquired, which meant an average of 6.4 strains per patient. Severely ill patients experienced frequent episodes of multiple species or even mixed blood cultures. The largest number of strains from one patient, who had acute bacterial endocarditis, was 33. Out of the 222 staphylococcus strains tested, 11 patients contributed 152 strains; the remaining 70 strains came from 35 patients. In 1973, only half as many strains, or 322, were tested as was the case in 1974,

with 649 strains. The proportion of strains from sputum cultures in 1973 was far larger than was the case with the 1974 group. This collection, being presented here, is almost exclusively from blood cultures. Only 4% of the strains came from other sources, and only 15, or 2.3%, came from sputum cultures. This is an unprecedented circumstance; in all previous compilations strains from sputum, wound and other sources made up a significant part of the total tested.

The battery of antibiotics used for sensitivity testing in 1974 was the same as that which was employed in the previous year. For gram positive organisms (chiefly staphylococci) it included nafcillin, oxacillin, methicillin, lincocin, clindamycin, minocin, garamycin (Gentamycin), and cephalothin (keflin). For gram negative organisms, garamycin, minocin, cephalothin, kanamycin, ampicillin, and collistemethate sulfate (Colymycin) were routinely used. Indol-negative Proteus sp were tested against penicillin G and Pseudomonas aeruginosa against carbenicillin. Recent developments in antibiotic dosage suggest that ampicillin and lincocin may be removed, to be replaced by more effective agents. The in vitro results with minocin and clindamycin have been very encouraging, but the side effects of these drugs have resulted in their receiving only minimal use.

RESULTS OF SENSTIVITY TESTS ON PRINCIPAL SPECIES OF BURN FLORA

Sensitivity of the major groups of microorganisms are presented in the following tables. The results are expressed in terms of cumulative sensitivity: each increment inhibits all strains that were inhibited by lesser amounts. An arbitrary level marking the upper level of sensitivity has been set at 6.5 ug/ml for gram positive organisms, and 12.5 ug/ml for gram negative bacilli (2).

Staphylococcus aureus. Sensitivity of 222 strains of Staph aureus tested in 1974 are summarized in Table 2. In view of this description in another section of this annual report (3) of a unique and dramatic reversal of the pattern of resistance to methicillin on the part of Staph aureus, the methicillin group of antibiotics were of particular interest in this stage of the study. The percentage of strains sensitive in 1974 to the least effective of these 3 antibiotics, methicillin, was 65.2%, and the other two methicillin type antibiotics, nafcillin and oxacillin, inhibited 83.3% and 82.6% of all strains tested at 6.5 ug/ml. Minocin and clindamycin were extremely effective, since they inhibited in the 96% range at 6.5 ug/ml, and the other antibiotics tested inhibited over 90% of isolates at this level. Complete cross-resistance, which had been observed with decreasing frequency in the past 3 years, did not appear in any strain. In 1972, 50 strains and in 1973, 5 strains, completely cross-resistant were recovered. This trend is of major significance in the biology of staphylococcal resistance to antibiotics, and it would be gratifying to ascribe to some

2. Finland M: Changing patterns of susceptibility of common bacterial pathogens to antimicrobial agents. Ann Int Med 76: 1009, 1972.

3. Lindberg RB, Contreras AA, Smith HOD, Jr, Plowey EC, Mason AD, Jr: Antibiotic sensitivity of current military burn patient flora. USA Inst Surg Res Ann Res Progress Rpt FY 1973, BAMC, Fort Sam Houston, Texas. Section 7.

Table 2. Cumulative Inhibitory Levels for 222 Strains of Staph aureus, ISR, 1974

Antibiotic ug/ml	Antibiotic and % of Strains Inhibited at Each Level							
	G	L	Sc	Ps	U	Kf	M	Cl
> 25	100	100	100	100	100	100	100	100
25	99.0	95.3	91.7	93.1	94.1	95.9	99.5	96.7
12.5	97.7	94.8	84.9	89.0	90.9	91.8	99.0	96.7
6.25	92.2	93.9	65.2	82.6	83.3	90.4	96.0	95.8
3.12	30.7	82.3	31.0	72.6	74.3	86.8	84.3	94.9
1.56	13.7	21.3	9.5	59.3	63.9	78.1	43.9	91.6
0.78	11.0	12.0	3.1	42.9	45.4	63.6	10.7	84.7
< 0.78	11.0	12.0	3.1	42.9	45.4	63.6	10.7	84.7
Total tested	218	215	214	219	222	220	205	216

No. of patients from whom strains were collected: 46

G: Gentamycin L: Lincocin Sc: Methicillin (Staphcillin) Ps: Oxacillin (Prostaphlin) U: Nafcillin (Unipen) Kf: Cephalothin (Keflin) M: Minocycline (Minocin) Cl: Clindamycin (Cleocin)

specific part of the treatment regimen this striking changeover from resistant to sensitive staphylococci; but no consistent alteration in therapeutic regimen could be recognized. The change may be correctly described as a reversal of sensitivity in a bacterial population continually subjected to antimicrobial agents, i.e., Sulfamylon or silver sulfadiazene, as topical agents. The use of systemic antibiotics was not altered in any significant degree during this period of changing sensitivity. The essential practice is to use them with caution and restriction to specific needs.

The comparison of the proportion of staphylococci inhibited by 6.25 ug/ml of antibiotic for the past 7 years underscores the dramatic change that took place in 1973 and 1974. These changes are summarized in Table 3. Since the change essentially started in the middle of 1973, the mean level of sensitivity was lower for that year than it was in 1974. Further, there was some fluctuation in sensitivity during the months that this staphylococcus population was changing in its sensitivity to antibiotics. Especially with methicillin, the proportion of resistant strains reached a peak in 1972; the shift to greater sensitivity was rapid in 1973 and the "sensitive" staphylococcal population is now very high.

Table 3. Antibiotic Sensitivity of Staph aureus, ISR, 1968-1974

Antibiotic	Year and % of Strains Inhibited by 6.25 ug/ml						
	1968	1969	1970	1971	1972	1973	1974
Lincocin	64.7	48.5	29.8	28.4	26.0	44.3	93.9
Methicillin	84.6	25.7	18.0	15.5	13.1	50.0	65.2
Oxacillin	80.0	33.0	22.4	20.1	18.8	69.7	82.6
Nafcillin	90.0	41.0	33.9	33.0	26.0	62.3	83.3
Gentamycin	-	52.0	32.0	50.0	35.0	67.9	92.2
Keflin	-	-	-	56.4	22.6	72.1	90.4
Minocin	-	-	-	-	-	84.1	96.0
Clindamycin	-	-	-	-	-	40.7	95.8

Staphylococcus epidermidis. There were 16 strains of Staph epidermidis recovered from 16 different patients in 1974. Five of these patients died, but none of the fatal cases had more than one recovery of Staph epidermidis and there is little reason to regard this species as a major burn pathogen. However, its ability to invade the blood stream makes its antibiotic sensitivity of significance. Table 4 summarizes the sensitivity levels observed. It was obvious that the strains were heterogeneous, since their antibiograms varied markedly, but equally obvious that a high level of sensitivity characterized this group of organisms. Nafcillin, gentamycin, and minocin were the most effective antibiotics, but all of the methicillin group, lincocin and keflin were also shown to be effective by in vitro testing. The sensitivity of strains of Staph epidermidis to this battery of antibiotics did not change markedly from that observed in 1973. The one preceding observation was in 1972; the 9 strains tested that year were markedly less sensitive than those observed since that time.

Pseudomonas aeruginosa. The continued prominence of Ps aeruginosa as an opportunistic invader in patients with severe burns is distressingly apparent despite the large volume of intensive effort devoted to control of this species. Successful control of burn wound sepsis, initially by Sulfamylon burn cream and more recently with topical silver compounds, has not reduced other infections caused by this organism. The scale on which this species appears in burn patients and especially as the cause of septicemia indicates that overall control has not been achieved. Antibiotic sensitivity is hence of critical importance, since when sepsis due to Pseudomonas does occur, the clinician is certain to need guidelines for initiating therapy before the individual strain can be tested. There were 35 patients from whom Pseudomonas strains were tested; of the 76 strains, 70 were recovered from the blood. Pseudomonas septicemia

Table 4. Staphylococcus epidermidis: Cumulative Sensitivity for 16 Strains from Blood Cultures on Burned Patients, ISR, 1974

Antibiotic ug/ml	Antibiotic and % of Strains Inhibited at Each Level							
	G	L	Sc	Ps	U	Kf	M	Cl
> 25	100	100	100	100	100	100	100	100
25	93.7	81.2	75.0	87.5	93.7	87.5	100	81.2
12.5	87.5	81.2	75.0	81.2	87.5	81.2	100	81.2
6.25	87.5	75.0	66.6	81.2	87.5	81.2	87.5	75.0
3.12	75.0	75.0	66.6	81.2	75.0	81.2	87.5	68.7
1.56	75.0	68.7	40.0	62.5	75.0	68.7	81.2	62.5
< 0.78	62.5	56.2	26.6	37.5	62.5	62.5	68.7	62.5

remained a major problem of severely burned patients.

The sensitivity of Ps aeruginosa strains in 1974 is summarized in Table 5. As was noted in the previous year, three antibiotics, keflin, ampicillin and kantrex, were virtually ineffective, and minocin was inhibitory to only 15% of the strains. Gentamycin, inhibiting 61.8% at 12.5 ug/ml, was for the first time markedly less effective than colymycin, which inhibited 70 strains at the 12.5 ug/ml level. Colymycin was proportionately more effective than gentamycin in lower concentrations. For the first time since gentamycin was used in this Institute, a significant decrease in sensitive strains occurred in 1974, while colymycin increased in the number of strains inhibited. The ratio of strains sensitive to the total tested reversed for these two antibiotics in 1974 from the relationship seen in 1973.

Carbenicillin remained, in 1974, a promising antibiotic for Pseudomonas; almost half of the strains were inhibited at 39 ug/ml. Carbenicillin is tested at a higher concentration than is the case for other antibiotics. It is the mainstay of the armamentarium for treating susceptible strains causing Pseudomonas sepsis.

Progressive, annual changes in antibiotic sensitivity of Ps aeruginosa are shown in Table 6, for 1969-1974. The behavior of Pseudomonas toward gentamycin has fluctuated, but it reached its lowest level of sensitivity since it has been observed, in 1974. 61.8 per cent of the strains tested were sensitive to this antibiotic. Conversely, colymycin has become more effective during this period. The proportion of sensitive strains reached its all time high of 93.3% of strains tested in 1974. Carbenicillin has increased in in vitro effectiveness

Table 5. Pseudomonas aeruginosa: Cumulative Inhibitory Concentrations for 76 Strains, ISR, 1974

Concentration ug/ml	Antibiotic and % of Strains Inhibited							
	G	M	K	Amp	Kf	Co	Conc ug/ml	Cb
> 25	100	100	100	100	100	100	>1250	100
25	63.1	61.8	13.3	1.5	1.3	94.6	1250	84.6
12.5	61.8	15.7	2.6	1.5	1.3	93.3	625	80.0
6.25	60.5	6.5	1.3	1.5	1.3	93.3	312	75.3
3.12	46.0	3.9	1.3	1.5	1.3	82.6	156	70.7
1.56	30.2	1.3	1.3	1.5	1.3	49.3	78	64.6
0.78	30.2	1.3	1.3	1.5	1.3	10.6	39	46.1
< 0.78	6.5	1.3	1.3	1.5	1.3	10.6	19	6.1

G: Gentamycin (Garamycin) M: Minocin K: Kantrex (Kanamycin) Amp: Ampicillin Kf: Keflin (Cephalothin) Co: Colistimethate sulfate (Colymycin) Cb: Carbenicillin (tested at higher concentrations than other antibiotics)

since 1972. Although resistant strains are recovered intermittently, the likelihood of carbenicillin being ineffective against Pseudomonas was far less in 1974 than it was in 1971. The increase in strains sensitive to this analogue of penicillin occurred at the same time that an increase in sensitivity to methicillin occurred in staphylococci. Carbenicillin was, in vitro, more effective in 1973 and 1974 than it had been at any previous time.

Klebsiella pneumoniae. Among species of the family Enterobacteriaceae, strains of Klebsiella were numerically second only to Enterobacter cloacae among strains tested for antibiotic sensitivity in 1974. The enteric organisms are ubiquitous in the immediate environment of the severely burned patient, and there is little likelihood of eradicating a species which can readily re-seed the burn from the patient's own gut or respiratory tract.

Cumulative sensitivity of 73 strains, from 29 patients, is shown in Table 7. Minocin and colymycin were the only antibiotics with a high degree of inhibitory action against this species. In 1973, gentamycin inhibited 83.3% of strains tested, kantrex 72% and keflin 60.8%. The

Table 6. Comparison of Antibiotic Sensitivity of *Pseudomonas aeruginosa*, 1969-1974

Antibiotic	Year and % Inhibited at 12.5 ug/ml					
	1969	1970	1971	1972	1973	1974
Kantrex	12.0	1.5	0	0	2	2.6
Keflin	5.4	0	5.8	0	0	1.3
Colymycin	61.0	63.4	73.3	70.0	86.2	93.3
Gentamycin	75.8	71.6	71.4	68.0	84.3	61.8
Ampicillin	-	-	-	-	0	1.5
Minocin	-	-	-	-	31.3	15.7
Carbenicillin 156 ug/ml	50.0	33.9	30.0	34.6	80.4	70.7

change to resistance to these antibiotics was abrupt; not one of them in 1974, showed a reasonably effective level of inhibition. Although they were not sought out, it is highly probable that episomal transfer factors have created these resistant strains from the previously heterogeneous and relatively sensitive population. This change has appeared after a relatively long period, since 1970, in which strains of Klebsiella pneumoniae changed little in sensitivity to antibiotics. This loss of sensitivity in a species which is relatively common in sepsis in burn patients is disquieting. The rate of extension of transmissable resistance factors appears to be increasing. New antibiotics will be sought to reverse this pattern, but the process could well repeat itself.

Enterobacter cloacae. It has been pointed out that Enterobacter cloacae has in a single year increased numerically in incidence in the burn patient from being a relatively inconspicuous and presumably benign part of the burn flora to being a major problem as a cause of sepsis. In 1973, 15 strains of Entero cloacae were tested for sensitivity. In 1974, 119 strains, all but 3 of which were recovered from the blood, were tested. It is evident that a new opportunistic pathogen presents itself here; in previous years, the numbers tested for antibiotic were so small that they did not merit tabulation.

Table 8 summarizes the sensitivity of the Entero cloacae strains collected from blood cultures in 1974. The strains were sensitive to minocin, from 3 ug/ml upward, to a very high degree. Colymycin was the other effective drug, and it was extremely effective. Minute amounts (<0.78 ug/ml) served to prevent growth in 72% of strains tested, and over 90% of isolates were inhibited by 3.1 ug/ml. The other antibiotics in the test battery: gentamycin,

Table 7. Klebsiella pneumoniae: Cumulative Sensitivity for 73 Strains, ISR, 1974

Concentration ug/ml	Antibiotic and % Inhibited					
	G	M	K	Amp	Kf	Co
> 25	100	100	100	100	100	100
25	43	91.7	8.2	5.4	19.4	95.8
12.5	15.2	90.4	8.2	1.3	13.8	95.8
6.25	12.8	83.5	4.1	1.3	12.5	95.8
3.12	12.5	58.9	1.3	1.3	4.1	95.8
1.56	4.1	4.1	0	1.3	0	79.4
0.78	2.7	0	0	1.3	0	43.8
< 0.78	2.7	0	0	1.3	0	43.8
Total tested	72	73	73	73	72	73

kantrex, ampicillin and keflin, were of little potential value against this newly prominent species of Enterobacteriaceae. Further search for antimicrobials effective against Enterobacter is urgently needed, since this ubiquitous opportunist so readily reaches the burn patient, and has now exhibited an unsuspected predilection for invasive proliferation in the burn wound.

Proteus mirabilis. There were 22 strains of Proteus mirabilis recovered from the blood stream of 9 patients during 1974. This is a minor part of the total picture of sepsis in the burn population at the Institute of Surgical Research, but in view of the fact that 8 of these 9 patients died, and that 5 of them had from 2 to 5 successive positive blood cultures, the capability of this species for causing significant infections is indicated. The sensitivity of these strains is shown in Table 9. In addition to the basic battery of 6 antibiotics, penicillin G was evaluated, since it has been described as effective against many indol-negative Proteus strains. Gentamycin, kantrex and keflin were by far the most effective antibiotics against these strains. All strains were inhibited by keflin at 12.5 ug/ml, all but one by gentamycin and over 60% of them by kantrex. The remaining antibiotics were so ineffective as to be beneath consideration. Four strains were inhibited at 12.5 ug/ml by penicillin G. When the behavior of these isolates was adjusted in terms of letting each contributor of several strains carry the average sensitivity as one strain, there was no essential change in the

Table 8. Enterobacter cloacae: Cumulative Sensitivity for 119 Strains, ISR, 1974

Concentration ug/ml	Antibiotic and % Inhibited					
	G	M	K	Amp	Kf	Co
> 25	100	100	100	100	100	100
25	48.7	96.6	6.7	6.7	2.5	95.7
12.5	13.4	94.9	4.2	4.2	1.7	94.9
6.25	13.4	94.1	4.2	3.3	0.8	93.2
3.1	11.7	70.5	1.6	2.5	0	91.5
1.5	8.4	5.0	0.8	0.8	0	87.2
0.78	4.2	3.3	0.8	0.8	0	72.0
< 0.78	4.2	3.3	0.8	0.8	0	72.0

No. of strains tested: 119

sensitivity ratios. None of these patients were examples of single species involvement; other species were also recovered.

Table 9. Proteus mirabilis: Cumulative Sensitivity for 22 Strains, ISR, 1974

Concentration ug/ml	Antibiotic and % Inhibited						
	G	M	K	Amp	Kf	Co	Pen G
> 25	100	100	100	100	100	100	100
25	95.2	54.5	81.6	18.1	100	0	18.9
12.5	95.2	4.5	63.5	18.1	100	0	18.9
6.25	72.5	4.5	27.2	18.1	81.5	0	4.7
3.12	58.9	4.5	4.5	18.1	40.7	0	0
1.56	40.8	4.5	4.5	13.6	22.6	0	0
0.78	4.5	4.5	4.5	0	9.0	0	0
< 0.78	4.5	4.5	4.5	0	9.0	0	0

No. of isolates tested: 22

Providencia stuartii. There were 29 patients from whom a total of 57 strains of Providencia stuartii were recovered. This is a somewhat lower incidence of Providencia infection than had been seen in previous years, but the organism has been a very dangerous burn wound pathogen and there is no assurance that it will diminish further. Out of the 29 patients with Providencia septicemia, 23, or 79.3%, expired. As had been described previously, the appearance of Prov stuartii, often after another species had previously been recovered, was very apt to be a terminal event.

There were no strains of Prov stuartii suppressed by 25 ug/ml of any of the 6 antibiotics tested. In 1969-1970, gentamycin, colymycin, tetracycline, kantrex and keflin were active at 12.5 ug/ml against a small part of the total population. This proportion decreased in 1970. Since 1970, no strains sensitive to the test battery have been recovered. This total cross resistance is not only serious from the standpoint of there being no effective antibiotic for this organism, but the implication of a large pool of transfer resistance factors which can be introduced into other Enterobacteriaceae is disturbing. As has been pointed out earlier, the Providencia strains behave in an epidemic manner, and are obviously capable of seeding a burn population completely. This pathogen remains a major concern in burn patients, and no good solution leading to its control has been advanced.

Escherichia coli. An ubiquitous enteric species, Escherichia coli, has a consistent but relatively low level of behavior as a systemic invading organism, and in 1974, 30 strains, all from blood cultures from 12 patients, were tested for antibiotic sensitivity. The cumulative sensitivity of these strains is shown in Table 10. The most potent antibiotics against these isolates were colymycin and minocin. Gentamycin inhibited two-thirds of the isolates, and the remaining antibiotics were effective against from one-third to one-fourth of the strains tested. Although the number of strains of E coli had not, in previous years, reached numbers to justify setting down sensitivity results in detail, the antibiotics of greatest effectiveness have been minocin (or formerly, tetracycline), colymycin and to a lesser extent, gentamycin.

Serratia marcessens. Serratia marcessens has, at times, caused serious outbreaks of sepsis on the burn ward, but in 1974 its incidence was low and sporadic, and it was not found in blood cultures after June 1974. There were 10 patients from whom 17 strains altogether were recovered from the blood. They were relatively resistant to antibiotics: all were inhibited by minocin at 6.25 ug/ml; gentamycin, ampicillin and kantrex respectively inhibited one-fourth or less of the strains at 12.5 ug/ml, and keflin and colymycin were totally inactive against these strains of S marcessens. The species continues to appear on burn wounds and on other sites, but has not recently been a matter of concern as a cause of sepsis.

EXPERIMENTAL ANTIBIOTICS TESTED, WITH REFERENCE TO PSEUDOMONAS AERUGINOSA AND PROVIDENCIA STUARTII

In view of the continued importance of Ps aeruginosa and of Prov stuartii

Table 10. Escherichia coli: Cumulative Sensitivity of 30 Strains, ISR, 1974

Concentration ug/ml	Antibiotic and % Sensitive					
	G	M	K	Amp	Kf	Co
>25	100	100	100	100	100	100
25	83.2	96.6	51.6	26.5	59.8	89.8
12.5	66.6	83.3	37.8	26.5	33.2	89.8
6.25	56.6	70.0	20.6	19.9	29.9	89.8
3.12	56.6	50.0	0	6.6	3.3	86.5
1.56	23.3	40.0	0	0	0	83.2
0.78	3.3	20.0	0	0	0	46.6
<0.78	3.3	20.0	0	0	0	46.6
No. strains tested:	30					

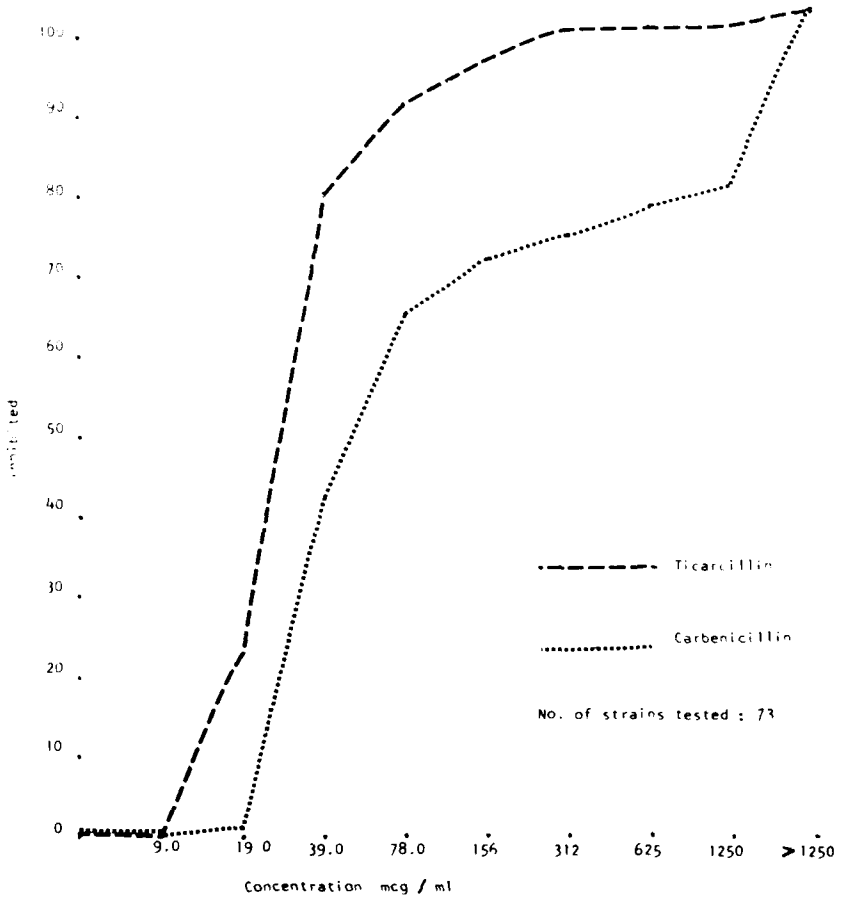
as opportunistic invaders, and of the relative to complete antibiotic resistance shown by these species, further in vitro study was made of two experimental antibiotics: BB-K8 (now designated as amikacin) and BRL-2288, or ticarcillin. In vitro tests of isolates of Ps aeruginosa were compared in ticarcillin and carbenicillin to which it is a close analogue. Tests were an extension of initial observations made in 1973.

The cumulative sensitivity of these strains is shown in Figure 1. The effective concentration range for ticarcillin, like carbenicillin, is higher than is the case with most categories of antibiotics, and concentrations up to 156 ug/ml are regarded as connoting a clinically meaningful sensitivity level.

Ticarcillin was more active than carbenicillin. Over 80% of the strains were inhibited by 39 ug/ml, and over 90% by 78 ug/ml. In contrast, 39 ug/ml of carbenicillin inhibited only 42% of the strains, and 78 ug/ml inhibited 72% of the strains. These promising results in vitro, together with laboratory evidence of the feasibility of controlling Pseudomonas burn wound sepsis in the experimental rat model, strongly indicate that ticarcillin should be considered, assuming that clinical trials have indicated its safety, in the not inconsequential number of cases of septicemia due to Ps aeruginosa which still occur in this Institute.

Amikacin, or BB-K8, produced by Bristol Laboratories, has been scrutinized with particular care because of its reported effectiveness against Prov stuartii.

Figure 1 Ticarcillin (BRL-2288) and Carbenicillin inhibitory effect with clinical isolates of Ps. aeruginosa - 1974



Tests were extended to include other species of Enterobacteriaceae capable of causing sepsis in burned patients. Twenty-six strains of Prov stuartii, primarily from blood cultures, were tested along with 54 strains of Klebsiella pneumoniae, 45 strains of Enterobacter cloacae, and 54 strains of Ps aeruginosa. The results are presented graphically in Figure 2. The most dramatic result occurred with Enterobacter cloacae, which has, in its enlarged role as a burn pathogen, been less than amenable to antibiotic therapy. At 3.12 ug/ml, over 95 of Enterobacter strains were inhibited. Among other antibiotics tested against Enterobacter, only colymycin and minocin are active in this range. Each of these two antibiotics has attributes that make it less than optimal as a therapeutic agent. The effectiveness of amikacin merits further investigation.

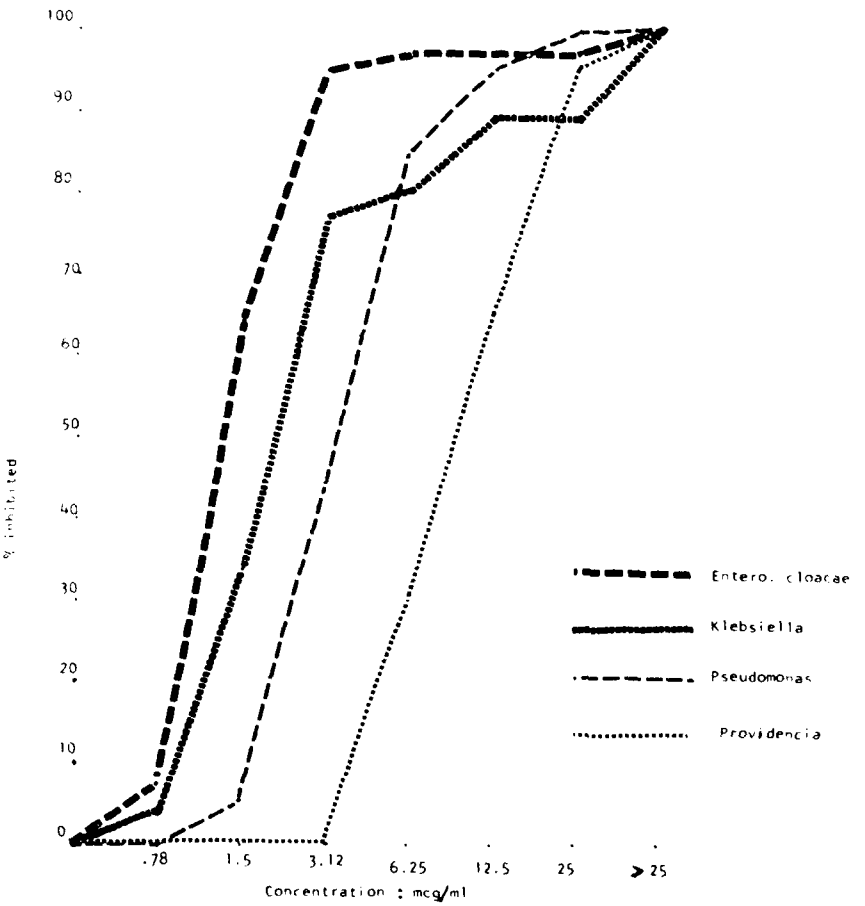
Klebsiella pneumoniae was also highly sensitive to amikacin, although not to the degree of Enterobacter. Pseudomonas strains were inhibited to the extent of 85% of those tested at 6.2 ug/ml. Prov stuartii, the initial reason for trying amikacin, was the least affected by the drug; 30.7% of the strains tested were inhibited by 6.25 ug/ml, and 65% by 12.5 ug/ml. This is almost identical to the first brief study of Providencia strains; the sensitivity is at least far more promising than the total lack of sensitivity to available antibiotics. Amikacin appears to hold real promise in therapy of sepsis occasioned by Enterobacteriaceae, and possibly by Ps aeruginosa as well.

DISCUSSION

Knowledge of the changes in sensitivity to antibiotics which occur with some species of bacteria in the Institute of Surgical Research burn patient population are of great importance in programming the most effective presumptive therapy for use in the early stages of sepsis in these patients. While sensitivity determinations are done routinely on all blood stream isolates and on request, from any other source, there is a relatively long delay between the first signs of septicemia and the acquisition of these data. In this interval the selection of antibiiotic by the clinician may be greatly aided by knowledge of the sensitivity pattern of recent isolates. Further, occasional dramatic profound changes in antibiotic sensitivity of strains of bacteria known to be endemic on the burn ward have occurred. Definitive explanation of these changes has not yet been achieved and only precise chronologic assessment of sensitivity will detect such changes so that we can at least have a a strarting point for assigning significance to this phenomenon.

The Staph aureus populations followed a new trend in sensitivity in 1974, by reaching a new high in sensitivity to the methicillin group of antibiotics. This pattern also extended to lincocin, minocin, clindamycin, gentamycin and keflin. Despite a mean sensitivity pattern which did not include even one strain with complete cross-resistance, in contrast to 50 cross-resistant strains observed in 1972, the incidence of sepsis due to staphylococci rose in 1974 over that in 1973. More people had prolonged staphylococcal septicemia than had been recorded previously. Thus, in the presence of a highly in vitro sensitive population of organisms, control of infection by antibiotic was less successful than it had been during a period of high antibiotic resistance.

Figure 2 Amikacin (BB-K8) Activity in vitro against major Gram-negative opportunists from burn patients, 1974



The major burn pathogen Ps aeruginosa, exhibited no marked change in the sensitivity of its population to antibiotics, with the possible exception of a decrease in sensitivity to minocin. The spectrum of effective safe antibiotics availabe for treatment of Pseudomonas sepsis is narrow, and the in vitro effectiveness of ticarcillin and of amikacin, assessed as part of a survey of potential therapeutic agents, strongly indicates the need for a trial of these agents in episodes of such a nature.

Appearance of resistance to antibiotic was obvious with Klebsiella pneumoniae from burn patients in 1974. Gentamycin, kantrex and keflin fell from 60 to 80% of isolates sensitive to a level of 8 to 15% sensitive. Again, the need for more effective antibiotics makes consideration of amikacin, to which the 1974 Klebsiella isolates were very sensitive, a subject for serious consideration. The same situation prevailed for Enterobacter cloacae, although there was no antecedent information on sensitivity of a comparable order, since until 1974 E cloace had not been a numerically significant cause of sepsis n burns.

Other species of Enterobacteriaceae were found in numbers too small to make their role in sepsis significant, with the exception of Providencia stuartii. This species remained resistant to all antibiotics tested with the exception of the experimental amikacin, with 65% of strains inhibited by 12.5 ug/ml. This is not an optimal inhibitory capability, but it is, currently, the best available and would appear to merit trial in cases of sepsis due to Providencia.

The control of established sepsis in burn patients by use of systemic antibiotics is more than ever one of finding a means of controlling broadly-resistant gram negative bacilli. The available antibiotics are on the bsis of in vitro inhibitory potential, far from an adequate answer. Further search for effective compounds will be implemented.

PRESENTATIONS

Lindberg RB, "Antibiotic Resistance and Nosocomial Infections" presented at American Public Health Association meeting, New Orleans, La, Oct 12, 1974.

PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1 AGENCY ACCESSION	2 DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OE 6955	75 07 01	DD-DR&E(AR)636

3 DATE PREV SUM'RY	4 KIND OF SUMMARY	5 SUMMARY SCTY	6 WORK SECURITY	7 REGRADING	8a DISP'N INSTR'N	8b SPECIFIC DATA-CONTRACTOR ACCESS	9 LEVEL OF SUM
74 07 01	D. CHANGE	U	U	NA	NL	X YES ☐ NO	A. WORK UNIT

10 NO. CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A161102B71R	01	304
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code) (U) Emergence of Methicillin-Resistant Staphylococcus Aureus Type 84 in Burned Military Personnel (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS
003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
69 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 75	.6	19
FISCAL YEAR — CURRENT 76	.5	14

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

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

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

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

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic institution)
NAME: Robert B. Lindberg, PhD
TELEPHONE: 512-221-2018
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: Ruth L. Latta, BS
NAME: DA

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) Intensive study of identity of staphylococcus strains was instituted because of increase in staphylococcus septicemia, recognition of large scale monotype epidemics in burn patients and extensive antibiotic resistance. Understanding epidemiology of outbreaks is essential to their ultimate control and reduction of infection in burned soldiers.

24. (U) Staph phage typing, MIC technic of sensitivity testing and epidemic pattern tracing are used.

25. (U) 74 07 - 75 06 A monotype epidemic of Staphylococcus aureus phage type 84 continued after 1973 but a relatively rapid conversion of a methicillin-resistant to a methicillin sensitive population occurred with other categories of antibiotic reaching new peaks of sensitivity. Although in vitro sensitivity reappeared there was no lessening of the severity or extent of staphylococcal sepsis as a problem in burned patients. Further assessment of pathogenic behavior of staphylococci is called for.

*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. 3A161102B71R-01, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: EMERGENCE OF METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS TYPE 84 AND 84,85 IN BURNED MILITARY PERSONNEL

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

1 July 1974 - 30 June 1975

Investigators:

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

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A161102B71R 01, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: EMERGENCE OF METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS TYPE 84 AND 84,85 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 1974 30 June 1975

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

Reports Control Symbol MEDDH 288(R1)

Evaluation of the very extensive Staphylococcus aureus colonization and infection problem by phage typing and antibiotic sensitivity revealed existence of a unique, 3-year long epidemic of multiply-resistant Staph. aureus, phage type 84. Resistance to methicillin was exceptionally high. Extensive cross-resistance occurred over the entire spectrum of antibiotics available for staphylococcal disease. Starting in mid-1973, a reversion to sensitivity began, which during 1974, reached a climax level with a greater sensitivity to the methicillin group, cephalothins, aminoglycosides and tetracyclines than had previously been seen. The phage type remained unaltered although a recent shift in type to 84,85 has been manifest. The extent of staphylococcal infection has not lessened with the improved status of sensitivity in this population.

Staphylococcus
Septicemia
Burns
Burn Infection

EMERGENCE OF METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS TYPE 84 AND 84,85 IN BURNED MILITARY PERSONNEL

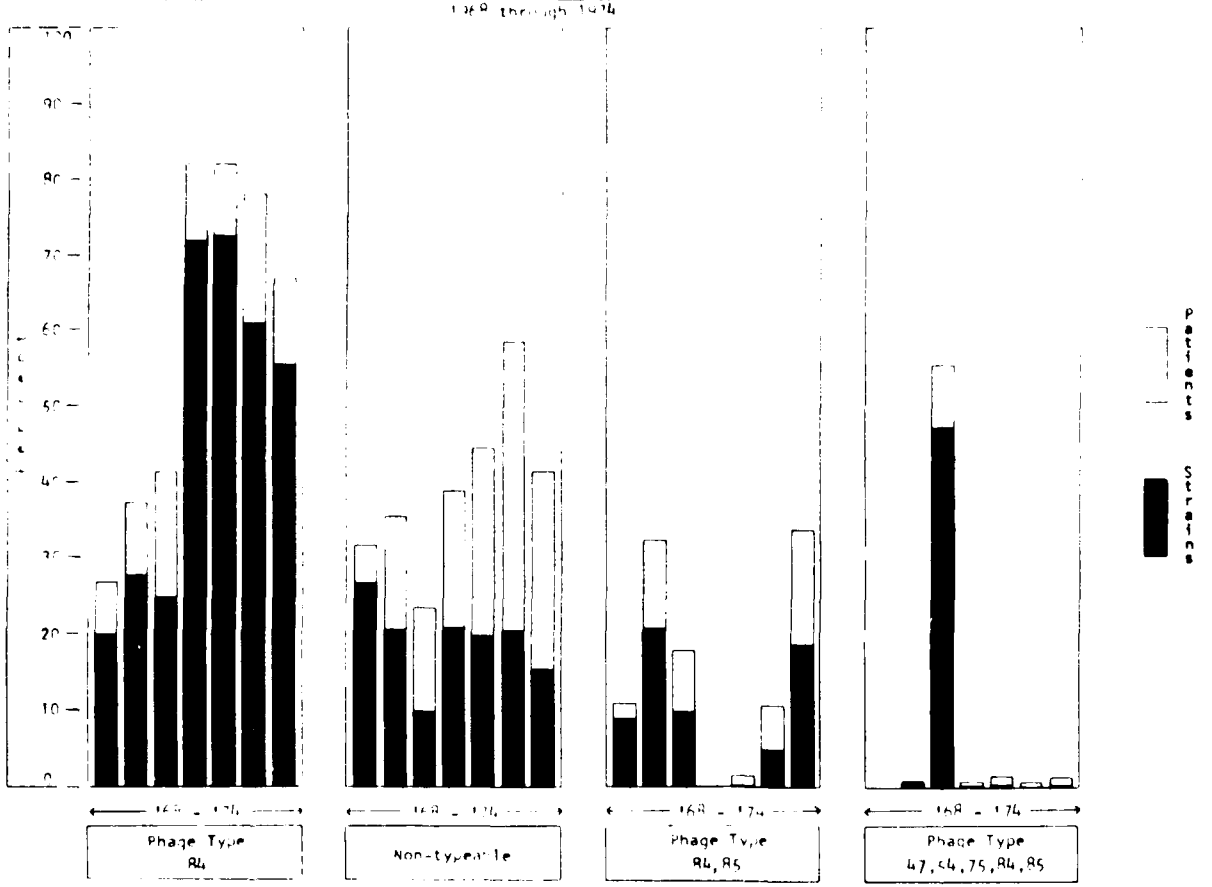
Staphylococcus aureus in burned patients has offered an anomalous picutre of a presumably controlled and controllable pathogen which, after a relatively quiescent period in 1962-1968, re-appeared as a major cause of morbidity and mortality in burned patients. In 1974, the sequence of initial staphylococcemia followed by gram negative sepsis was a conspicuous feature in the course of fatally burned patients. Staphylococcemia as a primary complication was seen more frequently, and staphylococcal colonization of burns was ubiquitous. The level of antibiotic susceptibility varied markedly during the period 1968-1974; the organisms went from a relatively susceptible population to an almost completely resistant one, then reverted, with a relatively unchanged phage type, to an antibiotic-sensitive series of strains, which is presently on hand. But such bizarre shifts in sensitivity offer no reassurance that the staphylococci will now remain sensitive, especially to the methicillin group of antibiotics.

Staph aureus was the species most frequently recovered in blood culture in 1974. This was in contrast to the previous year, when there was an absolute decrease in staphylococcemia. Most of the staphylococci recovered in blood were part of a mixed bacteremia, either preceded or followed by gram-negative bacilli in the blood stream.

Phage Types of Staph aureus Over the Period 1968-1974.

The incidence of the predominant phage types which made up this epidemic staphylococcus population are summarized over the period from 1968 through 1974 in Figure 1. The percentage of patients harboring a given strain is shown in the total proportion covered by the block outline. The solid black area represents the percentage of all strains that were of a given type for that year.

During the period from 1968 onward, type 84, type 84,85, the 47,54,75, 84,85 group and the nontypable strains were the most commonly encountered forms. No other types occurred in significant numbers. By 1970, type 84 was recovered from 40% of the patients. At this point the strain represented by type 84 preempted the patient population. During the next 3 years, it never fell in colonization rate below 78% of the patients. Next in frequency to type 84 were nontypable strains, which presumably represent a heterogeneous population. At the same time that an increase in incidence of type 84 was occurring, in 1970 a unique outbreak of a group of strains classified as 47,54,75,84,85 appeared and occasioned a striking peak incidence which abruptly disappeared at the end of that year. It has since been seen only often enough to assure that it still exists, but has never again exhibited the broad persistence that characterized its peak incidence. It is of particular interest that group I types, including the classic "hospital strain" of the 52,52A,80,81 group have not been found in numbers sufficient to have them appear in the compilation of types. Phage type 84,85 merits specific


Phage Type
84
Non-typeable
Phage Type
84,85
Phage Type
47,54,75,84,85
Patients
Strains

comment since it is closely related to type 84, but it has failed to reach numerical incidence comparable to type 84. It virtually disappeared in 1970 and 1971, and since then has been recovered with increasing frequency. Its proportionately high incidence in patients in contrast to its proportion of all strains recovered reflects the fact that it tends to appear once or twice in a patient's cultures, then disappear.

Staph Phage Types, ISR, 1974

There were 1152 strains of Staph aureus from 166 patients on the burn ward, typed in 1974. They were from all sources: wound, blood, sputum, urine catheters, etc. The overall frequency of the predominant types is shown in Table 1. The incidence of patients positive gives a truer picture of incidence in one respect, that it discounts the distortion of incidence which occurred when one or two patients contributed a disproportionate number of strains. The frequency of major groups was as it had been in recent years; type 84 from 67% of patients, nontypables from 41.6% of patients, and 84.85 from 34.3% of patients. Type WH-1, now re-designated as type 94, appeared in virtually identical proportion to its occurrence in 1973: 10.2% of the patients were positive at least once, although only 2.3% of the strains were WH-1. Type 75 is probably a member of the 47,54,75,84, 85 group. The categories shown accounted for 94.6% of all strains. The staphylococcus population, in terms of predominant forms, changed but little in 1974 over the picture seen in 1972 and 1973.

Table 1. Predominant Staphylococcus aureus Phage Types in ISR Burn Patients, 1974

Phage Type	Patients	Strains
	Per cent	
84	66.9	55.6
Non-typeable	41.6	15.6
84,85	34.3	19.2
WH-1	10.2	2.3
75	3.0	0.5
29	2.4	1.0
53		0.4

Staph aureus from Blood Stream Infections, 1974

The septicemic strains may readily be pictured as possessing invasive and pathogenic attributes not shared by all staphylococci. If this were true, phage type differentiation might show such a distinction, and the recognition of more virulent human strains could improve therapeutic approaches to their control. There were 204 strains from blood cultures on 42 patients typed during 1974. Table 2 summarizes the type distribution observed. The type distribution was in fairly close agreement with the overall type distribution, both in terms of patients positive for a given type and for the proportion of strains of each type. A higher proportion of nontypable strains were associated with a fatal outcome than would have been anticipated, and a proportionate lessening of lethal outcome in type 84,85 bacteremia. This reversal would not be sufficient to justify the assumption that a major difference in type behavior could be discerned. The percentage of patients with type 84 in the blood was 66.6%; 66.9% of patients had type 84 from all sources. Type 84,85 was recovered from the blood of 31% of patients with staphylococcemia, and in 34.3% of patients with staphylococci from all sources. Nontypable strains were found in 16.2% of patients with positive blood cultures and in 41.6% of patients with staphylococci from all sources.

Table 2. Phage Types of Staphylococcus aureus from Blood Stream of ISR Burn Ward Patients, 1974

Patient	No. of Patients-Strains	Phage Type			
		84	84,85	NT	Other Types
		No. of Patients-Strains Each Type			
Survived	12-44	8-25	3-11	1-7	1-1
Expired	30-160	20-94	10-44	10-19	3-3
Total	42-204	28-119	13-55	11-26	4-4

Staph aureus Types From Lung Tissue at Autopsy of Burn Patients

The predominant species of bacteria in the lung at autopsy can be of major significance in establishing the etiology of the pneumonia which, as a complication in severe burns, can contribute in a significant degree to fatal outcome. The phage type found among staphylococci recovered from lung at autopsy were hence cultured.

Twenty-seven patients yielded a total of 67 strains of Staph aureus from autopsy culture of the lung. The phage type distribution is summarized in Table 3. There were 20 patients with type 84 in the lung, 7 with 84,85, and 4 with nontypable strains. With due regard for the small sample size, these numbers are comparable to the type distribution of the whole staphylococcus collection. Nontypable strains were found in only 15% of the patients; they were 41.6% of all sources. Such a discrepancy is consistent with the interpretation that the most heterogeneous and mixed collection of staphylococci would indeed come from undifferentiated sources and would include the highest proportion of nontypable strains with the lowest proportion of tissue-invading strains.

Table 3. Phage Types of Staphylococcus aureus from Lung Tissues, 1974

Phage Type					
84	84,85	NT	WH-1	53	71
No. of Patients - Strains					
20-40	7-16	4-8	1-1	1-1	1-1

Antibiotic Sensitivity of Staphylococci, 1970-1974

The development of a population of staphylococci highly resistant to antibiotics of all major categories has been observed in the Institute of Surgical Research, with the peak of antibiotic resistance occurring in 1972. In the following year, 1973, there was a reversal of sensitivity, even though the predominant phage type did not change. At that time, a change in sensitivity to Oxaciilin and Nafcillin occurred, with a later change in the sensitivity of staphylococci to methicillin.

The sensitivity of the staphylococci since 1967 is summarized, on an annual basis in Table 4. Sensitivity is regarded as having an upper limit of 6.25 ug/ml; inhibition by this or a lower level qualifies the organism as sensitive.

It is obvious that the change in sensitivity that began in this epidemic population in 1973 progressed to the category of an extremely sensitive population of staphylococci by the beginning of 1974. The least active antibiotic, methicillin, was effective against 55.2% of the population; this figure had not changed since 1973. Every other antibiotic had a higher level of sensitive strains in 1974 than was seen in 1973. Whether the methicillin sensitivity will remain at this level cannot, at present, be foretold; the

Table 4. Antibiotic Sensitivity of Staph aureus
% of Strains Inhibited by 6.25 ug/ml or less

Year	Antibiotic and Per Cent Inhibited							
	G	L	Ps	Sc	U	Kf	M	Cl
1967	-	89.4	94.0	61.1	94.4	-	-	-
1968	-	64.7	80.9	84.6	90.0	-	-	-
1969	52.0	48.5	33.0	25.7	41.0	-	-	-
1970	32.0	29.8	22.4	18.0	33.9	-	-	-
1971	56.0	28.4	20.1	15.5	33.0	56.4	-	-
1972	35.6	26.0	18.8	13.1	26.0	22.6	51.5	-
1973	67.9	44.3	69.7	50.0	62.3	72.1	84.1	40.7
1974	92.2	93.9	65.2	82.6	83.3	90.4	96.0	95.8

G: Gentamycin; L: Lincocin; Ps: Oxacillin; Sc: Methicillin;
U: Nafcillin; Kf: Keflin; M: Minocin; Cl: Clindomycin

slight decrease between 1973 and 1974 could be a warning of further loss of activity, or could be a minor fluctuation.

The sensitivity of Staph aureus to the battery of test antibiotics is shown in graphic form to make more obvious the really extreme sensitivity that has been observed in this population (Figure 2) The proportion of all strains that are inhibited below the cut-off of 6.25 ug/ml is very high; methicillin is the only antibiotic that does not inhibit over 80% of strains at that level. The broad extension of increased sensitivity is shown in figure 3; here sensitivities to Lincocin, Keflin, Clindomycin and Minocin are summarized. Lincocin and Minocin were highly effective starting at 3.12 ug/ml; Keflin and Clindomycin were extremely active at the minimum test concentration of 0.78 ug/ml. This change of sensitivity in a population of staphylococci which remained of the identical phage type has no precedent in current literature. It is not a phenomenon that has been previously observed.

Chronologic Sequence of Staph aureus Phage Types, 1974

In view of the unique quality of the staphylococcus senstivity pattern, the sequence of events in the staphylococcus population with reference to succession of types was analyzed on a monthly basis. The sequence is illustrated graphically in figure 4.

Figure 2. CUMULATIVE SENSITIVITY OF STAPH. AUREUS TO ANTIBIOTIC METHICILLIN, OXACILLIN, NAFCILLIN AND GENTAMICIN - 1974

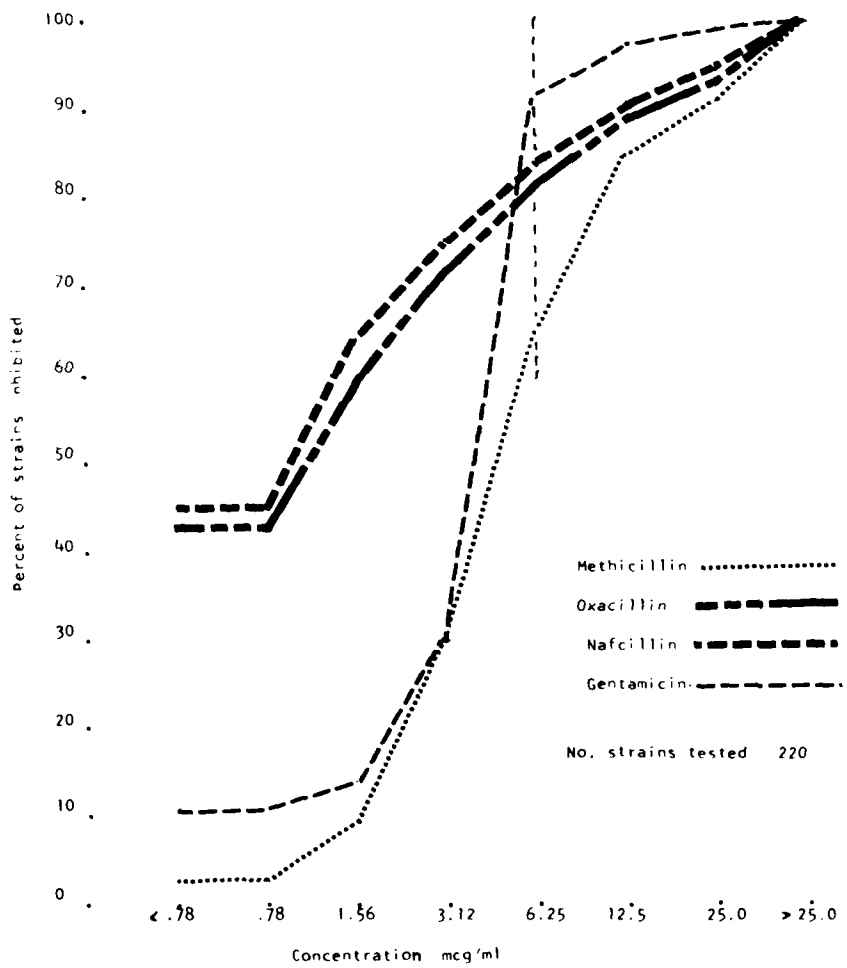


Figure 3. CUMULATIVE SENSITIVITY OF STAPH AUREUS TO ANTIBIOTIC LINCOCIN, KEFLIN MINOCIN AND CLINDAMYCIN - 1974

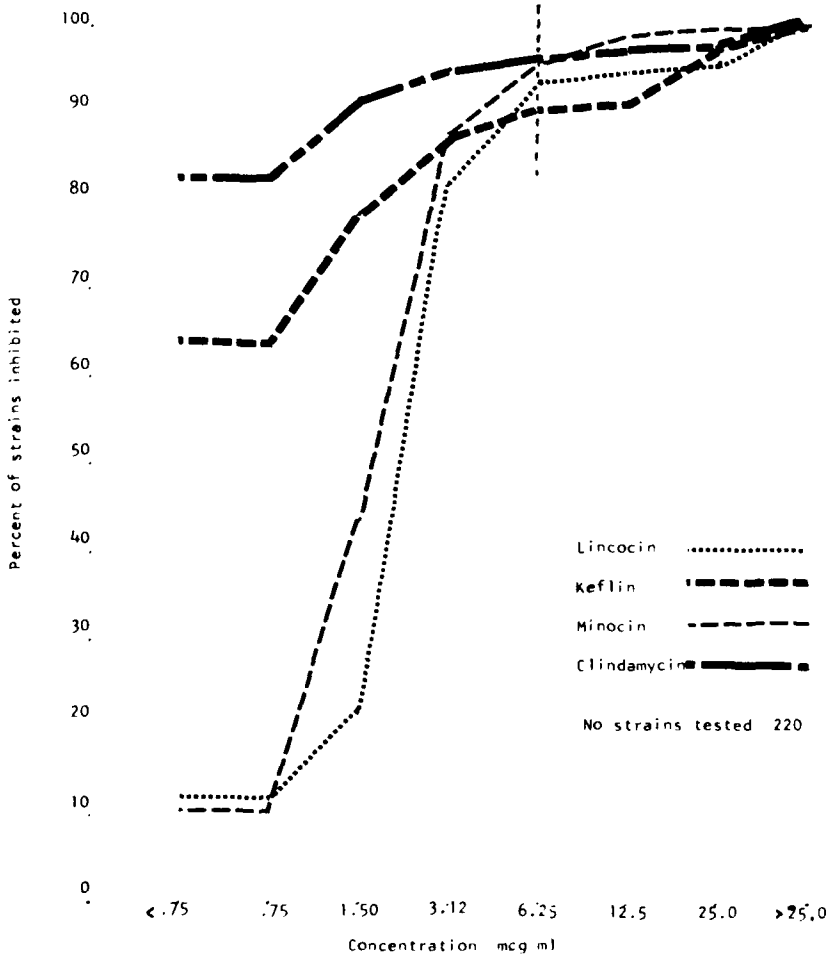


Figure 4. Monthly Distribution of *Staphylococcus aureus* Phage Types in 158 Burn Ward Patients, 1974

Phage Type	Month												Total Patients-Strains Each Type
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	
84	12-86	13-53	9-30	12-37	28-92	20-78	26-87	21-90	17-21	7-35	1-2	3-4	111-641
NT	4-12	4-8	6-11	7-12	9-26	6-15	13-23	16-32	4-13	4-18	4-6	2-4	69-190
84,85					5-14		9-13	14-27	2-3	15-34	18-70	13-60	57-221
WH-1		2-2	1-1	5-5	2-2	1-1	5-11	1-1	1-1	1-1	1-1		17-26
75		1-1		1-1	2-2		1-1	1-1					5-6
29		1-5	1-1			1-2		1-3	1-1				4-12
53					2-2	1-1		1-1					4-4
Total Patients-Strains Ea. Month	13-98	13-75	14-47	19-67	36-145	24-97	33-139	28-164	23-69	22-92	20-39	18-72	-1,090

Primary Phage Type Secondary Phage Type

Proceeding from left to right is the phage type; in blocks - the number of patients and strains during each month with a particular type, and last the total patients and strains for the year for each type. Listed at the bottom are the total patients and strains represented in each month's sampling. The predominant phage type for each month is illustrated by a solid black frame, the secondary type by a broken line frame, and other types by a single frame.

It is readily apparent that for the first nine months of the year, phage type 84 was predominant and nontypable strains were secondary. But during the last three months, there is a shift to phage type 84,85 strains with the type 84 and NT strains occupying second place. In fact, the substantial increase in the number of patients with type 84,85 strongly suggests that type 84 may be in the process of being superseded as the predominant type.

DISCUSSION

The sequence of development of extreme antibiotic cross-resistance in a monotype population of Staph aureus, and an abrupt shift to a highly sensitive population, was not only unexpected but, in terms of available literature, unprecedented. Cross-resistance of a high order has been reported as occurring only in a small proportion of isolates. The reversion to a level of sensitivity greater than had been seen since these antibiotics were first used is a development gratifying to the clinician but not explainable on the basis of available knowledge. Although the antibiotic sensitivity level is far higher than it was a year ago, staphylococcal sepsis still occurs, and the response to antibiotics which are highly active in vitro has not been extremely effective.

PUBLICATIONS

None

PRESENTATIONS

Lindberg RB: Microbiology of Hospital Infections. Presented at Conference on Cross-Infection Control in Health Care Facilities at the Univ. of Houston, Houston, Texas on November 14, 1974.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0C 6970	75 07 01	DD DR&E(AR)636

3. DATE PREV SUMMARY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
74 07 01	D. CHANGE	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

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

11. TITLE (Precede with Security Classification Code) (U) Sensitivity of Pseudomonas Aeruginosa Recovered from Burned Soldiers to Sulfamylon (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS
003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
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 75	.5	12
FISCAL YEAR CURRENT 76	.5	14

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

20. PERFORMING ORGANIZATION
NAME: US Army Institute of Surgical Research
Microbiology Branch
ADDRESS: Fort Sam Houston, Texas 78234
PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME: Robert B Lindberg, PhD
TELEPHONE: 512-221-2018
SOCIAL SECURITY ACCOUNT NUMBER:
ASSOCIATE INVESTIGATORS
NAME: Virginia C English, MA
NAME: DA

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

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

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

23. (U) Burns of serious extent continue to be extremely prone to colonization by Pseudomonas aeruginosa, and Pseudomonas sepsis and systemic involvement is still a major threat to survival in severely burned soldiers. Sensitivity to a major topical therapeutic agent is a vital part of controlling this lethal burn pathogen.

24. (U) Sensitivity to Sulfamylon is conducted by an agar dilution MIC technic. Correlation with phage type and epidemic patterns is carried out.

25. (U) 74 07 - 75 06 Sulfamylon sensitivity has varied around a median range, rather than progressing to a more resistant range. What has occurred is the appearance of relatively resistant epidemics; these clusters of resistant strains distinguished by phage type, can embody treatment resistant strains which are distinct from the whole, population of pseudomonads. Delineating these epidemic episodes may offer a beginning toward more effective control of Pseudomonas.

*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

ANNUAL PROGRESS REPORT

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

REPORT TITLE: SENSITIVITY OF *PSEUDOMONAS AERUGINOSA* RECOVERED FROM BURNED SOLDIERS TO SULFAMYLON (R)

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

1 July 1974 - 30 June 1975

Investigators:

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

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

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

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

Period covered in this report: 1 July 1974 - 30 June 1975

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

Reports Control Symbol MEDDH-288(R1)

Four hundred thirty-seven strains of Pseudomonas aeruginosa from burn patients were tested for sensitivity to Sulfamylon(R) (mafenide acetate) by a Minimum Inhibitory Concentration (MIC) technic. The strains were markedly more sensitive than a similar collection in 1973, and that, in turn, had extended from a period of relatively high resistance which occurred in 1972. The median sensitivity, 0.111% in 1972, fell to 0.086% in 1974. Resistant strains were concentrated in a new group of phage types, N7-3 and NT-16; there were 59 strains requiring 0.625% for inhibition. Strains reactive with dilute phages were all sensitive to mafenide acetate; strains lysing only with concentrated phage included the resistant types. Moderately resistant strains have not constituted a therapeutic or prophylactic problem, but the increase in Pseudomonas incidence accompanying use of topical silver-sulfadiazene increases the likelihood of this event occurring. Monitoring of types and sensitivities is the only available means for detecting such episodes.

Pseudomonas
Burns
Sulfamylon(R)
Topical therapy
Humans

SENSITIVITY OF PSEUDOMONAS AERUGINOSA RECOVERED FROM BURNED SOLDIERS TO SULFAMYLON(R)

The monitoring of sensitivity of Pseudomonas aeruginosa strains to Sulfamylon(R) (mafenide acetate) has been continued during 1974, with the added interest that was imparted by the introduction of silver-sulfadiazene as a topical agent in burn management. Mafenide acetate was still used on selected patients as the 10% burn cream and 5% mafenide acetate soaks were also used extensively, to expose the burn wound flora to an environment containing considerable mafenide acetate. The use of silver sulfadiazene might also be expected to alter the rate of emergence of strains resistant to mafenide acetate or even to enhance the reappaerance of a more susceptible population of pseudomonads, but knowledge of the influence of such environments on the persistence of pseudomonads of heightened drug resistance is virtually non-existent. Hence this scrutiny of the major part of the Pseudomonas population is very much indicated, in order to obtain precise information on the role of in vitro drug resistance on persistence, virulence and pathogenicity of invading strains of Ps. aeruginosa. Pseudomonas sepsis has, in 1974, remained one of the major causes of death in fatally burned patients, and the control of this burn pathogen remains an urgent facet of burn therapy.

The technics of assessing mafenide acetate sensitivity have been described in previous reports (1). The procedure using dilutions of mafenide acetate in agar, with seeding of 36 strains per plate; inhibitory end point is the absence of visible growth at 18-20 hours.

Sensitivity of Pseudomonas aeruginosa to mafenide acetate

There were 437 strains of Ps aeruginosa tested in 1974, a marked increase from the 285 strains assessed in 1973. The increase directly reflects the rise in incidence of pseudomonads on patients in 1974. The results of sensitivity tests for these strains are set down in Table 1, with sensitivity of strains annually since 1970 shown for comparison. There were more strains inhibited by 0.156% or less than had been the case in the 1973 collection. It was necessary to return to 1970 before finding a collection more sensitive than that recorded in 1974. The change in sensitivity of Ps aeruginosa, on an annual basis, is manifest in an uneven clustering of resistant strains. Such resistance reached a high point in 1972, when 38% of all strains were inhibited by 0.156% or less. 45% of the strains were clustered at 0.625%. Such a disporportion has not again been seen.

Cumulative sensitivity constitutes a more coherent picture of the inhibitory action of Ps aeruginosa. Table 2 presents this information for the

1. Lindberg RB, Calvert J, Brame RE, Dent R: Sensitivity of burn wound flora to Sulfamylon. USA Surg Research Unit, Annual Rpt FY 1965, BAMC, Fort Sam Houston, Texas, Section 15.

Table 1. Inhibiting Concentrations of Sulfamylon(R) for Pseudomonas Aeruginosa
1970 - 1974

Year	Concentration of Sulfamylon in % and Greater Inhibited								
	2.5	1.25	0.625	0.312	0.156	0.078	0.039	0.019	< 0.019
1970	0	0	0	65	83	83	53	6	0
% of total (296)	0	0	0	21.9	28.0	28.0	17.9	2.03	0
1971	0	0	48	41	56	57	45	13	0
% of total (280)	0	0	17.1	14.6	20.0	20.4	23.2	4.7	0
1972	0	29	212	46	88	31	37	15	5
% of total (463)	0	6.3	45.8	9.9	13.1	6.7	7.9	3.2	1.1
1973	0	4	14	85	35	62	32	12	1
% of total (295)	0	1.4	4.8	22.2	22.2	13.3	11.2	4.2	0.4
1974	0	5	59	78	97	97	96	11	4
% of total (437)	0	1.1	13.5	17.8	22.2	22.2	13.7	2.5	0.9
Total (1761)	0	38	333	315	409	320	279	57	10
% of total	0	2.2	18.9	17.8	23.2	18.1	15.8	3.2	0.6

Table 2. Cumulative Sensitivity to Sulfamylon(R) of Pseudomonas Aeruginosa 1967- 1974

Year	No.of Strains	Concentration* and % of 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	95.1	60.4	45.8	14.1	1.7
1969	385	100	100	96.5	50.0	26.9	7.7	0.5
1970	296	100	100	100	78.0	49.9	21.9	2.0
1971	280	100	100	82.9	68.3	48.3	27.9	4.7
1972	463	100	93.7	48.0	38.0	19.0	12.3	4.3
1973	285	100	98.1	81.3	57.0	33.5	16.1	3.2
1974	437	100	99.0	85.5	67.5	45.3	23.1	3.4

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

period from 1967 through 1974. A more comprehensive picture of the mean level of sensitivity and of the range of variation that may occur is achieved by the longer observation period. At 0.625% sensitivity was complete or virtually so, except in 1972 when the least sensitive series ever observed was collected. The proportion of strains inhibited by 0.312% of mafenide acetate represents an upper limit of sensitivity; until 1971, almost all strains were inhibited at that level. In 1971, this proportion fell to 82.9%, and in 1972,when mafenide acetate-resistant strains were at a maximum, only 48% of strains were inhibited by 0.312%. Sensitivity has increased since that time, and in 1974, 85.5% of isolates were inhibited by 0.312%. The proportion inhibited at still lower concentrations has fluctuated more widely; at 0.156%, the per cent inhibited dropped to a low 38% in 1972, then rose again to 67.5% in 1974.

These variations are visualized in a graphic summary shown in Figure 1. During 1970 and 1971 sensitivity levels were virtually identical through the major part of the sensitivity range, i.e.,between 0.039% and 0.312%. As was indicated in the tabulation of sensitivity, the 1972 curve shifted farther to the right, to the resistant range, than had any other annual collection of strains. In 1973, sensitive strains reappeared in numbers which moved the sensitivity curve back toward its typical range, and in 1974, the sensitivity curve coincided with the 1970 and 1971 pattern. It is not feasible to incorporate more years in this type of graph, but the 1969 curve fell very close to the 1973 curve; fluctuation of sensitivity from year to year seems to be the most rational explanation for this sequence of events, rather than a steady increase in the number of resistant strains.

A median level of sensitivity, or the value at which one-half are greater than, and the other less than the calculated value, has been determined for each annual group. These values are shown in Table 3. As was shown in the cumulative sensitivity data, the 1974 strains continued a shift to greater sensitivity to mafenide acetate after a period , two years ago, of markedly reduced sensitivity. The drop in median sensitivity continued a trend recognized in 1973.

Variation in sensitivity occurs in Ps aeruginosa in specific strains. Correlation between virulence and specific type has been sought unsuccessfully but the search has been continued, since establishing such correlation could be of great value in more rational control of sepsis. It would permit strenuous therapeutic effort in case of specific type colonization, but without correlation of virulence and type, diagnostic demonstration of Ps aeruginosa still does not warn of serious invasive potential associated with type identity. In type identification, a large number of strains nontypable by standard phage typing dilution technic have been effectively categorized using undiluted phage. A group of these "NT" types, i.e., strains typable only with undiluted phage in this system, have been tested for sensitivity. The sensitivity results are shown in Table 4. The identity patterns are designated by the prefix NT, with the number connoting a specific pattern.

Type NT-3 had a uniquely large number of resistant strains that required 0.625% mafenide acetate inhibition. This was the largest group of patients with

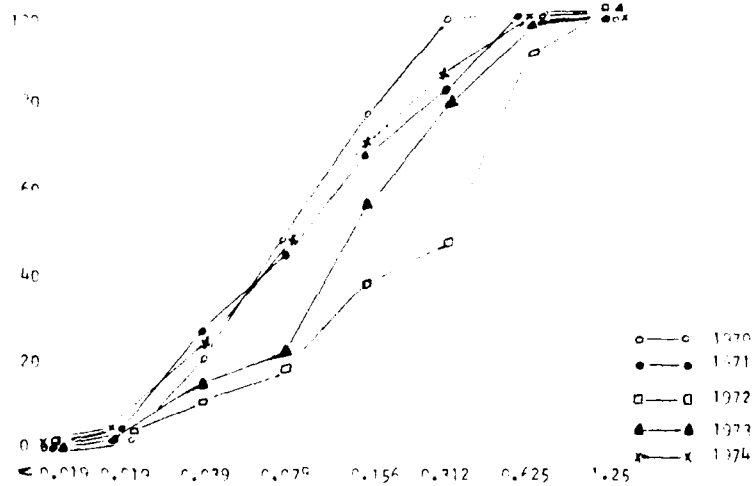
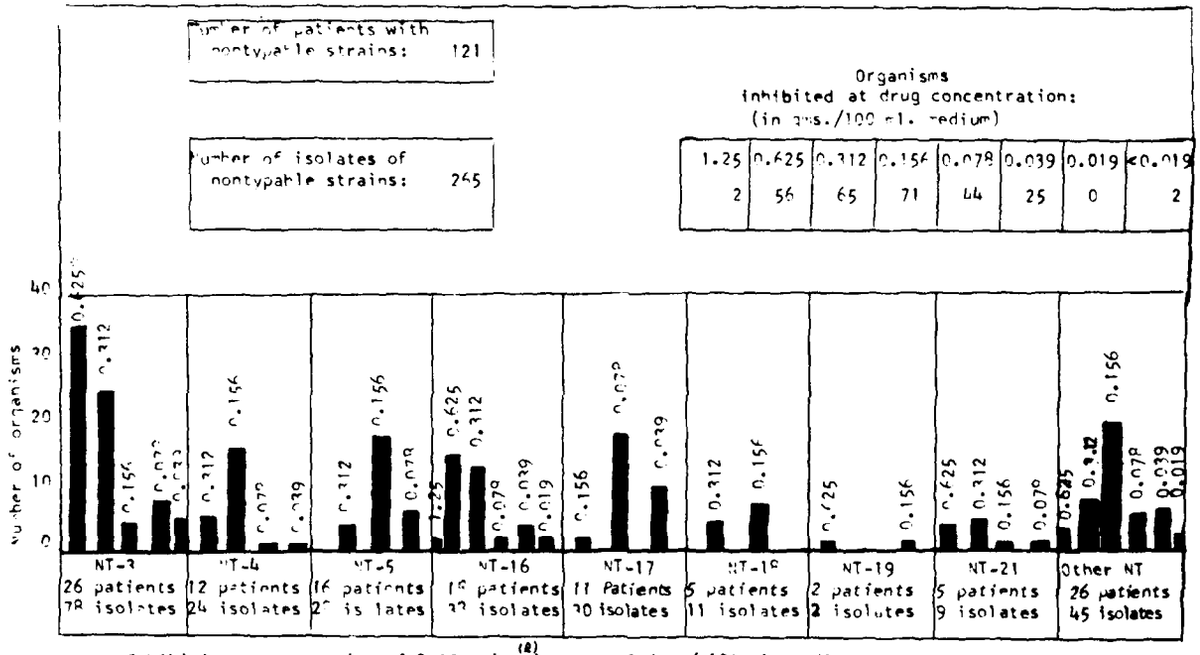


Figure 1. Sensitivity of *Pseudomonas Aeruginosa* to Sulfamylon(R)
1970 - 1974

Table 3. Median Value of Pseudomonas Aeruginosa Sensitivity to Sulfamylon(R)

Year	No. of Strain	Median Inhibitory Value (gms./100 ml.medium
1970	296	0.079
1971	280	0.125
1972	463	0.316
1973	295	0.111
1974	437	0.096
Total of 5 years	1761	0.143

Table 4. Sensitivity of Nontypable Groups of Pseudomonas Aeruginosa



* Inhibiting concentration of Sulfamylon[12] in gms. of drug/ 100 ml. medium

a specific strain, and the largest number of strains. Another strain, NT-16, also had a similar proportion of strains requiring 0.625% or 0.312% for inhibition.

In contrast, NT-4, NT-5 and NT-18 had the major part of the total of 33 patients' strains inhibited by 0.156%. NT-17 was even more sensitive; most of these strains were inhibited by 0.078%.

The overall pattern of sensitivity in these NT strains was that of a population markedly different from the total Pseudomonas population in sensitivity. Cumulative sensitivity percentages show this contrast.

	Concentration and % of Strains Inhibited					
	0.625	0.312	0.156	0.078	0.039	0.019
NT strains	99.2	78.1	53.5	26.7	10.1	0.7
All strains (1974)	99.0	85.5	67.5	45.3	23.1	3.4

Since the NT strains were 65% of all those tested, the difference in sensitivity in the 0.039% to 0.312% range is significant. The discrepancy would be far greater if all routinely typable strains were compared.

The sensitivity of typable strains (i.e., reactive at routine test dilutions) was markedly higher than the NT strains. The behavior of 5 typable groups is shown in Table 5. The median sensitivity was between 0.039% and 0.078%. It is quite evident that the in vitro resistance was markedly greater in the NT strains than with the typable strains.

The numerically predominant strains in 1974 were NT-strains, and specific types of these were numerous and also relatively resistant to mafenide acetate. The proportion of typable strains sensitive to mafenide acetate was markedly higher than that of the nontypable strains.

The total population of Ps aeruginosa was more sensitive to mafenide acetate than the NT-group of strains. This difference is explained by the sensitivity of those strains typable at high dilution with phage; these more sensitive strains in the total sensitivity value lowered the median inhibitory level.

The nontypable strains observed in 1973 were more sensitive to mafenide acetate than the 1974 strains. A strain-linked resistance to mafenide acetate was demonstrable in the 1974 isolates; such a pattern had not previously been discerned.

PUBLICATIONS AND/OR PRESENTATIONS

None

Table 5. Sulfamylon(R) Sensitivity Reaction of Various Phage Types - 1974

	PATIENT NO.	ISOLATES WITH INHIBITING CONCENTRATIONS* AT						
		0.125	0.625	0.312	0.156	0.078	0.039	0.019<0.019
Type C-25	7				1			
4 patients	25				1			
9 isolates	214			1	1	2		
	218			3				
Total each inhibited strain				4	3	2		
Type D-41	47						1	
	51						1	
5 patients	93						1	
	191		1					
5 isolates	221			1				
Total each inhibited strain			1	1			3	
Type D-9*	137					1	3	
	157					1		
7 patients	177					1		
	197					3	1	
	192					6		
21 isolates	194				1	1		
	205						1	
	223						2	
Total each inhibited strain					1	13	7	
Type E-11	233						1	1
3 patients	223							1
	237							2
5 isolates								
Total each inhibited strain							1	4
Type M2a	10			3	6			
3 patients	31			1				
	32						1	
11 isolates								
Total each inhibited strain				4	6		1	

*Concentration in gms. Sulfamylon/100 ml. media

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OB 6950	75 07 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
74 07 01	D. CHANGE	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO. CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A161102B71R	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 or Silver Sulfadiazene Treatment (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
65 07	Cont	DA	C. In-House

17. CONTRACT/GRANT: Not Applicable

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 75	.7	19
	CURRENT 76	.5	13

19. RESPONSIBLE DOD ORGANIZATION

NAME: US Army Institute of Surgical Research

ADDRESS: Fort Sam Houston, Texas 78234

RESPONSIBLE INDIVIDUAL

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

TELEPHONE: 512-221-2720

20. PERFORMING ORGANIZATION

NAME: US Army Institute of Surgical Research
Microbiology Branch

ADDRESS: Fort Sam Houston, Texas 78234

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

NAME: Robert B. Lindberg, PhD

TELEPHONE: 512-221-2018

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Anthony A. Contreras, MS

NAME: Daniel Zamora, SP6 DA

21. GENERAL USE

FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Burns; (U) Staph aureus; (U) Enterobacter cloacae; (U) Sepsis; (U) Humans

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

23. (U) The extreme susceptibility of patients with extensive thermal injury to infection requires a topical therapy barrier to permit healing. The use of such agents is itself a modifier of burn ward flora which is continuously assessed to maintain a valid picture of the current nature of the infection problem in burned military personnel.

24. (U) Surface cultures, blood, sputum, urine, biopsies and autopsies, qualitative and quantitative are characterized.

25. (U) 74 07 - 75 06 Increase in Staph aureus, Pseudomonas aeruginosa, and Enterobacter cloacae occurred; total of E. coli and Providencia stuartii decreased in incidence from 1974. Enterobacter cloacae became a new major epidemic type, never previously having shown such propensity in burn patients.

*Available to contractors upon originator's approval

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

ANNUAL PROGRESS REPORT

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

REPORT TITLE: PATHOGENESIS OF BURN WOUND INFECTION: BACTERIAL FLORA OF WOUNDS OF MILITARY PERSONNEL RECEIVING TREATMENT WITH SULFAMYLON OR SILVER-SULFADIAZENE

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

1 July 1974 - 30 June 1975

Investigators:

Robert B. Lindberg, PhD
Anthony A. Contreras, MS
Ruth L. Latta, BS
Harvey O.D. Smith, Jr, SP6
Daniel T. Zamora, SP6

Report Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

REPORT TITLE: PATHOGENESIS OF BURN WOUND INFECTION: BACTERIAL FLORA OF WOUNDS OF MILITARY PERSONNEL RECEIVING TREATMENT WITH SULFAMYLON OR SILVER-SULFADIAZENE

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

Period covered in this report: 1 July 1974 - 30 June 1975

Investigators: Robert B. Lindberg, PhD
Anthony A. Contreras, MS
Ruth L. Latta, BS
Harvey O.D.Smith, Jr, SP6
Daniel T. Zamora, SP6

Report Control Symbol MEDDH-288(R1)

Sepsis was the major cause of morbidity and death in burn patients in 1974, as it has been for at least the last 5 years. The incidence of principal pathogenic species resembled that of an epidemic, with high rates of explosive incidence interspersed with periods of reduced incidence. Species which had earlier been recognized as major contributors to wound infection and sepsis included Staphylococcus aureus, Pseudomonas aeruginosa and Providencia stuartii. However, species which had been relatively inocuous until last year continued to infect patients to an unprecedented extent: these included Klebsiella pneumoniae and Escherichia coli. A new pathogenic species was Enterobacter cloacae; it had not previously played a large role in burn infections, but it appeared abruptly in early 1974 and was prominent among the burn wound flora and in blood stream infections. Cross-resistance to most antibiotics was conspicuous among coliforms and pseudomonads; this attribute has increased in 1975. The altered flora accompanied extensive use of silver-sulfadiazene. As with any topical therapeutic agent, such alteration inevitably results in changes in the bacterial flora of the burn wound.

Burns
Staph aureus
Enterobacter cloacae
Sepsis
Humans

PATHOGENESIS OF BURN WOUND INFECTION: BACTERIAL FLORA OF WOUNDS OF MILITARY PERSONNEL RECEIVING TREATMENT WITH SULFAMYLON OR SILVER-SULFADIAZENE

The bizarre but lethal problem of a continued high level of wound infection and sepsis occurring in patients who were treated with topical prophylactic agents of proven value has continued during 1974. The armamentarium of effective broad spectrum antibiotics has increased, but still wound infection and pulmonary involvement are frequent, and proceed despite prophylactic therapy. Control of infection in the severely burned patient remains an incompletely solved problem. Classical burn wound sepsis, essentially a primary invasive disease of the burn wound due to Pseudomonas aeruginosa, was seldom seen during this reporting period, but extensive bacterial invasion and proliferation in tissues of severely burned patients, occurring 3 or more weeks postburn, has been a frequent occurrence. Protection of patients from this lethal complication has prompted the extensive use of 5% Sulfamylon solution, applied to the burn wound in the form of soaks (1). A further modification of treatment regimens practiced in recent years has been the use of silver-sulfadiazene burn cream on burned patients. This medication was used on alternate burns for the first half of 1974, and has since been used on most incoming patients as the topical antibacterial agent (2). Since Sulfamylon burn cream has been used on some patients, and 0.5% Sulfamylon soaks are used on many, an environment in which bacterial flora exists in contact with both of these agents now is present. The changes that have occurred in burn wound flora may reflect this altered chemotherapeutic environment. This possibility is of especial interest in the appearance of what is in essence a new burn pathogen, Enterobacter cloacae. This organism had been recovered, in previous years, from various sites on burned patients, but not to the extent that it emerged in 1974. Enterobacter cloacae presented virutally a new epidemic situation in burn patients in 1974.

ANTEMORTEM BACTERIOLOGY IN BURN PATIENTS

Total cultures. A summary of the bacterial flora recovered in clinical specimens from burn patients in 1974 is shown in Table 1. Candida sp are also included in this resume. The number of specimens collected reflects the severity of the problems in infection presented by the patient in relation to sites of involvement. Blood cultures were the largest single source of samples; this reflects the major preoccupation with sepsis that dominated the clinical atmosphere of the burn wards. Sputum cultures provided the largest number of isolates. Surface wound cultures and biopsies made up the major part of the remaining samples. Total isolates of species furnish an overall indication of the principal infectious agents present in the burn patients during the past year.

---

1. Erickson DR, Hunt JL, Pruitt BA, Jr: Five percent aqueous Sulfamylon soaks used in topical treatment of burned soliers. USA Inst Surg Res Ann Res Prog Rpt FY 1973, BAMC, Ft Sam Houston, Texas. Section 14.

2. Fox CL: Silver-sulfadiazene: A new topical therapy for Pseudomonas in burns. Arch Surg 96: 184, 1968.

Table 1 Antemortem Bacteriology of Burn Patients 1974

ORGANISM	SOURCE AND NUMBER OF ISOLATES							
	Wound Surface	Blood	Lukens Sputum	Urine	I.V. Cath	Foley Cath	Biopsy	Total Isolates
Staph aureus	339	236	235	18	61	17	154	1060
epidermidis	31	11	40	19	10	16	8	135
Alpha hemol-strep.	23	13	207	3	2	22	11	281
Beta hemol-strep.	1	0	11	0	0	1	3	16
Gp. A strep.	10	0	5	0	1	0	0	16
Non hemol-strep.	30	10	124	21	8	19	36	248
Corynebacterium sp.	11	0	5	0	0	0	6	22
Bacillus sp.	10	2	8	3	3	0	34	60
Pseudomonas sp.	185	56	428	46	22	28	55	820
Mima-Herellea gp.	7	0	20	2	2	0	1	32
Aeromonas sp.	1	0	0	0	0	0	0	1
K. pneumoniae	113	74	369	66	34	41	36	733
Ent. aerogenes	15	3	38	3	0	2	8	69
cloacae	122	109	99	70	50	49	177	676
hafnia	0	0	1	0	0	0	0	1
Serratia marcescens	18	16	49	4	8	3	7	105
E. coli	97	27	166	54	23	47	54	468
Citrobacter	5	0	1	0	0	1	0	7
Prot. mirabilis	70	20	102	36	5	15	28	276
morganii	0	0	1	1	0	2	0	4
Prov. stuartii	58	26	57	43	56	44	92	376
Neisseria sp.	7	0	19	0	0	1	0	27
Candida sp.	60	6	26	60	24	19	61	256
No. of Patients cultured	143	173	98	129	176	130	135	
No. of specimens	729	4654	888	443	550	187	585	
Total isolates								5689
Total specimens recived	8036							

Staphylococcus aureus, Providencia stuartii, Pseudomonas aeruginosa, Klebsiella pneumoniae and Escherichia coli were, in that order, predominant gram negative species recovered in years prior to 1974 and in 1973 they comprised 66.2% of all bacterial isolates from clinical specimens. In 1974, they made up 60.6% of all isolates, but a species previously of minor significance suddenly became a conspicuous part of the opportunistic invasive flora of the burn wound. This was Enterobacter cloacae, isolates of which totalled 11.8% of all strains recovered. Changes in species distribution are shown in Table 2.

Table 2. Predominant Species Among Isolates from Clinical Specimens, 1970-1974

Species	% of All Isolates From Clinical Specimens				
	1970	1971	1972	1973	1974
Staph aureus	12.6	15.0	13.8	19.6	18.6
Ps aeruginosa	13.6	12.4	13.2	10.4	14.4
K pneumonia	11.5	9.7	11.5	10.1	12.8
E coli	6.4	11.0	6.1	10.4	8.2
Prov stuartii	21.0	15.2	23.1	15.7	6.6
Enterobacter cloacae	not differentiated	2.0	3.8	4.3	11.8
% of all isolates	65.1	63.3	67.7	66.2	72.4
No. of isolates	3293	3179	6696	5672	5689

The incidence of Staph aureus rose in 1973 over that seen in previous years, and it remained higher in 1974. E coli and Klebsiella pneumoniae each remained in the relative incidence area that had been observed over the past 5 years. There was an increase in Ps aeruginosa in 1974, although this species has fluctuated in incidence in successive years. Providencia stuartii, which had been a predominant species over the past several years, dropped to an incidence lower by far than any seen in the past 5 years (3). Its peak incidence in 1972 showed it as the most frequently occurring species ever recorded in the Institute of Surgical Research, but since 1973, it has now become the least common of the 6 most common species. Its role as an opportunistic invader remained important out of proportion to its overall frequency of occurrence.

3. Lindberg RB, Mason AD, Jr, Pruitt BA, Jr: Providencia stuartii as a major factor in burn wound infections. (Abstract). Am Soc. Microbiol. 1973: 130.

The new species of numerical importance in the total burn flora was one not previously implicated as a significant part of the burn flora. Enterobacter cloacae had always been present in a small part of burn wounds, sputum, and urine, but in 1974 this small role was suddenly greatly enlarged. It was the fourth most commonly encountered organism in terms of total culture incidence. Enterobacter cloacae was not merely a very common contaminant, but instead, was frequently recovered from sites which indicated that it played a primary role in the septic state.

The total of strains recovered does not, of course, of necessity indicate how frequently patients were involved with a given species. There were 245 patients out of 284 admissions on whom at least one culture was taken. The sites cultured and the incidence of patients positive for a given site are summarized in Table 3. The largest group of patients on whom a given sample was examined were those in whom i.v. catheter tips were cultured. Almost as large was the group on whom blood cultures were taken. Surprisingly, the smallest category of patients sampled were those on whom sputa were examined. The major species in terms of patients harboring a given organism were Staph aureus, Enterobacter cloacae, Prov stuartii, Ps aeruginosa and E coli, in that order.

BACTERIOLOGY OF THE BURN WOUND

The contribution of burn wound infection to the development of sepsis in a severely burned patient has never been clearly delineated. It is plausible to assume that a heavily colonized wound offers the bacteria access to the blood stream, but only in the case of Pseudomonas burn wound sepsis has this sequence been established by study of experimental models. Control of invasive infection is the primary objective of topical antimicrobial therapy, but the exclusion of organisms other than Pseudomonas has been difficult at best. During the latter half of 1974, silver sulfadiazene was used as the primary topical agent on burn surfaces. Sulfamylon burn cream was also in use, at a reduced level, and the extensive use of 5% Sulfamylon soaks was a major component of antibacterial surface treatment. The relative distribution of principal species on the burn wound was based on culture results on 245 out of the 284 patients admitted in 1974. The remaining 39 patients had either no cultures taken at all, or had one or two negative cultures taken at the time of admission to the burn ward. Seven patients who had no cultures taken had very large, lethal burns, with an average area of 89.3% of body surface. Twenty-eight patients with small burns had an average burn area of 12.4%, and these patients exhibited an uneventful recovery that called for no bacteriologic procedures. Bacterial species recovered from wound surface or biopsy, and the number of patients colonized are summarized in Table 4.

Staph aureus was found on more patients than any other species. Even so, more than one-fourth of patients cultured did not acquire this species. The predominan type was type 84, as it had been in 1973 (4). The strains were

---

4. Lindberg RB, Latta RL, Pruitt BA,Jr,Mason AD,Jr: Emergence of methicillin-resistant Staphylococcus aureus type 84 in burn patients. USA Inst Surg Res Ann Prog Rpt FY 1974, BAMC, Ft Sam Houston, Texas. Section 7.

Table 3 Antemortem Burn Patients Cultured, 1974

ORGANISM	SOURCE AND NUMBER OF PATIENTS POSITIVE ON CULTURE						
	Wound Surface	Blood	Lukens Sputum	Urine	I.V. Cath	Foley Cath	Biopsy
Staph aureus	3	40	58	15	46	16	68
epidermidis	25	11	29	15	8	15	15
Alpha hemol-strep.	18	10	58	3	2	19	5
Beta hemol-strep.	1	0	7	0	0	1	2
Gp. A strep.	9	0	2	0	1	0	0
Non hemol-strep.	20	5	40	14	6	15	23
Corynebacterium sp.	6	0	3	0	0	0	4
Bacillus sp.	8	2	4	3	3	0	17
Pseudomonas sp.	56	26	62	23	18	20	31
Mima-Herellea gp.	7	0	6	1	2	0	1
Aeromonas sp.	1	0	0	0	0	0	0
K. pneumoniae	44	25	67	36	23	29	14
Ent. aerogenes	6	3	12	3	0	2	6
cloacae	50	35	35	42	36	41	59
hafnia	0	0	1	0	0	0	0
Serratia marcescens	8	8	12	3	5	2	6
E. coli	48	10	33	30	17	38	28
Citrobacter sp.	3	0	1	0	0	1	0
Prot. mirabilis	28	9	21	18	5	12	13
morganii	0	0	1	1	0	2	0
Prov. stuartii	58	26	57	27	29	30	44
Neisseria sp.	7	0	7	0	0	1	0
Candida sp.	33	4	18	19	13	16	28
Total patients Sampled	143	173	98	129	176	130	135

Table 4. Burn Wound Surface Flora in 245 Patients, ISR, 1974

Species	No. of Strains Recovered	No. of Patients Positive on Burn Surface	% of Cultured Patients Positive
Staph aureus	493	179	73.0
Staph epidermidis	49	40	16.3
Strep, non-hemolytic*	66	43	17.5
Klebsiella pneumoniae	149	63	25.7
Enterobacter cloacae	299	109	44.4
E coli	151	76	31.0
Proteus mirabilis	98	41	16.7
Providencia stuartii	263	102	41.6
Pseudomonas aeruginosa	240	87	35.5

* *These strains primarily group D.*

relatively sensitive to the major categories of antibiotics including the methicillin group of semi-synthetic penicillins. Nevertheless, prolonged septicemia occurred in some patients with strains sensitive in vitro to penicillin G. Seventy-three per cent of patients who were cultured harbored Staph aureus on the burn wound. This was a smaller incidence than occurred in 1973.

Staph epidermidis was recovered from burn wounds of 16.3% of patients cultured. This is a drop from 20.4% of all burn patients in 1973. At one time, it was postulated that this species was increasing in burn wounds, but this trend has not continued. No instances of wound sepsis due to Staph epidermidis have been uncovered.

Non-hemolytic Streptococci were recovered from wounds of 17.5% of patients cultured. As with Staph epidermidis, it was for a time thought that a marked rise in non-hemolytic streptococci was occurring in burn wounds. In 1973, 29.4% of burn wounds had harbored such streptococci. However, the drop in incidence in 1974 suggests that this peak of non-hemolytic streptococci may now be waning. These strains were, on the basis of cultural characteristics, all Lancefield group D.

Klebsiella pneumoniae was numerically less frequent in 1974 than in the preceding year; one-fourth of the burn wounds harbored the organism in contrast to one-third in 1973. It was still a major part of the wound flora, but

was overshadowed by the rapid rise in incidence of Enterobacter cloacae.

Continued observation on group A streptococci has been maintained, since this potentially dangerous burn wound invader can best be controlled with the penicillin group of antibiotics if its presence is detected promptly. The organism has varied between being rare in some years to relatively numerous in others After several years in which the species was a rarity, an upsurge of positive cultures occurred in 1972, followed in 1973 by its virtual disappearance (Table 5). In 1974, 30 strains of Streptococcus pyogenes Lancefield A were recovered primarily from wound surfaces and pulmonary secretions. No overt infection problems were seen, although wound infections at the time of autografting are a particularly hazardous potential. Fluctuations such as those seen in the past 3 years make monitoring of this pathogen of great importance.

Table 5. Incidence of Group A Streptococci on Burn Wounds

Year	No. of Strains of Group A Streptococci Recoverd
1969	8
1970	2
1971	1
1972	56
1973	3
1974	30

RESPIRATORY TRACT BACTERIAL FLORA IN BURNS

Pulmonary complications are a major problem in treating burn patients, even in the absence of inhalation injury. No specific etiologic agent has emerged as the primary cause of such pneumonia, and as in other infected sites, the burn patient's lung is most often invaded by the same group of organisms that invade the wound in opportunistic fashion. The number of sputum samples cultured , 888 for 1974,is the highest annual total for the Institute of Surgical Research. This need not reflect an increased incidence of repiratory tract problems, but it does reflect the extent of concern and interest in this problem. Ninety-eight patients had sputum cultures, for a ratio of over 9 samples per patient. In the previous year, 1973, 130 patients had contributed 846 cultures for an average of 5.5 samples per patient; the intensity of scrutiny had almost doubled. This trend of increased concern with pulmonary microbial flora has been apparent since 1972.

Results of sputum cultures for major species recovered are summarized in Table 6. The proportion of patients from whom principal pathogens were recovered is shown for the past 4 years; the proportion of cultures positive for

a given species may be indicative of its importance in pathogenesis of pulmonary disease in burned patients.

Table 6. Principal Species of Bacteria Recovered from Respiratory Tract of Burned Patients, 1971-1974

Species	% of Patients Exhibiting Positive Sputum on Culture			
	1971	1972	1973	1974
Staph aureus	43.0	38.5	56.9	59.2
Klebsiella pneumoniae	45.0	58.8	60.0	68.4
Enterobacter cloacae	11.0	27.0	23.8	35.7
E coli	27.2	40.9	53.8	33.7
Proteus mirabilis	-	19.0	10.8	21.4
Providencia stuartii	33.0	56.5	40.8	59.2
Ps aeruginosa	39.0	38.5	36.2	63.3
Patients cultured	94	122	130	98

Staph aureus increased in incidence significantly in 1973 and the proportion of positive cultures rose to almost 60% of patients in 1974. Staphylococci have continued to represent a major cause of pneumonia.

Klebsiella pneumoniae has steadily increased in incidence in sputum cultures, and was the predominant organism in sputum. Whether this incidence connotes an actual pathogenic role is not clear; using the criterion of predomoinant organism in lung tissue at autopsy, there were 13 out of 57 autopsies in which the predominant organism was Klebsiella; in 14 it was Ps aeruginosa and in 19, Providencia stuartii. Thus, although more patients harbored Klebseilla pneumoniae in sputum than any other bacterial species, the postmortem data suggest it was not the principal offender in clinical pneumonia.

Pseudomonas aeruginosa had remained relatively constant in its incidence in sputum cultures for the past 3 years, but in 1974 a sharp rise in its incidence from 36.2% to 63.3% of patients with positive sputum occurred. The rise in Pseudomonas incidence has been one of the unusual features of the bacteriologic changes which may be associated with a changed treatment regimen during 1974.

Enterobacter cloacae, which on an overall basis constituted a major microbiologic feature of the burn ward flora in 1974, increased its incidence in sputum from 23.8% to 35.7% of the patients cultured. This is a significant but not dramatic rise in incidence, and in view of the fact that only lung samples from 57 autopsies showed E cloacae as a predominant organism, it is probable that this

species was not a major pathogen in pneumonia in burn patients. E coli, present as the predominant organism in 7 out of 57 autopsies, was actually numerically as significant as E cloacae by this criterion. Despite the marked rise in incidence of E cloacae as part of the burn flora, it showed no significant predilection for the lung.

SEPTICEMIA AND BACTEREMIA IN BURN PATIENTS

Sepsis, with its concommitant blood stream invasion, remains the principal cause of death in severe burns. The microbial flora of blood cultures offers the most definitive information on the actual cause of death in large burn injuries, although there remains the essential problem of ultimate cause: the positive blood culture connotes a grave threat to survival, but most such positives are a terminal event rather than initial cause of the problem.

In 1974, 173 patients were sampled at least once by blood culture. Ninety-five patients, or 54.9%, had at least one positive blood culture. The rate of positive blood cultures in relation to the total of patients sampled has been remarkably consistent. In 1972, 47% of those cultured were positive, in 1973, 54.0%, and in 1974, 54.9%. This rate of bacteremia is at a level notably higher than prevailed previous to 1972. The rate of positives was 30% in 1971. There were 2327 sets of 2-bottle blood culture samples collected in 1974; this represented 13.4 cultures per patient sampled. This is the largest sampling that has been recorded in this Institute.

Blood cultures from 173 patients (of whom 95 had bacteria recovered) are summarized in Table 7. The species of major numerical importance are underlined. These ranged from 25 to 40 patients, and included Staph aureus, Ps aeruginosa, Kleb pneumoniae, Entero cloacae, and Prov stuartii. A group of significant but less common species included alpha hemolytic streptococci, Staph epidermidis, E coli, Prot mirabilis and Serratia marcessens. These were recovered from 8 to 10 patients each. Survival rates associated with the predominant species were far from encouraging; the recovery of Staph aureus connoted a survival rate of only 42.5%. Gram negative bacilli in blood cultures reflected mortality rates of 87.5% to 100% for Entero cloacae.

A more informative insight into the pathologic implications of a positive blood culture was obtained by scrutinizing the outcome of patients who had only one species recovered. Of course, such injuries were less extensive: 40 patients out of 95 with positive blood cultures yielded only one species on culture. The situation with reference to the group of patients is summarized in Table 8. The largest number of patients in this group harbored Staph aureus followed by those with Staph epidermidis and Entero cloacae. Mortality rate in patients with Staph aureus was 28.5%. Staph epidermidis alone was associated with no deaths, and Entero cloacae alone appeared in only one fatal burn. Klebsiella pneumoniae and Prov stuartii, each recovered as sole species from 4 patients, were associated with 50% mortality; the smallest incidence of species, S marcessens, Ps aeruginosa and E coli were each in the 100% mortality category. The average burn size in this group of 40 patients was 49.5%. The mortality figures for these single strain infections are in general markedly lower than

Table 7. Blood Culture Isolates from 173 Burn Patients, 1974
Relation of Species of Microorganism to Mortality

Species Recovered	No. Patients Positive	No. Isolates	% of All Patients Cultured Positive	No. Patients Expired	% of All Patients with Positive Cultures Who Expired
Staph coag pos	40	236	23.1	23	57.5
Staph coag neg	11	11	6.3	4	36.3
Strep, alpha	10	13	5.7	10	100.0
Strep, non-hemolytic	5	10	2.8	5	100.0
Bacillus sp	2	2	1.1	2	-
Pseudomonas sp	26	56	15.0	25	96.1
Kleb pneumoniae	25	74	14.4	21	84.0
Entero aerogenes	3	3	1.7	3	-
Entero cloacae	35	109	20.2	35	100.0
E coli	10	27	5.7	9	90.0
Prot mirabilis	9	20	5.2	9	100.0
Prov stuartii	26	59	15.0	23	88.4
Candida sp	4	6	2.3	3	75.0
Serratia marcessens	8	16	4.6	7	87.5
No of patients with positive blood culture	95				

Table 8. Bacteremia with Only One Species of Bacteria Recovered: Burn Patients, 1974

Species	No. Patients with One Species Recovered	Ave. No. of Positive Blood Cultures Per Patient	Deaths	% Mortality for One Species Bacteremia
Staph aureus	14	4.6	4	28.5
Staph epidermidis	7	1.0	0	0
Kleb. pneumoniae	4	3.0	2	50.0
Entero cloacae	5	1.2	1	20.0
E coli	1	4.0	1	100.0
S marcessens	3	2.0	3	100.0
Prov stuartii	4	1.2	2	50.0
Ps aeruginosa	2	2.0	2	100.0
Total	40	2.5	15	37.5

when 2 or more organisms have invaded the patient.

Blood cultures in 55 patients, or 57.8% of all patients with positive blood cultures, yielded more than one bacterial species in successive blood cultures, or in some instances, in a single blood culture. The mixed culture results are shown in Table 9. The combinations were very heterogeneous; the most frequent combination, Entero cloacae and Kleb pneumoniae, was found in only 4 patients, while at the extreme end of the spectrum, there were 8 patients who exhibited 6 different species in blood cultures altogether. Multiple blood stream invasion is a disturbing phenomenon, since it suggests the complete breakdown of defense measures which keep bacteria out of the blood stream of viable patients. The tabulation assigns priority to four major pathogens: Staph aureus, Prov stuartii, Entero cloacae, and Kleb pneumoniae, and other combinations are listed under these key organisms. Staph aureus was a prominent part of the flora of almost half of this group of patients, but no numerically significant pairing with another species could be discerned. Prov stuartii and Ps aeruginosa were encountered almost as often as staphylococci. However, Entero cloacae was the most frequently encountered species.

BIOPSY OF BURN WOUNDS

The use of biopsies as a guide in treatment of burn wound, and as a technic for assessing effectiveness of therapy, has become well established as a useful procedure. The species of microorganism recovered from burn

Table 9 Blood Culture Isolates in Patients with More than One Species Recovered

Species	No. of Patients
Staph aureus, Entero cloacae	1
Staph aureus, Entero cloacae, *Pseudomonas*	1
Staph aureus, Entero cloacae, alpha strep	2
Staph aureus, Entero cloacae, Prov. stuartii	1
Staph aureus, Entero cloacae, Pseudomonas, Prov stuartii	2
Staph aureus, Entero cloacae, Pseudomonas, alpha strep	1
Staph aureus, Entero cloacae, Pseudomonas, Staph epidermidis	1
Staph aureus, Entero cloacae, Pseudomonas, Prot mirabilis	1
Staph aureus, Entero cloacae, E coli, Kleb pneumoniae, Prov stuartii	1
Staph aureus, Entero cloacae, Pseudomonas, E coli, Prov stuartii, Kleb pneumoniae	1
Staph aureus, Entero cloacae, *E coli, non hemolytic* strep, Kleb pneumoniae, Prot mirabilis	2
Staph aureus, *Pseudomonas*	2
Staph aureus, Pseudomonas, Prov stuartii	1
Staph aureus, Pseudomonas, E coli	1
Staph aureus, Pseudomonas, alpha strep, Kleb pneumoniae, Staph epidermidis	1
Staph aureus, Prov stuartii, S marcessens, Candida	2
Staph aureus, alpha strep, non hemolytic strep, E coli, Kleb pneumoniae	1
Prov stuartii, Entero cloacae	2
Prov stuartii, Entero cloacae, Pseudomonas	3
Prov stuartii, Entero cloacae, Pseudomonas, Staph epidermidis	1
Prov stuartii, Entero cloacae, Pseudomonas, Kleb pneumoniae	2
Prov stuartii, Entero cloacae, S marcessens, Prot mirabilis, E coli	1
Prov stuartii, Entero cloacae, Entero aerogenes, Pseudomonas, alpha strep, non hemolytic strep	2
Prov stuartii, Entero cloacae, Pseudomonas, alpha strep, Kleb pneumoniae, Prot mirabilis	2
Prov stuartii, Prot mirabilis,	1
Prov stuartii, non hemolytic strep, Candida	1
Entero cloacae, Pseudomonas	3
Entero cloacae, Pseudomonas, Staph epidermidis, S marcessens	2
Entero cloacae, Kleb pneumoniae	4
Kleb pneumoniae, alpha strep	1
Total patients with 2 or more species	55

		% of all Positives
No. of patients with 2 species	17	17.8
No. of patients with 3 species	13	13.6
No. of patients with 4 species	13	13.6
No. of patients with 5 species	4	4.2
No. of patients with 6 species	8	8.4
No. of Patients with Entero cloacae	35	
Staph aureus	25	
Prov stuartii	24	
Ps aeruginosa	23	

wound biopsies offers a distinctive and essential parameter in understanding the role of infection in the burn wound, and is a reflection of the effect of topical therapy on bacterial colonization and invasion of the burn wound. The relation of invading bacterial species to mortality may be disclosed by summarizing the data on burn biopsies.

Table 10 shows the species recovered from biopsies of burn wounds of 135 patients in 1974. This is the largest number of patients that have been biopsied in a single year. Species of numerical importance were, in descending order, Staph aureus, Entero cloacae, Prov stuartii, Ps aeruginosa and E coli. The mortality rate associated with the predominant species of microogranisms was not proportionate to their frequency. Prov stuartii and Ps aeruginosa had the highest associated mortality rate, and Staph aureus the lowest. Kleb pneumoniae was not high in recovery rate, but patients harboring it had a very high mortality rate.

Comparison of annual incidence rates for biopsy flora and associated mortality rates is shown in Table 11. There is a suggestion that Staph aureus has become more dangerous as an invading organism, despite its relatively constant incidence. Kleb pneumoniae has never been frequent in biopsies, but deaths due to Klebsiella sepsis are disproportionately frequent. In terms of incidence in biopsied tissues, there was little change on an annual basis except for the striking increase in incidence of Entero cloacae. This species was so infrequent in biopsies that it was not tabulated prior to 1973. But when the mortality trend is viewed, there was an obvious very large up-turn in 1973 for all the major species recovered.

PROVIDENCIA STUARTII AND ENTEROBACTER CLOACAE IN BURN PATIENTS

Burn infection due to Prov stuartii was first described in patients in this Institute as a major new infectious process caused by a relatively uncommon enteric bacillus of usually minor interest as an opportunistic pathogen. It assumed a major role as the predominant organism in burn wounds, pulmonary infections and in septicemia. The extent to which Prov stuartii involved patients during 1974 is summarized in Table 12. The organism was a major part of the burn wound flora, sputum flora, and in biopsies. Septicemia due to Providencia was a major part of blood stream invasion, and the high percentage of wound tissue and lung samples positive at autopsy suggests that Providenica is especially prone to proliferate in the terminal stage of a burn patient's course.

Comparison of the proportion of patients who harbored the organism on burn wound, biopsy, in blood or in sputum is shown in Table 13. The incidence has fluctuated but it remained high enough so that the species remains a major concern as a burn wound pathogen. The organism is transmitted by contact in the burn ward; its eradication shows no promise of being achieved.

Enterobacter cloacae has emerged as a significant wound pathogen since 1973. Prior to that time, it had not occurred in a frequency that prompted its detailed scrutiny. The rate of occurrence in 1974 is summarized in Table 14.

Table 10 Bacterial Flora of Biopsies on Burn Wounds of 135 Patients, 1974

Species	No Patients Positive	% of Patients Positive	No Patients with Positive Cultures Who Expired	% of Patients Positive Who Expired
Staph aureus	68	50.4	31	45.6
Staph epidermidis	15	11.1	7	46.7
Alpha hemolytic Strep	5	3.7	4	80.0
Non-hemolytic Strep	23	17.0	12	52.2
Corynebacterium sp	4	3.0	3	75.0
Bacillus sp	17	12.6	8	47.1
Pseudomonas sp	31	23.4	21	67.1
Mima Herellea sp	1	0.7	1	100.0
Klebsiella pneumoniae	19	14.1	15	78.9
Enterobacter aerogenes	6	4.4	5	83.3
Enterobacter cloacae	59	43.7	35	59.3
E coli	28	20.7	19	67.9
Proteus mirabilis	13	9.6	7	53.8
Prov stuartii	44	32.6	31	70.5
Candida sp	28	20.9	15	53.6
Serratia marcessens	6	3.9	5	83.3

No of specimens collected 585

No of samples per patient (average) 4.3

Table 11. Burn Wound Biopsy Flora Species Incidence and Mortality 1969 as Compared with 1971-1974

Species	% of Patients Positive					% of Patients Positive who Expired				
	1969	1971	1972	1973	1974	1969	1971	1972	1973	1974
Staph aureus	42	44	41	51.9	50.4	22	38	22	50.9	45.6
Kleb pneumoniae	20	17	32	17.0	14.1	50	31	19	55.5	78.9
Enterobacter cloacae	-	-	-	19.8	43.7	-	-	-	61.9	59.3
E coli	14	19	27	25.5	20.7	47	33	16	66.6	67.9
Prov stuartii	51	40	56	36.8	32.6	14	58	36	51.0	70.5
Prot mirabilis	34	13	14	9.4	9.6	38	40	9	60.0	53.8
Ps aeruginosa	30	30	32	32.1	23.0	39	57	20	50.0	67.7

Table 12. *Providencia stuartii* Isolates from Clinical and Autopsy Specimens, 1974

Source	No. Isolates/ Total Specimens	% Positive	No. Patients Positive/ Total Patients Cultured	% of Cultured Patients Positive
Burn wound, swab, clinical	171/729	23.4	58/143	40.6
Biopsy, wound	92/585	15.4	44/135	32.6
Blood culture	59/2327	2.5	26/173	15.0
Sputum (Lukens)	426/888	47.9	57/98	58.2
Urine	43/443	9.3	27/129	20.9
Foley catheter tip	19/187	10.3	16/130	12.3
I.V. catheter tip	56/558	10.2	29/176	16.5
Autopsy, burn	128/269	47.2	43/80	53.8
Autopsy, lung	162/320	50.2	51/80	63.8

Table 13. Per Cent of Patients Cultured Who Harbored Providencia stuartii

Year	Site of Culture and % Positive			
	Wound	Biopsy	Blood	Sputum
1969	46	51	32.9	54.4
1970	43.7	45.6	14.0	67.7
1971	34.0	40.0	36.5	33.0
1972	49.5	55.4	23.9	56.6
1973	34.0	36.8	20.0	40.8
1974	40.6	32.6	15.0	40.8

Table 14. Enterobacter cloacae Isolates from Clinical and Autopsy Specimens, 1974

	No. Isolates / Total Specimens	% Positive	No. Patients Positive / Total Patients Cultured	% of Cultured Patients Positive
Wound swab	123/729	16.5	50/143	35.0
Sputum	177/585	30.1	59/135	43.7
Blood	109/465	23.4	35/173	20.2
Lukens-sputum	99/888	11.1	35/98	35.7
Urine	70/443	15.8	42/129	32.6
I.V. catheter tips	59/558	9.0	36/176	20.5
Foley catheter tips	49/180	26.2	41/130	31.5
Autopsy Lung	52/320	16.3	24/80	30.0
Autopsy Liver	19/80	23.8	22/80	27.5
Autopsy wound	71/269	26.4	32/80	40.0

[illegible] incidence in burn wounds, and especially in biopsies indicates that these organisms were colonizing the burn patient very heavily and that tissue penetration was even more marked. The incidence in blood cultures was relatively high, as was the case with sputum. Interestingly, at autopsy the lung was less extensively involved than would have been thought from the sputum culture data. The presence of Entero cloacae in liver tissues indicates that late appearing septicemia was a common occurrence for Entero cloacae, since the organisms, sequestered in liver macrophages, survive to appear in postmortem cultures.

I.V. CATHETER TIP CULTURES

Surveillance of indwelling i.v. catheters by culturing the tip when it is removed from the vein furnishes essential data on such catheters as a factor in the etiology of intravascular infections. There is, however, no assurance that a positive catheter tip culture means that concurrent or subsequent septicemia is the result of i.v. catheter transmission; indeed, the tip may well become seeded in the presence of a bacterial infection arising elsewhere. In any event, the i.v. catheter tip site merits detailed study, since it is a source of a potentially severe complication of burns.

Table 15 presents results of culturing 550 catheter tips from 178 burn patients. Out of the 178 patients, 100, or 56.1%, had bacteria recovered from at least one i.v. tip culture. The numerically important species, in descending order of frequency, were Staph aureus, Entero cloacae and Prov stuartii. This is in contrast to the incidence of opportunistic pathogens in wounds or septicemia; the relatively low incidence of Ps aeruginosa and Kleb pneumoniae was in contrast to the occurrence of these species in wounds or in septicemia.

DISCUSSION

The etiology of bacterial sepsis in burn patients is heterogeneous. The principal species colonizing burn wounds and lung, and recovered from blood cultures, include the species which have been important in previous annual summaries: Staph aureus and Ps aeruginosa. Prov stuartii was still a major opportunistic invader, but was distinctly less prominent than it had been during the previous 3 years. Entero cloacae, previously relatively uncommon, suddenly became a major infecting agent, and was a prominent part of the bacterial flora in septicemia and in burn wound biopsies. Limited epidemics due to S marcessens occurred, but between outbreaks, this potentially serious pathogen virtually disappeared.

Antibiotic resistance in Enterobacteriaceae was prevalent; this problem is discussed in more detail in another section of this report. The appearance of resistant strains is consistent with the presence of resistance transfer factors; although these have not yet been sought, their presence is virtually assured as reflected in the patterns of emerging resistance that have occurred. Elimination of colonization in burn patients under less than optimal isolation conditions is an unrealistic goal. The incidence of individual opportunistic invading species points out to the areas where major emphasis on control should be applied. The numerical incidence of some opportunistic invading species suggest that

Table 15. Bacterial Flora of I.V. Catheter Tips from 178 Burn Patients, 1974

Species	No. Patients Positive	% of All Patient Positive	% of Patients with Positive Cultures
Staph aureus	46	16.1	46
Staph epidermidis	8	4.5	
Non hemolytic strep	13	8.0	13
Bacillus sp	3	1.7	
Pseudomonas sp	18	10.2	18
Mima-Herellea gp	2	1.1	
Klebsiella pneumoniae	23	13.1	23
Entero cloacae	36	20.4	36
Serratia marcessens	5	2.8	
E. coli	17	9.7	17
Prot. mirabilis	5	2.8	
Prov. stuartii	29	16.5	29
Candida sp	13	7.4	13

No. catheter tips cultured: 550

Average catheter tips per patient: 3.1

No. of patients with positive cultures: 100

they may be playing a larger role in burn wound pathogenesis than had been assumed.

PUBLICATIONS

None

PRESENTATIONS

Lindberg RB: "The Control of Nosocomial Infections: Microbiologic Aspects", presented at Symposium on Control of Hospital Infections, Duquesne University, Pittsburgh, Pa, October 4, 1974.

Lindberg RB: "Providencia stuartii as a Significant Pathogen in Burn Wound Infections" presented at 4th International Congress for Study of Burn Injury, Buenos Aires, Argentina, September 21, 1974.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OB 6397	75 07 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
74 07 01	D. CHANGE	U	U	NA	NL	[X] YES [ ] NO	A. WORK UNIT

10. NO. CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A161102B71R	01	188
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede 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
65 07	Cont	DA	C. In-House

17. CONTRACT/GRANT: Not Applicable

a. DATES/EFFECTIVE: EXPIRATION

b. NUMBER:

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
FISCAL YEAR	PRECEDING 75	.7	20
	CURRENT 76	.5	15

19. RESPONSIBLE DOD ORGANIZATION

NAME: US Army Institute of Surgical Research

ADDRESS: Fort Sam Houston, Texas 78234

RESPONSIBLE INDIVIDUAL

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

TELEPHONE: 512-221-2720

20. PERFORMING ORGANIZATION

NAME: US Army Institute of Surgical Research
Microbiology Branch

ADDRESS: Fort Sam Houston, Texas 78234

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

NAME: Robert B. Lindberg, PhD

TELEPHONE: 512-221-2018

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Ruth L. Latta, BS

NAME:

21. GENERAL USE

FOREIGN INTELLIGENCE NOT CONSIDERED

DA

22. KEYWORDS (Precede EACH with Security Classification Code)

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

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

23. (U) Pseudomonas aeruginosa remains an important pathogen in burned soldiers despite use of effective topical therapy. Reports of widespread nosocomial infection due to Pseudomonas reflect a broadening scope for this opportunistic invader and its control requires precise epidemiologic strain identification which is the objective of this study.

24. (U) Phage typing procedures, plus modification to read strains untypable at the standard 1000 RTD strength. A concentrated phage typing fluid is used.

25. (U) 74 07 - 75 06 Reactors with dilute phage made up only 25% of all strains typed, but with dilluted phage, 94% of all isolates could be typed. Four major epidemic types - up epidemics of 5 to 8 months duration, with considerable overlap. The typing system was entirely effective in characterizing the epidemic patterns.

*Available to contractors upon requestor's approval

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

ANNUAL PROGRESS REPORT

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

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

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

1 July 1974 - 30 June 1975

Investigators:

Ruth L. Latta, BS
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. 3A161102B71R-01, RESEARCH IN BIOMEDICAL SCIENCES

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

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

Period covered in this report: 1 July 1974 - 30 June 1975

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

Reports Control Symbol MEDDH-288(R1)

Use of a mixed system of both 1000 Routine Test Dilution (RTD) and undiluted phages has made possible a typing of 94% of a collection of 1085 strains of Pseudomonas aeruginosa. Undiluted lytic patterns made up 8 of the prominent types; 4 more were typable at 1000 RTD. The most common type, found in 33% of all patients with Pseudomonas, was NT-3. It replaced NT-5, still present but not predominant as it was in 1973. NT-3 had not been observed in 1972 or 1973. The 1973 A-71 epidemic disappeared completely in 1974. Septicemic strains varied from the overall strain frequency, so that NT-4 involved the blood stream proportionately more often than in all sources combined, but essentially no septicemic type could be discerned. Since it was extremely infrequent in lung, NT-4 septicemia appeared most plausibly to arise from infected burn wounds. Epidemic episodes of NT-4, NT-5, NT-3, and NT-16 extended over all of 1974. The duration of a given epidemic was 6 to 8 months. The control of this complex Pseudomonas population with topical antibacterial agents is effective only at the individual host level.

Pseudomonas
Phage typing
Burn wounds
Topical chemotherapy
Humans

BACTERIOPHAGE TYPES OF PSEUDOMONAS AERUGINOSA FOUND IN BURNED SOLDIERS

The differentiation of infecting strains of bacterial species involved in epidemic infectious disease in a hospital population is an essential part of understanding the epidemiology of the disease. Burn wound infections are heterogeneous as to causative species, but the principal opportunistic invader in causing burn wound sepsis is Pseudomonas aeruginosa. Despite the extensive and diverse efforts made to eradicate this opportunistic pathogen from burn wards, it continually reappears, and it has been studied intensively in this Institute for the past 15 years. Phage typing has been selected as the differentiating method of choice(1,2) and a set of phages derived in this Laboratory have remained the mainstay of a strain-differentiating system.

METHOD

In two previous years, various experiments aimed at characterizing the increasing number of strains untypable with the standard 18-phage typing set were described (3,4). After extensive study, new typing phages, which would lyse strains at the Routine Test Dilution (RTD), and which were considered as replacements for original phages, were set aside, and a technic using undiluted phage fluids to type strains not lysed by the RTD was employed. Thus, the original typing designated with the addition of a prefix "NT" could be used. This precept was defined as a strain capable of being lysed with undiluted phage but not lysed by RTD.

Further elucidation of the nature of the NT series of typing reactions is needed. The technic works, and it is probable that it reflects the behavior of a pyocin component in the phage preparation, but final elucidation of this question awaits further study.

---

1. Lindberg RB, Latta RL, Brame RE, Moncrief JA: Definitive bacteriophage typing system for Pseudomonas aeruginosa. Bact Proc 1964, p. 81.

2. Lindberg RB, Latta RL, Pruitt BA, Jr: Stability of bacteriophage strains as epidemiologic markers for Pseudomonas aeruginosa. Bact Proc 1970, p. 79.

3. Latta RL, Lindberg RB, Pruitt BA, Jr, Mason AD, Jr: Bacteriophage types of Pseudomonas aeruginosa in burned soldiers. US Army Institute Surg Res Ann Rpt FY 1973, BAMC, Fort Sam Houston, TX. Section 12.

4. Latta RL, Lindberg RB, Pruitt BA, Jr, Mason AD, Jr: Bacteriophage types of Pseudomonas aeruginosa found in burned soldiers. US Army Institute Surg Res Ann Rpt FY 1974, BAMC, Fort Sam Houston, TX. Section 11.

Phage typing of Pseudomonas aeruginosa from Burn Ward Patients, 1974

There was a marked rise in the number of strains of Ps aeruginosa recovered in 1974. The principal ecologic change that was suspected in this period was the introduction of silver-sulfadiazene burn cream in topical treatment. Sulfamylon(R) was still used but a large group of patients did not receive it.

There were 1085 strains of Ps aeruginosa collected for typing from clinical specimens on 116 patients. Sources were diverse and included blood, sputum, biopsy tissue, urine, catheter tips, stool and post mortem tissues. Predominant phage types found are summarized in Table 1.

The Phage Type Code is listed to indicate the total phage type pattern shown in the "Phage type" column. As defined above, NT indicates strains not typable by RTD, but which will react with undiluted phage to give a usable, reproducible type reaction. Eight of the 12 type codes were NT reactions. The percentage of all Pseudomonas-positive patients, harboring a given type, and the percentage of all strains that were of that type are shown in the right hand columns. Any types found in fewer than 5 patients are not shown; otherwise the table becomes unwieldly with small numbers of types of little epidemiologic significance. The relative proportion of the total isolates tabulated is reflected in the fact that 74.3% of all isolates are accounted for on Table 1. The remaining 25.7 are distributed over a large number of types, many of which are unique.

NT-3 was the most frequently seen type; 32.8% of all Pseudomonas-positive patients harbored it and it made up one-fourth of all strains isolated. It is of particular interest that NT-3 was not seen even once in 1973 or 1972. Not previously had a new epidemic strain pervaded the ward population so completely and so consistently.

Type NT-16 was the next most prevalent type, with 18.1% of patients and 10.1% of all strains in this category. This, too, was a new, previously unreported type.

The third most frequently encountered type was NT-5; 14.7% of the patients harbored 8.1% of all strains recovered. This type had been found in 1973 as the predominant type for that year.

Type NT-4 was the fourth most common strain occurring in 12.1% of the patients and even more strains - 8.7% - than had NT-5. NT-4 was more common in 1973; then it was almost as common as NT-5.

Another previously unknown type, NT-17, was fifth in frequency in 1974. 8.2% of all strains were harbored on 8.6% of all patients concerned.

Type D-98, representing strains of pattern 21,31,119X, was the sixth most common pattern. It had not been seen in at least the preceding 4 years.

Next most common were types NT-22 and NT-21. Each of these was

Table 1. Predominant Phage Types of 1,085 *Pseudomonas* Strains from 116 ISR Burn Ward Patients, 1974

Phage Type Code	Phage Type	Per Cent Each Phage Type	
		Patients	Strains
NT-3	Non-typeable *(21),(24),(44),1214,(68),109,352,(F7),F8	32.8	24.3
NT-16	Non-typeable *(21),68,(F7)	18.1	10.1
NT-5	Non-typeable *(21),24,44,1214,(68),119x,(F7)	14.7	8.1
NT-4	Non-typeable *(21),24,31,44,1214,(68)	12.1	8.7
NT-17	Non-typeable *21,1214,68	8.6	8.2
D98	21,31,119x	6.9	4.1
NT-22	Non-typeable *(21),(1214),(F7),(F8)	6.0	3.5
NT-21	Non-typeable *(21),1214,68,(109),F8		2.3
M 2	119x	5.2	
NT-18	Non-typeable *24,44,1214		2.2
D41	21,68		0.6
C26	16,44,1214,68	4.3	2.2

* Phage Type using undiluted phage

( ) Variable reaction

found on 6% of the patients who harbored Pseudomonas. Neither of these type patterns had been seen previously.

Next in frequency, with strains recovered from 5.2% of patients in each case, were types M-2, NT-18 and D-41. The number of isolates was small in each case; they made up from 2.3% to 0.6% of the isolates in 1974. Of these, phage type M-2, (119X) is not new to the burn ward. It has been observed in previous years, and was as common in 1973 as in 1974. Phage type D-41 has been a very common "burn type", and in some years was overwhelmingly predominant. In recent years it has never been numerically conspicuous.

The final type included, as involving at least 5 patients, was C-26. This pattern has been seen in previous years but it has never been high in incidence.

The standard typing technic, working with RTD dilutions of at least 10^{-3}, was ineffective with 75% of the strains examined. Although this level was dismayingly high, the utility of the procedure, as far as differentiating strains was concerned, remained unchanged. When strains from other laboratories were submitted for typing, the proportion which reacted at RTD was very high, as it was when this sytem was first developed in this Laboratory. The indication is that the continued interchange of genetic material in the Institute of Surgical Research burn ward microbial population is generating a high level of strains which are not lysed by the phages in this collection. This tolerance is evidently local, since even Pseudomonas strains from Brooke General Hospital are typable by RTD concentrations of phage. The implication of locally concentrated strains resistant to antibiotics, and sorted by fortuitous contact as is evidently the case with phage susceptibility, is disturbing. No method of ridding a population completely of resident strains of an opportunistic pathogen is known.

Phage Types of Pseudomonas From the Blood Stream of ISR Burn Patients, 1974

The extremly high mortality of patients from whose blood stream Ps aeruginosa has been recovered makes the identification of such strains particularly important. If such strains represent a preponderance of particular types, not consistent with the overall incidence of Pseudomonas in the burn ward, then the existence of septicemic types might be established, and concentration on control of such lethal types would be merited.

There were 32 patients in whom Pseudomonas was recovered from the blood in 1974. Thirty of these, or 93.7%, died. Sixty-six strains from these 32 patients were studied. The incidence of types in the blood stream was not dissimilar to the pattern recovered from all sources. The comparison of these frequencies is shown in Table 2.

It will be seen that the most common type, NT-3, was first in incidence in blood and in overall incidence. Type NT-4, second in incidence in blood, was fourth in incidence in all cultures. The incidence of all remaining blood stream types was not markedly different from that of the total Pseudmonas

Table 2. Frequency of Occurrence of Pseudomonas Phage Types in Blood Cultures Compared to All Isolates ISR - 1974

Type	Frequency of Occurrence					
	Blood			All Sources		
	Patients	Strains	Incidence	Patients	Strains	Incidence
NT-3	9	19	1	38	355	1
NT-4	5	5	2	14	131	4
NT-17	4	7	3	10	93	5
NT-5	3	7	4	17	159	3
NT-16	3	5	5	21	196	2
NT-18	2	3	6	6	56	10
NT-22	2	3	7	7	65	7
M-2	2	2	8	6	56	9

population. Although differences occurred, they were not of a magnitude to suggest that a particular type was more commonly encountered in blood culture than would be expected on the basis of overall incidence.

There were 15 strains recovered from blood cultures representing 10 different types that were found each in only one or two patients.

The chronology of occurrence of Pseudomonas types shows those episodes in which an epidemic sequence may have taken place. This total incidence rate is shown in Figure 1.

The sequence of occurrence of bacteremia due to Pseudomonas makes it apparent that a given strain was involved in several outbreaks. Type NT-5 occurred in January, M-2 in March, each with two patients. NT-4 caused 4 episodes in May and June. The major epidemic episode was that caused by NT-3, in 9 patients from July through October. In September and October, NT-17 involved 3 patients, as did NT-16 in October-December. A 2-patient episode due to NT-18 occurred in November.

It was evident that no single phage type showed an invasive capacity out of proportion to its incidence, as would be evinced, by its disproportionate occurrence in bacteremia. What was equally clear was the succession of invasive episodes reflecting the dominance of a given type during a given period. There were 7 types which caused outbreaks, in from 2 to 7 patients, each during a circumscribed period. In addition, at least 9 other types caused bacteremia in patients either singly or at intervals too widely separated to constitute a sequence of infection in a single episode. This progression paralleled the changes in predominant types during a given period.

Figure 1. Phage Types of Pseudomonas from Blood Stream of ISR Burn Ward Patients, 1974

Month	Pat. No.	No. of Specs.	Phage Type Code										
			C26	D98	M 2	NT-3	NT-4	NT-5	NT-16	NT-17	NT-18	NT-22	Other
Jan	256	2						2					
	6	2						2					
Feb	30	1					1						
Mar	31	1			1								
	10	1			1								
Apr	61	4											NT -2 B71-2
May	68	1					1						
	80	1					1						
	62	3						3					
Jun	64	1					1						
	93	2					1						D18
Jul	126	1										1	
	117	1											F12
	100	1											D38
	121	1				1							
Aug	149	5				5							
	138	2											NT
	160	2				1							NT
	131	1				1							
	148	2				2							
	146	4				2						2	
Sep	143	1				1							
	135	1								1			
Oct	193	1								1			
	192	4		1						4			
	191	5				3			2				
	202	3				3							
Nov	214	2	2										
	213	1									1		
	210	2									2		
	177	3							2	1			
Dec	223	3							1				E11-2

Pseudomonas Phage Types Recovered From Lung Tissue at Post-Mortem, ISR - 1974

The bacteria recovered from lung tissue at autopsy furnish a definitive representation of the bacterial etiology of pneumonia complicating a severe burn. This problem of pneumonia has become a major part of the situation prevailing in the severely burned patient. The phage types of strains recovered from lung at autopsy are summarized in Table 3. It is apparent that the predominant type, NT-3, was also the predominant type in the overall type distribution. As with strains recovered in septicemia, the distribution roughly paralleled the overall incidence of phage types in 1974. NT-3 was again the most common type in autopsy lung samples. NT-5, second in incidence in lung, was third in overall distribution while the third type in frequency in lung tissue was fifth in overall distribution. The greatest discrepancy was with NT-4, seventh in frequency in lung samples but fourth in overall distribution. It is not plausible to expect exact correspondence in the lung samples; the discrepancies are not great enough to suggest a type with predetection for causing pneumonia. Instead, many types, if present, were obviously able to achieve this result.

Table 3. Phage Types of Pseudomonas aeruginosa from Lung Tissue of Burn Patients at Autopsy

Phage Type	No. Patients Positive	No. Strains
NT-3	11	27
NT-5	6	17
NT-17	6	18
NT-16	5	9
D-98	3	6
NT-22	3	5
NT-4	2	3
NT-18	2	3

The events summarized by patient for lung involvement by Pseudomonas during 1974 are shown in Figure 2. There were 6 types which showed the pattern of an epidemic outbreak for the burn population. NT-5 was the cause of 2 episodes in January, and appeared in 4 more patients in May and June. NT-3 caused pneumonia in 10 patients from July to October. NT-16, at more widely spaced intervals appeared in the same months, and NT-17 involved 5 patients in September and October. Remaining types were not involved in enough cases, spaced closely enough, to merit the designation of outbreak, with the possible exception of NT-22 in August and September.

Chronologic Sequence of Pseudomonas Phage Types, 1974

There is an apparent discrepancy between the designation of Pseudomonas

Phage Types of Pseudomonas from Post Mortem Lung Tissues of ISR Burn Ward Patients, 1974

Month	Pat. No.	No. of Specs.	Phage Type Code										
			C26	D98	M 2	NT-3	NT-4	NT-5	NT-16	NT-17	NT-18	NT-22	Other
Jan	1	4						4					
	6	2						2					
Feb													
Mar	10	4			4								
Apr	61	3											NT
May	90	2					2						
	62	3						2					D27-1 D42-1
	66	4						4					
	85	1						1					
Jun	93	1											A40
	48	1					1						
	87	4						4					
Jul	117	2											E13
	100	4											C38
	123	2				2							
	109	1											NT
	132	4				1			3				
	118	1				1							
Aug	160	3				3							
	131	1							1			1	
	148	4				4							
	146	4				3						3	
Sep	135	4								4		1	
Oct	193	4								4			
	192	4		4						2			
	191	4				2			2				
	202	4				4							
	176	3				2			2				
	185	4				4							
	205	4		1						4			
	137	2								2			
	194	4		1									C17-1 D23-1 D18-3
Nov	210	3									1		E13-3
	177	2								2			
	209	2				1			1				
	218	4	4								2		
Dec													

"epidemics" in the burn ward and the relatively small number of patients involved on an annual basis. Thus, NT 3 strains, the predominant type in 1974, were found in only 32% of the patients and made up only 24% of all isolates. There is, in fact, no single epidemic type but instead continually changing "epidemic" types. Thus, the number of patients harboring an epidemic type remains small in proportion to the large number of patients all positive for Pseudomonas. The 12 predominant types of Pseudomonas observed in 1974 were not found continuously during the year. One particular phage type appears, is seen perhaps for months, then disappears, never to be seen again. A few types appear sporadically over a long period of time. No explanation has yet been found for this unending succession of phage types.

Figure 3 illustrates the progression of predominant phage types as they occurred throughout the year. On the left is listed the Phage Type Code; then in blocks, the number of patients-number of strains of each phage type during each month of the year. On the far right is listed the yearly total of patient - strains for each particular phage type. At the bottom are the total patients and strains for each month. A solid dark frame in each month indicates the most prevalent phage type during that month and a partially dark frame, the secondary type. A double frame indicates the third most common type during that month. Types M-2, C-26 and D-41 were distributed throughout the year, and only M-2 had a month in which it was predominant. Type NT-4 was very numerous from January through July; then it virtually disappeared. NT-5 was similarly prominent for only five months; it then dwindled to a minimum level and after August was not seen again. With phage type NT-22, "new" types as they appeared and disappeared between May and October are shown. This type was the second most common type in August and September. NT 4 and NT-5 were carry-overs from 1973, when they were first found in April and May and persisted at a high incidence for the remainder of 1973, and into 1974, when they disappeared in August.

NT-3 was found on one patient in June and then explosively became the predominant type for 4 months. It was very much present in November and December. NT-16, starting with its appearance in July, became more numerous throughout 1974, and was the predominant type in December. NT-17 strains paralleled this distribution but were less numerous most of the time.

D-98, formerly a not uncommon pattern, appeared in small numbers from August to December. It was, in October, the third most common type. Types NT-18 and NT-21 appeared from September onward. Only one, NT 18, was a predominant strain in the month of November.

The complexity of a chronologic month-by-month summation of strain distribution of Pseudomonas is shown in this resume. Two major types, NT 4 and NT-5, had been for 16 months major types in causing Pseudomonas infection. They then disappeared totally, while two new epidemic types NT-3 and NT 16 played a major role in the Pseudomonas infection pattern for the remainder of 1974.

Figure 1. Monthly Distribution of Predominant Pseudomonas Phage Types in ISR Burn Ward Patients, 1974

Phage Type Code	Month												Total Patients-Strains Each Type
	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	
NT-4	3-14	3-15	1-2	1-3	5-39	5-13	2-7	1-1					14-94
NT-5	5-36	3-4	1-1	4-5	8-33	1-7	1-1	1-1					17-88
M 2	1-1	2-3	4-19					1-2					8-25
C26	1-1		1-1	1-1							2-9	1-12	5-24
D41			1-1	2-2		1-1				1-1		1-1	6-6
NT-22					1-1	1-2	2-5	3-21	3-5	1-4			7-38
NT-3						1-7	7-54	19-109	9-16	9-40	4-14	5-25	38-264
NT-16							3-16	3-13	1-3	4-24	4-9	7-43	21-110
NT-17							2-8	2-11	2-12	7-49	1-8	1-2	10-89
D98								1-1	2-12	6-26		1-6	8-45
NT-18									1-2		5-17	1-5	6-24
NT-21									1-2	3-12	1-3	2-7	7-25
Total Patients-Strains Each Month	8-54	11-29	10-34	12-58	19-114	10-59	20-127	24-179	18-65	17-177	13-68	13-121	- 832

PUBLICATIONS

1. Lindberg RB, Latta RL: Phage typing of Pseudomonas aeruginosa: Clinical and epidemiologic considerations. J Infec Dis 130:S33-S42, 1974.

PRESENTATIONS

Lindberg RB: "Typing of Pseudomonas aeruginosa" presented at seminar on Nosocomial Infections, Am Soc Microbiology in Chicago, Illinois. 1974.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA OC 6396	75 07 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
74 07 01	D. CHANGE	U	U	NA	NL	[X] YES [ ] NO	A. WORK UNIT

10. NO. CODES.*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A161102B71R	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

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
67 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 75	.6	19
FISCAL YEAR — CURRENT 76	.6	20

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

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

20. PERFORMING ORGANIZATION
NAME:* US Army Institute of Surgical Research
Microbiology Branch
ADDRESS:* Fort Sam Houston, Texas 78234

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME:* Robert B. Lindberg, PhD
TELEPHONE 512-221-2018
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: Virginia E. English, MA
NAME: Ruth L. Latta, BS DA

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 exhibits recurrent potential as an opportunistic invader. Its invasion of burn wounds has been reported from various sources. Characterizing of strains of nosocomial infecting agents is essential to understanding their spread and assessing effectiveness of control in burned soldiers.

24. (U) A phage typing set, derived from sewage, is propagated to a high titer yield to permit strain differentiation.

25. (U) 74 07 - 75 06 A comparison of annual typing results disclosed the two basic kinds of Serratia epidemics. In 5 of 7 years, multiple small epidemics in the form of successive type specific outbreaks occurred. In two annual increments, massive epidemics of a single phage type occurred. One major epidemic type appeared in successive years and finally appeared as a predominant epidemic.

*Available to contractors upon originator's approval

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, NO. 1, AND 1498-1, 1 MAR 68, FOR ARMY USE ARE OBSOLETE

ANNUAL PROGRESS REPORT

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

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

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

1 July 1974 - 30 June 1975

Investigators:

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

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

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 1974 - 30 June 1975

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

Reports Control Symbol MEDDH-188(R1)

An effective seven phage typing set has been used to differentiate over 850 strains of Serratia marcessens since 1967. During this time, numerous small epidemic episodes of a single strain were distinguishable, but in only two out of seven years did major outbreaks of a single type occur. The epidemiologic pattern of strain colonization, persistance and disappearance in a burn ward could be delineated precisely with this system, and epidemic outbreaks can be recognized. The importance of distinguishing the presence of epidemics of this potentially lethal opportunistic species are obvious and the value of phage typing with the Institute of Surgical Research set of phages has been shown.

Burns
Serratia
Bacteriophage

BACTERIOPHAGE TYPES OF SERRATIA MARCESSENS FROM BURN WOUNDS OF MILITARY PERSONNEL

The species of the family of Enterobacteriaceae capable of opportunistic colonization and invasion of burn wounds include a wide variety of enteric bacteria, and of these, Serratia marcessens displays an unpredictable but recurring aptitude for such invasion. Actual Serratia epidemics have been reported from some institutions, although this situation has not developed in the Institute of Surgical Research. Sporadic instances and small outbreaks of Serratia have occurred here, but a sustained epidemic outbreak has not been encountered. In view of its ability to infect burn patients, however, a systematic program for monitoring the strain identity of Serratia is maintained. This includes a cultural technic that concentrates on detection of non-pigmented forms, and a typing of isolates by use of a bacteriophage set collected in this Institute from sewage (1). The set of typing phages has been used to characterize strains recovered in the Institute of Surgical Research since 1967, and the nature of the seeding of burn patients by this opportunistic pathogen, its variability and the degree to which individual strains are selected by the patient population in which they are propagated, have been elucidated by this approach.

The typing system as evolved consisted of seven phages. As with any strain differentiating technic, the essential question was, whether a sufficiently detailed screening structure had been devised, so that strains varying in identity could be distinguished, and also whether identical strains would fall into a single group. This objective was achieved; in 5 out of the 7 years the number of type patterns was such that the average number of strains per pattern was quite small, i.e., 3.1 to 4.5 strains per pattern.

Table 1 summarizes the progression of Serratia infections that has occurred in burn ward patients since an effective typing system was available. Note that in 1967, as an illustration, 3 phage types recurred in enough patients to merit consideration as a sequential transmission, although the maximum number of patients with one type was 6 out of a total of 27 patients with positive cultures. Sixteen patients had one of 3 types as the infecting strain. The remaining 16 types that were recovered were distributed among 11 patients and also occurred among those patients who harbored the predominant type.

A similar pattern of type distribution occurred in 1968 through 1971. There would be a relatively large number of types that occurred each in a small number of patients, (often in only one patient); then there would appear a small epidemic pattern of 3 or 4 types which recurred in several patients, although any one of these predominant types would be found in only

---

1. English VC, Latta RL, Brame RE, Lindberg RB: Development of a bacteriophage typing system for organisms of the genus Serratia. USA Surgical Res. Unit Annual Rpt FY 1968, BAMC, Ft Sam Houston, Texas. Section 32.

Table 1. Phage Types of Serratia marcessens in Burned Patients, ISR - 1967-1973

Year	Total Strains	Total Types	No. Patients Positive	Predominant Type	Patients With This Type		Strains of This Type	
					No.	% of All Pts.	No.	% of all Strains
1967	59	19	27	3,5,7,9,11,15,18	6	22.2	8	13.5
				3,5,7,11,15	5	15.5	21	35.5
				3,5,7,11,15,18	5	15.5	7	11.8
1968	118	26	51	5,7,9,11,15,18*	7	13.7	18	15.2
				5,7,15	7	13.7	7	5.9
				11,15	7	13.7	16	13.5
				15	8	15.6	16	13.5
1969	168	37	78	5,7,9,11,15,18*	10	12.8	19	11.3
				5,7,9,15,18	4	5.1	4	2.3
				7,9,15	7	8.9	15	8.9
1970	117	26	49	5,7,9,11,15,18*	10	20.4	29	24.7
				5,7,9,15,18	8	16.3	9	7.6
				15	8	16.3	13	11.1
				18	5	10.2	7	5.9
1971	50	12	23	3,5,7,11,15,18	5	21.7	11	22.0
				5,7,9,11,15,18*	3	13.0	7	14.0
				15	3	13.0	3	6.0
1972	100	14	27	11	19	70.3	63	63.0
1973	139	10	24	5,7,9,11,15,18*	17	70.8	98	70.5
				15	4	16.6	7	5.0

* This type: 5,7,9,11,15,18, was the most frequently encountered predominant type

a small part of the total of patients positive for Serratia. In 1970, as an illustration, the predominant type occurred in 10 patients, but two other types occurred each in 8 patients, and one more occurred in 5.

There was one type, out of all the phage patterns distinguished, that recurred most often. This was type 5,7,9,11,15,18. If any type could be described as an endemic strain, this one would fit that category. It was the cause of one of the two unequivocal outbreaks of Serratia marcessens infection, when an epidemic episode of Serratia occurred in 1973. There were 24 patients who harbored Serratia in some site, and of these, 17 were positive for 5,7,9,11,15,18.

A monotype epidemic unique in the course of this study occurred in 1972, when type 11 was found in 19 out of 17 patients positive for Serratia.

A more detailed study of the strains that have incited epidemic type outbreaks is shown in Table 2. Type 5,7,9,11,15,18 was the most common type recovered in 3 years, and the next most common in 2 more years. Type 15 was a recurrent type of major importance: in 1968 it was the most important type numerically, in 1969, 1970, and 1973 it was second in occurrence and in 1969 it was third most frequently encountered.

A type which was numerically important in 2 years was 3,5,7,11,15,18 It was second in occurrence in patients in 1967; four years later, in 1971, it was found in more patients than any single type.

Type 11 has only once been numerically important. It was the cause of a major epidemic episode in 1972. Seventy per cent of all patients positive for Serratia harbored type 11; the remaining 14 types found in that year were scattered so that none were found in more than one patient.

The implications of this analysis of type distribution of Serratia marcessens are significant in their explanation of the epidemiologic potential of this opportunistic burn pathogen. Serratia in blood stream, lung and burn wound can act as a highly lethal organism, and its recovery as the causative agent in sepsis has been reported in this Institute on several occasions. It appears, however, to be most often present as a transient inhabitant of the burn ward; brief epidemic episodes of single type infections tend to die out, and a heterogeneous flora is often present. But the organism can establish epidemic infection patterns, as were seen in 1972 and 1973. One of these was with an exotic, seldom seen type, type 11. The other was a numerically predominant outbreak of a type that in 4 earlier years had occurred without once involving more than 12% of all the patients harboring Serratia in a given year.

Explosive persistent epidemics are prone to consist of strains which exhibit increasing virulence and increasing antibiotic resistance as they progress. Thus far they have been recognized in retrospect after they had subsided by whatever natural causes determine such ebb and flow of strains in the burn population. But the capacity for serious damage and

Table 2. Incidence of Four Epidemic Types of *Serratia marcessens*

Year	Type	Order of Frequency	Patients		Strains	
			No. Positive Pos. Pts.	% of all Serratia	No.	% of all Strains
1968	5,7,9,11,15,18	2	7	13.7	18	15.2
1969		1	10	12.8	19	11.3
1970		1	10	20.4	29	24.7
1971		2	3	13.0	7	14.0
1972		0	0		0	
1973		1	17	70.8	98	70.5
1968	15	1	8	15.6	16	13.5
1969		3	5	6.4	5	2.9
1970		2	8	16.3	13	11.1
1971		3	3	13.0	3	6.0
1972		-	0		0	
1973		2	4	16.6	7	5.0
1967	3,5,7,11,15,18	2	5	18.5	7	11.8
1971		1	5	21.7	11	22.0
1972	11	1	19	70.3	63	63.0

uncontrollable sepsis inherent in such transmissible epidemics makes their recognition of greater importance. The system here devised and tested can effect precise identification of epidemic types. Control measures in such situations are not necessarily obvious, but until the situation is recognized, little effective action can be taken.

PRESENTATIONS AND/OR PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION* DA OE 6382	2. DATE OF SUMMARY* 75 07 01		REPORT CONTROL SYMBOL DD DR&E(AR)636
3. DATE PREV SUM'RY 74 07 01	4. KIND OF SUMMARY D. CHANGE	5. SUMMARY SCTY* U	6. WORK SECURITY* U	7. REGRADING* NA	8a. DISP'N INSTR'N NL	8b. SPECIFIC DATA-CONTRACTOR ACCESS ☒ YES ☐ NO	9. LEVEL OF SUM A. WORK UNIT
10. NO. CODES:*	PROGRAM ELEMENT	PROJECT NUMBER		TASK AREA NUMBER	WORK UNIT NUMBER		
a. PRIMARY	61102A	3A161102B71R		01	317		
b. CONTRIBUTING							
c. CONTRIBUTING							

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

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*
003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
71 10	Cont	DA	C. In-House

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

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 75	.5	15
FISCAL YEAR CURRENT 76	.3	8

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

20. PERFORMING ORGANIZATION
NAME:* US Army Institute of Surgical Research
ADDRESS:* Burn Study Branch
Fort Sam Houston, Texas 78234
PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME:* Hugh D. Peterson, COL, MC
TELEPHONE 512-221-3301
SOCIAL SECURITY ACCOUNT NUMBER:
ASSOCIATE INVESTIGATORS
NAME: Daryl R. Erickson, 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) Five percent Sulfamylon acetate solution has been shown to be an effective topical antibacterial agent when used with wet to dry dressings during the latter stages of eschar separation and when used as a continuous soak covering areas which have been mesh grafted in soldiers with burn injury.

24. (U) Five percent Sulfamylon acetate is used as a debriding agent by applying gauze sponges soaked in the solution to the burn wound and wrapping the area. The gauze sponges are applied soaked with the solution, allowed to dry and removed dry every six or eight hours. The solution is also used to keep the dressing covering mesh grafted areas wet until there has been epithelial coverage of the open areas.

25. (U) 74 07 - 75 06 Five percent Sulfamylon acetate solution was used in 137 patients. In 128 cases it was used with wet to dry applications of coarse mesh gauze to facilitate debridement. In 110 cases it was used in conjunction with mesh grafting. There was one case of severe respiratory insufficiency and three cases of combined toxic encephalitis and respiratory insufficiency in these patients which if due to this agent, represents a complication incidence of 1.7%.

*Available to contractors upon originator's approval

ANNUAL PROGRESS REPORT

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

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

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

1 July 1974 - 30 June 1975

Investigators:

Hugh D. Peterson, DDS, MD, COL, MC
Daryl R. Erickson, MD, MAJ, MC
Basil A Pruitt, Jr, MD, COL, MC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

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

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

Period covered in this report: 1 July 1974 - 30 June 1975

Investigators: Hugh D. Peterson, DDS, MD, COL, MC
Daryl R. Erickson, MD, MAJ, MC
Basil A. Pruitt, Jr, MD, COL, MC

Reports Control Symbol MEDDH-288(R1)

Five per cent Sulfamylon acetate solution is used as the wetting agent in the wet to dry coarse mesh gauze debridement technique and for the continuously wet dressings which cover "meshed" autografts or excised areas in the burned soldier. Fifty-six point one per cent of all admissions had the agent used at least for one of the above mentioned techniques. Seventy-six per cent of the patients receiving the agent had it used for more than one of the treatment modalities. Only one patient had severe respiratory problems apparently related to the application of 5% Sulfamylon acetate solution for a presumed 0.7% complication rate. One patient had severe respiratory problems while being treated with the Sulfamylon acetate cream in addition to the 5% Sulfamylon acetate solution. Another patient had severe respiratory distress related to the topical application of the Sulfamylon acetate cream very early in her burn course and received the 5% Sulfamylon acetate solution on three separate occasions later in her course without manifesting any signs of toxicity.

Burn
Eschar separation
5% Sulfamylon acetate solution
Humans

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

Five per cent Sulfamylon acetate solution was used in 137 patients or 56.1% of all admissions. It was used on 128 patients as the wetting agent in the wet to dry coarse mesh gauze debridement technique. It was used as the antibacterial wetting agent for continuously wet dressings covering "meshed" autografts or excised areas in 110 patients. Most of these patients (76%) had the 5% Sulfamylon acetate solution used for both treatment modalities.

Toxic reactions manifested by pulmonary and/or cerebral signs were clinically diagnosed as being related to the use of 5% Sulfamylon acetate solution in four cases. The apparent pulmonary signs of Sulfamylon acetate toxicity were initially hyperventilation followed by pulmonary infiltrates seen on chest roentgenograms.

It appears as if one of these patients should be excluded even though he was receiving the agent because he had blood cultures positive for coagulase positive Staphylococcus on each of the three occasions in question. He responded on each occasion to having the agent discontinued; however, he also had multiple other therapeutic maneuvers carried out including changes in antibiotics.

Another of these patients did have hyperventilation, severe obstructive lung disease and marked pulmonary infiltrates beginning on post burn day six, the fifth day of Sulfamylon acetate cream therapy. She had no evidence of fluid overload nor of sepsis. She also was not receiving 5% Sulfamylon acetate solution - only the cream. Of interest is the fact that she went on to receive 5% Sulfamylon acetate solution on three separate occasions later in her course without manifesting any signs of toxicity.

One patient had marked pulmonary signs without any evidence of sepsis while receiving 5% Sulfamylon soaks to extensive areas in addition to the Sulfamylon acetate cream on other parts of his body. He recovered when all Sulfamylon therapy was stopped. This patient subsequently had 5% Sulfamylon acetate solution used on four separate occasions without any difficulty.

Only one patient had severe pulmonary problems with the 5% Sulfamylon solution that could not be related to sepsis or use of Sulfamylon cream. He had two separate episodes of respiratory distress, the last requiring tracheostomy. His pulmonary signs cleared within two days after stopping the use of the solution on both occasions.

In summary, there were three patients who had respiratory problems apparently related to the application of Sulfamylon acetate; however, only one was receiving the aqueous solution in the form of soaks. Since 5% Sulfamylon acetate solution was applied to 137 different patients, the incidence of severe complications apparently is only 0.7%.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OB 6982	75 07 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
74 07 01	D. CHANGE	U	U	NA	NL	[X] YES [ ] NO	A. WORK UNIT

10. NO. CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A161102B71R	01	223
b. CONTRIBUTING				
c. CONTRIBUTING				

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

12. SCIENTIFIC AND TECHNOLOGICAL AREAS
003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
65 06	Cont	DA	C. In-House

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

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
FISCAL YEAR PRECEDING 75	.5	14
FISCAL YEAR CURRENT 76	.5	15

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

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

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

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME: Robert B. Lindberg, PhD
TELEPHONE: 512-221-2018
SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS
NAME:
NAME:

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

DA

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Burns; (U) Sulfamylon; (U) Semi-synthetic penicillins; (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 and systemic agents in the control of burn wound sepsis in a controlled burn rat model indicates degree of effectiveness of established methods, reveals mechanisms of pathogenesis in new burn strains and aids in improving therapeutic approaches to improve survival of burned troops.

24. (U) Surveillance of infecting types and their response to topical therapy has uncovered categories of refractorness to therapy that had not been known.

25. (U) 74 07 - 75 06 Analysis has revealed possibly 12 to 14 type specific epidemics of Pseudomonas aeruginosa refractory to Sulfamylon (mafenide acetate) treatment. Systemic ticarcillin a semi-synthetic penicillin has effected recovery in otherwise unretrievable infections. Its behavior merits consideration for clinical trial.

*Available to contractors upon originator's approval

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE AND 1498A 1 MAR 68 IN ARMY USE ARE OBSOLETE

ANNUAL PROGRESS REPORT

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

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

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

1 July 1974 - 30 June 1975

Investigators:

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

Report Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

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

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

Period covered in this report: 1 July 1974 - 30 June 1975

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

Report Control Symbol MEDDH-288(R1)

The existence of epidemic outbreaks of relatively resistant strains of Pseudomonas aeruginosa on the burn ward was uncovered by phage typing of isolates. Although most of these outbreaks were with strains that responded in vivo to topical therapy with Sulfamylon[R], there were strains that could not be effectively treated with Sulfamylon. Epidemics of such strains pose a threat of invasive sepsis due to Ps aeruginosa that could be very dangerous to patients. Ticarcillin, a new experimental semi-synthetic penicillin, was markedly effective in preventing sepsis in animals in which other therapies had no effect. This drug merits serious consideration as an adjunct in burn therapy.

Burns
Sulfamylon
Pseudomonas
Semi-synthetic penicillins
Rats

DEVELOPMENT OF PROPHYLATIC TOPICAL THERAPY FOR USE ON BURN WOUNDS OF MILITARY PATIENTS. THE SEARCH FOR NEW FORMULATIONS

The role of Pseudomonas aeruginosa in burn wound infection has been a recurrent problem; therapy has been directed toward it for at least two decades; a succession of broad spectrum antibiotics and a semi-synthetic penicillin have offered a degree of specific antibacterial action undreamed of 20 years ago. Yet, severely burned patients still face a high likelihood of, at best, Pseudomonas colonization, and systemic involvement in such forms as pneumonia, urinary tract infection or invasive wound sepsis are frequent occurrences. Current literature is replete with allusions to the growing problem of Pseudomonas infection; the species is, if anything, continuing to expand its numbers and the frequency with which it involves traumatized or compromised hosts.

Ps aeruginosa is capable of setting up in properly burned and seeded rats, a burn wound sepsis lesion virtually identical with the disease in human burn victims, and this infection model has been of great value in developing formulations for topical therapy in burns. The Sulfamylon® formulation was initially derived using a lethal strain which could be effectively treated with daily topical application. As more strains were tested, the range of resistance of Ps aeruginosa was shown to extend from 0 to 100%. At first it was believed that all strains would respond to topical treatment with Sulfamylon, but it developed that treatment refractory strains exist [illegible] the search for definite treatment.

[illegible] resistant strains of Ps aeruginosa [illegible] was not initially recognized, but after [illegible] of this phenomenon with type A-21 in 1969, a search of [illegible] types to Sulfamylon revealed that this situation had [illegible] the past 3 years, the sensitivity of specific types had varied, [illegible] of [illegible] form in the burn ward. Table 1 shows the [illegible] incidence of strains which required 0.5% or more of Sulfamylon [illegible] this concentration did not, in itself, indicate [illegible] strains could in some instances [illegible] Sulfamylon [illegible].

Table 1. Epidemic Episodes of Ps aeruginosa due to in vitro Sulfamylon Refractory Types

Phage Type	Year and Number of Patients with Type						
	1967	1968	1969	1970	1971	1972	1973
E-2	11	14					
A-87	19						
M-2			13	22			
B-13			42				
F-3					17		
H-15				17			
M-4					12		
NT-2						57	
NT-1						14	
A-71							25
NT-5							21

under study were distinguished by relatively high virulence and refractoriness to treatment. All in vitro resistant strains were not like these; many were less than completely virulent and most of them responded to treatment. But these epidemic strains were resistant in vitro and treatment-refractory. These factors are summarized in Table 2.

Table 2. Response to Treatment with Sulfamylon of Experimentally Burned Rats Seeded with Sulfamylon-Resistant Epidemic Strains of Ps aeruginosa

Year	Strain	Mortality in Burned Seeded Rats - %	
		Sulfamylon Treated	Untreated
1967-8	E-2	92.5	100
1967	A-87	87.2	96.6
1969	B-13	97.7	100
1972	NT-2	94.4	92.5
	NT-1	90.2	93.7
1973	NT-5	89.5	94.4
	A-71	92.3	96.9
	Treatment-Responsive Epidemic Strains		
1969-1970	M-2	12.2	96.8
1971	F-3	7.6	100

Sensitivity to Sulfamylon: Ave. 0.48
Range 0 - 0.62

Seven strains, over this 7-year period, were highly virulent for the burned rat model and were relatively resistant to Sulfamylon in vitro. Two strains conformed to the in vitro part of the resistant epidemic strain, but they responded to Sulfamylon treatment in the classic manner.

Certain of the epidemic strains exhibited a behavior pattern that suggested they were indeed the cause of a disproportionately large part of specific Pseudomonas sepsis. An example was the epidemic outbreak due to type B-13 in 1969 (with a brief extension into 1970). Table 3 illustrates this sequence of events. The strain had been seen first on 4 patients in 1965, and 5 in 1966, on one in 1967 and on 4 in 1968. It rose abruptly to epidemic proportions, but more than any other widespread type, it was the cause of 12 cases of septicemia and 3 of burn wound sepsis. This type of epidemic outbreak is not necessarily recognized at once, since other strains were present at the same time and a widespread seeding of burn wounds occurred. Of these strains, there were 24 different types each of which involved at least 4 patients. However, in reporting on type B-13 at the time, the statement was made that "Type B-13, in relation to its incidence on wounds, strongly suggested that an exceptionally invasive or virulent strain was present....during this year (1969)".

Table 3. Sepsis due to Ps aeruginosa, Type B-13: Epidemic Strain, 1969

No. of patients with positive cultures:	21
No. of patients with positive blood cultures	12
Month of first positive blood culture:	
April	2 patients
May	2 patients
June	1 patient
July	2 patients
August	4 patients
September	1 patient
Patients developing burn wound sepsis	3

Topical therapy, in the experimental model, was clearly not an effective answer to the problem of the treatment-refractory strains. Antibiotics have for the most part been ineffective in controlling burn wound sepsis in the experimental animal. This was one of the basic reasons for embarking on research directed toward control of Pseudomonas burn wound sepsis by topical chemotherapy. In 1970, carbenicillin was described as possessing a degree of effectiveness in controlling burn wound sepsis, but the phenomenon was strain related, and this attribute tended to minimize the potential of this antibiotic approach (1).

1. Lindberg RB, Curreri PW, Pruitt BA, Jr: Microbiology of burns treated with carbenicillin: Experimental and clinical observations. J Infec Dis (S) 122 :534-539, 1970.

In view of the experimental and clinical evidence that specific strains of Ps aeruginosa were unaffected by topical Sulfamylon, and that a high incidence of sepsis occurred as a complication of colonization with such strains, other chemotherapeutic agents were investigated. A new semi-synthetic penicillin, BRL-2288, designated Ticarcillin, was obtained from Beecham-Massengill Pharmaceuticals. The in vitro behavior of this drug compared with carbenicillin, showed that the same population of Ps aeruginosa of which 80 would be inhibited by 20 ug/ml, would require 100 ug/ml to inhibit 70% of the same strains. The drug was administered i.p. in 100 ug/day doses in 200 gm rats, for a daily input of 0.5 ug/gm for 10 days. Parallel treatment with carbenicillin was used; with this drug the dose was 150 ug i.p./day.

Results of this comparative trial are summarized in Table 4. Six highly virulent strains were selected for test. One of them, A-87, responded with survival of 89% of the animals with both antibiotics. The strain was completely virulent. With each of the other 5 strains, survival was not affected by carbenicillin, but Ticarcillin was strikingly active. Only one strain, A-71, was completely suppressed by the Ticarcillin. In the remaining 4 strains from 12.5% to 25% of the Ticarcillin-treated animals succumbed, in contrast to the carbenicillin-treated animals, in which the same strains were lethal for from 89.7% to 95.7% of the treated animals.

Table 4. Carbenicillin and Ticarcillin in Control of Experimental Burn Wound Sepsis due to Sulfamylon-resistant, Treatment-refractory Ps aeruginosa

Strain	Mortality: % of Animals Tested		
	Carbenicillin	Ticarcillin	Control
87	11	11	100
13	91	27	89.7
T-2	80	25	95.7
T-1	100	20	95.0
-71	83.3	0	85.0
T-5	100	12.5	94.0

Treatment: Carbenicillin: 150 ug/i.p./day/10 days
Ticarcillin: 100 ug/i.p./day/10 days

Treatment begun 24 hours post-seeding

This result was unique in the entire course of study of therapy of Pseudomonas burn wound sepsis. No other therapeutic agent has, when administered parenterally, acted to arrest the process of burn sepsis and permit survival.

DISCUSSION

The existence of Sulfamylon-resistant strains of Ps aeruginosa which could incite lethal burn wound sepsis despite topical Sulfamylon was not previously established. The treatment failure is not simply a reflection of in vitro resistance, since there are even more strains which cannot be successfully treated in the experimental animal, even though the strains are sensitive in vitro. The fact is that in vitro sensitivity or resistance is not a criterion of susceptibility to in vivo treatment. There is, of course, a degree of correlation but in any given strain, only experimental trials will tell whether the organism is treatment responsive or not.

In view of the demonstration of occasional epidemic strains, resistant in vitro to a level of 0.312% or greater, it would appear to be advisable to assess the presence of epidemic strains on the burn ward more promptly, so that, if strains that are potentially dangerous and treatment refractory are present, alternative approaches to treating such sepsis may be developed. The behavior of Ticarcillin is particularly promising; if this antibiotic continues to show, on further investigation, a major capability for arresting the progress of invasive Pseudomonas infection, then its use in human illness as an investigative drug may well be merited.

PUBLICATIONS

Lindberg RB, Latta RL, Pruitt BA, Jr: Transfer and Control of Hospital Epidemics of Drug-resistant Pseudomonas aeruginosa. *Annual meeting Am Soc Microbiol.* (75th annual meeting). C.83, p. 40 (Abstract).

PRESENTATIONS

Lindberg RB: "Hospital epidemics of Pseudomonas aeruginosa resistant to topical therapy on burned patients". Presented at 7th Annual Meeting, Am Burn Assoc., March 21, 1975 in Denver, Colorado.

Lindberg RB: "Transfer and control of hospital epidemics of drug resistant Pseudomonas aeruginosa". Presented at Am Soc Microbiol. annual meeting 1 May 1975, in New York, New York.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA OB 6978	75 07 01	DD DR&E AR(6)7A

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
74 07 01	D. CHANGE	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO. / CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A161102B71R	01	219
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)* (U) The Role of Fungi in Burn Wound Infection: Observations on Biopsy and Autopsy Tissues from Seriously Burned Soldiers (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*
003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
66 02	Cont	DA	C. In-House

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

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 75	.6	18
FISCAL YEAR CURRENT 76	.4	7

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

20. PERFORMING ORGANIZATION
NAME:* US Army Institute of Surgical Research
Microbiology Branch
ADDRESS:* Fort Sam Houston, Texas 78234
PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME:* Robert B. Lindberg, PhD
TELEPHONE 512-221-2018
SOCIAL SECURITY ACCOUNT NUMBER
ASSOCIATE INVESTIGATORS
NAME: Anthony A. Contreras, MS
NAME: DA

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Fungi; (U) Mucor; (U) Rhizopus; (U) Fusarium; (U) Burns; (U) Phycomycosis; (U) Humans

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

23. (U) To determine species of fungi in burn patients and the importance of such opportunistic invaders in producing pathologic changes in burned soldiers.

24. (U) Routine cultivation of all biopsy samples and autopsy specimens. Continues search for improved substrates and technics for recovering fungi.

25. (U) 74 07 - 75 06 The rate of fungus colonization has diminished in terms of strains isolated in the last two years, although the range of species is little changed. Fusarium and Cephalosporium are the two principal genera in autopsy tissues. Candida sp. remained numerically the principal group recovered. Phycomycetes (Rhizopus and Mucor) were recovered in small numbers but invasive phycomycosis was not seen, at least in terms of cultivation of the organism. Eighty-three percent of patients autopsied had at least one tissue positive for yeast or fungi.

*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. 3A161102B71R-01, RESEARCH IN BIOMEDICAL

REPORT TITLE: THE ROLE OF FUNGI IN BURN WOUND INFEC
ON BIOPSY AND AUTOPSY TISSUES FROM SI
SOLDIERS

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

1 July 1974 - 30 June 19

Investigators:

Robert B. Lindberg, PhD
Anthony A. Contreras, MS
Harvey O.D. Smith, Jr., SP6

Report Control Symbol MEDDH 288(R1)

CONFID

ABSTRACT

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

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

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

Period covered in this report: 1 July 1974 - 30 June 1975

Investigators: Robert B. Lindberg, PhD
Anthony A. Contreras, MS
Harvey O.D. Smith, Jr, SP6

Reports Control Symbol MEDDH-288(R1)

Cultivation of fungi from biopsy and autopsy tissues is carried out routinely since fungal sepsis has been a serious clinical problem when it occurs. Seventeen genera of fungi were recovered. Candida spp was the predominant form, and among true fungi, Fusarium and Cephalosporium were most common. Phycomycetes (Mucor and Rhizopus) were recovered from these tissues in small numbers and were not involved in systemic mycoses. The role of fungi in burn wound infection has apparently lessened.

Fungi
Mucor
Rhizopus
Fusarium
Phycomycosis
Burns
Humans

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

Interest in the problem of fungal infection in burns has increased greatly in the past 5 years. With increasing frequency episodes of fungal or yeast invasion of burn wounds have been reported. Whether the problem is indeed growing progressively more severe may still be open to question: in the Institute of Surgical Research the incidence of severe wound invasion by non-septate fungi of the genus Mucor and Rhizopus has become virtually nil. However, yeast colonization is extremely common on burn wounds and continued monitoring of biopsy and autopsy tissues has revealed the organisms still to be present. Increased expertise at recovering fungi undoubtedly has helped to make the detection of a fungal infection more accurate and reliable.

Fungi in Biopsy Specimens from Burned Patients

There were 135 patients on whom biopsies were performed in 1974, and 585 samples (4.3 specimens per patient) were cultured. Fungus and yeast culture results are shown in Table 1. Similar data for 1972 and 1973 are shown for comparison. The total number of genera of fungi (excluding yeasts) recovered was 13 in 1972, 10 in 1973, and 7 in 1974. There appeared to be a distinct drop in diversity of species, as well as in number of strains; 90 were isolated in 1972, 64 in 1973 and 41 in 1974. Some genera have been consistent in appearing every year; others have been episodic in appearance. Consistent species included Aspergillus, Cephalosporium, Fusarium. Curvularium, Alternaria, and Penicillium. Genera collected in 2 out of 3 years were Helminthosporium, Rhizopus, Mucor and Geotrichum. Species which appeared in only one year included Sepedonium, Scopulariopsis, Diplosporium, and Stemphyllium. In biopsies, the most conspicuous fungal genus was Fusarium. Yeasts were more numerous, of course, than fungi. Candida spp. have, if anything, become more numerous in 1974.

Fungi Recovered in Autopsy of Burned Patients.

The recovery of fungi from burn wounds and viscera at autopsy is summarized in Table 2. Fungi were more frequently recovered from autopsy specimens than from biopsy tissues. There is an empirical basis for this result: the autopsy tissues have presented more opportunity for fungi to take root, and among these fatalities are a relatively large number of lethally injured patients whose defense mechanisms against infection are virtually non-existent.

The visceral samples were almost as frequently colonized as were wound samples. Most of these positive tissues were lung samples. There were 13 genera of fungi, plus two samples of non-fruiting "mycelia sterila" and Candida spp. recovered from wound samples. Twelve genera of fungi were recovered from viscera. Exactly as with biopsy specimens, the predominant genus for autopsy samples was Fusarium. Cephalosporium is a very closely related genus, and it was evident that this genus was the second most commonly

Specimens Positive for Fungi and
Years 1972-1974

	No. Patients Positive			No. Tissues Recovered		
	1972	1973	1974	1972	1973	1974
Aspergillus	8	8	8	11	17	5
Penicillium	5	4	5	15	7	6
Fusarium	13	12	11	33	22	17
Sepedonium	0	1	0	0	1	0
Curvularium	3	2	1	3	2	1
Scopulariopsis	11	0	0	11	0	0
Alternaria	3	2	3	7	4	3
Diplosporium	1	0	0	1	0	0
Penicillium	1	1	3	1	1	3
Helminthosporium	0	4	2	0	4	2
Rhizopus	2	2	0	3	2	0
Mucor	2	2	0	2	2	0
Stemphyllium	1	0	0	1	0	0
Syncephalastrum	1	0	0	1	0	0
Geotrichum	1	0	2	1	0	2
Candida sp.	28	57	34	40	161	100
No. Patients Cultured 1974	135					
No. Tissue Samples 1974	585					
Number of species recovered		13	10	7		

Table 2. Genera of Fungi Recovered from Viscera (Liver, Lung, Spleen) and Burn Wounds at Autopsy 1974

	Patients Positive Wounds	Total Isolates Wounds	Patients Positive Viscera	Total Isolates Viscera
Mucor	3	6	1	1
[illegible]	1	1	0	0
[illegible]	4	9	2	2
[illegible]	3	4	6	8
[illegible]	1	1	2	2
[illegible]	4	4	8	17
[illegible]	12	35	13	29
[illegible]	4	4	1	6
[illegible]	2	6	2	3
[illegible]	1	1	1	1
[illegible]	3	3	1	1
[illegible]	47	93	47	[illegible]
[illegible]	2	2	2	2
[illegible]		1		1
[illegible]	1		1	

[illegible]

recovered form from both biopsy and autopsy tissue. It is probable that some of the Fusarium-Cephalosporium identifications could be reversed; the morphologic criteria for the two genera are very close. The two genera with the most serious pedigree for severe deep invasion of tissue are Mucor and Rhizopus. Each of these genera were represented both in biopsy and autopsy samples.

Since autopsy fungus cultures offer a broader spectrum of recovery of genera, a comparison of recovery rates for 1971 through 1974 was made, as shown in Table 3. The number of genera, including Candida, varied from 17 in 1971 to 15 in 1974. Relatively rare species which have not been recovered in the past 2 or 3 years in autopsy specimens included Absidia, Paecilomyces, Nigrospora, Diplosporum, Fonsecaea, and Microsporum. There were, in all, 14 genera in biopsies and autopsies that occurred often enough to be considered a plausible part of the fungal flora on burn wounds, but of these, only 10 occurred with at least a moderate level of annual frequency.

Fungi were not routinely sought prior to 1969. The sharp rise in incidence in 1970 was consistent with concern about the increase in clinically significant fungal burn wound infections. The occurrence of this syndrome, however, diminished markedly in 1973 and 1974, and although the number of species and the frequency of occurrence has remained relatively constant, the rate of actual tissue invasion has fallen markedly. The frequency with which fungi are found in burn tissues appears to be diminishing. The program of monitoring fungi in biopsies and autopsies will be continued, but the occurrence of this group of organisms may well continue to diminish. The consequences of invasive sepsis are too severe to omit surveillance of the fungi in burn patients.

PRESENTATION AND/OR PUBLICATION

None

Table 1. Autopsy Tissues Cultured for Fungi
Fungi Recovered from Viscera (Lung, Liver, Spleen)
and Burn Wound at Autopsy, 1971 - 1974

Genus	Number of Patients Positive at Autopsy							
	Burned Wound				Viscera			
	1971	1972	1973	1974	1971	1972	1973	1974
Mucor	3	7	3	3	0	0	1	1
Rhizopus	1	[illegible]	2	1	1	1	0	0
Absidia	1	1	0	0	0	0	0	0
Aspergillus	6	11	19	4	3	2	5	2
Penicillium	8	1	0	1	1	2	0	5
Paecilomyces	0	0	0	0	1	2	0	0
Alternaria	2	3	6	1	0	0	0	2
Cephalosporium	2	4	3	4	0	4	4	8
Fusarium	9	30	25	12	7	20	19	8
Helminthosporium	4	1	4	4	0	0	2	1
Nigrospora	9	0	0	0	3	0	0	0
Scopulariopsis	5	3	1	2	1	2	0	2
Sepedonium	1	5	5	1	1	4	3	1
Diplosporum	1	2	0	0	0	0	0	0
Geotrichum	0	0	3	0	2	0	0	1
Fonsecaea	2	0	0	0	2	0	0	0
Curvularia	0	5	1	0	0	0	0	0
Microsporum	0	1	0	0	0	0	0	0
Cladosporium	0	0	1	0	0	0	3	0
Candida	13	32	67	41	11	25	50	42
Number patients positive	46	65	80	67				
Total patients cultured	61	89	91	80				
Number genera present	17	15	14	15				

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0F 6398	75 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
75 07 01	D. CHANGE	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO. CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A161102B71R	01	319
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code) (U) Non-Fermentative Gram-Negative Bacilli in Burned Soldiers: New Potential Opportunistic Pathogens (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS
003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
74 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 75	.8	26
	CURRENT 76	.7	18

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

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

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

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME: Robert B Lindberg, PhD
TELEPHONE: 512-221-2918
SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS
NAME: Virginia E English, MS
NAME: DA

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Burns; (U) Oxidative Gram-Negative Bacilli; (U) Pseudomonas; (U) Acinetobacter; (U) Alcaligenes; (U) Flavobactrum; (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) Infection in soldiers with thermal injury is a main cause of morbidity and death. The significance of an apparent rise in the number of non-fermentative gram-negative bacilli in wounds is to be sought by precise characterization of this poorly-understood group of bacteria.

24. (U) Special culture technics and substrates appropriate to the groups concerned will be set up and studied. Emphasis will be on improved recognition of colonies on primary isolation.

25. (U) 74 07 - 75 06 A significant increase in non-fermentative gram-negative bacilli in burn tissues on autopsy was established. Most of these were isolated as predominant organisms in quantitative dilution plates, which means that they were missed in primary isolation. Pseudomonas maltophilia, Alcaligenes odorans var viridans and Flavobacterium sp. were the prominent species but 15 additional species in these genera and in Acinetobacter were recovered. The epidemiology of these species and their degree of pathogenicity in the burn patient are to be studied.

*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. 3A161102B71R-01, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: NON-FERMENTATIVE GRAM-NEGATIVE BACILLI IN BURNED SOLDIERS: NEW POTENTIAL OPPORTUNISTIC PATHOGENS

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

1 July 1974 - 30 June 1975

Investigators:

Virginia C. English, MA
Robert B. Lindberg, PhD

Report Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

REPORT TITLE: NON-FERMENTATIVE GRAM-NEGATIVE BACILLI IN BURNED SOLDIERS: NEW POTENTIAL OPPORTUNISTIC PATHOGENS

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

Period covered in this report: 1 July 1974 - 30 June 1975

Investigators: Virginia C. English, MA
Robert B. Lindberg, PhD

Report Control Symbol MEDDH-288(R1)

Oxidative, non-fermentative gram-negative bacteria have been found with increasing frequency as predominant organisms in postmortem bacteriology of autopsy tissues, and in biopsies. A system for detecting and classifying these organisms was developed and assessed for completeness and accuracy. Pseudomonas maltophilia, Alcaligenes odorans var. viridans and Flavobactrum sp were the predominant types found, but 18 species including 11 of Pseudomonas were identified. Since most were recovered as predominant organisms in quantitative cultures, there is a strong likelihood that these species are still escaping detection. Their pathogenicity is not established in most instances, but they constitute a significant potential source of new opportunistic invaders.

Burns
Oxidative gram negative bacilli
Pseudomonas
Acinetobacter
Alcaligenes
Flavobactrum
Humans

NON-FERMENTATIVE GRAM-NEGATIVE BACILLI IN BURNED SOLDIERS: NEW POTENTIAL OPPORTUNISTIC PATHOGENS

The evaluation and development of medical microbiology showed, from its beginning in the mid-nineteenth century, an irregular sequence of emphasis based in part on the relationship of pathogenic microorganism to specific human or animal disease. Spore-forming aerobes were in focus with anthrax; acid fast bacilli with tuberculosis; sperochetes with syphilis, and fermentative gram-negative bacilli representing the enteric fevers, typhoid and paratyphoid, dysentery, and the whole realm of fecal contamination, control of which is a major pre-occupation of Western civilization. A group of organisms which, although early discerned, excited little medical interest for a hundred years, were the oxidative gram-negative bacilli. This neglect undoubtedly stemmed from their conspicuous absence from the roster of baceria that cause specific diseases of man. The fact that an oxidative pigment-former causes furunculosis in the trout, or that a chromogen causes red-leg in leopard frogs was not noteworthy. When, however, control of specific infecting organisms, by sanitation, vaccination and chemotherapy, became a reality, the stage was set for the performance of this little understood group in a new role - that of opportunistic pathogens. Pseudomonas aeruginosa is, of course, the conspicuous successful opportunist in burns, cancer wards, debilitated aged, newborns, and other compromised hosts. This organism can and does take a major toll yet primary infections with this ubiquitous opportunist are, in normal healthy individuals, virtually unknown.

The taxonomy, identification and ecology of non-fermenting gram-negative bacilli has become in the past 5 years a matter of increasing importance. The increase in infections caused by them is probably real, as the status of modern medicine presents them with patients suitable for colonization and invasion, while at the same time familiar pathogens are controlled if not eliminated. The other reason for the increased interest is an improvement in acceptable determinative technics that make their identification possible and feasible (1-6)

---

1. Gilardi GL: Characterization of non-fermentative non-fastidious gram negative bacteria encountered in medical bacteriology. J Appl Bact 34: 623, 1971.

2. Pickett MJ, Pedersen M: Salient features of non-saccharolytic and weakly saccharolytic nonfermentative rods. Can J Microbiol 16: 401, 1970.

3. Gilardi GL: Characterization of Pseudomonas species isolated from clinical specimens. Appl Microbiol 21: 414, 1971.

4. Gilardi GL: Pseudomonas maltophilia infections in man. Am J Clin Path 51: 58, 1969.

5. King EO, et al: The identification of unusual pathogenic gram negative bacteria. US Dept HEW, Center for Disease Control, Atlanta, Ga, Preliminary Rev. Sept. 1972.

6. Franklin M, Franklin M: A profile of Pseudomonas. Beecham Pharm. (Div. of Beecham, Inc), 1971.

METHODS

Routine microbial identification technic in the Institute of Surgical Research laboratory is designed to detect new species which are behaving in a manner that merits their intense scrutiny. Thus, routine diagnostic procedures categorize fermentative microorganisms: staphylococci, Enterobacteriaceae, and Pseudomonas aeruginosa readily and accurately, but detailed search for atypical uncommon strains is not routinely done. But all specimens from autopsies and biopsies are done with pour plate dilutions: the bacterial content of the specimen is determined and the highest dilution with bacterial growth is assessed for species identification in complete detail. The hypothesis is, that in the event that significant wound colonization or visceral involvement by a new species is occurring, this will be detected by the presence of the new strain as a predominant strain in these tissues. It is in these specimens that an increasing number of oxidative organisms have been found in the past 2 years.

RESULTS

Table 1 shows the basic work sheet of tests to which non-fermenting organisms are subjected. Utilization of the first 8 carbohydrates listed shows whether the organism is oxidative, monoscaccharolytic or weakly saccharolytic. If it is non-saccharolytic or weakly saccharolytic, additional carbohydrates are not needed; physiologic and nutritional attributes, listed in the right hand column are then used for further classification.

Table 2 presents infrequently isolated species recovered at autopsy, as predominant organisms. From 2 patients, unusual species were recovered antemortem from biopsy specimens, as well as postmortem. Twenty-three isolates of rarely encountered oxidative organisms were predominant strains in tissue from 13 autopsies. There were 4 species of Pseudomonas, one Aeromonas, one Flavobactrum, one Alcaligenes, and, for convenience in listing, one vibrio species (a fermentative genus). The biopsy of Pseudomonas cepacia was not followed by retrieval of cepacia at autopsy. Four patients who harbored Ps maltophilia, Herellea and Aeromonas antemortem did not show the species at autopsy.

In 1974, with improved technics and experience at characterizing oxidative organisms, 15 patients on the burn ward were found to harbor oxidative organisms, as shown in Table 3. Four of these 15 patients expired. In 2 of them, at autopsy, the organism which had been found in life was not again recovered. There were 9 different species recovered. The most common were Acintobacter (formerly Herellea) and Flavobactrum species. In view of its frequent occurrence in autopsy tissues, it is noteworthy that Ps maltophilia was recovered in only one antemortem sample.

Autopsy samples still yielded more oxidative isolates than did antemortem cultures. Table 4 shows oxidative species recovered from autopsies in 1974 These were all dilution plate isolations, and in consequence represent samples in which the strain was the predominant organism There were 32 patients from

Table 1. Bacteriology Worksheet

	24 hrs	48 hrs	72 hrs	7 da
Glucose				
Xylose				
Mannitol				
Lactose 1%				
Lactose 10%				
Sucrose Saccharose				
Levulose Fructose				
Maltose				
Sa				
Raffinose				
Trehalose				
Galactose				
Rhamnose arabinose				
Cellobiose				
Melibiose				
Raffinose				
Glycogen				
Inulin				
Adonit				
Dulc				
Glycerin				
Sorbitol				
Inositol				
Mannitose				
Media #				
Media #				

H2S	24 hrs	48 hrs	72 hrs	7 da
Ammonia Citrate				
Motility				
Methyl Red				
V/P				
Indole				
Urea				
Phenylalanine				
Lysine				
Malonate				
Nitrate gas				
Gelatin				
Serum gas				
ONPG				
Nutrient Broth 0% NaCl				
Nutrient Broth 2 5% NACl				
Nutrient Broth 6 5% NACl				
Lysine				
Arginine				
Ornithine				
Methionine				
Cetrimide				
Litmus Milk				
Gluconate				
SOA (pH 6 8)				
SS				
MAC				
DNA				
Lipase				
Starch				
Tyrosine				
5° 35°				
37° 42°				

BAMC Form 178 01
1 Apr 74

Table 2. Antemortem Isolates from Surface Swab Postmortem Organisms

Autopsy No.	Patient No.	Organisms Isolated Antemortem Only
[illegible]	127	R.L. [illegible] Pseudomonas maltophilia
A [illegible] 73	144	Left foot Pseudomonas maltophilia
A 58 73	157	R. sacral thigh bed sore Aeromonas
A [illegible] 73	176	Surface Tissue Bx Aeromonas sp.
A [illegible] 74*	259	Surface Bx [illegible] sore Pseudomonas maltophilia

		Organisms Isolated Postmortem Only
A 48 73	136	RLL Pseudomonas maltophilia
A 49 73	150	Spleen LLL LUL RLL RUL Pseudomonas maltophilia
A 53 73	148	RUL Pseudomonas maltophilia
A 59 73	138	RUL LUL Pseudomonas maltophilia
A 61 73	181	Spleen LUL Pseudomonas maltophilia
A 62 73	177	Surface Bx Aeromonas sp.
A 69 73	195	LLL Pseudomonas maltophilia
A 70 73	202	Liver Pseudomonas maltophilia Surface Bx # 2 & 5 Flavobacterium Type II B
A 83 73	248	LLL Alcaligenes odorans var. viridans
A 84 73	257	Spleen Pseudomonas fluorescens
A 90 73	244	Surface Bx #2 Pseudomonas maltophilia

Organisms Isolated Antemortem and Postmortem

Autopsy and Patient No.	Antemortem	Postmortem
A 23 73 206	Belly Pseudomonas cepacia	Spleen Pseudomonas maltophilia
A 80 73 225	Surface Bx RLL thighs Pseudomonas putrefaciens, Vibrio alginolyticus**	Surface Bx Nos. 1 & 5, RLL Pseudomonas putrefaciens Vibrio alginolyticus**

* Patient admitted and expired in 1973

** Fermentor, but not commonly isolated in this laboratory

Table 3. Nontermenting Species of Bacteria Recovered From Patients on Burn Ward, 1974

No. Patients Positive	Source	Species
3	Biopsy	Acinetobacter sp
1	Biopsy	Alcaligenes fecalis
1	Biopsy	Alcaligenes denitrificans
3	Biopsy, wound	Flavobactrum II-B
2	Wound	Aeromonas sp
1	Blood	Ps. maltophilia
1	Wound	Ps. fluorescens
1	Stool	Ps. testosteroni
2	Wound, sputum	Ps. aeruginosa, Apyocyanogenic

whom oxidative species were isolated; in 22 of these the organisms were recovered from autopsy material.

Table 4. Oxidative Organisms Isolated from Autopsies, 1974

Species	Total Patients Positive	Site of Isolation			
		Liver	Spleen	Lung	Wound
Alcaligenes fecalis	1				1
A. denitrificans	1	1			
A. odorans var. viridans	5	1	2	3	1
Acinetobacter sp	2	1			1
Mima polymorph var. oxydans	4	2	1	1	1
Ps. maltophilia	13	5	2	6	3
Ps. vesiculare	2	2			
Ps. diminuta	2	1	1		
Ps. putida	1		1		
Ps. stutzeri	1		1		
Ps. fluorescens	1				1
Ps. Pseudoalcaligenes	1			1	
Ps. Testosteroni	1	1			
Atypical Ps. aeruginosa	1			1	
Flavobact. II-B	5		3	2	
Flavobact. II-K-2	3			3	

The predominant species in this series was Ps. maltophilia. Next in frequency was Alcaligenes odorans var. viridans. The remaining species were

each relatively infrequent in occurrence.

A relatively high proportion of patients who harbored oxidative species had more than one species of nonfermenter present, i.e., 13 out of 22. The implications of this circumstance are provocative: the relatively rare oxidative species are concentrated in a small proportion of burn fatalities. It is as though, if the soil is suitable for one species, that other oxidative species flock to the site. The distribution of oxidative species on 13 patients is shown in Table 5. There were only 2 patients in whom antemortem and postmortem culture findings could be compared. It is hoped that with increasing effort and experience, more such cases will become available.

The high incidence of Ps maltophilia has been noted. In 1973, there were 12 patients from whom maltophilia was recovered: 3 of these were from biopsy of patients who survived, and 9 from viscera at autopsy. Of these 9, there were 5 with the lung positive, 2 with liver, 3 with spleen, and 2 with wound positive for Ps maltophilia.

In 1974, 15 patients, including one who survived, were positive for Ps maltophilia. In this series, liver samples were positive in 6 cases, spleen in 3, lung in 7, and wound in one. This is a total of 27 patients and 44 strains recovered from 171 autopsies. This is 16% of the total autopsies; it is a large enough figure to merit further observation of this oxidative form.

The oranism grows readily on the usual laboratory media and with experience can be seen in mixed cultures. It exhibits a thin, transparent colony with a yellow to brown non-diffusible pigment. This brown pigment can be enhanced on 1% Tyrosine in Tryptic Soy Agar. The characteristic recognition reaction is the ability of Ps maltophilia to split maltose. If it splits glucose the reaction is late and weak. The organism has been described elsewhere (4) as causing infections in post-surgical, periurethral and scrotal abcesses. The possibility of Ps maltophilia inciting burn wound sepsis in burned rats is being studied.

The incidence and site of recovery of other oxidative and 2 rate fermentative genera are shown in Table 6. Flavobacterum II-B was found in 9 patients; Alcaligenes odorans var.viridans in 8, and others in not more than 2 or 3 patients. Flavobacterium, obviously still subjudice as to speciation, produce a dramatic yellow to orange, water-insoluble pigment. Indole formation has been ascribed to them, but our strains did not form it.

The distribution of these organisms was episodic; periods of no recovery were interspersed with clusters of positive cultures. The pattern suggested that transmission from patient to patient might be occurring. Since these strains grow more slowly than Enterobacteriaceae, they tend to be overgrown by the gram negative flora which is so characteristic of burn wounds. Since the organisms were in most instances recovered as the predominant flora on a burn, there is a strong presumption that they are far

Table 5. Patients From Whom Multiple Nonfermenting Organisms Were Recovered

Patient No.	Antemortem Cultures	Postmortem Cultures
206 (1-'80)	Belly: Pseudomonas cepacia	Spleen: Pseudomonas maltophilia
223 (1-'81)	R & L Thighs: Pseudomonas putrefaciens, Vibrio alginolyticus*	Surface biopsies Nos. 1, 5, RLL: Pseudomonas putrefaciens, Vibrio alginolyticus*

Patients Whose Postmortem Tissue Samples Presented Multiple Nonfermenting Organisms

Patient No.	Surface Biopsy	Liver	Spleen	Lung
223 (1-'81)	Flavobacterium, Type II-B	Pseudomonas maltophilia		
27			Flavobacterium, Type II-B; Ps. maltophilia	Alcaligenes odorans var. viridans; Ps. putida; Ps. maltophilia
80			Ps. stutzeri	Ps. maltophilia; Flavobacterium, Type II-B
85		Ps. vesiculare; Alcaligenes denitrificans		
68			Flavobacterium, Type II-B	Ps. maltophilia
94				Flavobacterium Type II-B; Ps. maltophilia; CDC Gp. II-K-2
133		Ps. maltophilia; Ps. vesiculare		Ps. maltophilia
146		Ps. diminuta; Ps. testosteroni	Ps. diminuta	
187	Ps. maltophilia	Alcaligenes odorans var. viridans	Ps. diminuta	Ps. maltophilia; Alcaligenes odorans var. viridans
195		Alcaligenes odorans var. viridans	Alcaligenes odorans var. viridans	Alcaligenes odorans var. viridans; Ps. maltophilia
41		Ps. maltophilia	Ps. maltophilia	Ps. maltophilia; Ps. pseudoalcaligenes

* Although fermentative, requires special means of identification and is not commonly encountered in this laboratory

Table 6 Incidence of Soufermenting Organisms Not Commonly Recovered from Patients

Organism	Number of Patients	Number of Isolates	Antemortem Surface Bx	Antemortem Blood	Autopsy Surface Bx	Autopsy Viscera
Alcaligenes denitrificans	2	2	1			1
Alcaligenes faecalis	2	2	1		1	
Alcaligenes odorans var. viridans	8	14				14
Flavobacterium Gp. II B	9	12	3		2	7
CDC Gp. II K-2	2	3				3
Apyocyanogenic Ps. aeruginosa	3	5	(Hyken's)			2
Ps. cepacia	1	1	1			
Ps. diminuta	3	4				4
Ps. fluorescens	2	2	1			1
Ps. Pseudoalcaligenes	1	2				2
Ps. putida	2	3				3
Ps. putrefaciens	1	5	2		1	2
Ps. stutzeri	1	1				1
Ps. testosteroni	2	3	2 (1 tissue, 1 stool)			1
Ps. vesicularis	2	2				2
Ps. maltophilia	27	44	1	1	4	36
Vibrio alginolyticus*	1	5	2		1	2
Aeromonas Gp. *	5	5	1		1	1

* These groups are included, though fermentative, since they are not commonly isolated and require special techniques in identification.

more common in the burn wound than has thus far been recognized. In mixed cultures they could be difficult to detect. Further efforts will be directed toward uncovering this group of organisms in burn patients. They may well have a greater significance in burn wound infection than the present number of isolates would indicate.

PUBLICATIONS AND/OR PRESENTATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OE 6985	75 07 01	DD DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA: CONTRACTOR ACCESS	9. LEVEL OF SUM
74 07 01	K. COMP	U	U	NA	NL	[X] YES [ ] NO	A. WORK UNIT

10. NO. CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A161102B71R	01	083
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) Complement Components in the Thermally Injured Soldier (Total, C1, C3, C5 and C8 Levels) and their Relationship to Bacteremia (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
74 02	75 07	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: 75	.6	16
FISCAL YEAR CURRENT:		

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

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

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

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME: Willard A Andes, MD, MAJ, MC
TELEPHONE: 512-221-3411
SOCIAL SECURITY ACCOUNT NUMBER

21. GENERAL USE: FOREIGN INTELLIGENCE NOT CONSIDERED

ASSOCIATE INVESTIGATORS
NAME: Arthur D Mason, Jr, MD
NAME: James Murray DA

22. KEYWORDS (Precede EACH with Security Classification Code): (U) Burn Soldier; (U) Complement; (U) Thermal Injury; (U) Infection; (U) Host Resistance

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

23. (U) Study complement components and their relationship to infection in the thermally injured soldier.

24. (U) Obtain prospective serum samples on thermally injured patients. Measure total complement and C1, C3, C5, C8 components by hemolytic assay before, during and if possible, subsequent to proven septic episodes in those patients.

25. (U) 74 07 - 75 06 Serial assays of total hemolytic complement in five burned patients (mean burn size 56%) revealed that complement activity was normal or elevated in each patient prior to, during and subsequent to (in the one survivor) serious bacterial sepsis. Complement deficiency would not seem to be a serious factor in the susceptibility of the burned patient to infection.

*Available to contractors upon originator's approval

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

FINAL REPORT

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

REPORT TITLE: COMPLEMENT COMPONENTS IN THE THERMALLY INJURED SOLDIER (TOTAL, C_1, C_3, C_5, and C_8 LEVELS) AND THEIR RELATIONSHIP TO BACTEREMIA

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

1 July 74 - 30 June 1975

Investigators

Willard A. Andes, MD, Major, MC
Arthur D. Mason, Jr., MD
James Murray, SP4

Report Control Symbol MEDDH-288 (R1)

UNCLASSIFIED

ABSTRACT

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

REPORT TITLE: COMPLEMENT COMPONENTS IN THE THERMALLY INJURED SOLDIER (TOTAL, C_1, C_3, C_5, and C_8 LEVELS) AND THEIR RELATIONSHIP TO BACTERMIA

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

Period covered in this report: 1 July 1974 - 30 June 1975

Investigators; W. Abe Andes, MD, Major, MC
J. Murray, SP4
Arthur D. Mason, Jr., MD

Reports Control Symbol MEDDH-288 (R1)

The importance of total hemolytic complement activity in the thermally injured soldier's ability to resist infection is unknown. Such activity is however, required for optimal phagocytic function and host resistance in other disease states and experimental situations. In this study we measured total hemolytic complement activity in 5 burned patients before, during, and after bacterial sepsis manifested by positive blood cultures and clinical signs. Four patients (mean burn size = 57%) expired and one (49% total body surface burn) survived. Each patient had normal or elevated total complement activity prior to sepsis. Although complement activity fell after the onset of infection in 2 patients, at no time did the levels fall below those found in healthy normal people similarly assayed.

Thus, it would seem that complement activity in seriously burned humans is usually normal or elevated postburn. The levels of total complement found in this study would seem to make individual complement component deficiencies unlikely. A lack of total complement activity would not seem to be a significant factor in the increased frequency and severity of infectious complications such patients suffer.

Infection
Host Resistance
Burn Soldier
Complement
Thermal Injury

COMPLEMENT COMPONENTS IN THE THERMALLY INJURED SOLDIER (TOTAL, C_1,C_3,C_5, AND C_8 LEVELS) AND THEIR RELATIONSHIP TO BACTEREMIA

The role of complement in the decreased resistance to bacterial infections displayed by the burned soldier has been studied to a limited degree. The scarcity of such studies has been related to the complexity of assay procedures and the unavailability of reliable and reproducible reagents. With the introduction of sensitized sheep red cells and antibody (Cordis Laboratories, Miami, Florida) the measurement of hemolytic complement activity (as opposed to possibly nonfunctional, immunologically measured complement) has become feasible. These test reagents were used to study total hemolytic complement activity in five seriously burned patients (mean burn size =56% of the total body surface) by Nelson's modification of the method of Kabat and Mayer (1,2). Results are expressed as the complement activity which facilitates hemolysis of 50% (CH_{50}) of the sensitized sheep red cells in the test system in one hour.

Blood was drawn prospectively from the patients and allowed to clot in glass tubes at 25° C for 90 minutes. It was then centrifuged at 2000 G for 20 minutes and the serum stored at -70° C until assayed. Blood from 22 normal volunteers was drawn and tested similarly. An aliquot of serum from a single normal sample was run with each group of patient samples to serve as an internal control and to allow for the use of various lots of sheep red cells in the test system. Patients were judged to be septic when they had blood cultures positive for bacteria and symptoms or signs compatible with that diagnosis.

Complement levels and infections in individual patients are shown in Figures 1-5. Normal complement activity (with 95% confidence limits) are shown by stippling. Patients were septic on the days indicated by arrows or on each day within the bracketed arrows. The distribution of all complement levels related to the day postburn (Fig.6) or to the presence of infection (Fig.7) are also shown. Two of the four patients who expired at 15 and 75 days post burn respectively. Both were judged to have died with septic complications (Klebsiella and pseudomonas pneumonia, U.S. Army Institute of Srugical Research Autopsy Reports A-24-74 and A-35-74).

DISCUSSION

Complement activity has been more celarly defined in the last several years. The participation of complement in various aspects of inflammatory reactions has been partially characterized (3). This study focused on the

---

1. Nelson RA, Jensen J, Gigli I, Tamura N: Methods for the separation purification, and measurement of nine components of hemolytic components in guinea pig serum. Immunochem 3:111-135, 1966.

2. Kabat EA, Mayer MM: Complement and complement fixation, Chap 4, Experimental Immunochemistry. Second edition, Springfield, Illinois, Charles C. Thomas, 11:133-240, 1961.

3. Ruddy S, Gigli I, Austen KF: The complement system of man. N. Engl. J. Med. 287: 489-495, 1972.

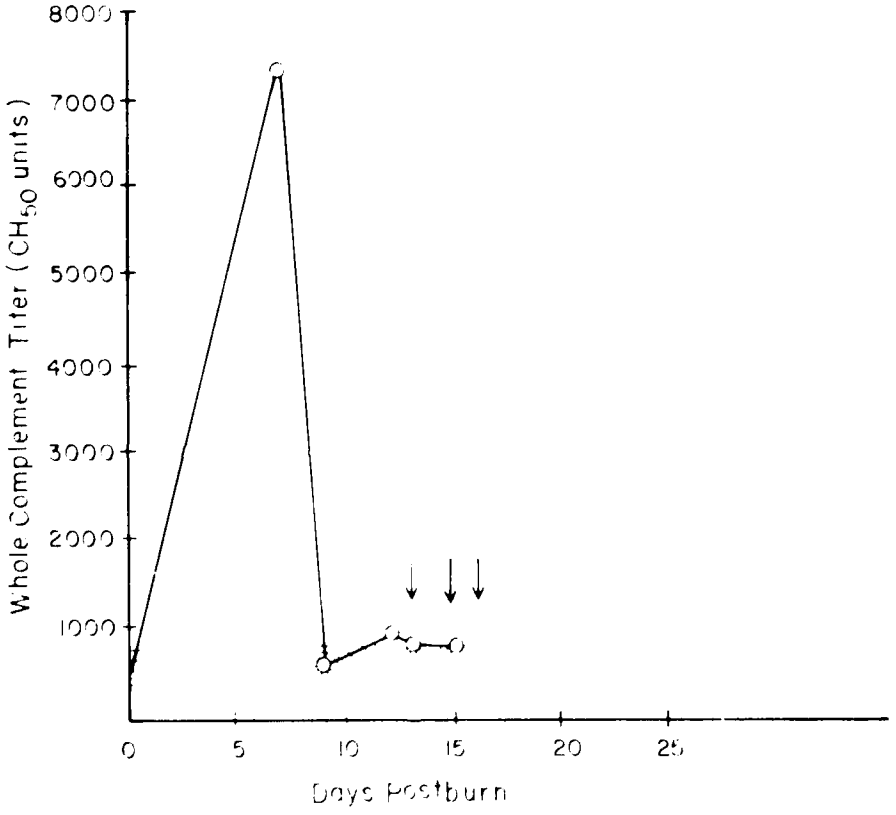


Figure 1. Total hemolytic complement following thermal injury. Septic episodes, manifested by clinical symptoms and bacteremia are indicated by vertical arrows.

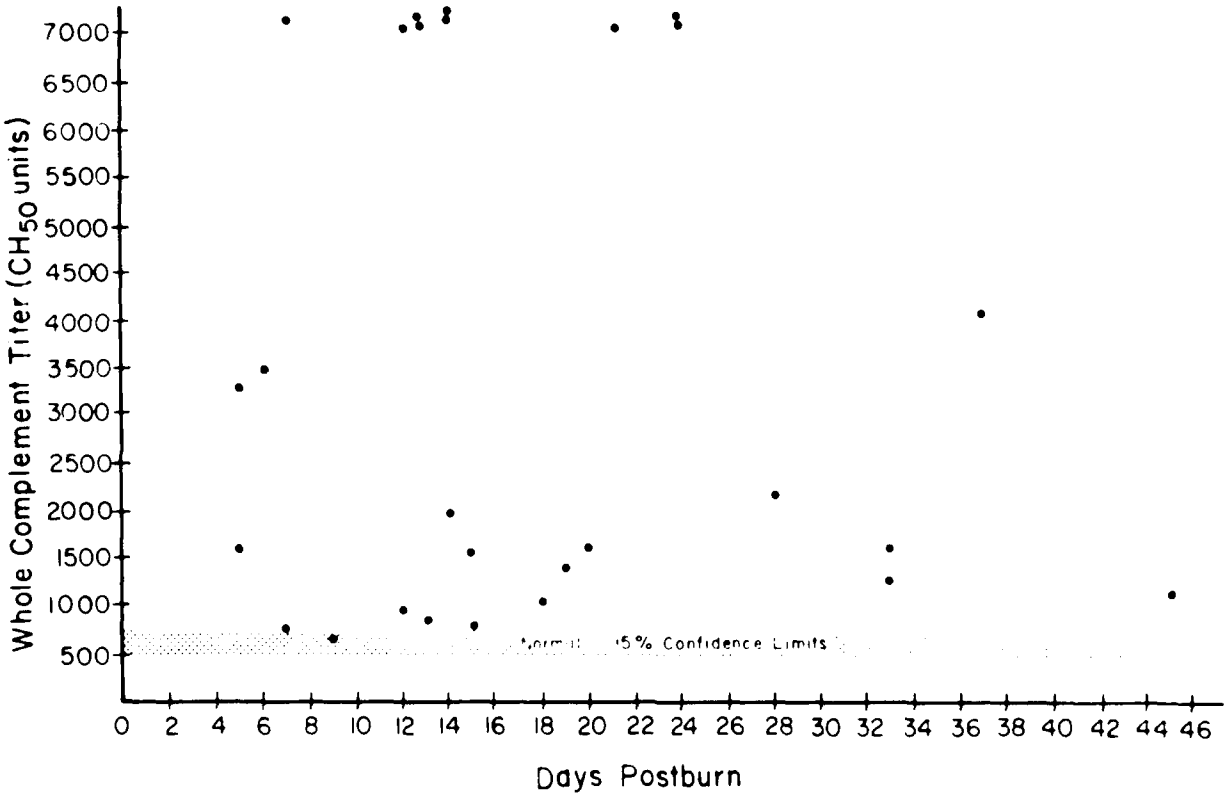


Figure 2. Total hemolytic complement following thermal injury. Septic episodes manifested by clinical symptoms and bacteremia are indicated by vertical arrows.

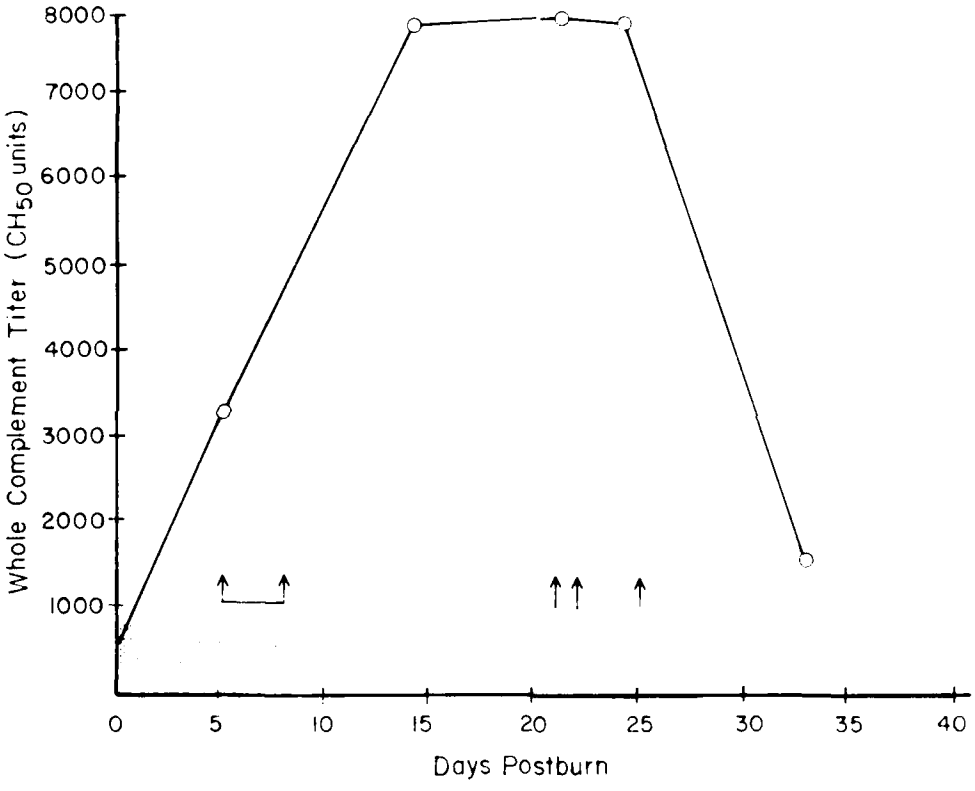


Figure 3. Total hemolytic complement following thermal injury. Septic episodes manifested by clinical symptoms and bacteremia are indicated by vertical arrows.

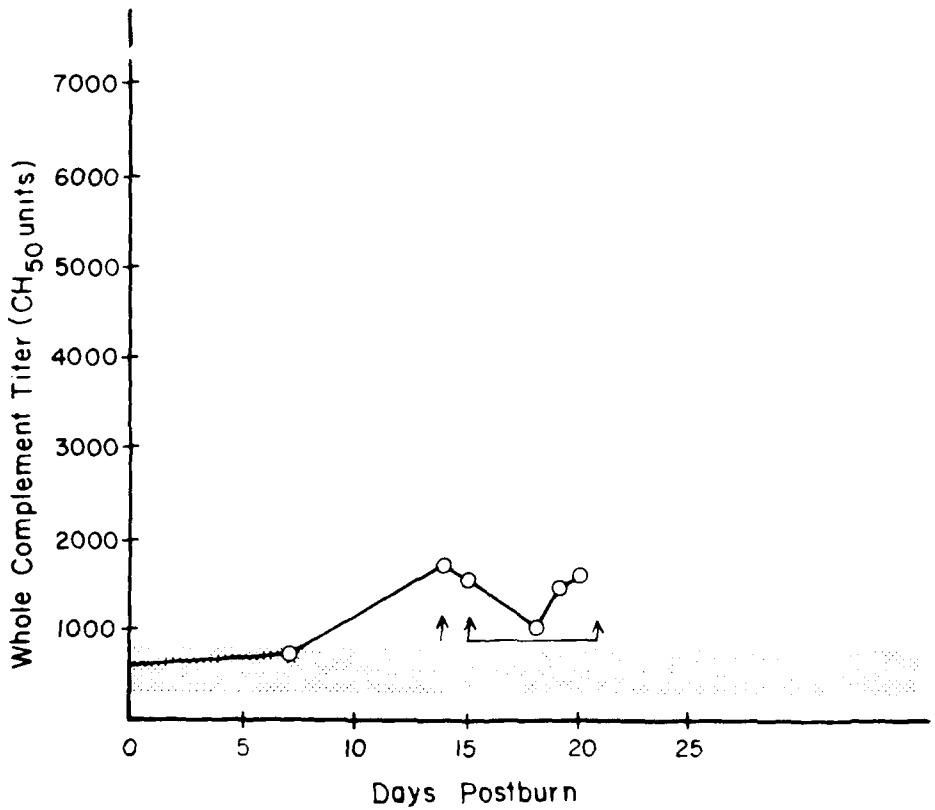


Figure 4. Total hemolytic complement following thermal injury. Septic episodes manifested by clinical symptoms and bacteremia are indicated by vertical arrows.

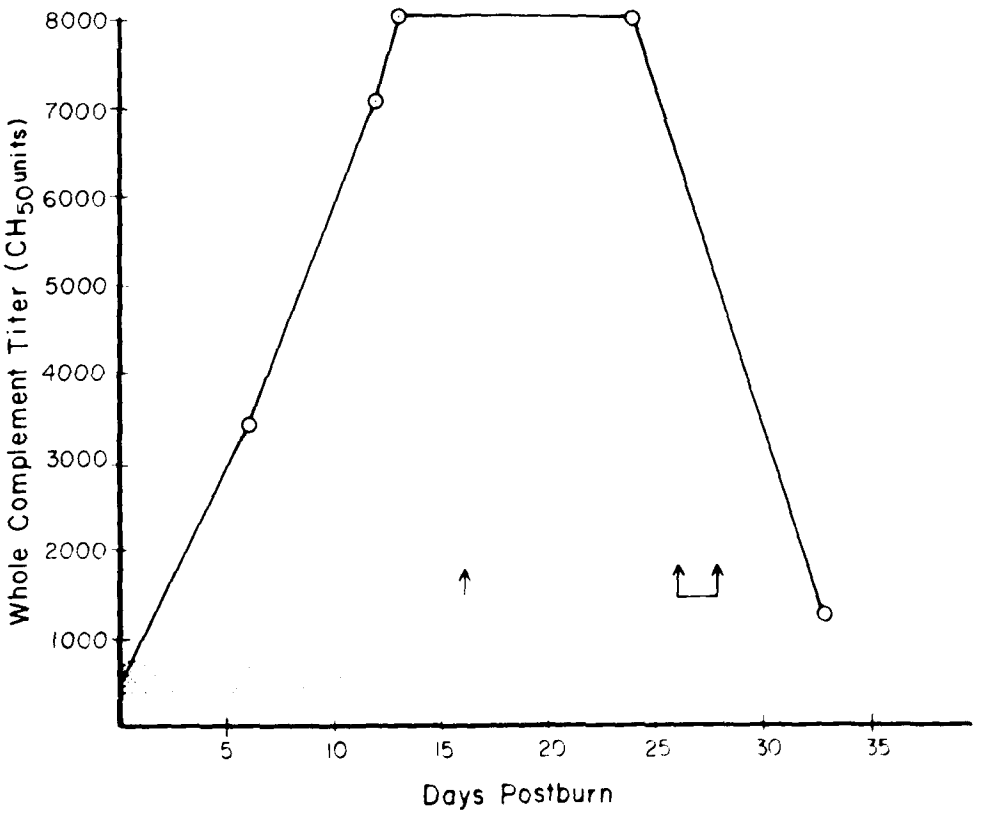


Figure 5. Total hemolytic complement titers during thermal injury. Septic episodes manifested by clinical symptoms and bacteremia are indicated by vertical arrows.

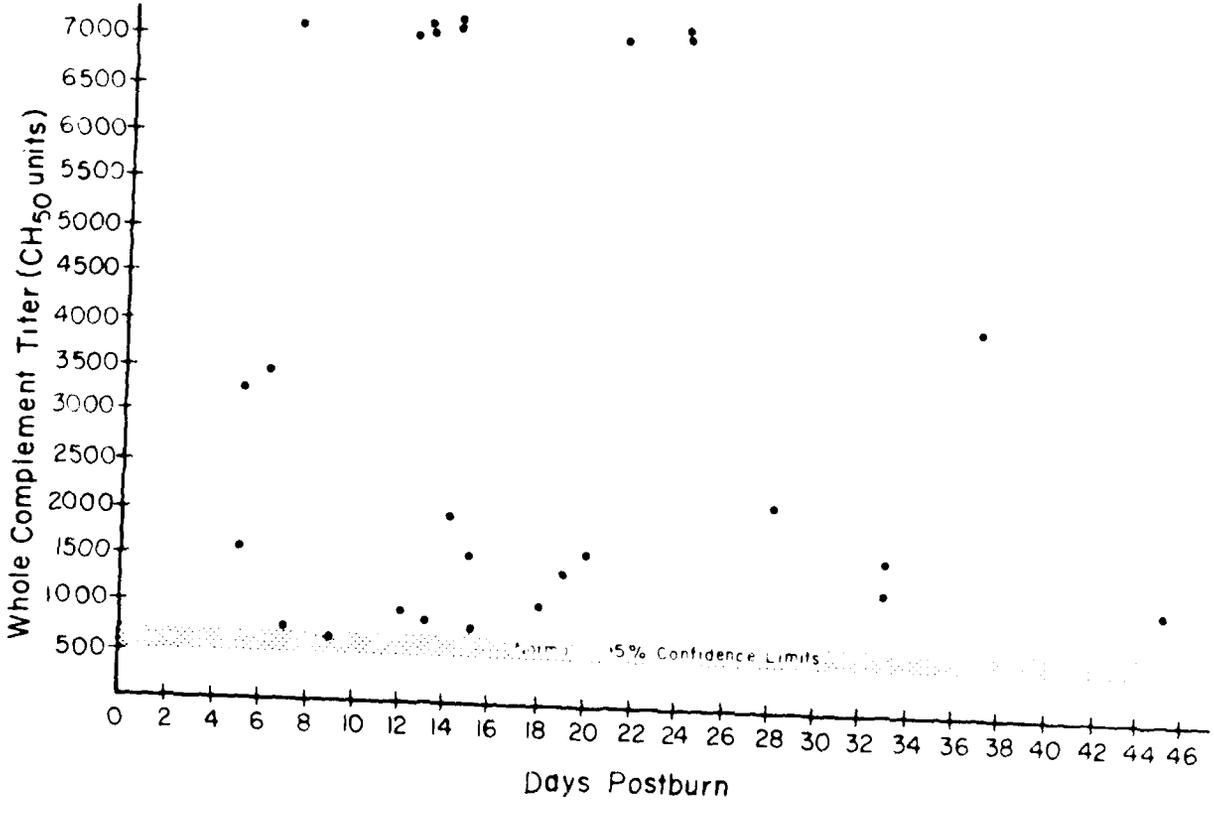


Figure 6. Serum Complement Following Thermal Injury in Five Patients Related to Day Postburn.

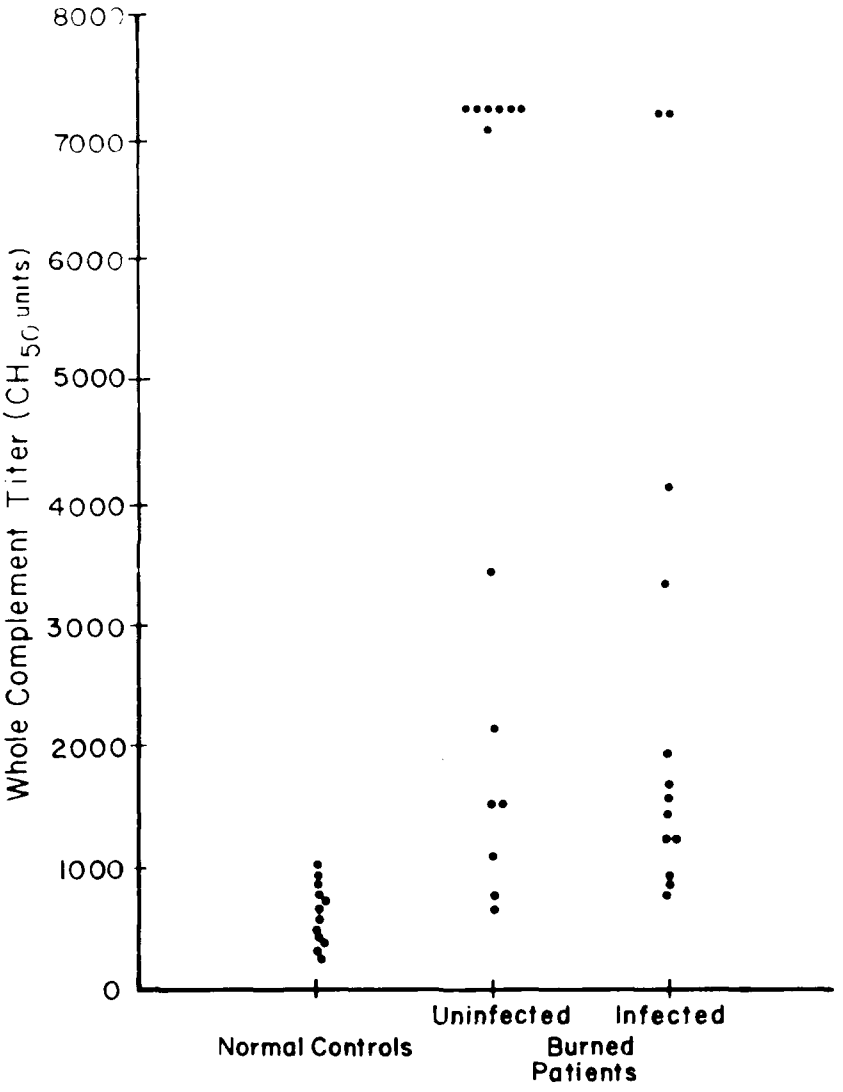


Figure 7. Complement activity in normal humans, burned or burned-infected patients.

functional levels of complement before, at the time of, and after serious bacterial infection. In none of our patients was there an overall deficiency of potential complement activity at any time during their hospital course. Although individual complement components might have been low with elevated compensatory levels of other components, this is unlikely and did not appear to be a factor contributing to the fatal infections afflicting 4 patients. In view of the markedly elevated total complement levels and the known synchrony by which the complement components activate one another (4), there was sufficient complement activity in these patients to allow optimal function of this important enzymatic system in their response to bacterial infections.

PUBLICATIONS AND/OR PRESENTATIONS:

None

4. Kohler PF: Editorial Note: Ann. Intern. Med. 82: 420-421, 1975.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA 0A 6980	75 07 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISS'N INSTR'N	8b. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
74 07 01	D. CHANGE	U	U	NA	NL	[X] YES [ ] NO	A. WORK UNIT

10. NO. CODES*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A161102B71R	01	165
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)* (U) Studies of Disturbance of Protein Turnover in Burned Troops-Use of An Animal Model (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*
003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
65 07	Cont	DA	C. In-House

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

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 75	.8	27
	CURRENT 76	.6	17

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

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

20. PERFORMING ORGANIZATION
NAME:* US Army Institute of Surgical Research
Renal Branch
ADDRESS:* Fort Sam Houston, Texas 78234

PRINCIPAL INVESTIGATOR (Furnish SEAN if U.S. Academic Institution)
NAME:* Wanda L Brown, MS
TELEPHONE: 512-221-4652
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: Eleanor G Bowler, PhM
NAME: Arthur D Mason, Jr, MD DA

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Protein; (U) Burn; (U) Trauma; (U) Turnover; (U) Rats; (U) Albumin

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

23. (U) To determine the cause of the dysproteinemia observed following burn injury and to determine if the more marked dysproteinemia seen in the presence of infection of the burn wound is an effect caused by some action of the bacteria. It is hoped that this will aid in understanding similar changes which are observed in burned soldiers.

24. (U) The amount of (2-14C) glycine incorporated into the serum proteins of burned, burned-infected, treated burned-infected, and control rats has been measured. The body distribution of albumin was determined by radioimmunoassay using extracts of tissues obtained on the sixth day postburn. Measurements of 14C incorporation at the subcellular level will be done to confirm the in vivo findings.

25. (U) 74 07 - 75 06 The albumin content has been measured in blood and tissues of groups of control, burned, and burned-infected rats. The albumin content of the burn wound tissue was much greater than control values of unburned skin. The albumin content of unburned skin and carcass of the injured rats were equal to control values. The increased albumin content of the burn wound persisted in spite of the lowered plasma albumin pool size. The tissue albumin had molecular size and immunoreactivity characteristic of native albumin. The study of protein turnover 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. 3A161102B71R-01, RESEARCH IN BIOMEDICAL SCIENCES

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

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

1 July 1974 - 30 June 1975

Investigators:

Wanda L. Brown, MS
Eleanor G. Bowler, PhM
Arthur D. Mason, Jr., MD
Basil A. Pruitt, Jr, MD, Colonel, MC

Report Control Symbol MEDDH-288 (R1)

UNCLASSIFIED

ABSTRACT

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

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

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

Period covered in report: 1 July 1974 - 30 June 1975

Investigators: Wanda L. Brown, MS
Eleanor G. Bowler, PhM
Arthur D. Mason, Jr, MD
Basil A. Pruitt, Jr, MD, Colonel, MC

Report Control Symbol MEDDH-288(R1)

Measurement of incorporation of (2-^{14}C)glycine into serum proteins of control, burned, burned-infected, and burned-infected rats which had been treated by topical application of 10% mafenide acetate ointment was used to estimate serum protein synthesis on the sixth day postburn. The relative specific activity of all of the protein fractions except gamma globulin was higher in serum from the injured rats. Mafenide acetate treatment reduced the mortality of the burned-infected rats but did not prevent a marked decrease in serum albumin concentration.

Whole body albumin was determined by radioimmunoassay using extracts of individual tissues of rats or of blood-free eviscerated rat carcasses. The albumin content of the burned rats' viscera was lower than that of the control rats. The albumin content of the skin from the burn wound contained 3.5 times as much albumin as that of the controls. Albumin contents of carcass and unburned skin were equal to control values. The total body albumin of the injured rats was higher than that of the control rats, despite their depleted plasma albumin pool. The tissue albumin was shown to be immunoreactive and of large molecular size.

This study shows that the low plasma albumin pool size of burned and burned-infected rats is not caused by a lowered synthetic rate but by changes in albumin compartmentation in the tissues. We propose that this is a result of disruption of the integrity of the burned tissue due to the rapidity of the influx of fluid immediately after burn injury.

Protein Turnover | Burn Rats | Trauma Albumin

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

The serum protein changes which occur in man following burn injury are well documented (14, 21, 23) and include a progressive decrease in the albumin, an increase in the alpha globulin, and either no change or a slight increase in the beta and gamma globulin concentrations. Similar changes occur in the serum protein concentrations of rats after burn injury; they are more extreme when the burn wound is seeded with Pseudomonas aeruginosa (1).

Elevated serum alpha globulin concentration after injury is in part due to increased synthesis of glycoproteins (9). The prolonged postburn decrease in serum albumin has been attributed to loss of albumin into or from the burn wound (6, 10, 20), to increased catabolism (5, 10), or to decreased synthesis (13, 27).

---

14. Lanchantin GF, Deadrick RE: Serum protein changes in thermal trauma: I. Electrophoretic analysis at pH 8.6. J Clin Invest 37: 1736-1745, 1958.

21. Perlman GE, Glenn WWL, Kaufman P: Changes in the electrophoretic patterns in lymph and serum in experimental burns. J Clin Invest 22: 627-633, 1943.

23. Prendergast JJ, Fenichel RL, Daly BM: Albumin and globulin changes in burns as demonstrated by electrophoresis. Arch Surg 64: 733-740, 1952.

1. Alexander JW, Brown WL, Mason AD, Jr, Moncrief JA: The influence of infection upon serum protein changes in severe burns. J Trauma 6: 780-789, 1966.

9. Chandler AM, Neuhaus OW: Synthesis of serum glycoproteins in response to injury. Am J Physiol 206: 169-173, 1964.

6. Birke G, Liljedahl S-O, Plantin L-O, Reizenstein P: Studies on burns. IX. The distribution and losses through the wound of ^{131}I-albumin measured by whole-body counting. Acta Chir Scand 134: 27-36, 1967.

10. Davies JWL, Ricketts CR, Bull JF: Studies of plasma protein metabolism. Part 1. Albumin in burned and injured patients. Clin Sci 23: 411-423, 1962.

20. Nylen B, Wallenius G: The protein loss via exudation from burns and granulating wound surfaces. Acta Chir Scand 122: 97-100, 1961.

5. Birke G, Liljedahl S-O, Plantin L-O, Reizenstein P: Albumin catabolism in burns and following surgical procedures. Acta Chir Scand 118: 353-366, 1960.

13. Kukral JC, Meadows DC: Synthesis of plasma protein fractions in burned patients. Surg Forum 15: 43-45, 1964.

27. Rothschild MA, Oratz M, Schreiber SS: Albumin synthesis (Part II). New Eng J Med 286: 816-821, 1972.

Studies which have determined synthetic and catabolic rates from plasma disappearance rates of injected labeled protein following injury are difficult to interpret since the subjects were not in a steady state. They are further complicated in burn patients by intravenous fluid and colloid replacement therapy which must be continued for some time after injury.

Our object was to determine the relative contributions of synthesis and body distribution to the prolonged depression of the plasma albumin pool following burn injury. In order to avoid some of the problems inherent in disappearance studies, we have used an experimental rat burn model in which incorporation of (2-^{14}C)glycine into serum proteins was used to estimate synthesis. The distribution of albumin in blood and tissues was determined by direct measurement using a radioimmunoassay technic.

MATERIALS AND METHODS

Standard Burn (32). Young male Sprague-Dawley rats (Holtzman, Madison, Wis.) weighing from 180-200 gm were anesthetized with sodium pentobarbital administered intraperitoneally (1 mg/25 gm). Burns were inflicted on the dorsum after the hair was clipped with a No. 40 blade in an Oster animal clipper. The animal was held in a protective template which limited the area of exposure while the area to be burned was immersed in boiling water for 10 seconds. This procedure produces a uniform full-thickness burn with sharp margins.

Groups of rats were subjected to the following treatments:

Group B: Full-thickness burn of 20% of the body surface; expected mortality rate less than 10% (16).

Group BI (Burned-Infected): An equivalent burn which was immediately seeded with one ml of an 18-hour Trypticase soy broth culture of Ps aeruginosa [(SRU-12-4-4-(59)] which contained approximately 10^8 organisms (31); expected mortality rate 85-90% (16).

Group BIS: Burned and infected as in Group BI but treated beginning 24 hours postburn with daily topical applications of 3.5 gm of 10% mafenide acetate (Sulfamylon® Cream, Winthrop Laboratories, New York); expected mortality rate less than 10% (16).

Group C: Stock rats of equivalent size and age were used as controls.

---

32. Walker HL, Mason AD, Jr: A standard animal burn. J Trauma 8: 1049-1051, 1968.

16. 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.

31. Walker HL, Mason AD, Jr, Raulston GL: Surface infection with Pseudomonas aeruginosa. Ann Surg 160: 297-305, 1964.

The rats were housed in individual cages and given free access to food (Purina Lab Chow) and water until sacrifice.

Radioactivity Measurements. A Tricarb Liquid Scintillation counter, a Model 3002 Auto-Gamma Counter, scintillation counting vials, and polystyrene counting tubes were obtained from Packard Instruments, Inc., Downer's Grove, Ill. Scintillator compounds were also obtained from this source. All other chemicals were reagent grade.

Toluene scintillator solution contained 4 gm 2,5-diphenyloxazole (PPO) and 100 mg 2,2-p-phenylenebis (5-phenyloxazole) (POPOP) made to one liter with toluene. For counting ^{14}C protein on paper electrophoresis strips, each paper strip was placed in 10 ml toluene scintillator solution. Counting efficiency was 20%.

Bray's scintillator solution (7) contained 60 gm naphthalene, 4 gm PPO, 0.2 gm POPOP, 100 ml absolute methanol, 20 ml ethylene glycol, made to a volume of one liter with dioxane. Aqueous samples (1 ml) were counted using 14 ml Bray's solution. Counting efficiency was 38%.

All samples for liquid scintillation counting were held in the refrigerated counting compartment for sufficient time to become dark adapted and temperature equilibrated before counting was begun.

^{125}I and ^{131}I content of radioimmunoassay samples and of tissue extracts were determined with the Auto-Gamma counter set to discriminate between the two isotopes. Counting efficiency for ^{125}I was 60%; for ^{131}I, 40%.

Statistical counting error for all samples was 2-5%.

(2-^{14}C)glycine Incorporation Experiments. On the fifth or sixth day post-burn, 20 uCi of (2-^{14}C)glycine (sp.act. 21-22 mCi/mM, New England Nuclear, Boston, Mass.) in 0.5 ml 0.15 M NaCl was injected into the tail vein of each rat. Blood samples were withdrawn from the tail vein or heart at 30, 60, or 150 minutes after the injection. The blood was allowed to clot; the serum was separated and stored at 4°C.

Paper Electrophoresis. Spinco Model R electrophoresis units, Model RB Analytrol scanning densitometer, paper strips (S&S 2043a mgl), and B-2 barbital buffer were products of Spinco Division, Beckman Instruments, Inc., Palo Alto, CA. The electrophoretic separation was performed in barbital buffer, pH 8.6, μ = 0.075, at a constant current of 3.5 mA per unit for 16 hours. Filter paper wetted with buffer was placed in the end plates of the tank cover to prevent drying of the strips during the run. At the end of the run, the strips were removed and dried in an oven at 110-120°C for 30 minutes. Two

---

7. Bray GA: A single efficient liquid scintillator for counting aqueous solutions in a liquid scintillation counter. Anal Biochem 1: 279-285, 1960

strips (10 ul/strip) prepared from each serum sample were stained with alcoholic bromphenol blue (1 gm/liter of absolute methanol), rinsed in 5% acetic acid, dried, and scanned in the Analytrol to determine the relative concentrations of the various fractions.

Two strips, to each of which 20 ul of serum had been applied, were washed extensively in 2% acetic acid solution to remove unincorporated ^{14}C before they were immersed briefly in a solution containing 20 mg nigrosin per liter of 2% acetic acid. The strips were subsequently carried through three more 10-minute 2% acetic acid rinses, blotted, and dried. Nigrosin does not readily penetrate dense bands of protein, so only the margins of the bands were stained. This was sufficient to assure accuracy in cutting the bands apart but did not decrease the efficiency of the scintillation counting. The radioactivity on the pieces of paper containing each protein fraction, was determined by liquid scintillation counting; a segment of the paper containing no protein was used for background correction. The cpm for the five fractions from each strip (corrected for background) were summed and the percentage of the total cpm in each fraction was calculated.

Total ^{14}C content of the serum was determined using 0.05 ml serum diluted to one ml with 0.15 M Nacl. The total ^{14}C incorporated was measured on an aliquot of the serum which had been passed through a 1 x 10 cm column of Sephadex G-25 (Pharmacia, Piscataway, NJ) equilibrated with 0.15 M NaCl to remove unincorporated ^{14}C. The protein concentration of the serum and eluate was determined by a biuret procedure (34). An aliquot of the eluate containing an amount of protein equivalent to that contained in 0.05 ml of the untreated serum was diluted to one ml with 0.15 M NaCl, Bray's solution was added and the samples were counted as described above. Standards prepared from the injection solution were included with each run.

The ratio of the protein concentrations in the serum and eluate was used to convert the cpm of the eluate to the equivalent cpm/ml serum. This value multiplied by the percentage of the total cpm present in each electrophoretic fraction gave the cpm/ml serum incorporated into each of the fractions.

The final results were expressed as relative specific activity (RSA) which is equal to

(cpm/100 mg protein) / (cpm injected per gm rat weight).

This provides a correction for small differences in the animals' weights (33).

---

34. Weichselbaum TE: An accurate and rapid method for the determination of proteins in small amounts of blood serum and plasma. Am J Clin Path 16 (Tech Sec 10): 46-49, 1946.

33. Walter H, Haurowitz F, Fleischer S, Lietze A, Cheng HF, Turner JE, Friedberg W: The metabolic fate of injected homologous serum proteins in rabbits. J Biol Chem 224: 107-119, 1957.

Total Body Albumin Experiments. Control, burned, and burned infected animals models were the same as those described above. Animals were sacrificed on the fifth or sixth day postburn.

Extraction of Albumin from Tissues. The following procedure is essentially the same as those described by Sellers, Katz, et al [12]

On the day before sacrifice, the hair was removed from the rat with clippers. The next day each rat was weighed, and 0.1 uCi ^{131}I-labeled serum albumin (Abbott Laboratories, Chicago, Ill.) was injected into the tail vein. The rat was anesthetized with methoxyflurane, the chest cavity was opened, and an 18-gauge intravenous catheter placement unit was inserted into the heart or aorta. As much blood as possible was withdrawn. Care was taken to obtain a sample within 3 to 6 minutes after the injection for determination of plasma volume by isotope dilution. Through the cannula, which had been left in place, 20-30 ml of heparinized saline (4000 units sodium heparin/liter 0.15 M NaCl) was alternately injected and aspirated. This procedure reduced the amount of residual plasma albumin in the tissues to minimal values.

For some experiments the rat's blood-free body was divided into viscera, whole skin, or burn eschar and unburned skin, and carcass (skinned-eviscerated body). In other experiments, the whole eviscerated blood-free body was processed as a single sample. The individual tissues were weighed before they were placed in containers in an ice bath.

The carcass or eviscerated body was ground twice through a meat grinder before a 25 gm portion was taken for extraction. The other tissues were minced and the entire tissue sample was homogenized. The tissues were homogenized in approximately 9 volumes of ice cold 0.15 M NaCl containing one gram deoxycholate per liter, pH 8.0, using a Polytron Model PT 10-30 and PT35-ST generator (Brinkmann Instruments, Inc., Westbury, NY). The sample container was kept in an ice bath while the homogenizer was operated at setting No. 7 for one minute. The container was swirled in the ice bath for two minutes before a second one-minute homogenization was done. The homogenates were then adjusted to an approximately 10% (w/v) suspension; the exact tissue content was calculated from the analytical weight measurements. The homogenates were transferred to polycarbonate tubes and immediately centrifuged for 30 minutes at 12,000 g at 4° C (Type 30 rotor, Beckman LS-50 preparative ultracentrifuge). A 2 ml sample of each extract was transferred to a polystyrene counting tube for determination of ^{131}I content. This value and the specific activity of the plasma albumin were used to correct for residual plasma albumin in the extracts. Several aliquots of each extract were quick-frozen by placing the tubes in an isopropyl alcohol-dry ice bath. The tubes were tightly capped and stored at -20°C.

12. Katz J, Bonorris G, Golden S, Sellers AL: Extravascular albumin mass and exchange in rat tissues. Clin Sci 39: 705-724, 1970.

28. Sellers AL, Katz J, Bonorris G, Okuyama S: Determination of extravascular albumin in the rat. J Lab Clin Med 68: 177-185, 1966.

Radioimmunoassay. Rat albumin for standards and labelling was isolated by an alcohol TCA extraction procedure (28) from freshly drawn normal rat serum. Albumin was labeled with ^{125}I (carrier free, New England Nuclear, Boston, Mass.) by an iodine monochloride procedure (18). Free ^{125}I was removed by passing the solution through a small column of Amberlite IR-4B which had been pretreated with histidine buffer and 0.15 M NaCl. Albumin concentration of the eluate was determined from its absorbance at 179 nm (22) before 0.1 volume normal rabbit serum was added to protect the albumin from radiation damage. Specific activity averaged 40-50 uCi per mg albumin and free ^{125}I was less than 2%. The ^{125}I-labeled rat albumin migrated with the control albumin band when mixed with normal rat serum. When crossover electrophoresis with rabbit antiserum to rat albumin was performed on cellulose acetate plate, the labeled albumin was retained in the gamma globulin area.

Radioimmunoassay buffer (RIA-buffer) was 0.05 M borate, 0.1 M NaCl, pH 8.5. A solution (NRS buffer) containing one volume normal rabbit serum (NRS) and 9 volumes RIA buffer was used as diluent for all reagents and samples used in the tests. Normal rat serum (Pentex, Miles Laboratories, Kankakee, Ill.), which had been standardized both by electrophoresis and by radioimmunoassay using rat albumin standards, was used routinely for the preparation of the standard curve.

The antigen binding capacity of the rabbit antiserum to rat albumin (Cappel Laboratories, Downingdon, PA) was determined by Farr's procedure in which the antiserum content is varied and the labeled antigen content is held constant (11).

On the day the RIA was to be performed, the frozen extracts were thawed in the cold and recentrifuged before analysis. The antiserum was diluted so that 0.05 ml would precipitate approximately 3 ugm albumin. The ^{125}I-labeled rat albumin was diluted so that 0.05 ml contained approximately 15,000 cpm. All tests were performed in triplicate and all reagents and samples were maintained at 0-4^{o}C until they were finally transferred to counting tubes.

Samples of the tissue extracts or diluted plasma samples, estimated to contain 5-15 ugm albumin, and standards containing 4, 6, 8, 12, and 16 ugm albumin were placed in 12 x 75 mm polystyrene test tubes. NRS-buffer was added to bring the volume of each to 0.2 ml. Precision syringes equipped with hand operated repeating dispensers (Hamilton Co., Reno, Nev.) were

---

18. McFarlane AS: In vivo behavior of ^{131}I-fibrinogen. J Clin Invest 42: 346-361, 1963.

22. Peters T, Jr: The biosynthesis of rat serum albumin. I. Properties of rat albumin and its occurrence in liver cell fractions. J Biol Chem 237: 1181 1185, 1962.

11. Farr RS: Determination of antigen binding capacity. In: Methods in Immunology and Immunochemistry. Reactions of antibodies with soluble antigens, Edited by C.A. Williams & M.W. Chase, New York Academic Press, 1971, Vol. III, p. 66 73.

used to dispense first 0.05 ml diluted ^{125}I-labeled rat albumin, and then 0.05 ml diluted antiserum to each tube. A normal serum control containing NRS instead of antiserum, and an antiserum control to which no unlabeled rat albumin was added, were included in each run. After mixing, the tubes were capped and incubated in the refrigerator. The next morning 0.5 ml of a 2.5 M $(NH_4)_2SO_4$ solution (prepared by mixing 64 ml of $(NH_4)_2SO_4$ solution, saturated at 4°C, with 36 ml of the RIA buffer) was added to each tube. The contents were mixed and allowed to stand for 30 minutes before they were centrifuged in a Sorvall RC-3 centrifuge at 1600 g for 30 minutes. The supernates were decanted, and 0.5 ml of each were transferred to polystyrene tubes. The ^{125}I content was measured in a Packard Model 3002 Auto-Gamma Spectrometer.

Calculations. Various transformations of the data were made in an attempt to extend the linear portion of the standard curve. The best fit was achieved using a logit transformation (25), and deriving separate regression equations for the lower and upper halves of the curve. These calculations were programed on magnetic cards for a Hewlett Packard 9810A calculator in such a way that the regression equations for the standard curve were first derived and stored. Upon entry of the ^{125}I cpm of the test supernate, the choice of the proper regression equation and the corrections for the control values were made automatically, and the mean albumin content of the tube was printed out. In subsequent steps, entry of the ^{131}I cpm/ml extract, ^{131}I cpm/ml plasma, ugm albumin/ml plasma, and gm tissue per ml extract yielded a value for the ugm albumin/gm tissue corrected for residual plasma albumin in the extracts. The final conversions to whole body albumin content and mg albumin/100 gm rat weight were performed manually.

Additional Procedures. Aliquots of representative tissue extracts were ultrafiltered by centrifugation at 4°C in Amicon Centriflo™ membrane ultrafilters (Amicon Corp., Lexington, Mass). These filters are rated to retain molecules above 50,000 molecular weight when forces of less than 1000 g are applied. Aliquots of the ultrafiltrates were tested in the standard RIA system.

The material retained on the filters was washed once with 0.15 M NaCl, recentrifuged, and then concentrated in Amicon Minicon™-B Concentrators (retention rating ~15,000 molecular weight). The proteins in the concentrated extracts were separated by electrophoresis on 78 x 98 mm Titan III cellulose acetate plate (Helena Laboratories, Beaumont, TX) in Helena HR buffer (Tris-barbital, pH 8.8, I - 0.04) at a constant current of 6 mA per plate for 30 minutes. Duplicate plates were electrophoresed and normal control rat serum was run with each group of extracts. After separation, one plate was stained with Ponceau S for protein visualization. Antisera to whole rat serum, rat albumin, and rat IgG were applied to the other plates; the plates were immersed in liquid petrolatum for 24-48 hours for immunodiffusion. The plates were rinsed in petroleum ether to remove the oil, then in buffered 0.15 M NaCl (25 ml electrophoresis buffer/liter 0.15 M NaCl) to remove unreacted protein, and were

---

25. Rodbard D, Bridson W, Rayford PL: Rapid calculation of radioimmunoassay results. J Lab Clin Med 74: 770-781, 1969.

stained with dilute Ponceau S or nigrosin.

Some of the concentrated extracts and serum samples were electrophoresed on 1 x 3 inch cellulose acetate strips (1.5 mA per strip for 30 minutes), stained with Ponceau S, cleared, and scanned in a Gelman integrating recording densitometer.

Statistical Procedures. The significance of the differences between the treatment groups was determined by analysis of variance. Using the procedure outlined in Steel and Torrie (29), a computer program was devised which permitted comparison, with or without transformation of the data, between groups of unequal size. Where noted below, the data were transformed to Naperian logarithms (ln) before analysis to minimize heterogeneity of variance (4). Data were analyzed from a remote teletype terminal, linked by an acoustic telephone coupler to a Honeywell 635 computer at Griffis AFB, Rome, NY.

RESULTS

(2-^{14}C)glycine incorporation. The mean serum protein concentrations of groups of control and injured rats are shown in Table 1. The albumin levels were slightly depressed in the burned rats and were markedly lower in the burned-infected rats. Although treatment with Sulfamylon^{R} decreased the mortality rate of burned-infected rats, the treated rats' serum albumin levels were almost as low as those of the untreated group. The alpha-1, alpha-2, and beta globulin concentrations were much higher in the serum of the infected rats. The total protein levels, and gamma globulin levels of the four groups of rats were not significantly different. The plasma volumes of the burned-infected rats were about 1.1 times those of the other groups, a small, but statistically significant increase.

The relative specific activity (RSA) of the serum proteins determined 150 minutes after injection (2-^{14}C)glycine is shown in Table 2. This time was first chosen for measurement because it had been reported to be the time of maximum incorporation. The RSA values for all fractions were greater than control values; those of the burned-infected group showed the greatest increase. Because there was little free ^{14}C remaining in the plasma of the injured rats at 150 minutes after injection we then measured RSA at 30 and 60 minutes to see if lack of labeled precursor had limited incorporation. In the meantime, the Institute had begun to test the effectiveness of topical Sulfamylon treatment on infection in burn injury and we added a group of treated burned-infected rats to the study.

---

29. Steel RGD, Torrie JH: Principles and Procedures of Statistics. New York, McGraw Hill, 1960, p. 112-115.

4. Bartlett MS, Kendall DG: The statistical analysis of variance heterogeneity and the logarithmic transformation. J Royal Stat Soc Supplement Vol. 8: 128-138, 1946.

Table 1. Rat Serum Protein Concentrations and Plasma Volumes

Serum Protein Fraction	Group: Control (C) N = 17	Burned (B) N = 20	Burned Infected (BI) N = 21	Burned Infected Sulfamylon® Rx (BIS) N = 17	ANOV Comparisons: B vs BIS	B + BIS vs BI p	B + BIS + BI vs C
Total Protein	5.78 (5.63 – 5.93)	5.61 (5.47 – 5.75)	5.53 (5.39 – 5.67)	5.82 (5.62 – 5.97)	N.S.	N.S.	N.S.
Albumin	3.50 (3.34 – 3.66)	3.09 (2.94 – 3.24)	1.52 (1.38 – 1.66)	1.66 (1.50 – 1.82)	<0.005	<0.001	<0.001
Alpha 1 Globulin	0.62 (0.53 – 0.71)	0.59 (0.51 – 0.67)	1.11 (1.03 – 1.19)	0.75 (0.66 – 0.84)	<0.005	<0.001	<0.001
Alpha 2 Globulin	0.48 (0.41 – 0.55)	0.61 (0.54 – 0.68)	1.06 (0.99 – 1.13)	0.76 (0.69 – 0.83)	<0.050	<0.001	<0.001
Beta Globulin	0.75 (0.67 – 0.83)	0.85 (0.78 – 0.92)	1.40 (1.33 – 1.47)	1.16 (1.08 – 1.24)	<0.001	<0.001	<0.001
Gamma Globulin	0.43 (0.36 – 0.50)	0.51 (0.45 – 0.57)	0.44 (0.38 – 0.50)	0.50 (0.43 – 0.57)	N.S.	N.S.	N.S.
Plasma Vol ml/100 gm	3.61 (3.43 – 3.79)	3.71 (3.44 – 3.98)	4.12 (3.85 – 4.39)	3.74 (3.54 – 3.94)	N.S.	<0.010	<0.050

Samples were drawn on the sixth day postburn. Values are means and (C.I. 95) confidence intervals of the means (Gm protein/100 ml serum). P values were determined by analysis of variance (ANOV)

Table 2. Mean Relative Specific Activity of Rat Serum Protein Fractions
150 Minutes Post-injection

Protein Fraction	Group			ANOV Comparisons	
	Control	Burned	Burned-Infected	B vs C	B+BI vs C
	(C) N = 9	(B) N = 13	(BI) N = 9	B: P	
Total	0.886	1.469	3.092	<0.001	<0.001
Protein	(0.751 - 1.045)	(1.271 - 1.697)	(2.624 - 3.654)		
Albumin	0.285	0.527	0.709	<0.325	<0.001
	(0.216 - 0.376)	(0.431 - 0.675)	(0.537 - 0.936)		
Alpha 1 Globulin	2.222	3.475	3.505	N.S.	<0.001
	(1.811 - 2.726)	(2.896 - 4.170)	(2.856 - 4.301)		
Alpha 2 Globulin	2.504	3.420	5.438	<0.001	<0.001
	(1.957 - 3.204)	(2.745 - 4.261)	(4.250 - 6.959)		
Beta Globulin	1.487	2.402	3.202	<0.005	<0.001
	(1.205 - 1.836)	(1.990 - 2.898)	(2.594 - 3.954)		
Gamma Globulin	1.225	1.684	3.097	<0.001	<0.001
	(1.002 - 1.589)	(1.435 - 2.005)	(2.546 - 3.766)		

(2-^{14}C)glycine was injected on the sixth day postburn. Values are means and 0.95 confidence intervals of the group means. Data were transformed to ln before analyses of variance (ANOV).

The RSA of the serum proteins measured at 30 and 60 minutes after injection of (2-^{14}C)glycine is shown in Tables 3 and 4. The RSA values of all the proteins except gamma globulin were 1.5 times higher in the burned rats than in the controls. The RSA of all the proteins of the burned-infected rats were 2.5 to 3 times control values; those of the Sulfamylon treated group were about twice control values. The limitations of using RSA values to estimate synthesis rates will be discussed below.

Table 5 shows the percentage of total serum (^{14}C)protein in each electrophoretic fraction. Not only was a greater quantity of ^{14}C incorporated into serum protein by the injured animals but its distribution among the fractions was changed; a greater proportion was channeled into the alpha and beta globulin fractions.

Total Body Albumin Experiments. The albumin contents of tissues of control and burned rats are shown in Table 6. The albumin content of the plasma and viscera were lower in the burned animals. The burned skin albumin content was 3.5 times that of the unburned skin of the rats of either group. The albumin contents of the carcass and of the unburned skin of the burned and control rats were essentially equal, as was the water content (Table 7) of these tissues. The water content of muscle samples taken from beneath the burn wound was slightly higher than that of muscle taken from an area away from the burn wound. Burned skin had a total water content 1.1 times that of the unburned skin.

The albumin contents of the plasma and eviscerated blood-free body of other groups of rats are shown in Table 8. The whole-body albumin content of the burned rats was 1.4 times the control value; that of the burned-infected rats was 1.2 times control. The values for the burned-infected rats may be biased upward because final calculations were expressed on a weight basis. Many of these rats had lost almost one-third of their original body weight and were moribund at the time of sacrifice. In the meantime, the burned rats had regained weight lost during the first two days postburn; at time of sacrifice their mean weight was equal to that of the control group.

No albumin was detected in the ultrafiltrates of tissue extracts when they were tested by radioimmunoassay. A concentrate of the portion of the extract retained by the ultrafilter showed precipitin bands with anti-rat serum which appeared to be identical to those of normal rat serum when tested by electrophoresis followed by immunodiffusion. With anti-rat albumin or anti-rat IgG, a single precipitin band was detected with each antiserum. No attempt was made to quantitate the precipitin reaction, nor to determine the composition of the components separated by ordinary electrophoresis procedures.

DISCUSSION

(2-^{14}C)glycine incorporation. Because we did not measure the glycine specific activity we cannot directly convert relative specific activity (RSA) values to synthetic rates. However, glycine specific activity estimated from published free glycine levels in plasma ultrafiltrate (Control, 0.527 uM/ml;

Table 3. Mean Relative Specific Activity of Rat Serum Protein Fractions at 30 Minutes Post-injection

Protein Fraction	Group				ANOV Comparisons		
	Control	Burned	Burned-Infected	Burned-Infected Sulfamylon^{R} Rx	B vs BIS	B + BIS vs BI	B + BIS +BI vs C
	(C) N = 8	(B) N = 9	(BI) N = 12	(BIS) N = 8		p =	
Total Protein	0.227 (0.176 - 0.293)	0.366 (0.289 - 0.463)	0.836 (0.689 - 1.015)	0.529 (0.410 - 0.682)	<0.025	<0.001	<0.001
Albumin	0.103 (0.079 - 0.135)	0.165 (0.129 - 0.213)	0.303 (0.245 - 0.375)	0.218 (0.166 - 0.285)	<0.050	<0.001	<0.001
Alpha - 1 *Globulin*	0.443 *(0.330 - 0.595)*	0.704 *(0.534 - 0.927)*	1.004 *(0.794 - 1.270)*	0.756 (0.562 - 1.015)	N.S.	<0.005	<0.001
Alpha - 2 Globulin	0.661 (0.456 - 0.959)	0.919 (0.650 - 1.301)	1.558 (1.160 - 2.092)	1.078 (0.743 - 1.563)	N.S.	<0.005	<0.001
Beta Globulin	0.297 (0.216 - 0.409)	0.457 (0.339 - 0.616)	0.924 (0.717 - 1.191)	0.645 (0.468 - 0.888)	<0.050	<0.001	<0.001
Gamma Globulin	0.263 (0.160 - 0.431)	0.275 (0.173 - 0.436)	0.518 (0.350 - 0.767)	0.362 (0.221 - 0.594)	N.S.	<0.010	<0.050

(2-^{14}C) glycine was injected on the sixth day postburn. Values are means and ( ) 95% confidence intervals of the group means. P values were obtained by analysis of variance (ANOV) on ln transformed data.

Table 4. Mean Relative Specific Activity of Serum Protein Fractions at 60 Minutes Post-Injection

Protein Fraction	Group				ANOV Comparisons		
	Control (C) N = 8	Burned (B) N = 9	Burned-Infected (BI) N = 12	Burned-Infected Sulfamylon^{R} Rx (BIS) N = 8	B vs BIS	B + BIS vs BI	B + BIS + BI vs C
					p =		
Total Protein	0.648 (0.530 - 0.791)	0.996 (0.828 - 1.198)	1.896 (1.628 - 2.208)	1.586 (1.298 - 1.937)	<0.001	N.S.	<0.001
Albumin	0.267 (0.211 - 0.338)	0.409 (0.328 - 0.509)	0.670 (0.556 - 0.807)	0.587 (0.464 - 0.742)	<0.005	<0.001	<0.001
Alpha - 1 Globulin	1.391 (1.086 - 1.781)	2.022 (1.605 - 2.547)	2.279 (1.873 - 2.773)	2.205 (1.722 - 2.823)	N.S.	N.S.	<0.001
Alpha - 2 Globulin	1.727 (1.294 - 2.305)	2.313 (1.766 - 3.029)	3.284 (2.611 - 4.130)	3.158 (2.366 - 4.216)	<0.025	N.S.	<0.001
Beta Globulin	0.821 (0.627 - 1.076)	1.277 (0.992 - 1.644)	1.931 (1.558 - 2.393)	1.773 (1.353 - 2.324)	<0.025	<0.025	<0.001
Gamma Globulin	0.771 (0.549 - 1.084)	0.789 (0.574 - 1.083)	1.468 (1.121 - 1.922)	1.201 (0.855 - 1.687)	<0.025	<0.005	<0.005

(2-^{14}C)glycine was injected on the sixth day postburn. Values are means and ( ) 95% confidence intervals of the group means. P values were obtained by analysis of variance (ANOV) on ln transformed data

Table 5. Distribution of (^{14}C)protein in Electrophoretic Fractions of Rat Serum

Group	Minutes Post-Injection	% of Injected Dose of ^{14}C Incorporated into Total Serum Protein	% of Total Serum (^{14}C)protein in				
			Albumin	Alpha-1 Globulin	Alpha-2 Globulin	Beta Globulin	Gamma Globulin
Control	30	0.47	27.7	23.0	24.5	18.7	7.4
	60	1.25	23.9	24.2	24.9	19.3	7.6
Burned	30	0.77	23.6	22.2	27.9	19.2	7.3
	60	1.87	21.6	23.9	27.3	19.6	7.6
Burned-Infected Sulfamylon^{R} Treated	30	1.21	15.2	21.6	30.2	26.7	6.0
	60	3.06	13.4	23.3	30.3	24.5	6.8
Burned Infected	30	2.10	9.7	22.5	34.9	27.8	5.1
	60	4.18	8.7	23.5	35.6	26.1	6.2

[illegible] injected on the sixth day postburn.

Table 6. Albumin Content of Blood-free Rat Tissues

Tissue	% of Total Rat Weight		mg albumin/gm tissue (wet weight)		Ratio B/C
	Control	Burned	Control (C)	Burned (B)	
Viscera	21.3	20.0	1.28 ± 0.07	0.90 ± 0.08	0.70
Carcass	54.7	53.9	2.14 ± 0.16	2.10 ± 0.21	0.98
Burned Skin	-	7.1	-	17.50 ± 1.50	
Unburned Skin	18.2	11.8	4.88 ± 0.28	4.83 ± 0.06	0.99
Total Skin	18.2	18.9	4.88 ± 0.28	9.59 ± 0.71	1.97

Mean and S.D. for 3 rats in each group. Mean rat weights: control, 196 gm; burned, 200 gm.
Carcass includes muscle and bone.

Table 7. Water Content of Blood-free Rat Tissues

Tissue	Per Cent Total Water	
	Control (C)	Burned (B)
Burned skin	-	70.5 ± 1.7
Unburned Skin	63.1 ± 2.5	62.5 ± 3.5
Muscle	72.1 ± 2.3	70.9 ± 2.1
Muscle beneath burn	-	73.3 ± 1.0

Mean and S.D. for 3 rats in each group. Total water was determined by drying tissues to constant weight.

Table 8. Plasma and Total Albumin Content of Rats

	Mg Albumin/100 gm Rat Weight				
	Group			ANOV Comparisons	
	Control (C) N = 9	Burned (B) N = 14	Burned infected (BI) N = 8	B vs BI	B + BI vs C
				p =	
Plasma	125.34 (109.38 - 143.64)	107.57 (97.13 - 119.14)	40.26 (34.73 - 46.66)	<0.001	<0.001
Eviscerated-blood free body	207.28 (191.35 - 224.55)	294.48 (277.33 - 312.68)	255.65 (234.42 - 278.80)	<0.005	<0.001
Σ	332.90 (308.72 - 358.98)	402.70 (380.56 - 426.13)	300.55 (276.96 - 326.14)	<0.001	<0.050

Rats were sacrificed on the sixth day postburn. Values are means and (C.I. 95% confidence) intervals of the means. P values were obtained by analysis of variance (ANOV) on ln transformed data.

Burned, 0.454 uM/ml) (26), and our measured free (^{14}C)glycine levels (not shown), was 1.16 times higher in the burned rats. This difference is not great enough to account for the observed RSA differences.

Because both the RSA and plasma pool sizes of the alpha and beta globulins of the injured rats were larger than those of the control rats it is reasonable to conclude that the synthetic rates of these proteins were increased. This was not unexpected since the concentrations of these serum globulins remain elevated for a very long time after injury. This response is not specific for burn injury but also occurs following other forms of trauma, and in the presence of infection or malignancy. The gamma globulin RSA and pool sizes were also greater in the injured rats but because all newly synthesized gamma globulin is not released into the plasma compartment a similar conclusion regarding synthetic rates from plasma measurements alone is not warranted.

The higher plasma albumin RSA in the injured rats was partly due to the differences in the plasma albumin pool sizes. If one should assume that this was the only cause and adjust the ^{14}C incorporation values accordingly, the amount of ^{14}C incorporated into albumin of the injured rats was still equal to (Group BI), or greater than (Groups B and BIS) that of the control rats. We believe that depression of synthesis alone cannot explain the marked depletion of the plasma albumin pool following injury.

Total Body Albumin. The increased whole body albumin pool size of the burned rats reported here is in accord with findings reported by others who measured labeled protein retention in burned animals and humans (6, 10, 17). In addition, we have shown that the tissue albumin pool in burned-infected rats is enlarged despite their severely depleted plasma albumin pool. Although the albumin content of the burned rats' viscera decreased, this absolute quantity contributed little to the overall change. The increase in the whole body albumin pool can be accounted for by the increased albumin content of the burn wound. The larger number of rats processed as eviscerated blood-free whole bodies confirmed the statistical significance of the observed differences in albumin pool sizes.

We can only speculate on the mechanism that maintains an increased albumin content in the burn wound in the presence of a depleted plasma albumin pool. In addition to the restriction of protein transcapillary escape by the capillary pore size and by the basement membrane, interstitial tissue restricts the movement of molecules. Although the compliance of the tissue can accommodate the gradual accumulation of large amounts of edema fluid, the integrity of the tissue can be disrupted by rapid entry of a lesser amount of fluid (19). The

Rosen B, Levenson SM. Non-protein nitrogen changes in serum and plasma of rats following thermal injury. [illegible]

Leach JB, Bray JP, Lewis SR. [illegible]

McMaster PD. An inquiry into the [illegible] and [illegible] of protein in the interstitial fluid of the skin. [illegible]

rate at which protein, water and electrolytes have been shown to enter the skin immediately after burning (2,15) probably exceeds the compliance of the skin and could lead to the formation of channels with little resistance to inward flow (19)or to formation of macroscopic fluid pools in the interstitium (8).Dilution of or damage to the matrix would decrease the tissue volume from which protein and fluid is normally excluded.

It is unlikely that the albumin pool in the burn wound is not exchanging because it has been shown that albumin continues to enter the burn wound and to return to the intravascular compartment through the lymph channels at accelerated rates for several weeks after burn injury (3,17,24).Also, calculation of transfer rates from Lynch, et al's (17) plasma albumin disappearance data (C= -0.385/day; B = - 0.267/day) and our values for pool sizes yielded identical masses transferred per unit time for burned and control rats.The prolonged half life of albumin in burned rats reported by them can be accounted for by the difference in the pool sizes. Our finding that the albumin in the wound had the characteristics of native albumin is further evidence that the pool is not stationary.

The detailed studies performed by Studer and her colleagues of transfer rates of albumin into tissues of normal and plasma volume expanded rats showed that regional differences of transfer rates are very large (30). The results of whole body measurements of retained labeled protein would thus be dependent on the relative masses of the tissues.This is borne out by the fact that our albumin ratios measured on whole bodies were much smaller than those measured on the separated burn wound tissue. This would also mean that wound losses calculated from plasma albumin specific activity would not be reliable.

SUMMARY

This study shows that the low plasma albumin pool size of burned and burned-infected rats is not caused by a lowered synthetic rate but by changes in albumin compartmentation in the tissues. Present compartmental models for

---

2. Arturson G, Mellander S: Acute changes in capillary filtration and diffusion in experimental burn injury.Acta Physiol Scand 62:457-463,1964.

15. Leape LL: Initial changes in burns:Tissue changes in burned and unburned skin of Rhesus monkeys. J Trauma 10:488-492.

8. Catchpole HR: Capillary permeability.In: The Inflammatory Process, 2nd ed. Zweifach BW,Grant L,McClusky RT (eds),New York,Academic Press, 1973,Vol.II,p. 141-144.

3. Arturson G,Soeda S:Changes in transcapillary leakage during healing of experimental burns.Acta Chir Scand 133:609-614,1967.

24. Roberts JC,Courtice FC:Measurements of protein leakage in the acute and recovery stages of a thermal injury.Aust J Exp Biol Med Sci 47:421-433, 1969.

30. Studer RK,Morgan J,Penkoske M,Potchen EJ: Regional vascular volume and extravascular accumulation of labeled protein during plasma volume expansion.Am J Physiol 224:699-704,1973.

measuring protein metabolism should be modified to treat the burn wound as an additional compartment if they are to have real meaning.

REFERENCES

1. Alexander, J. W., W. L. Brown, A. D. Mason, Jr., and J. A. Moncrief. The influence of infection upon serum protein changes in severe burns. J. Trauma 6: 780-789, 1966.

2. Arturson, G. and S. Mellander. Acute changes in capillary filtration and diffusion in experimental burn injury. Acta Physiol. Scand. 62: 457-463, 1964.

3. Arturson G. and S. Soeda. Changes in transcapillary leakage during healing of experimental burns. Acta Chir. Scand. 133: 609-614, 1967.

4. Bartlett, M. S. and D. G. Kendall. The statistical analysis of variance-Heterogeneity and the logarithmic transformation. J. Royal Stat. Soc. Supplement Vol. 8: 128-138, 1946.

5. Birke, G., S-O Liljedahl, L-O Plantin, P. Reizenstein. Albumin catabolism in burns and following surgical procedures. Acta Chir. Scand. 118: 353-366, 1960.

6. Birke, G., S-O Liljedahl, L-O Plantin and P. Reizenstein. Studies on Burns IX. The distribution and losses through the wound of ^{131}I-albumin measured by whole-body counting. Acta Chir. Scand. 134: 27-36, 1967.

7. Bray, G. A. A single efficient liquid scintillator for counting aqueous solutions in a liquid scintillation counter. Anal. Biochem. 1: 279-285, 1960.

8. Catchpole, H. R. Capillary permeability. In: The Inflammatory Process 2nd ed. edited by B. W. Zweifach, L. Grant and R. T. McClusky, New York: Academic Press, 1973, Vol. II, p. 141-144.

9. Chandler, A. M. and O. W. Neuhaus. Synthesis of serum glycoproteins in response to injury. Am. J. Physiol. 206: 169-173, 1964.

10. Davies, J. W. L., C. R. Ricketts, and J. F. Bull. Studies of plasma protein metabolism. Part 1. Albumin in burned and injured patients. Clin. Sci. 23: 411-423, 1962.

11. Farr, R. S. Determination of antigen binding capacity. In: Methods in Immunology and Immunochemistry. Reactions of antibodies with soluble antigens, edited by C.A. Williams and M.W. Chase, New York: Academic Press, 1971, Vol. III p. 66-73.

12. Katz, J., G. Bonorris, S. Golden, and A.L. Sellers. Extravascular albumin mass and exchange in rat tissues. Clin. Sci. 39: 705-724, 1970.

13. Kukral, J. C. and D. C. Meadows. Synthesis of plasma protein fractions in burned patients. Surg. Forum 15: 43-45, 1964.

14. Lanchantin G. F. and R. E. Deadrick. Serum protein changes in thermal trauma. I. Electrophoretic analysis at pH 8.6. J. Clin. Invest. 37: 1736-1745, 1958.

15. Leape, L.L. Initial changes in burns: Tissue changes in burned and unburned skin of Rhesus monkeys. J. Trauma 10: 488-492, 1970.

16. Lindberg, R.B., J. A. Moncrief, and A.D. Mason, Jr. Control of experimental and clinical burn wound sepsis by topical application of Sulfamylon compounds. Ann. N.Y. Acad. Sci. 150: 950-960, 1968.

17. Lynch, J.B., J. P. Bray, S.R. Lewis, and T.G. Blocker. Studies with radioisotope labeled albumin in experimental burn wounds. J. Surg. Res. 4. 226-232, 1964.

18. McFarlane, A. S. In vivo behavior of ^{131}I-fibrinogen. J. Clin. Invest. 42: 346-361, 1963.

19. McMaster, P. D. An inquiry into the structural conditions affecting fluid transport in the interstitial tissue of the skin. J. Exp. Med. 74: 9-28, 1941.

20. Nylen B., and G. Wallenius. The protein loss via exudation from burns and granulating wound surfaces. Acta Chir. Scand. 122: 97-100, 1961.

21. Perlman, G. E., W. W. L. Glenn, and P. Kaufman. Changes in the electrophoretic patterns in lymph and serum in experimental burns. J. Clin. Invest. 22: 627-633, 1943.

22. Peters, T., Jr. The biosynthesis of rat serum albumin. I. Properties of rat albumin and its occurrence in liver cell fractions. J. Biol. Chem. 237: 1181-1185, 1962.

23. Prendergast, J. J., R. L. Fenichel and B. M. Daly. Albumin and globulin changes in burns as demonstrated by electrophoresis. Arch. Surg. 64: 733-740, 1952.

24. Roberts, J. C. and F. C. Courtice. Measurements of protein leakage in the acute and recovery stages of a thermal injury. Aust. J. Exp. Biol. Med. Sci. 47: 421-433, 1969.

25. Rodbard, D., W. Bridson, and P. L. Rayford. Rapid calculation of radioimmunoassay results. J. Lab. Clin. Med. 74: 770-781, 1969.

26. Rosen, H. and S. M. Levenson. Non-protein nitrogen changes in serum and plasma of rats following thermal injury. Proc. Soc. Exp. Biol. Med. 83: 91-97, 1953.

27. Rothschild, M.A., M. Oratz, and S.S. Schreiber. Albumin synthesis (Part II). New Eng. J. Med. 286: 816-821, 1972.

28. Sellers, A.L., J. Katz, G. Bonorris, and S. Okuyama. Determination of extravascular albumin in the rat. J. Lab. Clin. Med. 68: 177-185, 1966.

29. Steel, R.G.D., and J.H. Torrie. Principles and Procedures of Statistics. New York: McGraw Hill, 1960, p. 112-115.

30. Studer, R.K., J. Morgan, M. Penkoske, and E.J. Potchen. Regional vascular volume and extravascular accumulation of labeled protein during plasma volume expansion. Am. J. Physiol. 224: 699-704, 1973.

31. Walker, H.L., A.D. Mason, Jr., and G. L. Raulston. Surface infection with Pseudomonas aeruginosa. Ann Surg. 160: 297-305, 1964.

32. Walker, H.L., and A.D. Mason, Jr. A standard animal burn. J. Trauma 8: 1049-1051, 1968.

33. Walter, H., F. Haurowitz, S. Fleischer, A. Lietze, H.F. Cheng, J.E. Turner, and W. Friedberg. The metabolic fate of injected homologous serum proteins in rabbits. J. Biol. Chem. 224: 107-119, 1957.

34. Weichselbaum, T.E. An accurate and rapid method for the determination of proteins in small amounts of blood serum and plasma. Am. J. Clin. Path. 16 (Tech Sec 10): 46-49, 1946.

PRESENTATIONS AND/OR PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION* DA OC 6978	2. DATE OF SUMMARY* 75 07 01		REPORT CONTROL SYMBOL DD-DR&E(AR)636
3. DATE PREV SUM'RY 74 07 01	4. KIND OF SUMMARY D. CHANGE	5. SUMMARY SCTY* U	6. WORK SECURITY* U	7. REGRADING* NA	8. DISP'N INSTR'N NL	8b. SPECIFIC DATA-CONTRACTOR ACCESS ☒ YES ☐ NO	9. LEVEL OF SUM A. WORK UNIT

10. NO. CODES*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A161102B71R	01	300
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)* (U) Evaluation of Gastrointestinal Absorption and Nutritional Efficacy of Standard High Protein Diet in Burned Soldiers (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS* 003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
69 07	Cont	DA	C. In-House

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

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 75	.6	17
	CURRENT 76	.8	22

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

20. PERFORMING ORGANIZATION
NAME:* US Army Institute of Surgical Research
ADDRESS:* Burn Study Branch
Fort Sam Houston, Texas 78234
PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME:* Douglas W. Wilmore, MD
TELEPHONE: 512-221-5712
SOCIAL SECURITY ACCOUNT NUMBER:
ASSOCIATE INVESTIGATORS
NAME: John F. Peterson, 1LT, MSC
NAME: DA

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Gastrointestinal absorption; (U) High protein diet; (U) Trace elements; (U) Humans

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

23. (U) To evaluate the nutritional efficacy of standard high protein diets, nutritional supplements and elemental diets in burned patients. To determine the hormonal and dietary factors which influence nitrogen balance in thermally injured troops.

24. (U) The effect of fat and carbohydrate calories on nitrogen excretion and nitrogen balance is presently being evaluated in normal and injured man. Metabolic diets containing 15 g nitrogen per meter square and varying quantities of fat (0 to 900 kcal/m2) and carbohydrate (0 to 1600 kcal/m2) have been fed to 18 burn patients and eight normal individuals for 5 to 31 days per individual (a total of 278 study days to date). Nitrogen loss, metabolic rate, and body weight are measured, and nitrogen balance, caloric balance, and alterations in body weight are determined. Similar studies have been performed at lower(5-12 g/m2) and higher (18-22 g/m2) levels of nitrogen intake. Serial serum amino acid patterns have been determined in eight patients receiving the standard diet during their hospitalization.

25. (U) 74 07 - 75 06 Studies to date suggest that nitrogen excretion and nitrogen balance are influenced primarily by metabolic rate, carbohydrate calories, and the quantity of nitrogen in the diet. Amino acid profiles demonstrate a decrease in serum levels of all essential amino acids except phenylalanine. An elevation in the phenylalanine to tyrosine ratio exists following thermal injury. Further studies are now in progress to evaluate this enzymatic block and to provide therapeutic diets which could provide adequate precursors for catecholamine synthesis.

*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 68 (FOR ARMY USE) ARE OBSOLETE

ANNUAL PROGRESS REPORT

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

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

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

1 July 1974 - 30 June 1975

Investigators:

Douglas W. Wilmore, M.D.
John P. Peterson, 1LT, AMSC

Reports Control Symbol MEDDH-288(R1)

Unclassified

ABSTRACT

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

REPORT TITLE: EVALUATION OF GASTROINTESTINAL ABSORPTION AND NUTRITIONAL EFFICACY OF STANDARD HIGH PROTEIN DIETS 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 1974 - 30 June 1975

Investigators: Douglas W. Wilmore, M.D.
John P. Peterson, ILT, AMSC

Reports Control Symbol MEDDH-288(R1)

Thermal injury is a short-term hypercatabolic stress, which severly erodes body tissue stores. Adequate food intake, with or without supplemental hormonal therapy, which serves to stimulate insulin output, will reverse erosion of lean body mass and provide optimal function of organ systems until closure of the burn wound can be achieved. Further modification and improvement of the diet for the burn patient may be possible.

Intestinal absorption
Hospital diet
Protein sparing
Insulin

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

Modification of the accelerated rate of tissue breakdown and loss of protoplasmic mass is a major priority following extensive injury. This report reviews the role of nutritional support following major injury, and emphasizes those factors which have maintained or improved organ system function during the hypercatabolic state.

The Effect of Fat and Carbohydrate Calories on Nitrogen Excretion and Nitrogen Balance in Enteral Feedings

Because of the dominant effect exerted by carbohydrate calories administered by the intravenous route in reducing nitrogen excretion, this study was undertaken to evaluate the influence of fat and carbohydrate calories on nitrogen excretion and nitrogen balance in the standard high-protein hospital diets. Metabolic diets were prepared in the metabolic kitchen from known constituents. Nitrogen administration was held constant at 15 g nitrogen/m^2, but the quantity of nonprotein calories varied, with fat administration ranging from 0 to 900 calories/m^2 and carbohydrate calorie administration ranging between 0 and 1600 calories/m^2. Eighteen burned patients and eight normal individuals have been studied to date for five to 31 study days per individual (a total of 278 study days with an average of eight days per person). Nitrogen loss, metabolic rate, and body weight are measured daily, and nitrogen balance, caloric balance and alterations in body weight are determined. In addition, eight patients have been studied at lower levels of nitrogen intake (five to 12 g/m^2), and five individuals have been evaluated at higher levels of nitrogen intake (18 to 22 g/m^2).

The studies completed to date suggest that nitrogen excretion and nitrogen balance are influenced primarily by metabolic rate, carbohydrate caloric intake, and the quantity of nitrogen in the diet. Increasing carbohydrate calories in the hospital diet, which were administered as a constant dietary intake for a mean of eight days per individual, showed a gradual reduction of nitrogen excretion as the dose of carbohydrate calories increased (Table 1). A similar reduction in nitrogen excretion was not observed with step-wise increases in fat calories.

Serum Amino Acid Concentrations Following Thermal Injury

To determine the effect of the standard hospital high-protein, high-caloric diet on post-traumatic amino acid metabolism, serial amino acid patterns were determined on seven patients, with a mean age of 32

TABLE 1

NITROGEN EXCRETION IN G/m^2/Day AT VARIOUS LEVELS OF CARBOHYDRATE INTAKE

Carbohydrate Intake ($kcal/m^2$/Day)	0-100	400-700	800-1300
Metabolic rate			
<1100 $kcal/m^2$/day	13.7	11.8	6.1
>1200 $kcal/m^2$/day		15.0	9.5

years and a mean burn size of 53.5% total body surface (range 28-74%). Samples were obtained within 72 hours of injury, and then repeated in a serial manner until wound closure was achieved.

All essential amino acids were decreased in the serum except phenylalanine (Table 2). Phenylalanine was elevated compared with normal man, and the phenylalanine to tyrosine ratio was increased, suggesting a block in the enzymatic conversion steps from phenylalanine to tyrosine. Branch chain amino acids were decreased, as were the gluconeogenic precursors.

Consistently low levels of almost all amino acids occur in the blood sample of these catabolic patients, reflecting a state similar to chronic malnutrition starvation, in spite of the fact that adequate calorie and nitrogen support was provided. The exception to this general observation is that a high level of phenylalanine occurs, resulting in an elevated phenylalanine/tyrosine ratio. The physiological significance of this abnormality in stressed patients requiring metabolic precursors for catecholamine synthesis is presently being evaluated. The implications of these findings support the suggestion that high caloric, high protein food intake is required in critically ill patients. However, some variation in the diet may be necessary in order to bypass enzymatic blocks which may occur. For example, if hydroxylation of tyrosine could proceed at normal rates, then tyrosine loading would provide sufficient amino acid precursors for catecholamine synthesis. Branch chain amino acids are not elevated, suggesting adequate insulinization of skeletal muscle, which removes these amino acids from the blood stream. Gluconeogenic precursors are likewise decreased, presumably by deamination of these amino acids in the liver. Further work on the metabolism of specific amino acids following injury is required.

PUBLICATIONS AND/OR PRESENTATIONS

None

TABLE 2

AMINO ACIDS FOLLOWING THERMAL INJURY
(Micromoles/L; Mean ± S.E.)

	Burn Patients (n=41)	Normals (n=8)
Theonine	7.7 ± 0.4*	12.0 ± 0.5
Valine	11.9 ± 0.8*	22.2 ± 1.0
Methionine	2.1 ± 0.4	2.4 ± 0.3
Isoleucine	4.5 ± 0.3*	6.2 ± 0.4
Leucine	9.7 ± 0.5*	13.3 ± 1.0
Phenylalanine	5.8 ± 0.3*	4.8 ± 0.2
Lysine	11.6 ± 0.6*	16.6 ± 0.1
Histidine	6.0 ± 0.5*	7.6 ± 0.1
Tyrosine	3.9 ± 0.2*	5.2 ± 0.2
Glycine	11.9 ± 1.0*	20.4 ± 0.4
Alanine	17.5 ± 0.7*	23.2 ± 1.6
Ornithine	2.7 ± 0.4*	4.0 ± 0.2

*$p < 0.05$

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA OE 6982	75 07 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
74 07 01	D. CHANGE	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A161102B71R	01	119
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)* (U) A Therapeutic Trial of Antacid in Prevention of the Clinical Complications Associated With Gastric Mucosal Disease in Burned Soldiers (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*
003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
74 01	Cont	DA	C. In-House

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

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 75	.7	20
FISCAL YEAR CURRENT 76	.6	15

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

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

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

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME:* Joseph C. McAlhany, Jr, MD, MAJ, MC
TELEPHONE: 512-221-2943
SOCIAL SECURITY ACCOUNT NUMBER:

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

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Curling's Ulcer; (U) Burned soldiers; (U) Gastritis; (U) Antacid

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

23. (U) To determine if neutralization of hydrogen ions by antacid administration to burned soldiers who manifest disruption of the gastric mucosal barrier will prevent progressive gastric mucosal damage, gastric hemorrhage or perforation.

24. (U) All patients admitted to the Institute of Surgical Research within 72 hours after sustaining greater than 35% total body surface injury will be considered for this study. A lithium flux test will be performed as has been previously described within the 72-hour postburn period. The patients will then be randomly assigned to receive a standard antacid preparation or no neutralization of gastric acid. The patients with disruption of the gastric mucosal barrier who receive antacid therapy will be statistically compared as regards hemorrhage and perforation to those with disruption of the gastric mucosal barrier who receive no antacid therapy.

25. (U) 74 07 - 75 06 This project is still in progress and the data will be analyzed at the conclusion of fifty patients total. Preliminary review suggests that antacid therapy in burn patients reduces clinically significant complications of acute upper gastrointestinal mucosal ulceration and may limit progression of mucosal damage.

*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. 3A161102B71R-01, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: A THERAPEUTIC TRIAL OF ANTACID IN PREVENTION OF THE CLINICAL COMPLICATIONS ASSOCIATED WITH GASTRIC MUCOSAL DISEASE IN BURNED SOLDIERS

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

1 July 1974 - 30 June 1975

Investigators:

Joseph C. McAlhany, Jr., M.D., Major, MC
Albert J. Czaja, M.D., Major, MC
Basil A. Pruitt, Jr., M.D., Colonel, MC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

REPORT TITLE: A THERAPEUTIC TRIAL OF ANTACID IN PREVENTION OF THE CLINICAL COMPLICATIONS ASSOCIATED WITH GASTRIC MUCOSAL DISEASE IN BURNED SOLDIERS

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

Period covered in this report: 1 July 1974 - 30 June 1975

Investigators: Joseph C. McAlhany, Jr., M.D., Major, MC
Albert J. Czaja, M.D., Major, MC
Basil A. Pruitt, Jr., M.D., Colonel, MC

Reports Control Symbol MEDDH-288 (R1)

A lithium flux technique has been utilized to assess the integrity of the gastric mucosal barrier (GMB) following thermal injury. Within 72 hours post burn, disruption of the GMB was correlated with endoscopic progression of gastric mucosal disease, gastric hemorrhage or perforation in 7 of 8 patients. No instance of gastric hemorrhage or perforation was encountered in 10 patients with a normal GMB. This data suggests a lithium flux technique could be a useful index of clinical gastric complications occurring after thermal injury.

Patients admitted to the U.S. Army Institute of Surgical Research within 72 hours after sustaining greater than 35% total body surface injury will be considered for a therapeutic trial of antacid in prevention of the clinical complications associated with gastric mucosal disease after burns. A lithium flux test will be performed, as previously described, within 72 hour post burn period. The patient population will then be randomly assigned to receive a standard liquid antacid preparation or no intragastric neutralization of acid.

The data generated by the study will allow statistical comparison as regards the incidence of hemorrhage and perforation. The patients with disruption of the gastric mucosal barrier who receive antacid therapy will be contrasted to those with disruption of the gastric mucosal barrier who receive no antacid therapy. These comparisons will clarify the effectiveness of antacid in prevention of clinical complications associated with progressive gastric mucosal disease after burns.

Curling's ulcer
Burned soldiers
Antacid
Gastritis

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA OE 6978	75 07 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISP'N INSTR'N	8b. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
74 07 01	D. CHANGE	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A161102B71R	01	120
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)* (U) Cell Renewal Kinetics of the Gastric Mucosa 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
74 02	Cont	DA	C. In-House

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

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 75	.5	15
	CURRENT 76	.6	16

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

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

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

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME:* Albert J. Czaja, MD, MAJ, MC
TELEPHONE: 512-221-6532
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: Thomas A. Rizzo, MD, MAJ, MC
NAME: Joseph C. McAlhany,Jr,MD,MAJ,MC DA

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Mucosal regeneration rate; (U) Curling's ulceration; (U) Healing; (U) Microautoradiography; (U) Humans

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

23. (U) To determine the proliferative activity of the gastric mucosa in burned soldiers immediately after burn and during the second week after injury when the manifestations of acute gastroduodenal disease are most pronounced. Changes in the proliferative activity of the mucosa will be correlated with changes in the endoscopic appearance of the mucosa. Decreased proliferative activity in the presence of progressive mucosal injury would indicate an impaired healing process which might increase the mucosa's susceptibility to ulceration, hemorrhage, and perforation.

24. (U) Mucosal biopsies will be obtained from the antrum and body of the stomach through the gastroscope on the 3rd day postburn. This procedure will be repeated on the 14th day postburn and biopsies will be obtained from areas adjacent to mucosal lesions. Tissue specimens will be incubated in tritiated thymidine and then processed for autoradiogrphy. The proliferative index will be determined by a "blinded" investigator.

25. (U) 74 07 - 75 06 Preliminary animal studies have demonstrated that mucosal biopsies, incubation procedures, and autoradiography techniques are adequate. Ten patients have been completely studied to date. Autoradiography results are pending.

*Available to contract. rs 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. 3A161102B71R-01, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: CELL RENEWAL KINETICS OF THE GASTRIC MUCOSA IN THE THERMALLY INJURED SOLDIERS

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

1 July 1974 - 30 June 1975

Investigators:

Albert J. Czaja, MD, Major, MC
Thomas A. Rizzo, Jr., MD, Major, MC
Joseph C. McAlhany, Jr., MD, Major, MC
Paulette Langlinais
Basil A. Pruitt, Jr., MD, Colonel, MC

Reports Control Symbol MEDDH-288(R1)

Unclassified

ABSTRACT

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

REPORT TITLE: CELL RENEWAL KINETICS OF THE GASTRIC MUCOSA IN THE 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 1974 - 30 June 1975

Investigators: Albert J. Czaja, MD, Major, MC
Thomas A. Rizzo, Jr., MD, Major, MC
Joseph C. McAlhany, Jr., MD, Major, MC
Paulette Langlinais
Basil A. Pruitt, Jr., MD, Colonel, MC

Reports Control Symbol MEDDH-288(R1)

To determine changes in the proliferative activity of the gastric mucosa following thermal injury, and to correlate these changes with the development of acute gastroduodenal disease, fiberoptic gastroduodenoscopy, with visually directed tissue biopsy, was performed in 23 burn patients who had sustained burns of more than 35% of their total body surface. Endoscopic examinations of the stomach and duodenum were performed on the third day postburn, and were repeated on the 14th day after injury in each patient. Tissue specimens were obtained at each endoscopy from the area adjacent to mucosal lesions. Samples were procured from the corpus and the antrum of the stomach in each case. Tissue specimens were incubated in tritiated thymidine and processed for autoradiography. The ratio of labelled to unlabelled cells was determined by a blinded investigator and was expressed as the proliferative index. Forty two endoscopic procedures were performed without complication in the 23 patients, and 262 mucosal specimens were obtained. All thermally injured patients irrespective of the presence of gastric mucosal disease had elevated proliferative indices on the third day after burn. Patients with gastric mucosal disease had a higher proliferative index than patients without mucosal disease, indicating a mucosal response to injury. The poliferative index of the fundus was consistently greater than the proliferative index of the antrum in all patients on the third day postburn. By the 14th day after burn, four patterns of mucosal reaction were identified. Patients who had a normal gastric mucosa on Day 3, and who had maintained an intact mucosa by Day 14, demonstrated a decrease in the proliferative index to a level which has been reported as normal. Patients

with an abnormal gastric mucosa on Day 3, who had persistent or progressive gastric mucosal disease by Day 14, maintained an elevated, if not further increased, proliferative index. Patients with an abnormal gastric mucosa on Day 3, who subsequently healed their gastric mucosa by Day 14, demonstrated a lowering of their proliferative index to a level consistent with the patients who had maintained a normal gastric mucosa throughout their convalescence. Four patients who had abnormal gastric mucosa on Day 3, and whose gastric mucosal disease progressed by Day 14, demonstrated a marked fall in proliferative index into the lower normal range, even though their mucosal disease had worsened. The clinical course of each of these patients had been complicated by septicemia and hypotension.

Conclusion

The majority of thermally injured patients demonstrate a normal mucosal response to injury by increasing the proliferative activity of the mucosa in the normal healing process. Patients who deteriorate clinically tend to have progression of their gastric mucosal disease in association with an impaired healing response of their gastric mucosa.

Mucosal regeneration rate
Curling's ulceration
Healing
Microautoradiography
Humans

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA 0E 6969	75 07 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
74 07 01	D. CHANGE	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO. / CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A161102B71R	01	080
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Procede with Security Classification Code)* (U) The Effect of Epinephrine and Glucagon on the Rate of Heme Catabolism and Bilirubin Production in the Burned Soldier (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*
003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
73 03	Cont	DA	C. In-House

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

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 75	.7	20
FISCAL YEAR CURRENT 76	.4	11

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

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

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

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME:* Albert J Czaja, MD, MAJ, MC
TELEPHONE: 512-221-6532
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: Willard A Andes, MD, MAJ, MC
NAME: Douglas W Wilmore, MD DA

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Procede EACH with Security Classification Code)
(U) Bilirubin; (U) Heme Catabolism; (U) Epinephrine; (U) Glucagon; (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) In order to better understand the metabolic, hepatic, and hematologic derangements of the burned soldier, the controls of bilirubin production from heme precursors are examined immediately after thermal injury and during convalescence. The rate of bilirubin synthesis is correlated with red blood cell survival, levels of erythroid and nonerythroid heme substrate, glucagon and epinephrine levels.

24. (U) The injured soldier is studied acutely postburn and then during convalescence. Routine hemolytic studies, BSP retention, bilirubin, glucagon and epinephrine levels, and liver function studies are obtained during each study period. The chromium-51 RBC survival time and the rate of endogenous carbon monoxide production are measured simultaneously as a reflection of the rate of hemoglobin destruction and bilirubin production. Convalescent patients and normal controls are studies similarly during infusions of epinephrine, glucagon, or amino acids. The effects of epinephrine, glucagon, hemolysis, ineffective erythropoiesis, and increased non-erythroid heme catabolism on the rate of bilirubin production are determined.

25. (U) 74 07 - 75 06 Gas chromatography is being utilized to determine carbon monoxide concentrations in normal subjects. The sensitivity and reliability of this method is being established. A satisfactory rebreathing system has been constructed and the determination of the rate of endogenous carbon monoxide production is currently being measured in normal volunteers before and after epinephrine infusion. When consistent normal responses have been established, patients will be studied under similar circumstances.

*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. 3A161102B71R-01, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: THE EFFECT OF EPINEPHRINE AND GLUCAGON ON THE RATE OF HEME CATABOLISM AND BILIRUBIN PRODUCTION IN THE BURNED SOLDIER

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

1 July 1974 - 30 June 1975

Investigators:

Albert J. Czaja, MD, Major, MC
Willard A. Andes, MD, Major, MC
Edwin W. Hander, MS, Captain, MSC
Robert J. Lull, MD, Lieutenant Colonel, MC
Douglas W. Wilmore, MD

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

REPOPT TITLE: THE EFFECT OF EPINEPHRINE AND GLUCAGON ON THE RATE OF HEME CATABOLISM AND BILIRUBIN PRODUCTION 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 1974 - 30 June 1975

Investigators: Albert J. Czaja, MD, Major, MC
Willard A. Andes, MD, Major, MC
Edwin W. Hander, MS, Captain, MSC
Robert J. Lull, MD, Lieutenant Colonel, MC
Douglas W. Wilmore, MD

This investigation was designed to evaluate the initial step in bilirubin metabolism in patients with thermal injury, i.e., the conversion of ferroporphyrin to bilirubin. The object was to examine some of the controls of bilirubin production from heme precursors and to correlate changes in the rate of bilirubin synthesis with fluctuations in the levels of heme substrate, glucagon, and epinephrine under the acute and convalescent conditions of thermal injury. The contributions of erythroid and nonerythroid heme substrate to the bilirubin pool of these patients could also be determined. The data would provide insight into red blood cell survival after thermal injury and permit speculation about the humoral influences on bilirubin metabolism. The rate of heme catabolism and bilirubin production in the burn patient was determined by measuring the rate of endogenous carbon monoxide production shortly after thermal injury, during convalescence, and after stimulation of heme oxygenase activity by controlled intravenous infusion of epinephrine. The difference between the amount of carbon monoxide produced and actually measured in each patient, and that calculated from the rate of hemoglobin destruction, would allow estimation of the contribution of nonerythroid and erythroid heme catabolism to the bilirubin pool. The rate of endogenous carbon monoxide production was measured while patients were in a closed-rebreathing system, in which oxygen was continuously administered to maintain an oxygen tension of approximately 150 mmHg, and carbon dioxide was continuously removed from the system by a CO_2 absorber. Gas chromotography was utilized to determine carbon monoxide concentrations. The project was terminated when it became apparent that a closed-rebreathing system could not be devised which was airtight and which insured patient comfort during the four to six hour period of

rebreathing. The metabolic hood, the total body box, and gas mask rebreathing system were unable to provide reproducible measurements and patient comfort.

Bilirubin
Heme catabolism
Epinephrine
Glucagon
Humans

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OD 6985	75 07 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
74 07 01	D. CHANGE	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A161102B71R	01	311
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)

(U) Inhalation Injuries-Pathogenesis and Treatment in Burned Troops (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
72 05	Cont	DA	C. In-House

17. CONTRACT/GRANT: Not Applicable

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 75	.4	10
FISCAL YEAR CURRENT 76	.1	3

19. RESPONSIBLE DOD ORGANIZATION

NAME: US Army Institute of Surgical Research

ADDRESS: Fort Sam Houston, Texas 78234

RESPONSIBLE INDIVIDUAL

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

TELEPHONE: 512-221-2720

20. PERFORMING ORGANIZATION

NAME: US Army Institute of Surgical Research
Clinical Division

ADDRESS: Fort Sam Houston, Texas 78234

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

NAME: Gary W. Welch, MD, MAJ, MC

TELEPHONE: 512-221-5712

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Peter A. Petroff, Jr, MD, MAJ, MC

NAME: Edwin W. Hander, CPT, MSC DA

21. GENERAL USE

FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Inhalation injury; (U) Xenon lung scan; (U) N204; (U) Surfactant; (U) Goats

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

23. (U) To create a pulmonary injury by inhalation of nitrogen oxides and to evaluate the effect of various treatment modalities on such an injury as a means of developing more effective treatments for burned soldiers.

24. (U) At the present time, 18 goats have received instillation of N204 into their tracheobronchial tree. Four of these goats received methylprednisolone pre- and post-instillation. 133Xe lung scans, arterial blood gases, and lung compliances were obtained in all animals.

25. (U) 74 07 - 75 06 Inhalation of N204 resulted in a marked fall in pulmonary compliance and produced abnormal lung scans in all goats studied. There were no differences in the blood gases pre- or postinjury. Steroids did not affect any of the parameters investigated.

* Available from [illegible] 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. 3A161102B71R-01, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: INHALATION INJURIES--PATHOGENESIS AND TREATMENT IN BURNED TROOPS

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

1 July 1974 - 30 June 1975

Investigators:

Gary W. Welch, MD, Ph.D., Lieutenant Colonel, MC
Peter A. Petroff, MD, Major, MC
Edwin W. Hander, MS, Captain, MSC
John W. Sagartz, DVM, Captain, VC
Robert J. Lull, MD, Lieutenant Colonel, MC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

REPORT TITLE: INHALATION INJURIES--PATHOGENESIS AND TREATMENT 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 1974 - 30 June 1975

Investigators: Gary W. Welch, MD, Ph.D., Lieutenant Colonel, MC
Peter A. Petroff, MD, Major, MC
Edwin W. Hander, MS, Captain, MSC
John W. Sagartz, DVM, Captain, VC
Robert J. Lull, MD, Lieutenant Colonel, MC

To determine the effect of steroids in the treatment of inhalation injury, eight anesthetized goats were divided into two equal groups and subjected to inhalation of nitrogen tetroxide, a gas known to produce inhalation injury. Pulmonary compliance, xenon lung scan, and blood gases (on both room air and 100% oxygen) were obtained before and after injury. Following endotracheal instillation of nitrogen tetroxide, one group of goats received methylprednisolone (1500 mg given in three divided doses over 12 hours). The animals were sacrificed between 18-24 hours and the lungs were examined.

In the steroid-treated group, pulmonary compliance decreased 31.6%. In untreated controls, compliance decreased 41.3% (N.S.).

	ROOM AIR			100% O_2		
	Before	After		Before	After	
		Steroid	Control		Steroid	Control
PO_2	49.5	49.0*	46.5	369	327*	304
PCO_2	39.3	33.2*	36.8	41.8	49*	50.8
pH	7.37	7.47*	7.40	7.27	7.31*	7.23

*No statistical difference between controls and steroid treatment.

Preburn xenon lung scans were normal in all goats, and abnormal in all goats postburn. Three of the four goats treated with methylprednisolone cleared ^{133}Xenon more rapidly from the lungs when compared to the nonsteroid-treated group, but the difference was not significant. Pathological examination of the lung in both groups was consistent with a nitrogen tetroxide inhalation injury, showing obliterative emphysema and mild focal bronchopneumonia in a diffuse pattern throughout the lung fields. No gross or microscopic difference of decreased injury or inflammation could be identified by blinded observers. In conclusion, steroids appear to be of no benefit in the treatment of nitrogen tetroxide inhalation injury in goats.

Inhalation injury
Xenon lung scans
Surfactant
Goats

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION* DA OD 6980	2. DATE OF SUMMARY* 75 07 01		REPORT CONTROL SYMBOL DD-DR&E(AR)636
3. DATE PREV SUMRY 74 07 01	4. KIND OF SUMMARY K. COMP	5. SUMMARY SCTY* U	6. WORK SECURITY* U	7. REGRADING* NA	8A. DISP'N INSTR'N NL	8B. SPECIFIC DATA-CONTRACTOR ACCESS ☒ YES ☐ NO	9. LEVEL OF SUM A. WORK UNIT

10. NO. CODES*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A161102B71R	01	309
b. CONTRIBUTING				
c. CONTRIBUTING				

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

12. SCIENTIFIC AND TECHNOLOGICAL AREAS* 003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
75 06	75 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 75	.8	24
FISCAL YEAR CURRENT		

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

20. PERFORMING ORGANIZATION
NAME:* US Army Institute of Surgical Research
Burn Study Branch
ADDRESS:* Fort Sam Houston, Texas 78234
PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME:* Robert N. Agee, LTC, MC
TELEPHONE: 512-221-4440
SOCIAL SECURITY ACCOUNT NUMBER:
ASSOCIATE INVESTIGATORS
NAME: James M. Long, III, LTC, MC
NAME: John L. Hunt, MD DA

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Inhalation injury; (U) 133 Xenon Lung Scan; (U) Humans; (U) Burns

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

23. (U) To determine the reliability of the 133 Xenon Lung Scan in detecting inhalation injury in combat wounded personnel.

24. (U) All patients with flame, steam or blast injuries and suspected inhalation injury admitted to the USAISR receive a 133 Xenon Lung Scan on admission. The results of the scan are compared with clinical and pathologic diagnosis of inhalation injury to determine the reliability of the 133 Xenon scintiphotography.

25. (U) 74 07 - 75 06 Use of the 133 Xenon Lung Scan over the past year has demonstrated a high correlation of radiation densities with subsequent clinical pathological findings and changes on standard roentgenograms.

* Mandatory for contractors upon originator's approval

DD FORM 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE AND FORMS 1498-1 AND 1498-2 ARE OBSOLETE

FINAL REPORT

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

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

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

1 July 1974 - 30 June 1975

Investigators:

Robert N. Agee, M.D., Lieutenant Colonel, MC
James M. Long, III, MD, Lieutenant Colonel, MC
John L. Hunt, M.D., Lieutenant Colonel, MC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

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

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

Period covered in this report: 1 July 1974 - 30 June 1975

Investigators: Robert N. Agee, M.D., Lieutenant Colonel, MC
James M. Long, III, M.D., Lieutenant Colonel, MC
John L. Hunt, M.D., Lieutenant Colonel, MC

Reports Control Symbol MEDDH-288(R1)

Eighty-six patients with burns admitted to the US Army Institute of Surgical Research in 1974 were studied within 72 hours by ^{133}Xenon lung scan. Seventy-three were males and 13 were females. The average burn size was 50% total body surface, with a range of 7 to 96%.

Of the 86 scans performed, 43% were positive and 56% were negative for inhalation injury. One scan was incorrectly performed and was considered inadequate.

Thirteen per cent of the scans performed were considered erroneous based on all available clinicopathological evidence. Eight per cent were considered falsely positive and 5% falsely negative. The apparent accuracy of the test was 86%.

Confirmatory studies included bronchoscopy and pulmonary function testing. Fiberoptic bronchoscopy was performed in 51% of the patients and pulmonary function testing was performed in 50% of the patients. All three tests were performed in 27 patients or 31% of the total.

The estimate of accuracy of the various tests were scan alone 87%, bronchoscopy alone 86%, pulmonary function testing alone 91%; scan and bronchoscopy 93%, scan, bronchoscopy and pulmonary function testing 96%.

Inhalation injury
^{133}Xenon lung scan
Burns
Humans

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

Injury to the respiratory tract from products of incomplete combustion is a common accompanyment of cutaneous thermal injury. Physical signs and radiographic evidence of tracheobronchial or pulmonary damage are usually absent or equivocal during the first 4 or 5 days after injury when the initiation of specific therapy may be most beneficial. A satisfactory method with which to establish the diagnosis of inhalation injury in the early postburn period has not been available until the recent past.

Moylan and associates in 1972 reported the use of ^{133}Xenon perfusion-ventilation lung scan to establish the diagnosis of inhalation injury during the first 72 hours postburn (1). Among 50 consecutive admissions to the US Army Institute of Surgical Research for thermal injuries due to flame, 15 were found to have delayed isotope "washout", suggesting inhalation injury. Based on clinicopathological evidence, these investigators were unable to find any falsely positive or falsely negative lung scans in their series. They concluded that ^{133}Xenon scintiphotography prior to the 4th postburn day is an easily performed, accurate diagnostic test for inhalation injury prior to the onset of symptoms.

The present study was undertaken to further delineate the reliability of ^{133}Xenon lung scanning to detect inhalation injury. Two additional clinical tools, fiberoptic bronchoscopy and pulmonary function studies, have also been evaluated.

METHODS

Eighty-six patients admitted for burns to the US Army Institute of Surgical Research during 1974 were studied within 72 hours postburn by ^{133}Xenon lung scan (Table 1).

Table 1. Xenon Lung Scans - 1974

Patient Data

Total admissions	244
Xenon scans	86
Males	73
Females	13
Mean burn size	50% TBS
Range	7-96% TBS

The procedure involves positioning the patient beneath a counter in which a diverging collimator is used to obtain an anterior view of both lungs.

---

1. Moylan JA, Jr, Wilmore DW, Mouton DE, Pruitt BA, Jr.: Early diagnosis of inhalation injury using ^{133}Xenon lung scan. Ann. Surg. 176: 477-484, 1972.

Six to 10 millicuries of a ^{133}Xenon saline solution are injected as an IV bolus. Sequential scintiphotos are obtained every 6 seconds for 30 seconds to monitor bolus arrival. Then, 30-second scintiphotos are obtained until washout is complete. Exhaled Xenon gas is collected by vacuum for safe disposal.

Criteria for a normal study are absence of local radioisotope trapping and complete washout by 90 seconds. Scans demonstrating regional washout delay or generalized isotope delay beyond 90 seconds are seen with inhalation injuries and other pulmonary pathology including asthma, chronic obstructive pulmonary disease and pulmonary blebs.

Among the 86 patients who had ^{133}Xenon lung scans, 44 also had bronchoscopy and 43 had pulmonary function testing. Twenty-seven patients were studied by all three methods (Table 2).

Table 2. Confirmatory Studies

Total scans	86	
Bronchoscopy	44	(51%)
PFT	43	(50%)
Bronchoscopy + PFT	27	(31%)
Clinical data	86	(100%)
Autopsy data	38	(44%)

The clinical course and autopsy data, where obtained, were used in the overall assessment of diagnoses of inhalation injury.

RESULTS

Among the 86 scans, 37 were interpreted as positive and 48 as negative for inhalation injury. One scan was performed incorrectly and was considered technically inadequate (Table 3).

Table 3. Xenon Lung Scans - 1974

Total scans	86	
Positive scans	37	(43%)
Negative scans	48	(56%)
Inadequate scans	1	(1%)

Of the patients scanned, 74 (86%) were considered to have appropriate scan results based on all available clinicopathological evidence. The remaining 11 scans were felt to be erroneous. Of these, 7 were falsely positive and 4 were falsely negative (Table 4).

Table 5 lists the results obtained from 7 patients who had falsely positive Xenon lung scans. Six of the 7 had normal appearing tracheobronchial mucosa by fiberoptic bronchoscopy, confirmed by pathoscopy. Four of the 7 had

Table 4. Xenon Lung Scans - 1974

Appropriate scans	74	(86%)
Erroneous scans	11	(13%)
Falsely positive	7	(8%)
Falsely negative	4	(5%)
Inadequate scans	1	(1%)

no evidence of obstructive disease by pulmonary function testing. None had clinical evidence of inhalation injury during the early postburn period. Chest x-rays remained clear and early respiratory problems did not occur. One patient, #4, died one month postburn and had pneumonitis at autopsy, but no specific histopathological evidence of inhalation injury.

Table 5. Falsely Positive Xenon Lung Scans - 1974

Patient #	Scan	Bronchoscopy	PFT	Clinical	Pathologic
1	+	0	-	-	0
2	+	-	0	-	0
3	+	-	0	-	0
4	+	-	-	-	-
5	+	-	-	-	0
6	+	-	-	-	0
7	+	-	-	-	0

Four patients had falsely negative ^{133}Xenon lung scans (Table 6). All 4 had evidence of inhalation injury by bronchoscopy and pulmonary function testing. Three had clinical courses consistent with inhalation injury, and the one who died had autopsy evidence of inhalation injury.

Table 6. Falsely Negative Xenon Lung Scans - 1974

Patient #	Scan	Bronchoscopy	PFT	Clinical	Pathologic
1	-	+	+	-	0
2	-	+	+	+	+
3	-	+	+	+	0
4	-	+	+	+	0

Of the 44 patients undergoing bronchoscopy, none had falsely positive findings. Six patients later determined to have inhalation injury by all available criteria, had falsely negative bronchoscopic findings, i.e.,no carbonaceous material or tracheobronchial mucosal edema, erythrema, hemorrhage or ulceration (Table 7).

Pulmonary function testing employed maximum expiratory flow volume loops. Flow rates reduced out of proportion to volume were considered indicative of obstructive disease. Four patients determined to have inhalation

Table 7. Falsely Negative Bronchoscopy (13%) - 1974

Patient #	Scan	Bronchoscopy	PFT	Clinical	Pathologic
1	+	-	0	-	+
2	+	-	+	+	+
3	+	-	+	+	0
4	+	-	+	+	+
5	+	-	-	+	+
6	+	-	0	+	+

injuries had no evidence of obstructive disease. These were considered falsely negative pulmonary funtion tests (Table 8).

Table 8. Falsely Negative Pulmonary Function Tests (9%) - 1974

Patient #	Scan	Bronchoscopy	PFT	Clinical	Pathologic
1	+	+	-	+	0
2	+	-	-	+	+
3	+	+	-	+	+
4	+	0	-	+	0

DISCUSSION

From the available data, an attempt has been made to determine the reliability of ^{133}Xenon lung scanning as well as fiberoptic bronchoscopy and pulmonary function testing in the early detection of inhalation injury. Of the 86 lung scans performed, the results of 74 were considered appropriate for an accuracy of 87% (Table 9). These 74 included several scans that were neither confirmed nor refuted by the available clinicopathological evidence and were, therefore, considered appropriate. For example, a scan that was interpreted as "mildly positive" in a patient who had not had bronchoscopy or pulmonary function testing and who had a benign early clinical course was considered appropriate and consistent with a mild, subclinical inhalation injury.

Table 9. Diagnostic Technics for Inhalation Injury - 1974

	Estimate of Accuracy	
Scan alone	(74/86)	87%
Bronchoscopy alone	(38/44)	86%
PFT alone	(39/43)	91%
Scan + bronchoscopy	(41/44)	93%
Scan + bronchoscopy + PFT	(26/27)	96%

Eighty-six per cent of the 44 bronchoscopies performed were accurate in detecting the presence or absence of inhalation injury. The involvement of small airways in the absence of gross evidence of tracheobronchial injury

apparently occurs in some cases. Evidence for the presence or absence of obstructive disease by pulmonary function testing was accurate in the early detection of inhalation injury in 91% of those patients studied. Several patients with obvious inhalation injuries were not tested because of the presence of an endotracheal tube at admission.

To evaluate the relative effectiveness of the 3 tests employed to secure early diagnosis of inhalation injury, 27 patients were selected in whom all 3 diagnostic tests had been performed at an appropropriate interval following injury. Table 10 demonstrates the frequency of occurrence of falsely negative and falsely positive tests within this group. Falsely negative scans, which we consider more dangerous because they deny therapy where it may be useful, were virtually eliminated by using any pair of tests, while any single test alone failed in 10-15% to make the diagnosis when injury was present. Falsely positive tests occurred most frequently with Xenon scan.

Table 10. Errors in 27 Patients Undergoing all Three Studies

	Negative Test Positive Patient	Positive Test Negative Patient
Scan	4	3
Bronchoscopy	4	0
PFT	3	1
Scan + bronchoscopy	0	3
Scan + PFT	0	4
Bronchoscopy + PFT	1	1
All	0	4

The apparent effect of inhalation injury on survival is depicted in Table 11. Nearly three-fourths of those with inhalation injury died while two-thirds of those without inhalation injury survived. These numbers are similar to those reported by Moylan, et al. This is not unexpected, however, as larger burns more often have associated inhalation injuries than smaller burns.

Table 11. Effect of Inhalation Injury on Survival

Inhalation injury	33	
Survivors	9	27%
Non-survivors	24	73%
No inhalation injury	53	
Survivors	33	62%
Non-survivors	20	38%

Probit analysis of our last 10 years' experience was used to partition each group according to expected mortality. Exact solutions for the expected total number of deaths in each group were then obtained, using a computer

program for the serial expansion. From these calculations, 95% confidence intervals for total expected mortality were obtained; in each group, the observed number of deaths lay within these intervals (Table 12).

Table 12. Expected and Acutal Mortality
With and Without Inhalation Injury

	Inhalation Injury	No Inhalation Injury	Total
Deaths, expected	21	24	45
95% Confidence limits	17 - 24	19 - 29	39 51
Deaths, observed	24	20	44

Chi square analysis of these totals of observed and expected mortality revealed no significant deviation from expectancy, in agreement with the exact solutions. Similar comparison of mortality within each of the groups revealed good overall agreement between observation and expectation. Thus, each of these groups might, within 95% assurance, have been obtained by random selection from a single patient population and the observed differences between proportional mortality in the two groups be due to upward bias of severity of injury in the group having inhalation injury (Table 13).

Table 13. Frequency of Inhalation Injury in
Relation to Severity of Injury

Expected Mortality	Inhalation Injury	No Inhalation Injury	With Inhalation Injury
0 - 39	8	25	24
40 - 59	5	8	33
60 - 100	20	20	50

[illegible]

Table 14. Effect of Inhalation Injury Upon Mortality

Expected Mortality %		Inhalation Injury	No Inhalation Injury	
0 - 39	L	8	22	X^2 = NS
	D	3	3	
40 - 59	L	0	7	X^2 = 9.48**
	D	5	1	
60 - 100	L	4	4	X^2 = NS
	D	16	16	

during 1974. Inhalation injury was indicated by the 37 (43%) positive scans.

Based on all available clinicopathological evidence, 11 (13%) of the scans were erroneous with 7 (8%) falsely positive and 4 (5%) falsely negative. Eight-six per cent of the scans were "appropriate".[1]

Addition of bronchoscopy and/or pulmonary function testing appeared to improve diagnostic accuracy.

PRESENTATION

Agee RN, Long JM, III, Pruitt BA, Jr. Xenon^{133}Lung Scan for Early Diagnosis of Inhalation Injury. American Burn Assoc. Seventh Annual Meeting, Denver, Colorado, March 20-22, 1975.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA OF 6396	75 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
75 07 01	D. CHANGE	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO. CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A161102B71R	01	316
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)* (U) Evaluation of Dopamine (3,4-Dehydroxypheylethylamine) For Treatment of Septic Shock in Burned Troops (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*
003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMP. FTION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
75 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 75	.6	18
FISCAL YEAR CURRENT 76	.6	15

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

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

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

20. PERFORMING ORGANIZATION
NAME:* US Army Institute of Surgical Research
Clinical Division
ADDRESS:* Fort Sam Houston, Texas 78234

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

ASSOCIATE INVESTIGATORS
NAME: Allister K. Morris, MAJ, MC
NAME: Douglas W. Wilmore, MD DA

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Dopamine; (U) Septic shock; (U) Burn injury; (U) Cardiac output; (U) Humans

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

23. (U) To evaluate the use of dopamine in treatment of septic shock in burned soldiers.

24. (U) Patients meeting the criteria of reduced urine output, hypotension, and evidence of reduced peripheral perfusion will have a Swan-Ganz thermal dilution catheter inserted for determination of cardiac output. After baseline studies are obtained, a pressor infusion of either isoproterenol or dopamine will be started. Changes in cardiac output, blood pressure, urine output and perfusion will again be evaluated. This is a double blind study.

25. (U) 75 02 - 75 06 To date, one patient has met the criteria and been entered in the study. Infusion of dopamine resulted in an increase in cardiac output and improvement in urine flow without an increase in pulse rate. The study is continuing.

* Available from [illegible]

DD FORM 1498

ANNUAL PROGRESS REPORT

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

REPORT TITLE: EVALUATION OF DOPAMINE (3,4-DEHYDROXYPHENYLETHYLAMINE) FOR TREATMENT OF SEPTIC SHOCK IN BURNED TROOPS

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

1 July 1974 - 30 June 1975

Investigators:

Gary W. Welch, MD, PhD, Lieutenant Colonel, MC
Robert W. J. Baird, MD, Major, MC
Douglas W. Wilmore, MD

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

REPORT TITLE: EVALUATION OF DOPAMINE (3,4-DEHYDROXYPHENYLETHYLAMINE) FOR TREATMENT OF SEPTIC SHOCK 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 1974 - 30 June 1975

Investigators: Gary W. Welch, MD, PhD, Lieutenant Colonel, MC
Robert W. J. Baird, MD, Major, MC
Douglas W. Wilmore, MD

Patients suffering from septic shock usually manifest hypotension, tachycardia, and oliguria in spite of an elevated cardiac index. This is probably secondary to their markedly reduced systemic vascular resistance. Dopamine, in doses of 400 to 1800 μg/min, will increase cardiac index further while also producing an increase in peripheral resistance and maintaining renal and splanchnic perfusion. In spite of its beneficial effects on the cardiovascular system, dopamine did not appear to influence ultimate survival rate.

Dopamine
Septic shock
Burn injury
Cardiac output
Humans

EVALUATION OF DOPAMINE (3,4-DEHYDROXYPHENYLETHYLAMINE) FOR TREATMENT OF SEPTIC SHOCK IN BURNED TROOPS

Of the many possible postoperative and post-trauma complications, septic shock can be one of the most complex and life-threatening problems to arise. The patient is found to be tachycardic, hypotensive, and oliguric, but with evidence of adequate peripheral perfusion. The administration of a fluid challenge may result in overt cardiac failure and pulmonary edema. Administration of catecholamines may then be resorted to. Depending on the sympathomimetic agent chosen, however, there may be a worsening of the hypotension, tachycardia, or oliguria.

With these factors in mind, it was decided to evaluate dopamine in the treatment of patients with septic shock subsequent to thermal injury.

METHODS

All patients who met the criteria of hypotension and oliguria unresponsive to volume loading were studied. All patients studied had positive blood cultures. Prior to the administration of any pressor agents, a Swan-Ganz 7f flow-directed thermal dilution cardiac output catheter was inserted either percutaneously or via a cutdown. If the patient's condition allowed, a preinfusion two-hour creatinine clearance was done. All pressures were tranduced with a Trantec pressure transducer and displayed on a Tektronix 412 physiologic monitor. Cardiac outputs were done using the Olsen thermal dilution cardiac output computer. Arterial pressures were determined by either a direct intra-arterial catheter or the Infrasonde blood pressure monitor. Simultaneous systolic time intervals were recorded on an eight-channel Electronics for Medicine recorder, using standard limb leads for the EKG, a Statham strain gauge for the carotid pulse tracing, and a Hewlett Packard microphone for the phonocardiogram. Following baseline studies, dopamine infusion was begun at 400 µg/min. This is an average dose of 5 µg/kg/min. The studies were repeated as stated above. The dopamine infusion was then increased in 400 µg increments until the desired response was obtained, another agent was used, or the patient expired.

RESULTS

Nearly all the patients had a markedly reduced systemic vascular resistance, reduced pulmonary vascular resistance, and an elevated cardiac index. Pulse rate varied from 95 to 120 beats per minute. The initial infusion of dopamine reduced systemic vascular resistance and pulmonary vascular resistance. The effect on cardiac output was variable. The ratio PEP/LVET was reduced by the infusion and $1/PEP^2$ was

prolonged. In those patients in which it was possible to perform pre- and postinfusion creatinine clearances, creatinine clearance was improved. As the rate of dopamine infusion was increased, systemic resistance and cardiac output increased. Pulmonary resistance was usually unchanged. In no case did systemic vascular resistance achieve normal values, in spite of cardiac indices as high as 7.31 L/min/m^2.

DISCUSSION

Faced with the problem of a patient in septic shock who has a normal or elevated central venous pressure and pulmonary capillary wedge pressure, one has a choice of several sympathomimetic amines which can be used to elevate the blood pressure and, hopefully, maintain renal, coronary, and cerebral perfusion. The use of such drugs is not without complication. Epinephrine and norepinephrine contain alpha and beta stimulating properties, epinephrine having more beta effect than norepinephrine, particularly at low doses. In doses high enough to raise systemic pressure in septic shock, the alpha-stimulating effect may predominate. This results in reduced splanchnic and renal perfusion. Coronary blood flow rises secondary to increased myocardial metabolism. Isoproterenol, a potent beta stimulator, will produce vasodilation. This may result in increased hypotension and reduced cerebral and renal perfusion, in spite of increased cardiac output. In addition, the infusion of isoproterenal is associated with arrhythmias and prolonged use may result in myocardial microinfarcts.

Dopamine is a precursor of norepinephrine and as such is a naturally occurring catecholamine. In the last several decades, it has been extensively compared to the other sympathomimetic amines. More recently, it has undergone considerable investigation in the treatment of several clinical conditions associated with reduced myocardial function.

Goldberg has reviewed the pharmacology of dopamine in normal and pathologic conditions.[1,2] He cites studies which show dopamine has both alpha and beta adrenergic properties. Dopamine was shown to have 1/13 to 1/25 the vasoconstricting properties of norepinephrine.

In addition, Goldberg states dopamine has both an indirect and direct cardiac action which produces both positive inotropic and

---

1. Goldberg LI: Cardiovascular and renal actions of dopamine: potential clinical applications. Pharm Rev 24:1-29, 1972.
2. Goldberg LI: Dopamine - clinical uses of an endogenous catecholamine. New Eng J Med 291:707-710, 1974.

chonotropic effects. The former effect is less than that of epinephrine or norepinephrine while the latter is less for an equal increase in cardiac contractility than other catecholamines.

Dopamine also improves renal perfusion through its direct effect on dopaminergenic receptors in the renal vessels.[3] Dopamine has also been shown to increase renal sodium clearance.

Central to the consideration of any pressor in the therapy of shock is its effect on coronary blood flow. Brooks, et al, felt that dopamine was a potent coronary vasodilator but that the increase in blood flow was secondary to increased oxygen consumption.[4] Naylor and coworkers showed dopamine either increased or decreased coronary resistances in isolated heart preparations depending upon the dose administered.[5] Vatner and Higgins[6] have shown a direct coronary vasodilating effect of dopamine in intact awake dogs which had undergone combined alpha and beta receptor blockade. Hence, it would appear dopamine has a direct effect on the coronary arteries through its alpha and dopaminergenic effects and an indirect effect via the increase in myocardial oxygen consumption.

Because of its beneficial effect on the renal and splanchnic circulation in addition to its ability to improve myocardial action, dopamine has been evaluated in several types of shock. Carvalho and colleagues[7] evaluated these effects of dopamine infusion in traumatic, hemorrhagic, and cardiogenic shock. In dogs subjected to traumatic shock, dopamine increased cardiac output 108 per cent, mean coronary

---

3. Yeh BK, McNay JL, Goldberg LI: Attenuation of dopamine renal and mesenteric vasodilation by haloperidol: Evidence for a specific dopamine receptor. J Pharm Exp Therap 168:303-309, 1969.

4. Brooks HL, Stein PD, Matson JL, Hyland JW: Dopamine-induced alterations in coronary hemodynamics in dogs. Circ Res 24:699-704, 1969.

5. Naylor WG, McInnes I, Stone J, Carson V, Lowe TE: Effect of dopamine on coronary vascular resistance and myocardial function. Cardiov Res 5:161-168, 1971.

6. Vatner SF, Milland RW, Higgins CB: Coronary and myocardial effects of dopamine in the conscious dog: Parasympatholytic augmentation of pressor and inotropic actions. J Pharm Exp Therap 187:280-295, 1973.

7. Carvalho N, Vyden JK, Bernstein H, Gold H, Corday E: Hemodynamic effects of 3-hydroxytyramine (dopamine) in experimentally induced shock. Am J Cardiol 23:217-223, 1969.

blood flow increased nearly three times while coronary resistance fell 31 per cent. Both renal and mesenteric arterial flow were increased and resistance in both systems fell. Dopamine increased mean arterial pressure while decreasing peripheral resistance. Coronary blood flow doubled. Mean renal blood flow increased from 132 to 164 ml/min^{-1} while resistance decreased slightly. Superior mesenteric flow was increased but resistance was unchanged.

In cardiogenic shock, dopamine increased mean systemic pressure, cardiac output, cardiac work, renal and splanchnic blood flow. Peripheral resistance was unchanged.

MacConnell, et al,[8] examined the effect of dopamine on shock secondary to sepsis, myocardial infarction, and neurologic trauma. Both patients with sepsis expired although one died of neurologic complications.

The vast majority of investigations have examined the effects of dopamine infusion on shock secondary to depressed myocardial function following myocardial infarction or open-heart surgery. Rosenblum and Frieden[9] administered dopamine to 15 patients suffering from hypotension following cardiopulmonary bypass. The dosage used varied from 3 to 25 $\mu g/kg$ for periods up to 332 hours. Two thirds of the patients showed improvement in blood pressure, urine flow, and peripheral vasoconstriction. Six of those responsive to dopamine were discharged from the hospital. All five of the patients not responsive to dopamine were unresponsive to other agents and died.

Holzer and coworkers[10] also examined the effects of dopamine in patients with cardiogenic shock. They were able to show a significant increase in urinary output and decrease in left ventricular filling pressure in survivors. They concluded that dopamine either alone or together with other agents was useful in the treatment of cardiogenic shock.

---

8. MacConnell KL, McNay JL, Meyer MB, Goldberg LI: Dopamine in the treatment of hypotension and shock. New Eng J Med 275:1389-1398, 1966.

9. Rosenblum R, Frieden J: Intravenous dopamine in the treatment of myocardial dysfunction after open-heart surgery. Amer Heart J 83: 743-748, 1972.

10. Holzer J, Karliner JS, O'Rourke RA, Pitt W, Ross J: Effectiveness of dopamine in patients with cardiogenic shock. Amer J Cardiol 32:79-84, 1973.

Marchetti and coworkers[11] studied the use of dopamine in experimentally induced endotoxin shock. E. coli endotoxin produced a decrease in aortic pressure, dp/dt, aortic flow, cardiac work, coronary, mesenteric, and renal blood flow. Twenty µg/kg/min of dopamine raised left ventricular pressure, dp/dt, cardiac index, and coronary flow. Aortic pressure, mesenteric, renal, and femoral flows remained below normal. The effect of dopamine in this experimental model is consistent with those reported in our study in which cardiac index was raised. One would have to assume, however, that an increase in cardiac index with either no change or a decrease in vascular resistance represents increased peripheral blood flow. Winslow, et al,[12] compared norepinephrine, dopamine, and isoproterenol in patients with septic shock. There was no difference in the hemodynamic response to the various agents between survivors and nonsurvivors. With dopamine, there were significant increases in mean arterial pressure, heart rate, cardiac index and stroke volume. Thirty-two per cent of the patients in the dopamine series were adequately resuscitated.

The only parameter available to evaluate renal blood flow was the two hour creatinine clearance. Although increased in those patients who were oliguric, the creatinine clearance in burn patients not in shock has shown a 100 per cent variation. The findings of improved creatinine clearance are supported by Beregovich and coworkers,[13] who reported an average increase in creatinine clearance of 53 per cent above control values in patients suffering from congestive failure. In this study, dopamine was infused at a rate of 10 µg/kg/min.

The effect of dopamine infusion on the pulmonary resistance may be an important consideration in patients suffering from respiratory insufficiency and hypotension. An increase in resistance may increase ventilation-perfusion mismatch by redistribution of flow. Low dose dopamine produced a fall in pulmonary resistance. That was true even though pulmonary resistance was below normal prior to dopamine infusion.

---

11. Marchetti G, Longo T, Nurdo L, Noseda V: The effects of dopamine on cardiogenic and endotoxin experimental shock. Europ Surg Res 5:175-185, 1973.

12. Winslow EJ, Loeb HS, Rahimtoola S, Kamath S, Gunnar R: Hemodynamic studies and results of therapy in 50 patients with bacteremic shock. Amer J Med 54:421-432, 1973.

13. Beregovich J, Bianchi C, Rubler S, Lomnitz E, Cagin N, Levitt B: Dose-related hemodynamic and renal effects of dopamine in congestive heart failure. Amer Heart J 87:550-557, 1974.

This was in contrast to the findings reported by Rosenblum, Tai, and Lawson,[14] who reported no change in pulmonary resistance in patients with a normal initial value.

Although no patients admitted to this study ultimately survived, this was most likely due to an inability to eliminate the source of sepsis rather than the ineffectiveness of dopamine. This study has shown that dopamine is capable of improving cardiac performance and at the same time maintaining peripheral perfusion without producing arrhythmias or hypotension.

---

14. Rosenblum R, Tai AR, Lawson D: Dopamine in man: Cardiorenal hemodynamics in normotensive patients with heart disease. J Pharm Exp Therap 183:256-263, 1972.

REFERENCES

1. Goldberg LI: Cardiovascular and renal actions of dopamine: potential clinical applications. Pharm Rev 24:1-29, 1972.

2. Goldberg LI: Dopamine - clinical uses of an endogenous catecholamine. New Eng J Med 291:707-710, 1974.

3. Yeh BK, McNay JL, Goldberg LI: Attenuation of dopamine renal and mesenteric vasodilation by haloperidol: Evidence for a specific dopamine receptor. J Pharm Exp Therap 168:303-309, 1969.

4. Brooks HL, Stein PD, Matson JL, Hyland JW: Dopamine-induced alterations in coronary hemodynamics in dogs. Circ Res 24:699-704, 1969.

5. Naylor WG, McInnes I, Stone J, Carson V, Lowe TE: Effect of dopamine on coronary vascular resistance and myocardial function. Cardiov Res 5:161-168, 1971.

6. Vatner SF, Milland RW, Higgins CB: Coronary and myocardial effects of dopamine in the conscious dog: Parasympatholytic augmentation of pressor and inotropic actions. J Pharm Exp Therap 187:280-295, 1973.

7. Carvalho N, Vyden JK, Bernstein H, Gold H, Corday E: Hemodynamic effects of 3-hydroxytyramine (dopamine) in experimentally induced shock. Am J Cardiol 23:217-223, 1969.

8. MacConnell KL, McNay JL, Meyer MB, Goldberg LI: Dopamine in the treatment of hypotension and shock. New Eng J Med 275:1389-1398, 1966.

9. Rosenblum R, Frieden J: Intravenous dopamine in the treatment of myocardial dysfunction after open-heart surgery. Amer Heart J 83: 743-748, 1972.

10. Holzer J, Karliner JS, O'Rourke RA, Pitt W, Ross J: Effectiveness of dopamine in patients with cardiogenic shock. Amer J Cardiol 32:79-84, 1973.

11. Marchetti G, Longo T, Nurdo L, Noseda V: The effects of dopamine on cardiogenic and endotoxin experimental shock. Europ Surg Res 5:175-185, 1973.

12. Winslow EJ, Loeb HS, Rahimtoola S, Kamath S, Gunnar R: Hemodynamic studies and results of therapy in 50 patients with bacteremic shock. Amer J Med 54:421-432, 1973.

13. Beregovich J, Bianchi C, Rubler S, Lomnitz E, Cagin N, Levitt B: Dose-related hemodynamic and renal effects of dopamine in congestive heart failure. Amer Heart J 87:550-557, 1974.

14. Rosenblum R, Tai AR, Lawson D: Dopamine in man: Cardiorenal hemodynamics in normotensive patients with heart disease. J Pharm Exp Therap 183:256-263, 1972.

PRESENTATIONS AND/OR PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA OF 6395	75 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
75 07 01	D. CHANGE	U	U	NA	NL	[X] YES [ ] NO	A. LORK UNIT

10. NO. CODES*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A161102B71R	01	305
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)* (U) Prolongation of Skin Allograft Survival By Immunosuppressive Therapy (Upjohn ATG) in Soldiers With Massive Thermal Injury (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*
003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
75 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 75	.6	20
	CURRENT 76	.9	27

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

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

20. PERFORMING ORGANIZATION
NAME:* US Army Institute of Surgical Research
ADDRESS:* Clinical Division
Fort Sam Houston, Texas 78234

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME:* Hugh D. Peterson, COL, MC
TELEPHONE: 512-221-3301
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: Gary Welch, MAJ, MC
NAME: Douglas W. Wilmore, MD DA

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Autograft; (U) Mesh graft; (U) Excision of fascia; (U) Antithymocyte globulin; (U) Isolation; (U)Laminar flow room; (U)Homograft; (U)Humans

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

23. (U) The objective of this protocol is to see if burned soldiers with extensive injuries can be effectively treated with antithymocyte globulin causing suppression of cellular immunity and allowing prolongation of allograft take in areas of excision.

24. (U) Patients in the 15 to 40 age group with full thickness thermal injury greater than 60% of their body surface and without significant pulmonary injury will be immunosuppressed with antithymocyte globulin and serially excised and grafted with available autograft and then with fresh homograft. The homograft will be allowed to remain in place. The patient's immunosuppression will continue and as donor sites become available the allograft will be surgically excised and replaced with autograft. Immunosuppression will continue until all but 15% of the grafted surface is covered with autograft.

25. (U) 75 04 - 75 06 To date one patient has been studied; a 25-uear old white female with an 85% thermal injury underwent immunosuppression and excision of the arms, legs and a portion of the chest. There was a good take of the sheet allograft on the arms and the meshed autograft on arms, the meshed allograft on the excised legs did not take well. The patient expired with sepsis 10 days after beginning immunosuppression.

*Available to contractors upon originator's approval

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

ANNUAL PROGRESS REPORT

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

REPORT TITLE: PROLONGATION OF SKIN ALLOGRAFT SURVIVAL BY IMMUNOSUPPRESSIVE THERAPY (UPJOHN ATG) IN SOLDIERS WITH MASSIVE THERMAL INJURY

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

1 April 1975 - 30 June 1975

Investigators:

Hugh D. Peterson, DDS, MD, Colonel, MC
Douglas W. Wilmore, MD
*John Whelchel, MD, Lieutenant Colonel, MC

*Wilford Hall Air Force Hospital, Lackland AFB, Texas

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

REPORT TITLE: PROLONGATION OF SKIN ALLOGRAFT SURVIVAL BY IMMUNOSUPPRESSIVE THERAPY (UPJOHN ATG) IN SOLDIERS WITH MASSIVE THERMAL INJURY

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

Period covered in this report: 1 April 1975 - 30 June 1975

Investigators: Hugh D. Peterson DDS, MD, Colonel, MC
Douglas W. Wilmore, MD
John Whelchel, MD, Lieutenant Colonel, MC*

Reports Control Symbol MEDDH-288(R1)

The objective of this protocol is an attempt to prolong the survival of allograft, not tissue typed, in massive thermal injury where the thermal injury is excised in stages, covered with allograft, and the patient immunosuppressed allowing the graft to take for periods of 40 to 60 days while donor sites become available for recropping. To date one patient has been studied, a 25 year old white female with an 85% thermal injury, mostly third. She had excision of burn wounds of the arms, legs and a portion of the anterior chest. She was maintained in reverse isolation and immunosuppressed for 10 days. However, the patient had a positive blood culture prior to starting her immunosuppression. She had an inhalation injury documented upon admission and she died of sepsis 10 days after starting the excisions and immunosuppression.

No other patients have been done to date. We are awaiting further candidates with 70% third degree injury and installation of a laminar flow capability where the patient can be more successfully isolated. However, the laminar flow may not play a large role and we will continue to excise and immunosuppress large burns in an attempt to prolong homograft survival.

Autograft
Mesh graft
Excision of fascia
Antithymocyte globulin
Isolation
Laminar flow room
Homograft
Humans

*Wilford Hall Air Force Hospital, Lackland AFB, Texas

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OD 6978	75 07 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISP'N INSTR'N	8b. SPECIFIC DATA CONTRACTOR ACCESS	9. LEVEL OF SUM
74 07 01	D. CHANGE	U	U	NA	NL	[X] YES [ ] NO	A. WORK UNIT

10. NO. CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A161102B71R	01	194
b. CONTRIBUTING				
c. CONTRIBUTING				

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

12. SCIENTIFIC AND TECHNOLOGICAL AREAS
003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
70 07	Cont	DA	C. In-House

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

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
FISCAL YEAR — PRECEDING 75	.1	1
FISCAL YEAR — CURRENT 76	.1	2

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

20. PERFORMING ORGANIZATION
NAME: US Army Institute of Surgical Research Clinical Division
ADDRESS: Fort Sam Houston, Texas 78234
PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME: Basil A. Pruitt, Jr., COL, MC
TELEPHONE: 512-221-2720
SOCIAL SECURITY ACCOUNT NUMBER
ASSOCIATE INVESTIGATORS
NAME: Robert B. Lindberg, PhD
NAME: DA

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Military burn unit; (U) Operating room based infections; (U) Surgical drapes; (U) Surgical gowns

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

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

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

25. (U) 74 07 - 75 06 Repeat studies of the bacterial barrier properties of the synthetic sheeting have confirmed a variable, irregular penetration by a variety of organisms. The creation of small defects during the manufacture appears to be a likely cause for this "barrier breakdown". New material samples are to be tested with organisms penetration related to microscopic sheeting perforations if possible.

*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. 3A161102B71R-01, RESEARCH IN BIOMEDICAL SCIENCES

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

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

1 July 1974 - 30 June 1975

Investigators:

Basil A. Pruitt, Jr., M.D., Colonel, MC
Robert B. Lindberg, Ph.D.

Reports Control Symbol MEDDH-288 (R1)

UNCLASSIFIED

ABSTRACT

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

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

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

Period covered in this report: 1 July 1974 - 30 June 1975

Investigators: Basil A. Pruitt, Jr., M.D., Colonel, MC
Robert B. Lindberg, Ph.D.

Reports Control Symbol MEDDH-288(R1)

Available surgical drapes are either uncertain bacterial barriers or possess undesirable physical properties which limit their usefulness and clinical acceptance. A synthetic sheeting of Spunbonded Olefin was initially evaluated in both laboratory and clinic and found to be a reliable bacterial barrier but to drape poorly and to permit quantitative fluid run-off.

At present, a thin layer of Spunbonded Olefin is sandwiched between two layers of cellulosic material with the resulting laminate mechanically softened in the manufacturing process. The cellulosic material is absorbent and thereby diminishes fluid run-off and the softening has improved the draping characteristics of the sheeting. Unfortunately, however, this processing appears to have destroyed the reliability of the bacterial barrier of the resulting sheeting. Repeat testing as reported last year has confirmed the synthetic sheeting as currently produced to be an unreliable bacterial barrier with irregular penetration by a variety of test organisms including Pseudomonas aeruginosa, Klebsiella pneumoniae, Serratia marcescens, Escherichia coli and Staphylococcus aureus. Since individual discs of the material tested showed no bacterial penetration, the possibility that bacterial passage occurs through microscopic sheeting defects generated by the manufacturing process is being evaluated. Sheeting, as prepared by revised processing, is also being forwarded for evaluation of bacterial barrier properties, using the test procedures developed in the course of this study. The unreliability of the previously tested sheeting as a bacterial barrier militates against its use as a surgical drape or surgical gown material.

Military burn unit
Operating room-based infections
Surgical drapes
Surgical gowns

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION: DA 0D 6976
2. DATE OF SUMMARY: 75 07 01
REPORT CONTROL SYMBOL: CSRD-1(AR)(U)

3. DATE PREV SUMMARY: 74 07 01
4. KIND OF SUMMARY: D. CHANGE
5. SUMMARY SECTY: U
6. WORK SECURITY: U
7. REGRADING: NA
8. DISTRIB INSTRN: NL
9. SPECIFIC DATA (CONTRACTOR ACCESS): X YES NO
10. LEVEL OF SUM: A. WORK UNIT

11. NO. OF PES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A16110A91C	00	083
b. CONTRIB (1)				
c. CONTRIB (2)				

12. TITLE (Precede with Security Classification Code): (U) Studies of The Effect of Variations of Temperature and Humidity on Energy Demands of the Burned Soldier in a Controlled Metabolic Room (U)

13. SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 Clinical Medicine

14. START DATE: 71 07
15. ESTIMATED COMPLETION DATE: Cont
16. FUNDING AGENCY: DA
17. PERFORMANCE METHOD: 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	PRECEDING	A. PROFESSIONAL MAN-YRS	B. FUNDS (Thousands)
FISCAL YEAR	75	1.0	48
	CURRENT 76	1.0	20

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

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

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

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

ASSOCIATE INVESTIGATORS
NAME: Arthur D. Mason, Jr., MD
NAME: Basil A. Pruitt, Jr., COL, MC DA

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code): (U) Metabolism; (U) Heat loss; (U) Evaporative water loss; (U) Controlled environment; (U) Humans; (U) Critical temperature

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

23. (U) To define the relationship between surface cooling and hypermetabolism in a controlled ambient environment, to determine the mediator of the profound hypercatabolic response following thermal injury and the mechanisms of stress-induced heat production in burned soldiers.

24. (U) The use of a controlled environmental study room to measure metabolic rate at various temperatures; concomitantly, measurements of water loss, heat production, core temperature, mean skin temperature, and calculation of heat transfer coefficients and routes of heat loss. Simultaneously, measurements of blood substrate, urine and plasma catecholamines, blood hormone levels, and correlation of total body metabolism with energy demand are performed.

25. (U) 74 07 - 75 06 Studies of patients and normal individuals following central nervous system injury, denervation of the burn wound by topical or regional anesthesia, and administration of central nervous system narcotics, salicylate, [illegible], and atropine have been performed. The central nervous system is essential to evoke the metabolic response to injury. Interruption of afferent nervous input from the burn wound did not modify the post-traumatic response. Metabolic rate decreased with morphine administration in burn patients. Inhalation of inert gas in normals diminished the hypermetabolic response to cold stress. Other pharmacologic attempts to modify the stress-induced reflex arc at the hypothalamic level have not been effective. Studies of glucose kinetics and glucose flow demonstrate a close correlation between glucose flow through the extracellular space and oxygen consumption. Glucose flow studies in patients with gram negative septicemia show a diminution in the gluconeogenesis and heat production, suggesting an inter-relationship between substrate cycling and heat production following stress and injury.

* Available to contractors upon originator's approval

DD FORM 1498

ANNUAL PROGRESS REPORT

PROJECT NO. 3A161101A91C, 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 1974 - 30 June 1975

Investigators:

Douglas W. Wilmore, MD

Arthur D. Mason, Jr., MD

James W. Taylor, MD Major, MC

James M. Long, MD, Lieutenant Colonel, MC

Edwin W. Hander, First Lieutenant, MSC

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

Reports Control Symbol MEDDH-288(R1)

Unclassified

ABSTRACT

PROJECT NO. 3A161101A91C, 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 1974 - 30 June 1975

Investigators: Douglas W. Wilmore, MD
Arthur D. Mason, Jr., MD
James W. Taylor, MD, Major, MC
James M. Long, MD, Lieutenant Colonel, MC
Edwin W. Hander, First Lieutenant, MSC
Basil A. Pruitt, Jr., MD, Colonel, MC

Reports Control Symbol MEDDH-288(R1)

The afferent and efferent limbs of the reflex arc which initiates the hypercatabolic response to thermal injury were evaluated. Hypermetabolism was not affected by denervation of the injured area or with application of topical anesthesia to the burn wound. Administration of drugs known to affect central temperature regulation did not diminish the hypercatabolic response. However, oxygen consumption fell with central nervous system narcosis, and hypermetabolism was absent in a patient with brain death, demonstrating the importance of the central nervous system to the stress response. Glucose flow was elevated in noninfected, hypermetabolic, burn patients, and was related to oxygen consumption. Gram negative sepsis in burn patients resulted in a decrease in glucose flow and oxygen consumption. The etiology of the increased heat production in injured man appears to be related to increased gluconeogenesis and accelerated glucose cycling, directed by increased activity of the sympathetic nervous system.

Metabolism
Hypermetabolism
Heat loss
Evaporative water loss
Controlled environment
Critical temperature
Burned soldiers

STUDIES OF THE EFFECT OF VARIATIONS OF TEMPERATURE AND HUMIDITY ON ENERGY DEMANDS OF THE BURNED SOLDIER IN A CONTROLLED METABOLIC ROOM

The reflex arc which initiates the post-traumatic metabolic response to injury consists of nervous and/or hormonal afferent signals to the central nervous system, with homeostatic readjustment in the hypothalamus resulting in pituitary and sympathoadrenal discharge. This endocrine environment then directs the hypermetabolic response to injury and mediates alterations in flow of energy substrate. This report emphasizes the importance of the hypothalamus to this reflex arc and evaluates afferent stimuli and central nervous system mechanisms which could affect the stress response to thermal injury. In addition, interrelationships between glucose flow and heat production suggest that the biochemical etiology of heat production in man can be explained by interaction between substrate cycling, gluconeogenesis, and increased oxygen consumption following stress and injury.

MATERIALS AND METHODS

A variety of patients have been studied, most with burns greater than 35% of their body surface area. None of the patients had blood stream infection at the time of study, and most were evaluated during the second or third week postinjury, during the height of their hypercatabolic response to thermal injury. All patients were studied in an environmental chamber, at comfort temperature between 30 and 33° C unless otherwise noted. Oxygen consumption was measured using Douglas bag technique, and core temperature was monitored continuously from indwelling probes placed in the rectum and external auditory canal. Mean skin temperature was calculated from multiple surface temperatures, measured from burned and unburned areas, not in contact with the mattress. These measurements were weighted mathematically by surface area to determine the overall contribution to mean skin temperature, as previously described.[1] In selected studies, urinary catecholamines, glucose, insulin, and growth hormone were measured.

THE METABOLIC RESPONSE TO COMBINED THERMAL AND CENTRAL NERVOUS SYSTEM INJURY

Four burn patients with associated injuries of the central nervous system have been studied to date (Table I). Two individuals who sustained cerebral contusion, in association with flame injury, demonstrated measured metabolic rates which were greater than those predicted

---

1. Wilmore DW, Mason AD Jr, Johnson DW, Pruitt BA Jr: Effect of ambient temperature on heat production and heat loss in burn patients. J Appl Physiol 38:593-597, 1975.

TABLE 1

EFFECT OF CNS INJURY ON POST-TRAUMATIC HYPERMETABOLISM

CNS Injury	Burn Size (% BSA)*	Age (Years)	Postburn Day Studied	Metabolic Rate (kcal/m^2/hr)	
				Measured	Predicted
Cerebral contusion	26	15	15	65.6	53.0
Cerebral contusion	48	23	8	88.2	68.0
Cerebral contusion T-11 spinal cord transection	60	27	3	92.0	74.4
Cerebral edema (flat EEG)	23	19	3	30.8	56.1

*BSA - Body Surface Area

from the size of burn injury alone, suggesting that head trauma exerts an additive effect to the metabolic response to thermal injury. A patient with thoracic spinal cord injury and paraplegia, 60% total body surface burn, and cerebral contusion was markedly hypermetabolic, consistent with the degree of his extensive trauma. This metabolic response occurred despite denervation of a major portion of his cutaneous injury, which was over his lower trunk and lower extremities, and despite denervation of a large portion of his muscle mass. Finally, a patient with a 23% burn, and brain death resulting from cerebral edema (determined by flat EEG) was hypometabolic, less than normal predicted basal levels for uninjured man and below 56.1 kcal/m^2/hour predicted for individuals with thermal injury of comparable size.

INTERRUPTION OF PERIPHERAL NERVOUS STIMULATION FROM THE INJURY

To evaluate the role of peripheral nervous stimulation from the injured area, metabolic rate, core temperature, and urinary catecholamines were measured in three patients during the resting state, following four hours of equilibration in ambient comfort conditions. One per cent viscous lidocaine (Xylocaine^R) was then applied to the burn wound to achieve total anesthesia of the second degree area of injury and to insure that no afferent nervous stimulation from the other areas of cutaneous injury occurred. Additional studies were then performed at 2, 4, and 6 hours following the initial application of the topical anesthetic. Supplemental topical medication was applied periodically throughout the study period. Although the patients were remarkably free of pain following application of the topical anesthetic and slept most of the time during the investigation, topical anesthesia exerted no effect on metabolic rate or urinary catecholamines, measured serially throughout the study (Table 2). In a single patient with a 33% burn over his lower extremities, fracture of the right femur, right tibia, and fracture dislocation of the right ankle, a spinal anesthetic was administered and maintained for four hours at the T4 to T6 level. No significant effect on metabolic rate or core temperature was detected following total denervation of the injured area (Table 2).

ADMINISTRATION OF AGENTS WHICH INTERACT WITH CENTRAL TEMPERATURE REGULATION

Previous studies have demonstrated that burn patients sustain elevated core and skin temperatures following injury,[2] and this hyperpyrexia of thermal injury is a reflection of hypermetabolism and alterations in substrate flow (Fig. 1). To determine if the hyperpyrexia and hypermetabolism of thermal injury could be affected at the hypothalamic level by administration of antipyretics, 20 grains of

2. Wilmore DW, Long JM, Mason AD Jr, Skreen RW, Pruitt BA Jr: Catecholamines: mediator of the hypermetabolic response to thermal injury. Ann Surg 180:653-669, 1974.

TABLE 2

EFFECT OF BLOCKADE OF AFFERENT NERVOUS STIMULI FROM THE WOUND ON METABOLISM (MEAN, RANGE OR ± SE)

Treatment	N	Burn Size (% Body Surface Area)	Age (Years)	Postburn Day Studied	Metabolic Rate (kcal/m^2/hr)	
					Before	After
Topical anesthesia	3	66 (53-78.5)	28 (25-30)	13 (10-17)	77.8 ± 4.2	77.5 ± 2.5
Spinal anesthesia	1	33 (Multiple Fractures)	29	33	57.3	63.8

*Mean of two six-hour measurements.

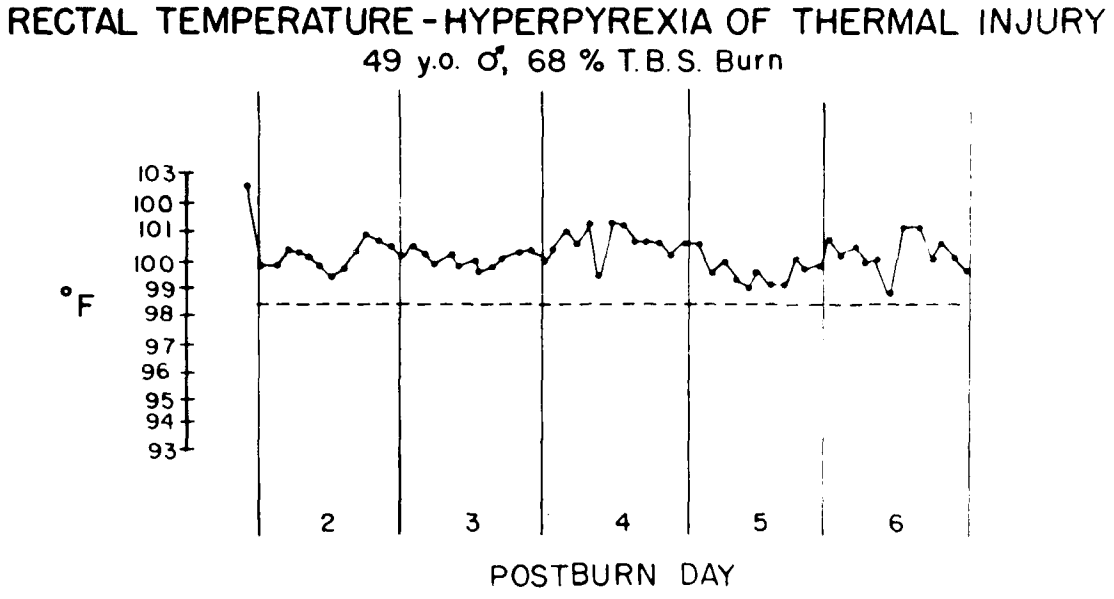


Figure 1. The hyperpyrexia of thermal injury is reflected by the persistent elevation of rectal temperature in this patient treated without dressing in a 25-27° C ambient temperature. A "normal" temperature (37° C or 98.6° F) would reflect an abnormal response in heat production following injury, and is usually associated with oversedation, anesthesia, gram negative sepsis, or inadequate oxygenation or circulation.

aspirin were administered orally every four hours for two days to three patients; resting metabolic rate was measured daily. Core temperature was measured every two hours throughout the period of study. Aspirin exerted no detectable effect on metabolic rate, and core temperature during the study period was also unchanged (Table 3).

L-dopa rapidly crosses the blood brain barrier, and increases concentrations of dopamine and norepinephrine within the central nervous system. L-dopa has been reported to reduce core temperature in 10 of 24 normal men studied in a cool environment.[3] L-dopa was administered by mouth in a 1.5 gram dose to seven individuals in the early morning following basal metabolic studies. Five thermally injured and two normal individuals participated in this study. L-dopa was absorbed in all individuals as demonstrated by an increase in serum growth hormone level (from a mean of 2.9 ng/ml to 15.7 ng/ml), which occurred two to three hours after ingestion of the drug. However, metabolic rate and core temperature were unchanged in all individuals (Table 3). In fact, subsequent studies demonstrated a 10 to 15 per cent increase in metabolic rate in two patients following administration of 2-2 1/2 grams of the amino acid.

It has been proposed that calcium exerts a braking effect on the temperature center, which is subject to ionic modulation.[4] Three patients, with a mean burn size of 60%, were studied before and after induction of a dose of calcium known to evoke an endocrine response. Calcium chloride was given intravenously as a loading dose of 4 mg calcium/kg body weight, and maintained as a constant infusion over four hours. In the three patients studied, mean calcium increased from pre-infusion values of 7.2 mg/100 ml to 11.7. However, calcium infusion did not exert an effect on metabolic rate or core temperature monitored throughout and following the infusion.

Atropine is known to inhibit cholinergic receptors in the central nervous system, and animal studies indicate that atropine may decrease heat production by blocking central inhibitory cholinergic mechanisms.[5] Atropine acts on the periphery to diminish evaporative water loss, but

---

3. Boyd AE, Mager M, Angoff G, Lebovitz HE: Effect of acute administration of L-dopa on body temperature in man. J Appl Physiol 37:675-678, 1974.

4. Myers RD: Primates in Comparative Physiology of Thermoregulation, edited by GC Whitton, New York & London, Academic Press. p. 283.

5. Kirkpatrick WE, Lomax P: The effect of atropine on the body temperature of the rat following systemic and intracerebral injection. Life Science 6:2273-2278, 1967.

blockade of insensible water loss does not occur in the burn patient because of the increased vaporizational heat loss across the injured integument. Atropine sulfate was administered as a single intravenous dose of 0.04 mg/kg body weight. No alteration in the metabolic rate or core temperature was noted with atropine administration. In one patient with persistent extrapyramidal movements, the atropine diminished muscle tremors, and this was associated with a decrease in metabolic expenditure from 73.3 to 62.6 kcal/m^2/hour. Atropine, however, exerted no effect on metabolism in the other patients, and no significant differences in metabolic rate could be distinguished following atropinization.

EFFECT OF CNS NARCOSIS ON THE SYMPATHETIC RESPONSE TO STRESS

Agents known to influence the sympathetic outflow from the hypothalamus were subsequently evaluated to determine their effects on the metabolic response to stress. Inert gases exert central narcotic effects,[6] and the metabolic and respiratory response to three hours of cold exposure (14° C) was measured in 14 studies in five normal males, wearing only light cotton shorts and breathing room air, 79% helium-21% oxygen, or 79% argon-21% oxygen. Pulse rate, oxygen consumption, core temperature, urinary catecholamines, blood glucose, insulin, and HGH were serially measured. Eight additional comparison studies between room air and the helium-oxygen mixture were performed in normal individuals in a thermal neutral environment (28° C), and six other studies compared the response to intravenous infusion of epinephrine (6 µg/min for one hour) during inhalation of He-O_2 and room air.

Heat production was significantly lower at the end of three hours of cold exposure during the helium-oxygen inhalation when compared to the period of cold exposure while breathing room air (Table 4). Core temperature fell to a greater extent during cold exposure, associated with inhalation of He-O_2. Similar effects were noted with the inhalation of argon-oxygen mixture. No effects on metabolism were noted in the thermal-neutral nonstressed studies in the normal individuals. Metabolic rate was unchanged following epinephrine infusion while breathing the helium and oxygen mixture, suggesting that helium does not act as a peripheral blocking agent but dampens central sympathetic nervous system outflow. Hypermetabolism did not decrease with inhalation of the inert gases in the burn patients, and the effects of more potent CNS narcotics were then evaluated.

Five studies in burn patients with a mean burn size of [illegible] evaluated the effect of intravenous morphine on the metabolic and respiratory response following thermal injury. An average dose of [illegible] g

6. Schreiner HR: General biological effects of the helium-xenon series of elements. Fed Proc 27:872-878, 1968.

TABLE 4

EFFECT OF CNS NARCOSIS ON THE METABOLIC RESPONSE TO STRESS

(Mean, Range or ± S.E.)

Narcotic Agent	Study Condition	N	Burn Size (% Body Surface Area)	Age (Years)	Postburn Day Studied	Metabolic Rate (kcal/m^2/hour)		Core Temperature (°C)	
						Control	Narcotic	Control	Narcotic
79% Helium-21% Oxygen	Comfort (28° C)	4		28 (25-33)		37.8 ± 1.5	35.2 ± 3.0	36.9 ± 0.1	36.9 ± 0.1
79% Helium-21% Oxygen	Cold stress (14° C)	5		31 (24-37)		58.8 ± 5.5	43.8 ± 3.3*	36.7 ± 0.1	36.5 ± 0.1*
79% Helium-21% Oxygen	Comfort Epinephrine infusion	3		29 (24-37)		47.2 ± 1.0	48.3 ± 0.9	37.1 ± 0.1	37.1 ± 0.1
79% Argon-21% Oxygen	Cold stress	3		33 (28-37)		56.1 ± 10.3	41.7 ± 3.8	36.8 ± 0.1	36.7 ± 0.1*
79% Helium-21% Oxygen	Patients	4	47 (42-57)	43 (36-48)	62 (30-104)	57.8 ± 1.5	56.4 ± 2.5	38.4 ± 1	38.3 ± 1
79% Argon-21% Oxygen	Patients	2	38 (32-43)	46 (43-48)	56 (26-87)	80.4 ± 7.7	80.8 ± 1.2	38.2 ± 0.0	38.6 ± 0.2
Morphine	Patients	5	74 (59-87)	26 (21-38)	16 (7-29)	77.4 ± 5.9	55.0 ± 3.7*	38.2 ± 0.1	37.3 ± 0.1*

*$p < 0.05$

morphine sulfate per kg body weight was given over one hour following basal studies, and serial oxygen consumption, pulse rate, minute ventilation, core temperature, blood pressure, and blood gases were monitored. Two of the patients on ventilators received the largest doses of the drug. Morphine administration significantly decreased oxygen consumption, pulse rate, core temperature, and minute ventilation, while blood pressure and partial pressure of oxygen and carbon dioxide in the blood remained unchanged (Table 5, Fig. 2).

ALTERATIONS IN GLUCOSE KINETICS

Blood glucose elevation occurs following injury and infection, and alterations in glucose dynamics appear central to the metabolic response to stress. Long and associates[7] demonstrated an increased rate of glucose turnover in traumatized and septic patients, and Gump, et al,[8] studied hepatic glucose production in similar patients and related increased hepatic glucose production to hyperglycemia in four of the nine patients studied. Glucose disappearance and glucose flow were determined in 21 burn patients with a mean burn size of 45% total body surface, between the 6th and 77th postburn day, and compared with findings in 12 normal individuals of comparable age and body weight. Intravenous glucose tolerance tests were performed in the early morning, following a four to six hour fast, with nine serial samples for serum glucose and insulin obtained over three hours following the injection of 25 grams glucose. The best curve describing the discrete data points was determined by computer fitting, and a mathematical expression written defining the proportionality constant for glucose disappearance, the asymptote which the curve approached, and the size of the glucose space. The flow through the glucose space was calculated as described by Hlad and associates.[9]

Glucose flow was significantly elevated in the 17 burn patients studied between the 6th and 16th day postburn when compared with normal individuals or the recovered patients (Table 6). Glucose flow during the second postburn week was related to the extent of injury (Fig. 3), and fell with time in a curvilinear manner to normal values with closure of the burn wound (Fig. 4). The increased glucose flow and elevated fasting serum glucose observed following injury did not result from prolonged glucose disappearance (Fig. 5) or from alterations in the

---

7. Long CL, Spencer JL, Kinney JM, Geiger JW: Carbohydrate metabolism in man: Effect of elective operations and major injury. J Appl Physiol 31:110-116, 1971.

8. Gump FE, Long C, Killian P, Kinney JM: Studies of glucose intolerance in septic injured patients. J Trauma 14:378-386, 1974.

9. Hlad CJ Jr, Elrick H, Witten TA: Studies on the kinetics of glucose utilization. J Clin Invest 35:1129-1149, 1956.

TABLE 5

ALTERATIONS WITH MORPHINE ADMINISTRATION TO FIVE BURN PATIENTS
(Mean ± S.E.)

	Before	After*
Minute volume (L/min)	21.5 ± 2.5	12.0 ± 1.0
Frequency (breaths/min)	20.4 ± 1.9	13.8 ± 1.6
Metabolic rate (kcal/m^2/hr)	77.4 ± 5.9	55.0 ± 3.7
Ventilatory equivalent (L/L)	41.2 ± 6.7	32.0 ± 4.1
Pulse rate (beats/min)	115 ± 4	104 ± 5
Core temperature (°C)	38.2 ± 0.1	37.3 ± 0.1

*$p < 0.05$

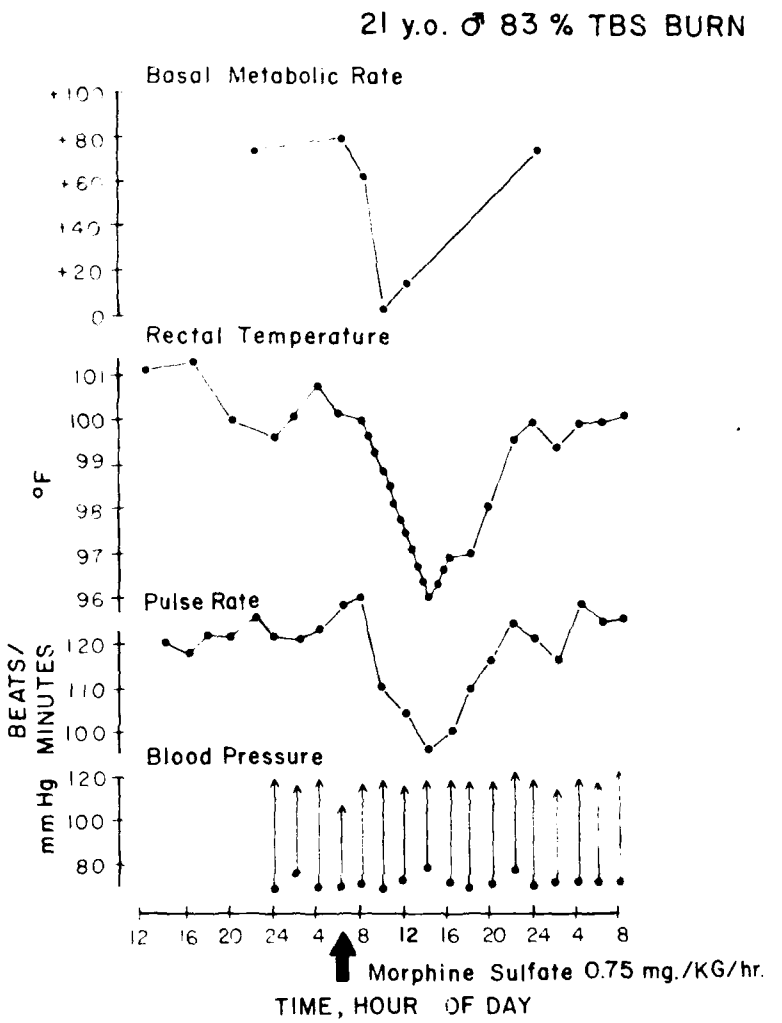


Figure 2. A prompt decrease in oxygen consumption and core temperature occurs following morphine administration. Oxygenation was normal throughout this study in this patient maintained on a ventilator.

TABLE 6

GLUCOSE FLOW STUDIES (Mean ± S.E.)

	Normals	Burn Patients*
N	12	17
Age (years)	26 ± 2	29 ± 3
Weight (kg)	66.4 ± 3.9	67.3 ± 2.7
Glucose space (L/kg)	0.152 ± 0.010	0.177 ± 0.010
Asymptote (mg/100 ml)	70 ± 2	113 ± 5**
K (100 min^{-1})	4.01 ± 0.56	5.27 ± 0.51
Q (mg/kg/min)	3.92 ± 0.32	10.12 ± 0.95**

*9th postburn day average day of study; **$p < 0.001$

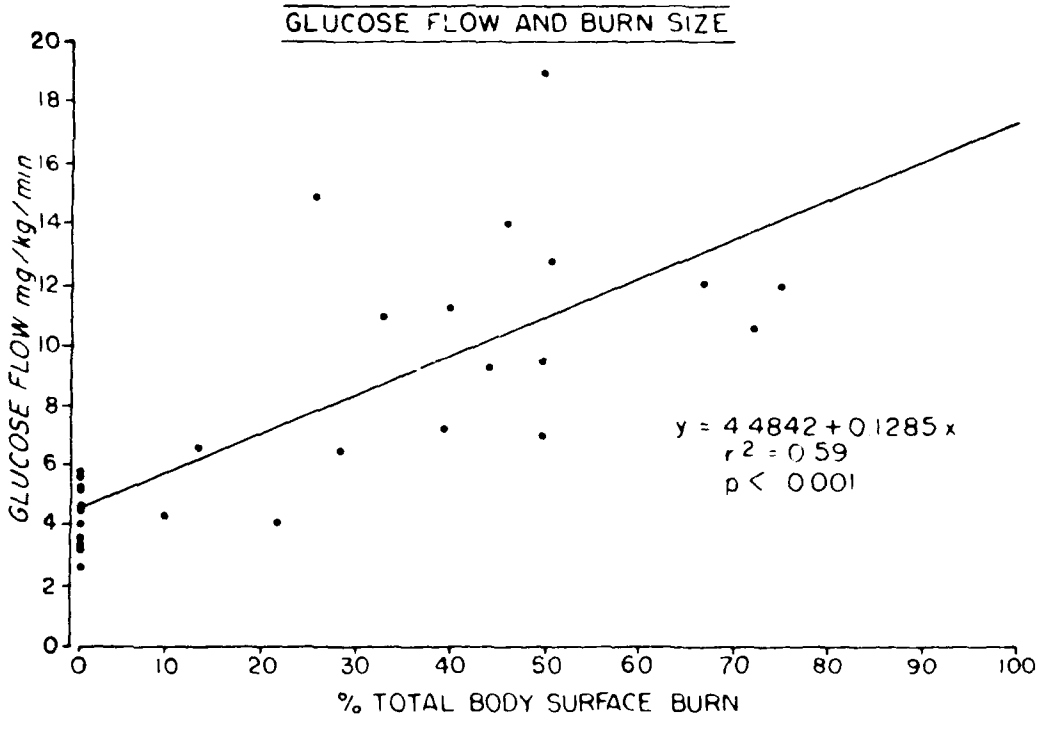


Figure 3. The relationship between glucose flow through the extracellular fluid compartment and the size of injury. Comparable fits were obtained expressing glucose flow/unit time using body surface area or body weight$^{3/4}$.

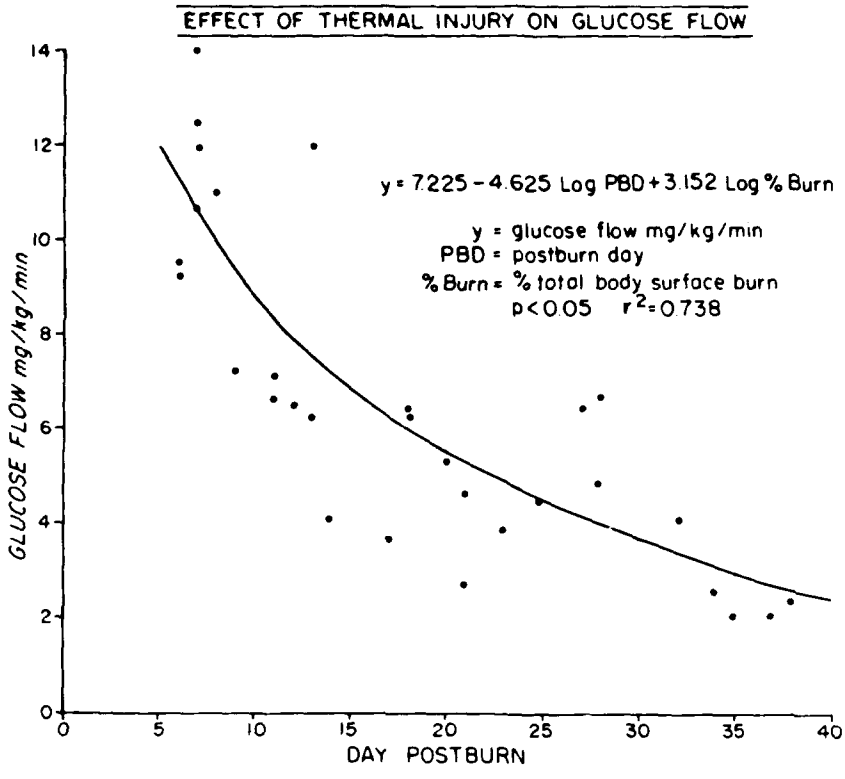


Figure 4. Glucose flow returns to normal with closure of the burn wound, as demonstrated by the regression curve calculated for the average burn size. Note that glucose flow is related to total body weight, time postinjury, and size of injury, and returns to normal as glucagon and catecholamine levels decrease (See Fig. 1).

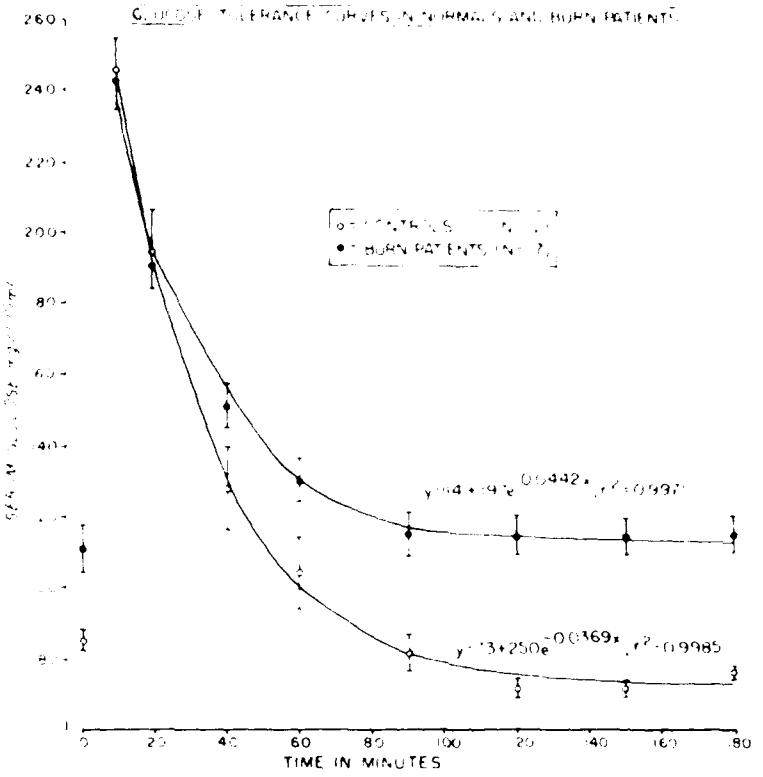


Figure 5. Comparable glucose disappearance occurred following an intravenous injection of 25 g glucose in burn patients and controls (proportionality constant is exponent in equations). The persistent hyperglycemia of injury (asymptote the curves approach is first term of the equations) is a result of increased hepatic production of glucose, not abnormal peripheral disposal. Curves comparable to those obtained from the burn patients can be obtained by infusion of 10% dextrose to normal men, equilibrating at a steady state, and then injecting a 25 g glucose dose. Points represent mean values ± S.E.

glucose space, which was equivalent to the extracellular fluid compartment. Thus, following resuscitation in burn patients, the elevated blood glucose is related to the increased entry of glucose into the extracellular fluid compartment (increased hepatic production), at a time when the proportionality constant for glucose disappearance is normal.

Simultaneous studies of glucose flow and oxygen consumption were performed in 10 normals and 17 uninfected burn patients to determine the relationship between heat production and glucose flow. A close relationship between glucose flow and oxygen consumption occurred (Fig. 6). However, the patients were not utilizing glucose as a primary fuel substrate, as demonstrated by low respiratory quotients (0.70-0.75) reflecting fat oxidation.

METABOLIC ALTERATIONS IN SEPTIC BURN PATIENTS

Hypermetabolism and loss of intracellular constituents have been commonly associated with infection in man, and it appears that infection exerts its catabolic alteration in body metabolism by way of the sympathetic-mediated stress response. However, patients with large thermal injury continue to die from the infectious complications of their injury, and the interaction between the extensive stress of injury and superimposed blood stream infection has not previously been described. Eighteen studies of heat production and heat loss were performed in 10 septic burn patients, all with proven bacteremia, demonstrated by positive blood culture obtained at the time of study. All patients maintained an adequate urine output at the time of study and did not demonstrate hypotension or signs of cardiovascular instability. Metabolic rate, core and skin temperature, urine and plasma catecholamines, were measured as previously described. Glucose kinetics were measured in 11 patients with positive blood stream cultures for gram negative organisms. These patients were considered to have mild or moderate infection at the time of study, and none had cardiocirculatory instability, although a decrease in core temperature was frequently noted in all individuals.

Metabolic rate was significantly decreased in the 10 septic patients studied. The mean metabolic rate was 50.3 ± 2 kcal/m^2/hour, compared with predicted or measured rates in nonseptic intervals which averaged 73 ± 1.2 kcal/m^2/hour. Urinary catecholamines were markedly elevated in these patients, averaging 910.8 ± 406 μg/hour, and this level of catecholamine excretion was inappropriately high for the metabolic response measured. Glucose kinetics were markedly deranged in the septic patients when compared with the nonseptic group of thermally

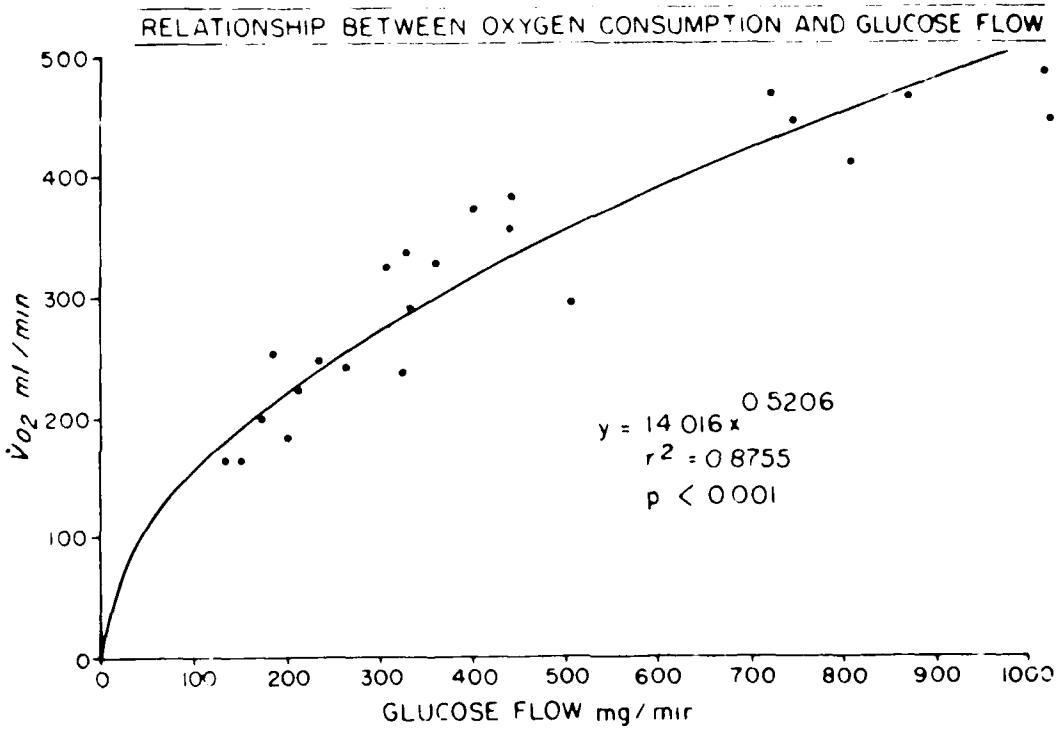


Figure 6. The relationship between total glucose flow and oxygen consumption in normal and nonseptic injured men.

injured individuals (Table 7). A consistent finding was the significantly decreased proportionality constant for glucose disappearance into the periphery, as demonstrated by the diabetic-like glucose tolerance curves following sepsis (Fig. 7). In addition, glucose flow through the extracellular compartment decreased in the patients with gram negative infection. Simultaneous measurements of both oxygen consumption and glucose kinetics demonstrated that burn patients with gram negative infection have a simultaneous decrease in glucose flow through the extracellular space and a fall in oxygen consumption.

DISCUSSION AND CONCLUSIONS

The reflex arc which initiates the post-traumatic metabolic response to injury consists of nervous or hormonal afferent signals to the central nervous system, with homeostatic readjustment in the hypothalamus resulting in pituitary and sympathoadrenal discharge. This endocrine environment then directs the metabolic response which mediates the increased heat production and alterations in substrate flow. The metabolic response to injury was absent in a patient with brain death, and studies using anesthetics and narcotizing agents demonstrated a marked reduction of the metabolic rate and catecholamine excretion associated with central nervous system narcosis. Spinal cord trauma, which interrupts the afferent nervous input from the injured area; the use of spinal anesthesia above the injury; and application of topical anesthetics to the injured area did not affect the metabolic response to injury. In addition, a variety of drugs, which are thought to play a central role in temperature regulation, such as salicylates, L-dopa, calcium, and atropine, exerted no detectable effect on the hypermetabolic response to thermal injury evaluated in the short-term studies described.

Hypermetabolism, negative nitrogen balance, and weight loss characterize the metabolic response to thermal injury. Increase in sympathetic activity appears to mediate this response by elaboration of catecholamines, increasing energy production and interacting with insulin and other hormones to exert direct cellular effects on heat production and to alter substrate flow. Cold, pain, anxiety, and hypovolemia are potent afferent stimuli which augment the catechol response. These factors may be minimized by careful clinical management. However, the basic reset in metabolic activity appears to be initiated by the burn injury, and the metabolic events do not return to normal until permanent closure of the cutaneous wound has been achieved.

Glucose flow through the extracellular space is elevated in burn patients during the peak of their hypermetabolic response to thermal injury. Hyperglycemia and increased glucose flow is a result of increased glucose production, not impaired glucose disappearance, and the

TABLE 7

GLUCOSE FLOW IN BURN PATIENTS
(Mean ± S.E.)

	Nonseptic	Gram Negative Sepsis
N	17	11
Age (years)	29 ± 3	28 ± 3
Weight (kg)	67.3 ± 2.7	78.1 ± 2.8+
Burn size (% B.S.)	42 ± 5	74 ± 3*
Postburn study day	9 ± 1	8 ± 1
Glucose space (L/kg)	0.177 ± 0.010	0.201 ± 0.012
Asymptote (mg/100 ml)	113 ± 5	113 ± 12
K (100 min^{-1})	5.27 ± 0.51	2.64 ± 0.59*
Q (mg/kg/min)	10.12 ± 0.95	4.96 ± 0.72*

+$p < 0.01$; *$p < 0.001$

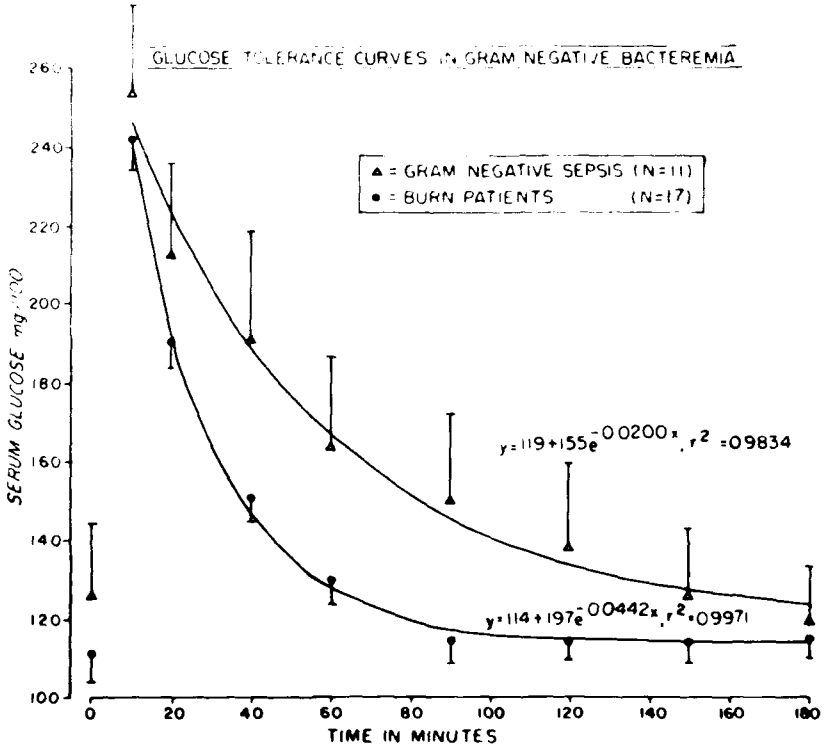


Figure 7. Glucose tolerance curves obtained from burn patients with positive blood stream culture for gram negative organisms demonstrate a decreased proportionality constant for disappearance of glucose into the periphery. The fasting blood glucose level and curve asymptote appeared to be related to the severity of the infection and virulence of the gram negative organism, with Klebsiella and Enterobacter species causing hyperglycemia, and Pseudomonas aeruginosa associated with lower blood glucose levels (several individuals had measured fasting blood glucose levels of 70 mg/100 ml).

accelerated rate of gluconeogenesis is associated in time with hyperglucagonemia, the increased elaboration of catecholamines, and normal fasting insulin levels. These findings are consistent with the hypothesis of Unger and Orci[10] that insulin primarily regulates peripheral glucose disposal while glucagon controls hepatic glucose production. Increased flow of gluocse to three carbon fragments, and conversion of these intermediates back to glucose, appears to occur following injury. Entry of glucose into the tricarboxylic acid cycle is limited, and fat is oxidized as the primary fuel source, a finding consistent with earlier studies which suggest a partial block in the metabolic pathways leading from three carbon to two carbon fragments during the convalescent stage of trauma.[11] Enzymes which favor conversion of three carbon intermediates to glucose are pyruvate carboxylase and phosphoenopyruvate carboxykinase; increased synthesis of these substances in the liver occurs in the presence of high levels of glucagon, catecholamines, glucocorticoids, and low levels of insulin,[12] precisely the hormonal environment present during the catabolic phase of injury.

Similar enzymatic adaptation occurs following prolonged starvation, but the major difference between the hormonal adaptation to starvation and the response to injury is the presence of increased sympathetic activity resulting in elaboration of catecholamines, which characterizes the response in the stressed state. Catecholamines, therefore, may not only participate in directing three carbon fragment flow back to six carbon synthesis but also determine body glucose mass and/or the extent of glucose cycling.

Heat production at the cellular level appears to be regulated by ATP hydrolysis and ADP stimulated substrate oxidation. In heat generating biologic systems, ADP is the most critical substance for "setting" the respiratory rate in mitochondria, a regulatory process known as acceptor control.[13] The cycling of glucose is an energy requiring process and hence utilizes ATP and generates ADP. Oxygen consumption is closely related to the rate of glucose flow through the extracellular space, and this relationship occurs at the time when glucose is not the major fuel source being oxidized. One explanation for this relationship is suggested by the following hypothesis: The ADP generated by the "futile cycle" of glucose controls the oxidation of fuel,

---

10. Unger RH, Orci L: The essential role of glucagon in the pathogenesis of diabetes mellitus. Lancet 1:14-16, 1975.

11. Drucker WR, Craig J, Kingsbury B, Hofmann N, Woodward H: Citrate metabolism during surgery. Arch Surg 85:557-563, 1962.

12. Exton JH: Gluconeogenesis. Metabolism 21:945-990, 1972.

13. Hochachka PW: Regulation of heat production at the cellular level. Fed Proc 33:2162-2169, 1974.

hence heat production and oxygen consumption can be related to glucose cycling through the ATP-ADP shuttle (Fig. 8).

Increased glucose flow through the extracellular compartment is interrupted in the injured patient by gram negative infection. Our preliminary investigations suggest the metabolic block occurs at the level of glucose outflow from the liver (i.e., failure of hepatic gluconeogenesis), findings consistent with the effects of gram negative infection in animals.[14] With diminution of glucose cycling, there is a simultaneous decrease in oxygen consumption. Although administration of glucose and insulin will provide available substrate for the periphery, this therapy is not effective in relieving the specific metabolic block which interferes with hepatic production of glucose, and further therapy should be aimed toward specific correction of the altered physiology.

Finally, it should be re-emphasized that both substrate flow and heat production are controlled by the central nervous system, acting by way of the sympathetic nervous system (Fig. 9). Increased sympathetic outflow from the hypothalamus carefully regulates mobilization of body fuel, flow rates of substrate, and final oxidation, and this finely orchestrated metabolic response to injury is controlled and directed by the elaboration of catecholamines.

---

14. LaNoue KF, Mason AD Jr, Bickel RG: Carbohydrate metabolism in Pseudomonas infection. Computers and Biomedical Research 2:51-67, 1968.

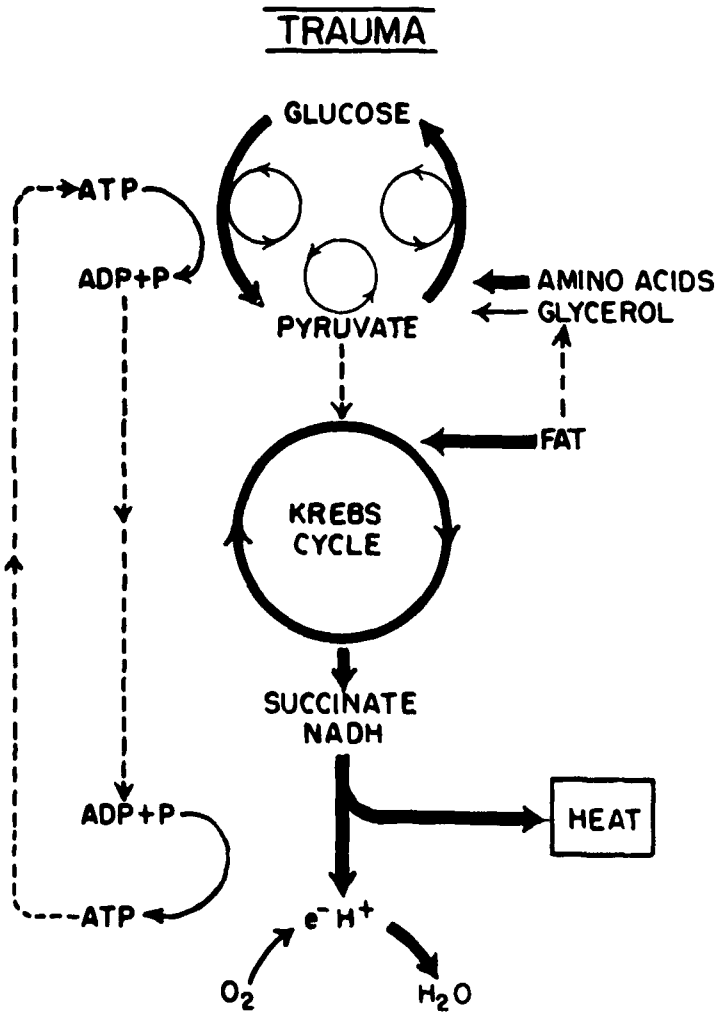


Figure 8. Heat production in traumatized man appears to be related to a separation of the glucose three-carbon cycle from the Krebs two-carbon cycle, because of the hormonal environment which directs three-carbon intermediates back to glucose. The rate of the Krebs cycle oxidation is regulated by the quantity of ADP generated by the glucose cycle, and an exothermic reaction results; hence, heat production and oxygen consumption are related to glucose recycling (the rate of spin of the upper cycle and the production of ADP), in spite of the fact that glucose is not the primary oxidized fuel. Gram negative infection interferes with gluconeogenesis and glucose recycling which occur following injury, and oxygen consumption decreased with failure of hepatic glucose production.

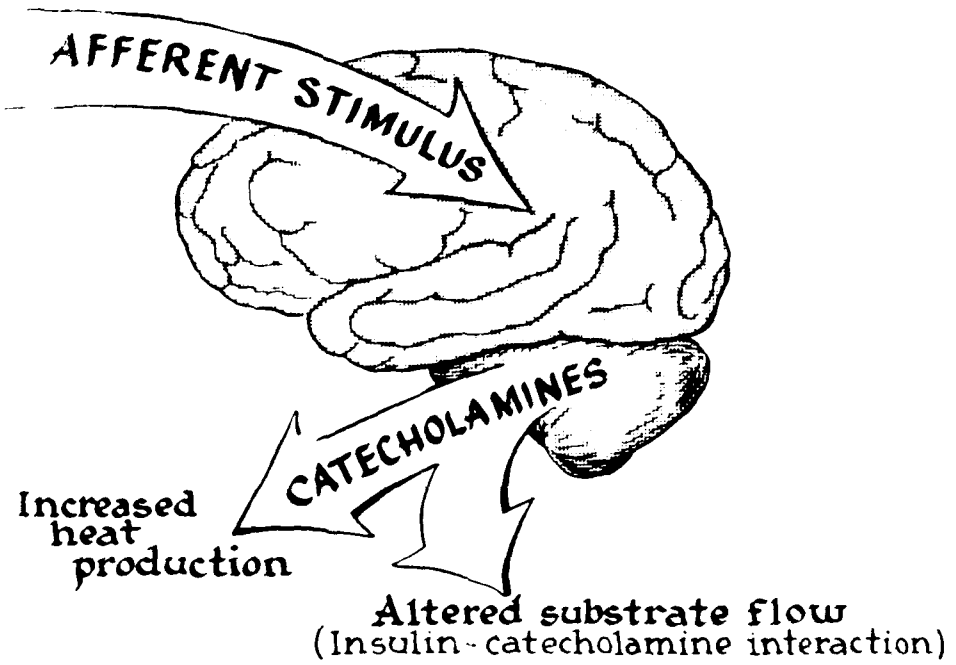


Figure 9. Afferent stimuli cause readjustment within the hypothalamus, which increases sympathetic outflow from the central nervous system. Catecholamines interact with insulin and other hormones to alter substrate flow, but have direct effects on cellular heat production and hence increase metabolic rate. Feeding the patient will alter substrate flow, but the degree or extent of energy or nitrogen balance exerts little effect on heat production, which returns to normal with closure of the burn wound.

REFERENCES

1. Wilmore DW, Mason AD Jr, Johnson DW, Pruitt BA Jr: Effect of ambient temperature on heat production and heat loss in burn patients. J Appl Physiol 38:593-597, 1975.

2. Wilmore DW, Long JM, Mason AD Jr, Skreen RW, Pruitt BA Jr: Catecholamines: mediator of the hypermetabolic response to thermal injury. Ann Surg 180:653-669, 1974.

3. Boyd AE, Mager M, Angoff G, Lebovitz HE: Effect of acute administration of L-dopa on body temperature in man. J Appl Physiol 37:675-678, 1974.

4. Myers RD: Primates in Comparative Physiology of Thermoregulation, edited by GC Whitton, New York & London, Academic Press, p. 283.

5. Kirkpatrick WE, Lomax P: The effect of atropine on the body temperature of the rat following systemic and intracerebral injection. Life Science 6:2273-2278, 1967.

6. Schreiner HR: General biological effects of the helium-xenon series of elements. Fed Proc 27:872-878, 1968.

7. Long CL, Spencer JL, Kinney JM, Geiger JW: Carbohydrate metabolism in man: Effect of elective operations and major injury. J Appl Physiol 31:110-116, 1971.

8. Gump FE, Long C, Killian P, Kinney JM: Studies of glucose intolerance in septic injured patients. J Trauma 14:378-388, 1974.

9. Hlad CJ Jr, Elrick H, Witten TA: Studies on the kinetics of glucose utilization. J Clin Invest 35:1129-1149, 1956.

10. Unger RH, Orci L: The essential role of glucagon in the pathogenesis of diabetes mellitus. Lancet 1:14-16, 1975.

11. Drucker WR, Craig J, Kingsbury B, Hofmann N, Woodward H: Citrate metabolism during surgery. Arch Surg 85:557-563, 1962.

12. Exton JH: Gluconeogenesis. Metabolism 21:945-990, 1972.

13. Hochachka PW: Regulation of heat production at the cellular level. Fed Proc 33:2162-2169, 1974.

14. LaNoue KF, Mason AD Jr, Bickel RG: Carbohydrate metabolism in Pseudomonas infection. Computer and Biomedical Research 2:51-67, 1968.

PRESENTATIONS AND/OR PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6971	75 07 01	DD-DR&E-AR(6)

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
74 07 01	D. CHANGE	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO. CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A16110A91C	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
69 07	Cont	DA	C. In-House

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

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
PRECEDING FISCAL YEAR 75	.7	16
CURRENT FISCAL YEAR 76	.5	15

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

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

20. PERFORMING ORGANIZATION
NAME: US Army Institute of Surgical Research
Pulmonary Section
ADDRESS: Fort Sam Houston, Texas 78234

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME: Peter A. Petroff, MAJ, MC
TELEPHONE: 512-221-4224
SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS
NAME: Edwin W. Hander, CPT, MSC
NAME: DA

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Pulmonary diffusion; (U) Shunt; (U) PV work; (U) Burns; (U) Lung mechanisms; (U) Blood gases; (U) Wounded soldiers; (U) Ventilation/perfusion abnormalities; (U)Humans

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

23. (U) To identify the types of pulmonary lesions in burned soldiers; to define their pathophysiology. Study is relevant to military situations since most burn deaths are due to pulmonary disease.

24. (U) Classify patients into inhalation and noninhalation injury. In those with inhalation injury, study serially lung volumes (static and dynamic), flow-volume loops, single breath nitrogen tests for closing volumes, static and dynamic compliance, and diffusion capacity. Compare with results of xenon scan and bronchoscopy. In non-inhalation injuries, continue prior work with attention to CO_2 response curve, better measurement of static lung volumes and use of flow-volume loop, single breath tests, and cardiac output.

25. (U) 74 07 - 75 06 Patients with positive xenon scans (inhalation injury) were compared to patients of similar age and burn size but negative xenon scan (no inhalation injury). The former patients showed evidence of obstructive lung disease (decreased flow rates, elevated pulmonary resistance). In addition, nine patients with negative xenon scans were studied shortly after admission and again prior to discharge. The patients showed evidence of minor to moderate restrictive disease (decreased vital capacities), and the patients who had anterior trunk injury had slightly elevated pulmonary resistance.

*Available to contractors upon originator's approval

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

ANNUAL PROGRESS REPORT

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: 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 1974 - 30 June 1975

Investigators:

Peter A. Petroff, MD, Major, MC
Edwin W. Hander, MS, Captain, MSC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: PULMONARY PATHOPHYSIOLOGIC CHANGES FOLLOWING THERMAL INJURY 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 1974 - 30 June 1975

Investigators: Peter A. Petroff, MD, Major, MC
Edwin W. Hander, MS, Captain, MSC

Reports Control Symbol MEDDH-288(R1)

We studied several aspects of pulmonary mechanics in burned patients, including the effects of inhalation injury and anterior trunk burns. We also examined the effect of nebulized gentamicin in normal subjects to determine its safety for use in the burned patient.

The patient with an inhalation injury has a restrictive-obstructive disease characterized by decreased flow rates at all lung volumes and an increased pulmonary resistance. The patient who sustains an anterior trunk injury has an elevated pulmonary resistance which is directly proportional to the extent of his injury. There is no difference in vital capacity between patients with and without anterior trunk injury.

Shunt
PV work
Burns
Lung mechanics
Pulmonary diffusion
Blood gases
Wounded soldiers
Ventilation/perfusion abnormalities
Humans

PULMONARY PATHOPHYSIOLOGIC CHANGES FOLLOWING THERMAL INJURY IN BURNED SOLDIERS

Since most burn deaths are due to pulmonary disease, this study was designed to identify the types of pulmonary lesions in burned patients and define their pathophysiology. The work on this project during the last fiscal year included 1) the effect of inhalation injury on pulmonary function, 2) the effect of anterior trunk burn on pulmonary function, 3) tests of small airway disease, and 4) the effect of nebulized gentamicin on pulmonary function in normal man.

Effect of Inhalation Injury on Pulmonary Function

Seven patients with positive ^{133}Xenon lung scans were studied and compared to eight patients (of similar age and burn size) with negative ^{133}Xenon lung scans. Maximum expiratory flow volume (MEFV) curves were obtained with an Ohio Model 840 dry spirometer, with output displayed on a Tetronix Model D-13 oscilloscope. Compliance, both static and dynamic, and pulmonary resistance were determined, using the Ohio spirometer for flow and volume measurements and an esophageal balloon (with its tip placed 42 cm from the nares) connected to a Statham differential transducer for pressure measurements. Arterial blood gases were measured with an IL gas analyzer.

No statistically significant differences in pH, PCO_2, vital capacity, static or dynamic compliance were observed. PO_2 was reduced, as were the flow rates, while pulmonary resistance was elevated in patients with a positive ^{133}Xenon lung scan (Table 1). This indicated that obstructive disease was associated with the ventilation-perfusion abnormalities.

Effect of Anterior Trunk Burn on Pulmonary Function

In order to determine the effect of abdominal-thoracic burns on pulmonary mechanics, we studied four patients without inhalation injury (negative ^{133}Xenon lung scan) and eight patients with inhalation injury (positive ^{133}Xenon lung scan) within 96 hours of the burn injury and again immediately prior to discharge. Results are shown in Table 2, with pulmonary function studies expressed as per cent of final values. The two groups did not differ in age or per cent total body surface burn. The average total body surface burn size for those patients with anterior trunk injury was 58.1 ± 24.8. Vital capacity and flow rates were similar for the two groups; however, the static compliance (C_{stat}) was significantly increased as was the pulmonary resistance (R_{pulm}) in the group with anterior trunk injury. In addition, the increased pulmonary resistance was directly

TABLE 1

COMPARISON: PATIENTS WITH POSITIVE ^{133}XENON LUNG SCANS

TO PATIENTS WITH NEGATIVE ^{133}XENON LUNG SCANS

	Positive ^{133}Xenon Lung Scan	Negative ^{133}Xenon Lung Scan
pH	7.38	7.39
PCO_2	34.6	33.9
PO_2	69.4	85.2
Vital Capacity (% Predicted)	80.8	85.3
Peak Flow (% Predicted)	61.9	99.1
Flow (50% Vital Capacity) (% Predicted)	41.6	98.7
Static Compliance	.28	.32
Dynamic Compliance	.22	.27
Pulmonary Resistance	4.8	3.1

TABLE 2

EFFECT OF ANTERIOR TRUNK BURN ON PULMONARY FUNCTION*

	Age	Per Cent Total Body Surface Burn	Vital Capacity
Anterior injury	30.8 (13.9)	35.0 (11.1)	91.6 (9.1)
No anterior injury	26.5 (8.4)	39.5 (3.3)	90.9 (5.7)
	Functional Residual Capacity	**Peak Flow Rate**	**Flow Rate at 50 Per Cent Vital Capacity**
Anterior injury	101.0 (12.3)	94.2 (17.7)	107.3 (18.3)
No anterior injury	92.5 (9.7)	84.4 (13.6)	112.3 (22.6)
	Static Compliance	**Dynamic Compliance**	**Pulmonary Resistance**
Anterior injury	99.7 (11.2)	107.0 (30.2)	165.4 (50.5)
No anterior injury	81.6 (13.1)	95.4 (49.9)	96.0 (19.2)

*Results are given in mean values; standard deviations in parentheses.

related to the extent of anterior trunk injury [Y = [illegible] (initial/final x 100) = 95.1 + 1.29 x (per cent anterior trunk burn)] (p < .01), whereas change in vital capacity was not.

The scope of this study was limited in that the patients had predominantly second degree injury. There were no previous studies with large third degree burns, and it is hypothesized that third degree anterior trunk burns would result in more restrictive disease. Nevertheless, until more conclusive data are obtained, it is felt that the patient with anterior trunk injury with severe pulmonary restrictive disease should be evaluated for other causes of this fact. When thoracic wall escharotomy is indicated in such a patient, pulmonary function measurements should be performed before and after the procedure.

The presence of elevated pulmonary resistance in the absence of anterior thoracic injury suggests that this test will be of little value in the diagnosis of inhalation injury. However, an elevated pulmonary resistance in patients without anterior thoracic injury is suggestive of innalation injury.

Tests of Small Airway Disease

The presence of obstructive pulmonary disease [illegible] patient with inhalation injury. The site of obstruction may be large or small airway or both. In some patients, bronchoscopy shows clear evidence of laryngeal and tracheal damage, edema, hemorrhage and obstruction; other patients have minimal changes. Biopsy findings often reveal bronchiolitis with peribronchial [illegible].

In an attempt to localize the disease process, several tests of pulmonary function were performed in smokers and nonsmokers, including closing volume, flow-volume loop, and helium-volume loop. Closing volume was measured in the following manner. The subject breathed out to residual volume (RV) and was then switched to breathe from a demand valve. Lungs were filled to total capacity and expired to RV; the exhalation was done through an orifice flow meter and was less than .5 l/sec. Inhalation was not [illegible], but the subject was instructed to inhale slowly. Volume was recorded on an Ohio Model 840 dry spirometer, with nitrogen recorded on a Perkin-Elmer Model 1100 membrane gas analyzer. Both signals were displayed on a Tetronix oscilloscope.

Flow volume loops were obtained in the usual manner. For the helium flow-volume loop, the subject breathed [illegible] minutes prior to the performance of three maximum flow-volume (MEFV) loops. These MEFV loops were performed [illegible]

flow-volume curves with the same procedure used for controls. The percent increase in flow at 50% VC and 25% VC was measured as well as the volume at which the flow rates from both the control and helium tests were equal $\dot{V}isoV$ (volume of isoflow).

Twenty-two healthy subjects were studied. There were 14 nonsmokers and eight smokers. The results of the tests are shown in Table 3 (given in mean values; standard deviations in parentheses). As can be seen, the flow rates at 50% VC and 25% VC were comparable. However, the ratio of $\dot{V}25$ to $\dot{V}50$ was decreased significantly in the smokers, a finding to be expected if the $\dot{V}25$ is determined by the small airway since tobacco usage is associated with small airway disease. The closing volume was increased (although not significantly) in the smokers. The volume of isoflow correlated better with the subject's smoking history. Although the delta flows 50 and 25 were not significantly different in the two groups, the ratio of the two was the most highly significant test.

Several tests correlate with each other. Volume of isoflow and delta flow 25 (response to helium at 25% VC) were correlated with the closing volume. In addition, delta flow 25 and 50 were correlated as was delta flow 50 with flow 50. Several of the tests were age-related, the closing volume, flow 25/50 ratio, and delta flow 25/50. We are now in the process of applying these tests to the patient with inhalation injury.

Effect of Nebulized Gentamicin on Pulmonary Function in Normal Man

Three young healthy adults were given a total of five five-day courses of nebulized gentamicin. For the study, 80 mg of gentamicin was nebulized t.i.d. using a Bird pressure ventilator with a Bard Parker nebulizer, Mark 7. Pulmonary function was measured prior to start of the treatment and after the first treatment, and then pre- and post-treatment on Day 1 or 2 and Day 4 or 5 postonset of the regimen. The pulmonary function testing included maximum expiratory flow volume loops, measurements of static and dynamic compliance, pulmonary resistance, and a single-breath nitrogen test.

The results are shown in Table 4. As can be seen, there was no consistent change in the postinhalation studies, and, in addition, the final study did not differ from the initial study. There is no evidence in these subjects of pulmonary changes due to the inhalation of gentamicin, either acutely or over a period of four to five days.

PRESENTATIONS AND/OR PUBLICATIONS

None.

TABLE 3

PULMONARY FUNCTION TESTS ON SMOKERS AND NONSMOKERS*

	Nonsmokers	Smokers	
Number	14	8	
Age	26.9 (4.2)	29.9 (4.7)	NS
$\dot{V}50$ (% Predicted)	108.1 (29.2)	110.5 (36.2)	NS
$\dot{V}25$ (% Predicted)	111.6 (28.4)	104.5 (38.9)	NS
$\dot{V}25/V50$	42.1 (6.2)	36.5 (4.9)	$p = .05$
Closing Volume (% of VC)	10.7 (3.6)	13.2 (5.9)	NS
Volume of Isoflow (% of VC)	14.0 (6.7)	19.4 (5.3)	$p > .1$
Delta $\dot{V}50$ (% Increase)	42.3 (13.6)	48.3 (25.4)	NS
Delta $\dot{V}25$ (% Increase)	30.1 (14.8)	21.6 (16.2)	NS
Delta $\dot{V}25$/Delta $\dot{V}50$	73.2 (31.9)	30.7 (23.2)	$p > .01$

*Results are given in mean values; standard deviations in parentheses.

TABLE 4

PULMONARY FUNCTION TESTS ON THREE YOUNG HEALTHY ADULTS
PRE- AND POST-NEBULIZED GENTAMICIN STUDY*

	Pre-Gentamicin	Post-Gentamicin
Vital Capacity	5.60 (.43)	5.64 (.45)
$\dot{V}50$	5.08 (1.23)	5.17 (1.41)
$\dot{V}25$	2.19 (.74)	2.18 (.78)
Static Compliance	.346 (.112)	.384 (.109)
Dynamic Compliance	.262 (.056)	.313 (.158)
Pulmonary Resistance	1.88 (.70)	2.13 (.63)

*Results are given in mean values; standard deviations in parentheses.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA OF 6384	75 07 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8A. DISP'N INSTR'N	8B. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
74 07 01	D. CHANGE	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	090
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)* (U) Inhalation Injury: Development of an Animal Model of Pulmonary Injury as it Occurs in Burned Soldiers (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*
003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
73 10	Cont	DA	C. In-House

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

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 75	.1	2
	CURRENT 76	.2	6

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

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

20. PERFORMING ORGANIZATION
NAME:* US Army Institute of Surgical Research
Metabolic Branch, Pulmonary Section
ADDRESS:* Fort Sam Houston, Texas 78234

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME:* Peter A. Petroff, MAJ, MC
TELEPHONE: 512-221-4224
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: Edwin W. Hander, CPT, MSC
NAME: Arthur D. Mason, Jr., MD DA

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Smoke inhalation; (U) Pulmonary; (U) Goats; (U) Animal model

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

23. (U) To produce an animal model which represents the pathological and physiological findings of smoke inhalation injury in order to identify the major offending agent and the effect of various modes of therapy. Because of the use of tanks in modern warfare, closed space injuries have become exceedingly important and the proper care of these soldiers critical.

24. (U) Goats will be exposed to smoke of a burning mattress for 10-20 minutes. Blood gases, static compliance, dynamic compliance, and pulmonary resistance will be measured before and after the inhalation, CO, CO_2, O_2, No_x, SO_2, TDI, HCN, and hydrocarbons will be measured during the inhalation. The goats will be sacrificed three hours to 72 hours after injury and their lungs studied pathologically.

25. (U) 74 07 - 75 06 To date, four exposures, totalling 25 goats, have been undertaken. Because of difficulties in reproducing the animal model, the last exposure utilized a combination of wood and urethane foam. In that exposure, all of the goats had an immediate fall in PaO_2 and one sustained evidence of inhalation injury at post-mortem examination.

*Available to contractors upon originator's approval

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

ANNUAL PROGRESS REPORT

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: INHALATION INJURY: DEVELOPMENT OF AN ANIMAL MODEL OF PULMONARY INJURY AS IT OCCURS IN BURNED SOLDIERS

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

1 July 1974 - 30 June 1975

Investigators:

Peter A. Petroff, MD, Major, MC
Gary W. Welch, MD, PhD, Lieutenant Colonel, MC
Edwin W. Hander, MS, Captain, MSC
Robert J. Lull, MD, Lieutenant Colonel, MC
Arthur D. Mason, Jr., MD

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: INHALATION INJURY: DEVELOPMENT OF AN ANIMAL MODEL OF PULMONARY INJURY AS IT OCCURS IN BURNED SOLDIERS

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

Period covered in this report: 1 July 1974 - 30 June 1975

Investigators: Peter A. Petroff, MD, Major, MC
Gary W. Welch, MD, PhD, Lieutenant Colonel, MC
Edwin W. Hander, MS, Captain, MSC
Robert J. Lull, MD, Lieutenant Colonel, MC
Arthur D. Mason, Jr., MD

Reports Control Symbol MEDDH-288(R1)

A large animal model of smoke inhalation injury has been produced by exposing goats with tracheostomies to smoke produced from a burning pyre of wood (pine) and urethane foam. In the most recent experiment, four goats were studied. One goat died during the exposure, and the other goats developed a fall in PaO_2, $PaCO_2$, and pH immediately post-injury. By 24 hours, these values had returned to normal. However, all goats had evidence of an inhalation injury at postmortem examination. During the forthcoming year, we will 1) determine that the system provides reproducible data, and 2) evaluate the effect of steroids on the pathological changes, blood gases, and xenon scans.

Smoke inhalation
Pulmonary
Goats
Animal model

INHALATION INJURY: DEVELOPMENT OF AN ANIMAL MODEL OF PULMONARY INJURY AS IT OCCURS IN BURNED SOLDIERS

The injury produced by inhalation of smoke remains one of the most critical problems in the care of the burn patient. In order to gain an understanding of the pathophysiology of this disorder, an animal model is essential. A small animal (rat) model has been developed by Dressler, et al.[1] However, the animals are exposed in a closed system so that hypoxia limits the amount of smoke to which the animals are exposed. In addition, the predominant lesion seen, an interstitial edema, may be due to the hypoxemia. Recently, the same group showed that steroids are effective in improving the mortality and pathological changes in the rat model.

Since it was felt that a more open-system model with larger animals would allow more flexibility, a goat model was developed. Initially, goats were exposed to smoke from a burning mattress; however, reproducible lesions were not obtained. In the last two experiments, a pyre consisting of 90 pounds of pine and eight pounds of urethane was burned, and the smoke produced from this material resulted in pathological changes in all of the animals (Tables 1 and 2).

The area used for the experimental burns consists of two chambers compartmented by a sliding door. Both chambers are vented with warm air. The material to be burned is placed in the larger chamber, and the animals are placed in the smaller chamber. Temperature, oxygen, CO_2, and C_0 concentrations, SO_2, NO_x, Hcl, and TDI are measured in both chambers. By using an open system, the oxygen concentration remains above 15%.

All of the animals exposed for more than 17 minutes had pathological changes at postmortem examination consisting of tracheal and bronchial lesions, and pneumonia was present in three of the four animals from the second experiment.

During the forthcoming year, we will repeat the same experiment to insure that the lesions are reproducible, and, in addition, will evaluate the effectiveness of steroids in treatment of the inhalation injury produced.

---

1. Bloom SB, Skornik WA, Dressler DP: Toxicity of smoke produced by combustion of home decorating material. Presented at the American Burn Association meeting, Denver, Colorado, March 1975.

TABLE 1

BLOOD GASES, EXPERIMENT I

	Pre	10 Minutes Post	2 Hours Post
Goat No. 1			
pH	7.396	7.433	7.452
pCO_2	36	29	32.7
PO_2	54.4	43.1	63.9
Goat No. 2			
pH	7.436	7.297	7.465
pCO_2	34.1	37.1	33.6
PO_2	73.4	53.2	74.4
Goat No. 3			
pH	7.290	7.173	7.404
pCO_2	37.1	15.4	17.6
PO_2	81.5	41.5	80.3

TABLE 2

BLOOD GASES, EXPERIMENT II

	Pre	10 Minutes Post	2 Hours Post
Goat No. 1			
pH	7.455	7.487	7.404
pCO_2	26.3	22.8	30.3
PO_2	81.9	63.0	67.7
Goat No. 2*			
pH	7.366		
pCO_2	33.4		
PO_2	79.9		
Goat No. 3*			
pH	7.426		
pCO_2	34.9		
PO_2	58.5		
Goat No. 4			
pH	7.502	7.385	7.418
pCO_2	26.3	23.4	31.2
PO_2	83.6	73.2	66.8

*Died with 25-minute exposure

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OA 6382	75 07 01	DD DR&E/AR/636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8b. DISP'N INSTR'N	8c. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
74 07 01	D. CHANGE	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO. CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A16110A91C	00	080
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) Hemodynamics and Pulmonary Vascular Studies in The Early Postburn Period of Burned Military Personnel (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
72 01	Cont	DA	C. In-House

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

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 75	.2	7
FISCAL YEAR CURRENT 76	.5	15

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

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

20. PERFORMING ORGANIZATION
NAME: US Army Institute of Surgical Research
Clinical Division
ADDRESS: Fort Sam Houston, Texas 78234

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

ASSOCIATE INVESTIGATORS
NAME: James M. Long, LTC, MC
NAME: Basil A. Pruitt, Jr., COL, MC DA

21. GENERAL USE: FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code): (U) Burn; (U) Wedge pressure; (U) Cardiovascular hemodynamics; (U) Resuscitation of fluids; (U) Humans

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

23. (U) To evaluate systemic and pulmonary hemodynamics during the resuscitation phase of thermal injury in burned military personnel.

24. (U) Selected patients are studied by means of hemodynamic flow and pressure measurements which are correlated with fluid intake and output.

25. (U) 74 07 - 75 06 At this time, one additional patient has been studied. Cardiac index was 2.56 L/m2. Total peripheral resistance was elevated as was pulmonary vascular resistance. Urine output was reduced. Infusion of a liter of Ringer's lactate over 20 minutes caused cardiac index to rise to 3.3 L/min and peripheral resistance to fall. The study is in progress.

*Available to contractors upon originator's approval

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

ANNUAL PROGRESS REPORT

PROJECT NO. 3A161101A91C-00, IN HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: 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 1974 - 30 June 1975

Investigators:

Gary W. Welch, MD, PhD, Lieutenant Colonel, MC
James M. Long, MD, Lieutenant Colonel, MC
Basil A. Pruitt, Jr, MD, Colonel, MC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A161101A91C-00, IN HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: 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 1974 - 30 June 1975

Investigators: Gary W. Welch, MD, PhD, Lieutenant Colonel, MC
James M. Long, MD, Lieutenant Colonel, MC
Basil A. Pruitt Jr, MD, Colonel, MC

Reports Control Symbol MEDDH-288(R1)

During the last six months, three burn patients have been studied using the Swan-Ganz thermal dilution output catheter during the acute resuscitation phase. Two of the three had elevated systemic vascular resistances and normal pulmonary resistances while the third had markedly lower systemic and pulmonary vascular resistance. In all three cases, cardiac index was below normal. Two of the three patients developed renal failure and continued to have low cardiac indices. The third patient developed a supranormal cardiac index within 72 hours of his initial injury. The study is continuing.

Burn
Wedge pressure
Cardiovascular hemodynamics
Resuscitation of fluids
Humans

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA OE 6954	75 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
74 07 01	D. CHANGE	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO. CODES*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161102A91C	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)
PRECEDING FISCAL YEAR 75	.2	1
CURRENT FISCAL YEAR 76	.2	3

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

20. PERFORMING ORGANIZATION
NAME* US Army Institute of Surgical Research
Burn Study Branch
ADDRESS* Fort Sam Houston, Texas 78234
PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME* Norman S. Levine, LTC, MC
TELEPHONE: 512-221-2943
SOCIAL SECURITY ACCOUNT NUMBER:
ASSOCIATE INVESTIGATORS
NAME: Hugh D. Peterson, COL, MC
NAME: DA

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Cryosurgery; (U) Liquid nitrogen; (U) Laser; (U) Escharotomy; (U) Excision; (U) Eschar; (U) Humans

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

23. (U) To investigate the use of a carbon dioxide laser to excise burns with a view towards minimizing blood loss involved in such excisions in burned soldiers.

24. (U) Symmetrical excisions have been performed on patients who are candidates for excision. Laser excisions were compared to both scalpel and electrocautery excisions from the standpoint of blood loss, time for excision, graft take, and pre-and post excision quantitative microbiology. The efficacy of tangential excisions for third-degree burns also has been studied.

25. (U) 74 07 - 75 06 The laser, although superior to the scalpel, did not show any substantial superiority over the electrocautery. The laser excisions involved somewhat less blood loss and somewhat greater operative time than the electrocautery excisions. Tangential debridements of third-degree burn wounds appear to be useful, particularly in combating delayed eschar separation. Formation of granulation tissue appears hastened following tangential excision so that grafting can be achieved at an earlier time. Tangential excision of burns of the hand results in earlier function and less hyptertrophic scar formation. Late functional results are being 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 68 AND 1498-1, 1 MAR 68, FOR ARMY USE, ARE OBSOLETE

ANNUAL PROGRESS REPORT

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: EXCISION OF ESCHAR IN BURNED SOLDIERS

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

1 July 1974 - 30 June 1975

Investigators:

Norman S. Levine, MD, Lieutenant Colonel, MC
Roger E. Salisbury, MD, Major, MC
Hugh D. Peterson, DDS, MD, Colonel, MC
Basil A. Pruitt, Jr., MD, Colonel, MC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: EXCISION OF ESCHAR IN BURNED SOLDIERS

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

Period covered in this report: 1 July 1974 - 30 June 1975

Investigators: Norman S. Levine, MD, Lieutenant Colonel, MC
Roger E. Salisbury, MD, Major, MC
Hugh D. Peterson, DDS, MD, Colonel, MC
Basil A. Pruitt, Jr., MD, Colonel, MC

The use of a 100 watt carbon dioxide laser for surgical excisions of burn eschar was evaluated in 13 patients with symmetrical burns. In each patient, laser excision was compared with either scalpel or electrocautery excision of a comparable area. Graft "take," blood loss, and operative speed were measured. Graft "take" following laser excisions was comparable to that obtained when either scalpel or electrocautery was used. Regression analysis indicates that laser excisions involved 29% of the blood loss encountered with scalpel excisions and that laser speed was 1.49 times scalpel speed. Although laser blood loss was 60% of electrocautery blood loss, laser speed was significantly slower: 73% of electrocautery speed. These differences, however, were of small clinical consequence. It is felt that the surgical arm of this laser is at present quite awkward and is in need of technological refinement if the full potential of the laser is to be realized.

Cryosurgery
Liquid nitrogen
Laser
Escharotomy
Excision
Eschar
Humans

EXCISION OF ESCHAR IN BURNED SOLDIERS

Early surgical excision of third degree burns combined with prompt skin grafting has for many years been an accepted form of therapy in carefully selected patients. The rationale for early excision closely parallels the basic tenets of modern burn therapy: 1) that the principal problem in the care of burned patients is infection; 2) that the origin of this infection is frequently in the burn eschar; and 3) that burned patients remain liable to infection until the eschar has separated from the underlying tissue and skin coverage is achieved by grafting.

Spontaneous separation of the burn eschar may not occur for 21 to 35 days, or even longer when topical chemotherapy is used. During this time, infection may develop in the eschar and, in fact, microorganisms are partly responsible for the sloughing of the eschar from the underlying tissue. Surgical excision offers the advantages of removing the burn wound before serious infection occurs and of permitting prompt skin coverage. Physical therapy and rehabilitation thus may be commenced at an early time postburn. MacMillan[1] has shown that in certain patients, hospitalization time can be shortened when burn wound excision is judiciously employed.

However, several factors limit the efficacy of surgical excision. First, a major operation and an anesthetic are required. Second, the blood loss can be massive, involving multiple transfusions and their accompanying risks. Third, skin grafts immediately applied to freshly excised surfaces do not always "take," and such graft loss may necessitate additional surgical procedures and the further expenditure of donor sites.

The blood loss in large-scale excisions can be extensive - over 20 units of blood for a single patient. Because of this, we have investigated the use of the carbon dioxide laser for the excision of third degree burns. In a previous report,[2] it was shown that laser excisions of experimental third degree burns involved one third the blood loss of scalpel excisions. The laser used experimentally, however, was very slow, and, to correct this, several technical modifications were suggested. Such changes have been incorporated in the laser used presently; and this study compares the second-generation laser to the scalpel and electrocautery for the excision of third degree burns.

---

1. MacMillan BG: Early excision. J Trauma 7:75-79, 1967.
2. Levine N, Ger R, Stellar S, et al: Use of a carbon dioxide laser for the debridement of third degree burns. Ann Surg 179:259, 1974.

MATERIALS AND METHODS

The surgical laser we have employed is a 100 watt, CO_2 laser made by American Optical Corporation. The laser beam is derived from vibrational and rotational energy levels in the CO_2 molecule, with a wavelength of 10.6 microns. These waves are resonated and amplified in a vacuum tube so that they become coherent in both space and time. This property allows the beam to traverse long distances without losing power. The beam is then passed through a surgical arm, consisting of seven jointed-reflecting surfaces, and delivered to a handpiece. A lens in the handpiece focuses the laser energy into a point of less than one millimeter diameter at a distance of one centimeter from the end of the handpiece. The power level may be regulated from 0-100 watts with a rheostat, conveniently located on the laser housing. A foot-pedal regulates "on" and "off" delivery of the laser beam to the operating field. Use of the laser is accompanied by a considerable production of smoke or "plume-fragmentation" caused by the photovaporization of tissue. A vacuum cleaner, equipped with a sterile nozzle, is used to remove this smoke.

During laser operations, plate glass spectacles or ordinary corrective eye-glasses were worn by everyone in the operating room theater to protect the eyes from reflected infra-red radiation. Signs stating "Do Not Enter, Laser in Use" were conspicuously displayed to minimize traffic into the operating room. A fire extinguisher was always available but never was needed.

Fifteen laser excisions were performed in patients who were candidates for excision. These patients fell into three groups: 1) patients with localized third degree burns of less than 20% body surface area; 2) carefully selected patients with burns of up to 70% in whom limited surgically-manageable areas were third degree; and 3) patients with third degree burns of over 80% body surface area. Excisions were performed at the level of the deep fascia. Care was taken to cut the subcutaneous margins of the excision in an oblique fashion to avoid creating an overhanging wound edge.

In 13 cases, symmetrical burns were excised; e.g., both legs, both arms, the anterior surface of the chest, etc.; in six such cases, the laser was compared to the scalpel; in seven, the laser was compared to the "cutting" (high frequency, undamped current) electrocautery. Pre- and postoperative photographs were routinely taken. Blood loss was measured by weighing the sponges and drapes used during the excisions. Graft "take" was determined either by the "take" of autografts immediately applied to the freshly excised tissue or by the adherence of immediately applied allograft or porcine xenograft in patients with massive burns.

For large excisions, a four-man surgical team was employed to excise the symmetrical areas simultaneously in order to minimize overall operative time. Arterial tourniquets were occasionally used when surgically feasible on extremity burns. In scalpel excisions, adjunctive use of an electrocautery was allowed to control small capillary bleeding points. It was felt that a "pure" scalpel excision, involving the individual clamping and ligation of even tiny vessels would increase operative time and blood loss beyond a reasonable limit. Similarly, in electrocautery excisions, although the "cutting" electrocautery was used for dissection, the "coagulating" electrocautery was employed to control bleeding.

RESULTS

Although both the laser and the electrocautery caused focal areas of microscopically visible damage to the underlying tissue, this was not sufficient to interfere with the "take" of skin autografts immediately applied to the freshly excised surface. Autograft "take" was equal for laser and scalpel excisions and for laser and electrosurgical excisions. In patients with massive burns, human cadaver allograft or porcine xenograft was used for initial wound coverage. The adherence of these biologic dressings was good regardless of the method of excision.

In comparing the three modalities of excision in terms of blood loss and operative speed, it was apparent that such indices varied considerably from patient to patient. In general, blood loss per unit area was less in children than in adults, and also was influenced by obesity and the time postburn at which the excisions were performed. Such patient-to-patient variation did not allow meaningful comparisons by simple averaging. Regression curve analysis of our data was therefore employed.

Laser Vs. Scalpel: Blood loss with the laser varied from one third to one sixth of scalpel blood loss in all cases. These data fit a linear regression function, defined in part by laser blood loss equalling 29% of scalpel blood loss. The reduction in blood loss was statistically significant.

Laser operative speed was also significantly faster than scalpel speed. Regression curve analysis indicates that laser speed equalled 1.49 times scalpel speed. When tourniquets were employed, the speed of excision, per se, was faster with the scalpel than with the laser. This was extended, however, by the time required to secure hemostasis in scalpel excisions after the tourniquets were released.

Laser Vs. Electrocautery: In all but one case, laser blood loss was less than electrocautery blood loss, though both were much less than scalpel blood loss. Regression curve analysis indicates that this difference in blood loss was significant, and is best described by a linear function wherein laser blood loss equalled 60% of electrocautery blood loss.

Operative speed, however, for the laser was generally slower than that of electrocautery excisions. Regression analysis indicates that laser speed was approximately 73% of electrocautery speed and that this difference was significant.

DISCUSSION

Early surgical excision of the burn eschar combined with prompt grafting has a well-established place in the treatment of localized third degree burns of limited size. The extension of this technique to larger area burns[3-8] has been limited by the ability of critically injured patients to withstand the operations and blood loss involved, and by the problems of attaining adequate skin coverage in patients with limited donor sites. The carbon dioxide laser has been advanced as a means of reducing operative blood loss during this procedure.

Previous experimental work performed with a less refined laser indicated that laser excisions involved one third the blood loss of scalpel excisions and that the take of skin grafts immediately applied to the freshly lased tissue was excellent. The early clinical experience of Fidler[9] confirmed these findings. Because the speed of this "first generation" laser was inordinately slow, two major technical

---

3. Artz CP, Thompson NJ: Early excision of large areas in burns. Surgery 63:868-870, 1968.
4. Cramer LM, McCormack RM, Carroll DB: Progressive partial excision and early grafting in lethal burns. Plast Reconstr Surg 30: 595-599, 1962.
5. Haynes BW Jr: Early excision and grafting in third degree burns. Ann Surg 169:736-746, 1969.
6. MacMillan BG: Early excision of more than twenty-five per cent of body surface in the extensively burned patient. Arch Surg 77:369-375, 1958.
7. Switzer WE, Jones J, Moncrief JA: Evaluation of early excision of burns in children. J Trauma 5:540-546, 1965.
8. Taylor PH, Moncrief JA, Pugsley LQ, et al: The management of extensively burned patients by staged excision. Surg Gynec Obstet 115:347-352, 1962.
9. Fidler JP, Low E, Rockwell J, et al: Carbon dioxide laser excision of acute burns with immediate autografting. J Surg Res 17: 1-11, 1974.

alterations were made: laser power was increased from 40 watts to 100 watts and the focal point of the laser was shortened. The combination of these factors resulted in a much higher power density at the focal point - a "sharper" laser, which we have used in this study.

For surgical excisions of burn eschar, the laser was clearly superior to the scalpel. In every comparison, laser blood loss was less than 30% of scalpel blood loss, and the laser excisions were significantly faster than scalpel excisions. The improved speed and reduced blood loss were both attributable to laser photocoagulation of small blood vessels within the path of the beam.

The theoretical advantage of using a carbon dioxide laser over other cauterizing instruments is based on the principal that infrared waves are very readily absorbed by tissue which has a high water content. Because the energy is so well absorbed, the laser beam produces a very fine line of damage, with injury to adjacent tissue confined to a zone measured in microns from the laser beam.[10] The "coagulation" electrocautery employs a low frequency, damped current which causes a relatively wide zone of tissue damage. The "cutting" electrocautery, however, utilizes a high frequency, undamped electric current which causes less tissue damage. It seemed logical, therefore, to compare the laser to this latter modality.

Neither the laser nor the "cutting" electrocautery caused enough damage to the underlying tissue to jeopardize the "take" of skin grafts immediately applied to the freshly excised tissue. Laser excisions involved significantly less blood loss (60% of electrocautery blood loss), but were significantly slower (73% of the speed of electrosurgical excisions). These differences, though significant statistically, were not impressive. The speed of the laser excisions was reduced primarily because of the awkwardness involved in handling the surgical arm. The optics and power delivery of the laser were excellent; on broad-planar surfaces, the speed of excision was limited only by the surgeon's capacity to control the laser beam. The laser, however, was considerably more difficult to maneuver in less accessible areas, such as the posterior surface of the thighs or the arms.

It is important to emphasize that the use of the carbon dioxide laser for burn wound excisions is a technique still in development. The optical changes incorporated in this "second generation" laser represent a dramatic improvement over the optics of earlier machines. For burn wound excisions, it is better than the scalpel. Comparison of this laser to the electrocautery, however, does not reveal a clear

---

10. Goldman L, Rockwell RJ Jr: Laser in Medicine, New York, Gordon and Breach Science Publishers, Inc., 1971.

advantage. The laser involves less blood loss, but laser excisions presently require more operating time. It is possible that the modifications required to make the laser less awkward, and therefore a superior instrument for this and other surgical procedures, are within the reach of modern day technology.

REFERENCES

1. MacMillan BG: Early excision. J Trauma 7:75-79, 1967.

2. Levine N, Ger R, Stellar S, et al: Use of a carbon dioxide laser for the debridement of third degree burns. Ann Surg 179:246-259, 1974.

3. Artz CP, Thompson NJ: Early excision of large areas in burns. Surgery 63:868-870, 1968.

4. Cramer LM, McCormack RM, Carroll DB: Progressive partial excision and early grafting in lethal burns. Plast Reconstr Surg 30: 595-599, 1962.

5. Haynes BW Jr: Early excision and grafting in third degree burns. Ann Surg 169:736-746, 1969.

6. MacMillan BG: Early excision of more than twenty-five per cent of body surface in the extensively burned patient. Arch Surg 77:369-375, 1958.

7. Switzer WE, Jones J, Moncrief JA: Evaluation of early excision of burns in children. J Trauma 5:540-546, 1965.

8. Taylor PH, Moncrief JA, Pugsley LQ, et al: The management of extensively burned patients by staged excision. Surg Gynec Obstet 115:347-352, 1962.

9. Fidler JP, Low E, Rockwell J, et al: Carbon dioxide laser excision of acute burns with immediate autografting. J Surg Res 17: 1-11, 1974.

10. Goldman L, Rockwell RJ Jr: Laser in Medicine, New York, Gordon and Breach Science Publishers, Inc., 1971.

PRESENTATIONS

Levine NS. Clinical Evaluation of the Carbon Dioxide Laser for Burn Wound Excisions. American Association for the Surgery of Trauma, Hot Springs, Virginia, 18 October 1974.

Levine NS. A Comparison of Laser, Scalpel, and the Electrocautery in Burn Wound Excisions. American Association of Plastic and Reconstructive Surgeons, Houston, Texas, 30 October 1974.

Levine NS. Limitations in Applying Tangential Excision to Patients with Large Burns, American Burn Association, Denver, Colorado, 20 March 1975.

PUBLICATIONS

Levine NS, Salisbury RE, Peterson HD, Pruitt BA Jr. Clinical Evaluation of the Carbon Dioxide Laser for Burn Wound Excisions: A Comparison of the Laser, Scalpel, and Electrocautery. In press, J Trauma.

Levine NS, Peterson HD, Salisbury RE, Pruitt BA Jr: Laser, Scalpel, Electrosurgical, and Tangential Excisions of Third Degree Burns: A Preliminary Report. In press, J Plast Reconstr Surg.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OF 6386	75 07 01	DD-DR&E-AR(6)6

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISS'N INSTR'N	8b. SPECIAL DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
75 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	3A16110A91C	00	077
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)

(U) Use of a Synthetic Dressing on Denuded Wounds in Burned Patients (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
75 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 75	.1	1
	CURRENT 76	.1	5

19. RESPONSIBLE DOD ORGANIZATION

NAME: US Army Institute of Surgical Research
ADDRESS: Fort Sam Houston, Texas 78234

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

21. GENERAL USE

FOREIGN INTELLIGENCE NOT CONSIDERED

20. PERFORMING ORGANIZATION

NAME: US Army Institute of Surgical Research
Burn Study Branch
ADDRESS: Fort Sam Houston, Texas 78234

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME: Norman S. Levine, LTC, MC
TELEPHONE: 512-221-2943
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: Hugh D. Peterson, COL, MC
NAME: Arthur D. Mason, Jr., MD DA

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Humans; (U) Wound dressing; (U) Biologic dressing; (U) Synthetic skin substitute; (U) Allograft

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 a synthetic laminate on open wounds of burned soldiers.

24. (U) Comparative testing of synthetic laminate (S), gauze (G), allograft (A), and porcine xenograft (X) on open wounds.

25. (U) 75 03 - 75 06 Wound appearance and quantitative wound bacteriology was studied on open granulation tissue wounds which were divided into four subareas and treated for 48 hours with either S,G,A, or X. Allograft was the only dressing to cause a significant decrease in wound flora and did so only when it "took". When allograft took, its use resulted in a wound appearance which was as good as that obtained by treatment with gauze or the synthetic dressing. For wounds which did not "take" allograft, the synthetic or gauze resulted in the best wound appearance. Porcine xenograft treatment resulted in the worst wound appearance.

*Available to contractors upon originator's approval

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

ANNUAL PROGRESS REPORT

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: USE OF A SYNTHETIC DRESSING ON DENUDED WOUNDS IN BURNED PATIENTS

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

1 July 1974 - 30 June 1975

Investigators:

Norman S. Levine, MD, Lieutenant Colonel, MC
Hugh D. Peterson, DDS, MD, Colonel, MC
Arthur D. Mason, Jr., MD

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: USE OF A SYNTHETIC DRESSING ON DENUDED WOUNDS IN BURNED PATIENTS

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

Period covered in this report: 1 July 1974 - 30 June 1975

Investigators: Norman S. Levine, MD, Lieutenant Colonel, MC
Hugh D. Peterson, DDS, MD, Colonel, MC
Arthur D. Mason, Jr., MD

Reports Control Symbol MEDDH-288(R1)

A new synthetic wound dressing made of nylon and expanded teflon was evaluated on modest sized areas of granulation tissue by comparing its effect on wound appearance and surface quantitative microbiology to that of human cadaver cutaneous allograft, porcine cutaneous xenograft, and coarse-mesh gauze during a 48 hour treatment period. A significant decrease in wound surface bacterial counts was observed only with allograft treatment and only when allograft "took." No other form of treatment significantly altered bacterial colonization of the burn wound. On wounds on which allograft "took," there was no significant difference in wound appearance between areas treated with cutaneous cadaver allograft, coarse-mesh gauze, or the synthetic dressing. Areas treated with porcine cutaneous xenograft appeared worst. On wounds on which cutaneous allograft did not "take," gauze and the synthetic dressing resulted in the best wound appearance; cutaneous allograft was third best and porcine cutaneous xenograft was worst.

The dressing may be used conveniently and safely; it conformed well to irregular surfaces and permitted motion of joints covered with the dressing. If suppuration under the dressing occurred, it was easily recognized. The principal advantage of this dressing is to provide membrane function and debridement for denuded wounds which require the removal of small amounts of surface debris before grafting can be achieved. For graftable wounds, it ranked second to human cadaver allograft as a temporary skin substitute.

Wound dressing
Biologic dressing
Synthetic skin substitute

USE OF A SYNTHETIC DRESSING ON DENUDED WOUNDS IN BURNED PATIENTS

Temporary skin substitutes, including uman cadaver cutaneous allografts, porcine cutaneous xenografts, and amniotic membranes have become widely used in the treatment of thermally injured patients. The principal use of these biologic dressings is for temrorary coverage of full-thickness burn wounds, after the necrotic eschar has separated from the underlying tissue. In addition, the use of such dressings has been advocated for the early treatment of partial-thickness burns, for temporary coverage of surgically excised burn wounds, and for other traumatic wounds involving full-thickness skin loss.

We believe that the beneficial effects of cutaneous allograft, and perhaps the other biologic dressings, are related to two interrelated properties. First, membrane function is provided by the epidermal surface of the graft, which limits fluid, electrolyte, and colloid losses from the wound and acts as a barrier against external infection. The second property relates to wound closure: With graft "take," the dermal surface adheres closely to the underlying tissue and is rapidly invaded by fibroblastic and vascular ingrowth from the wound. This interaction is thought to create a favorable environment in which cellular and other defense mechanisms may decontaminate the wound.

The use of viable biologic dressings has been limited by their availability, cost, difficulties of procurement (for allograft), limited shelf-life even under optimum storage conditions, antigenicity, and the fact that they are not readily sterilized. Further, unless strict asepsis is practiced in the harvesting of allogeneic or xenogeneic skin, iatrogenic infections may complicate the use of such grafts. The development of a clinically effective, synthetic, temporary skin substitute which is constructed from biologically inert materials, has indefinite shelf life, is readily autoclaved, and is inexpensive, would eliminate many of these problems. The testing of many different synthetic wound dressings has been performed at several institutions in recent years. From these studies has evolved the concept of a bilaminate dressing composed of an external surface to provide membrane function and a scaffold-like inner surface to allow fibroblastic and vascular ingrowth from the open wound.

A previous report from this institution described the development of a promising bilaminate which consisted of a nylon matrix adhered to a teflon membrane. Empirical studies determined that a minimum thickness of 0.025 inch was required to obtain consistent fibroblastic ingrowth into the scaffold-like surface. The dressing conformed well to irregular surfaces, and was somewhat stretchable in

two dimensions. Histologic studies of this material five days after application to experimental wounds demonstrated a close approximation of the nylon matrix to the underlying tissue and minimal foreign body reaction to the nylon. Fibroblastic and vascular ingrowth extended two-thirds of the distance from the inner surface to the teflon membrane. By 10 days, organized collagen was present within the nylon matrix. On animals with experimentally infected wounds, the dressing limited microbial colonization and prevented death from invasive infection. The membrane effects of the dressing were documented by its ability to promote survival in animals subjected to 60% body surface area excisions.

This study summarizes a clinical evaluation of the dressing and is divided into two sections: 1) a formal comparison of the effects of the synthetic dressing, human cadaver allograft, porcine xenograft, and coarse-mesh gauze on the appearance and quantitative microbiology of modest sized areas of granulation tissue; and 2) a descriptive report of use of the synthetic material to prepare wounds of larger body surface area for the acceptance of autografts.

METHODS

Formal Comparison on Granulation Tissue

Forty three granulation tissue wounds of uniform appearance were selected on 21 patients with burns ranging from 30% to 66% body surface area. Each wound was divided into four subareas. By randomized selection, each subarea was assigned treatment with a 48 hour application of either the synthetic dressing, human cadaver allograft, porcine xenograft, or 24 layers of coarse-mesh gauze. Both the synthetic dressing and the coarse-mesh gauze were soaked in sterile 0.9% saline solution before application. Forty eight hours later, the dressings were removed and the appearance of each subarea was graded on a "best," "second best," "third best," and "worst" basis by experienced burn surgeons who were not told which area received which treatment.

Quantitative aerobic wound bacteriology for each subarea was studied before and after treatment by using the quantitative swab technique. A sterile throat swab was twirled on the wound for five seconds, with sufficient pressure to cause a small amount of bleeding in the underlying tissue. The swab was then removed and shaken in 1.0 ml of transport medium (transport medium contained 3 g Bacto-Peptone and 0.3 g Agar Reagent in 300 ml distilled water; all obtained from DIFCO Laboratories, Inc., Detroit, Michigan), from which serial dilutions were made in trypticase soy broth and cultured on trypticase soy agar plates.

Human cadaver allograft was harvested within 10 days of use, and stored without antimicrobial agents in sterile Petri dishes at 4° C. Porcine xenograft was obtained weekly from a commercial source (Burn Treatment Skin Bank, Phoenix, Arizona 85034), refrigerated at 4° C, and used within 10 days after procurement. The synthetic dressing was sterilized with ethylene oxide prior to use. No antimicrobial agents were used in conjunction with the dressing in the formal comparative study.

Clinical Use

Since completion of the formal clinical comparison, the dressing has been used at our institution on patients with large areas of exposed granulation tissue, including several applications on 15 patients with burns of from 30% to 77% body surface area. The dressing was used to cover wounds ranging in size from 1% to 30% body surface area. It has been used primarily on granulation tissue to prepare the tissue for autograft acceptance. In some cases, heavily contaminated wounds with small foci of residual necrotic tissue were treated, and such wounds generally required daily changes of the dressing. In other cases, when the granulation tissue appeared to be ready for graft acceptance, a single application of the dressing was used and left in place for 2-3 days, following which grafting was performed.

In all cases, staining of the semitransparent dressing (vide infra) was interpreted as an indication to change the dressing on a daily basis. If no staining occurred, the dressing was left in place until autografting was performed.

In this part of the study, the synthetic dressing was sterilized by either ethylene oxide gas or steam autoclaving prior to use. The laminate was soaked in a 5% mafenide acetate solution immediately prior to clinical usage (vide infra).

RESULTS

Formal Comparison on Granulation Tissue

The clinical ranking of wound appearance was assigned a numerical value from 1 to 4: with 1 = "best," 2 = "second best," 3 = "third best," and 4 = "worst." Ties were allowed and these were assigned an intermediate numerical value. The ranking of wound appearance is tabulated for the 23 wounds on which allograft "took" (Table 1), for the 20 wounds on which allograft did not "take" (Table 2), and for all 43 wounds.

TABLE 1

RANKINGS OF WOUND APPEARANCE AFTER TREATMENT FOR WOUNDS ON WHICH ALLOGRAFT "TOOK" (N = 23)

	Number of Subareas in Each Rank			
Rank	Human Cadaver Allograft	Porcine Xenograft	Synthetic Dressing	Coarse-Mesh Gauze
1	7	0	8	5
1.5	1	0	1	0
2	1	2	9	11
2.5	1	1	1	1
3	9	3	2	5
3.5	2	2	0	0
4	2	15	2	1
Total Rank Value	55+	82.5*	44.0+	48.5+

+No significant difference, $X^2_{R-2} = 1.83$ by Friedman two-way analysis of rank among these three treatment modalities.

*Appearance of wounds following treatment with porcine cutaneous xenograft was "worst," $X^2_{R-3} = 23.33$, $p < 0.001$ by Friedman two-way analysis of rank.

TABLE 2

RANKINGS OF WOUND APPEARANCE AFTER TREATMENT FOR WOUNDS ON WHICH ALLOGRAFT DID NOT "TAKE" (N = 20)

	Number of Subareas in Each Rank			
Rank	Human Cadaver Allograft	Porcine Xenograft	Synthetic Dressing	Coarse-Mesh Gauze
1	4	0	6	8
1.5	0	0	2	2
2	2	0	11	4
2.5	1	0	0	1
3	6	10	1	2
4	7	10	0	3
Total Rank Value	56.5*	70**	34+	39.5+

**Human cadaver cutaneous allograft treatment resulted in worse wound appearance than did synthetic dressing or coarse-mesh gauze, $X^2_{R-2} = 6.93$, $p < 0.05$.

**Porcine cutaneous xenograft treatment resulted in worse wound appearance than did the other three treatment modalities, $X^2_{R-3} = 24.26$, $p < 0.001$.

+No significant difference, $X^2_{R-1} = 0.2$.

For wounds on which allograft "took" (Table 1), there was no significant difference in wound appearance between those areas treated with allograft, the synthetic dressing, or coarse-mesh gauze. Areas treated with porcine xenograft had an appearance which was significantly worse ($p < 0.001$) by Friedman's two-way analysis of variance for nonparametric functions.

For wounds on which allograft did not "take" (Table 2), areas treated with porcine xenograft again had the worst clinical appearance ($p < 0.001$). Areas treated with cadaver allograft had a significantly ($p < 0.05$) worse appearance than those treated with synthetic dressing or coarse-mesh gauze. There was no significant difference in wound appearance between those areas treated with coarse-mesh gauze or synthetic dressing.

For all 43 wounds (Table 3), areas treated with porcine xenograft appeared "worst" ($p < 0.001$), and areas treated with allograft "next worst" ($p < 0.05$). There was again no significant difference between the appearance of wounds treated with synthetic dressing or coarse-mesh gauze.

The wounds were colonized with a wide variety of organisms. Pseudomonas aeruginosa and Staphylococcus aureus were most frequently encountered. Klebsiella aerobacter, E. coli, Corynebacterium, Providencia stuarti, Enterobacter cloacae, and Candida were also found. These organisms were present in densities of from 0 to 10^8 organisms per swab. On a given wound, there was generally a less than 1 log variation between subareas in the quantitative microbial counts. The mean log bacterial counts before treatment were quite similar for all four treatment groups (Table 4).

Quantitative paired cultures were successfully retrieved from 41 of the wounds. The log changes in the organism counts fit a normal distribution. Nonparametric statistical analysis was performed on the direction of change (chi-square) (Tables 5,6) and on the log changes by the Wilcoxon matched pair signed-ranks test. Parametric analysis was performed on the log changes in microbial counts for paired cultures (Table 4, Fig. 1,2).

For wounds on which allograft "took," the number of organisms colonizing the wound after treatment with allograft was less than the number of organisms colonizing the wound before treatment on 17 of 23 occasions (Table 5). This is significantly different from the performance of other treatment modalities on wounds which "took" allograft ($p < 0.01$ by chi-square analysis). The average decrease in microbial densities following allograft "take" was greater than 1 log (Fig. 1), and is significant by both parametric (t-test) and

TABLE 3

RANKINGS OF WOUND APPEARANCE AFTER TREATMENT FOR ALL WOUNDS (N = 43)

	Number of Subareas in Each Rank			
Rank	Human Cadaver Allograft	Porcine Xenograft	Synthetic Dressing	Coarse-Mesh Gauze
1	11	0	14	13
1.5	1	0	3	2
2	3	2	20	15
2.5	2	1	1	2
3	15	13	3	7
3.5	2	2	0	0
4	9	25	2	4
Total Rank Value	111.5*	152.5**	78.0+	88.0+

*Appearance of wounds treated with human cadaver cutaneous allograft was worse than that of wounds treated with synthetic dressing or gauze, $X^2_{R-2} = 7.20$, $p < .05$.

**Appearance of wounds treated with porcine cutaneous xenograft was "worst," $X^2_{R-3} = 45.93$, $p < 0.001$.

+No significant difference, $X^2_{R-1} = 1.49$.

TABLE 4

PARAMETRIC ANALYSIS OF THE CHANGE IN LOG BACTERIAL COUNTS FOR ALL WOUNDS

	Human Cadaver Cutaneous Allograft	Porcine Cutaneous Xenograft	Synthetic Dressing	Coarse-Mesh Gauze
	WOUNDS ON WHICH ALLOGRAFT "TOOK" (N = 23)			
Mean log bacterial count before treatment	4.72	4.57	4.81	4.61
Mean log change in count after treatment*	-1.20	+0.13	+0.02	-0.25
Standard error for mean log change	0.38	0.39	0.35	0.40
95% confidence limits for log change	-0.44 to -1.96	-0.65 to +0.91	-0.68 to +0.72	-0.65 to + 1.05
Significance of change (t-test)	$p < 0.01$	None	None	None
	WOUNDS ON WHICH ALLOGRAFT DID NOT "TAKE" (N = 18)**			
Mean log bacterial count before treatment	4.23	4.77	4.24	4.72
Mean log change in count after treatment*	+1.00	+0.57	+0.95	+0.47
Standard error for mean log change	0.54	+0.40	0.47	0.43
95% confidence limits for log change	-0.09 to +2.09	-0.23 to +1.37	0 to +1.90	-0.39 to +1.29
Significance of change (t-test)	$p < 0.1$	$p < 0.2$	$p < 0.1$	$p < 0.3$

*Negative sign indicates decrease in mean log bacterial count after treatment. Positive sign indicates increase.

**For the combined results of all four treatments for wounds on which allograft did not "take," there was a mean log change of +0.70 (p 0.01, N = 72).

TABLE 5

CHANGE IN BACTERIAL COUNTS FOLLOWING TREATMENT FOR WOUNDS ON WHICH ALLOGRAFT "TOOK" (N = 23)

	Human Cadaver Allograft*	Porcine Xenograft	Synthetic Dressing	Coarse-Mesh Gauze
Number increased	6	14	11	12
Number with no change	0	1	3	1
Number decreased	17	8	9	10

*Direction of change is different for allograft than for the other treatment modalities by χ^2: $p < 0.05$ with "no change" counted as "decrease;" $p < 0.01$ with "no change" counted as "increase."

TABLE 6

CHANGE IN BACTERIAL COUNTS FOLLOWING TREATMENT FOR WOUNDS ON WHICH ALLOGRAFT DID NOT "TAKE" (N = 18)

	Human Cadaver Allograft	Porcine Xenograft	Synthetic Dressing	Coarse-Mesh Gauze
Counts increased	11	11	11	10
No change	1	0	1	1
Counts decreased	6	7	6	7

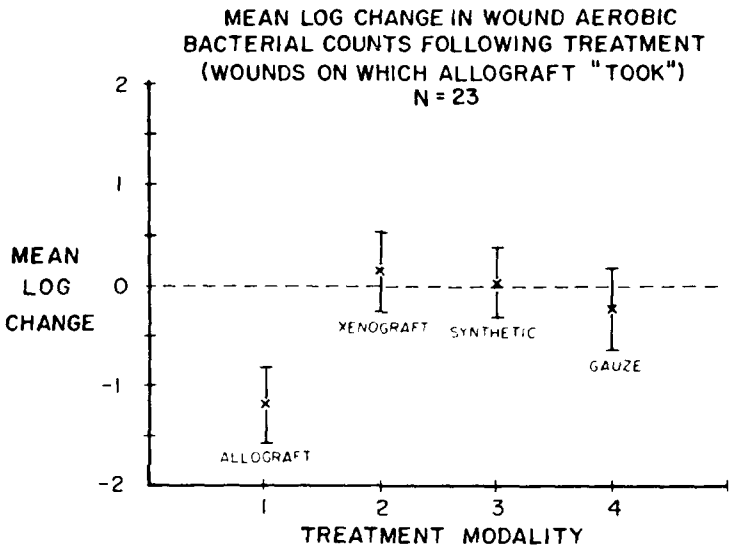


Figure 1. Mean log change in wound surface bacterial colonization following a 48 hour application of each of the four treatment modalities for wounds on which cutaneous allograft "took." Vertical lines represent the standard error. The only dressing which caused a significant change in the log bacterial count was human cadaver allograft which, when it "took," was associated with a mean change of greater than 1 log.

nonparametric analysis. For wounds on which allograft "took," there was no significant change in log of the organism counts with any of the other treatment modalities.

For wounds on which allograft did not "take," none of the individual treatment modalities resulted in a significant change in the log bacterial count. Indeed, over 50% of the time, the bacterial counts increased following a 48 hour application of any of the four treatment modalities (Table 6). Parametric analysis of the mean log change also failed to reveal any significant change following any of the four individual treatments. However, the combined effect of all four treatments was a significant increase in the log bacterial count (Fig. 2).

When all 41 wounds were pooled, there was no significant directional or numerical change in the quantitative wound cultures following treatment with any of the dressings.

Use on Larger Wounds

A series of subjective observations are set forth which are amplified in the discussion. First, the dressing could be used to cover large areas of granulation tissue which required the removal of surface debris before grafting could be performed. These wounds were successfully prepared for graft acceptance by daily changes of the dressing, soaked in 5% mafenide acetate solution prior to use. No desiccation of tissue beneath the dressing was noted. The dressing conformed well to irregular surfaces, but required external support (Surgiflex^{R}) when used on dependent wounds or wounds which were mobilized soon after application of the dressing. The presence of blood or purulence under the dressing was easily recognized and was interpreted as an indication to change the dressing. No fragmentation of portions of the dressing into the wound was observed.

DISCUSSION

The clinical evaluation of a new temporary skin substitute requires a concomitant re-evaluation of the commonly used temporary substitutes for cutaneous allograft and other accepted measures for treating such wounds. In this study, the new synthetic dressing was compared to the most frequently used autograft substitutes, human cadaver cutaneous allograft and porcine cutaneous xenograft. It was also compared to coarse-mesh gauze soaked in saline, an alternate method for treating open wounds. The results of our study not only indicate certain differences among the treatment modalities but also clarify the indications for using the different forms of treatment.

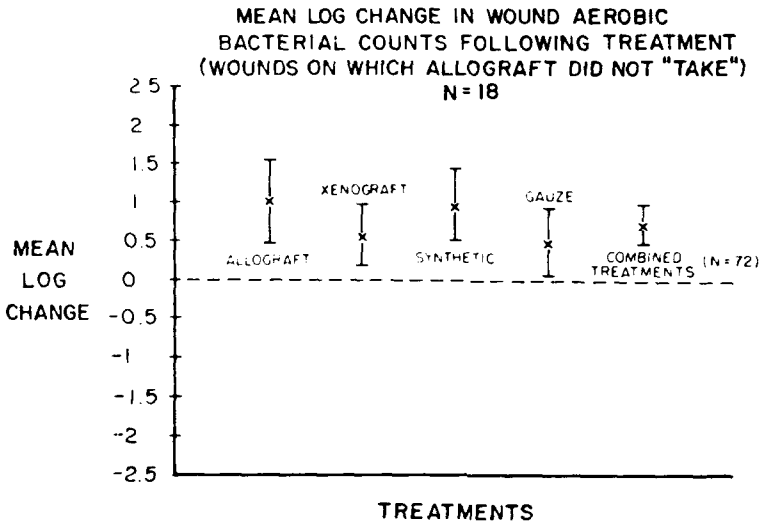


Figure 2. Mean log change in wound surface bacterial colonization following a 48 hour application of each of the four treatment modalities for wounds on which cutaneous allograft did not "take." Although there was an increase in bacterial counts following treatment with any of the dressings, the increase for any individual dressing was not significant. The combined effect of all 72 dressing applications was to significantly increase wound colonization ($p < 0.01$). This suggests that wounds which are not graftable should be treated with dressing changes more frequently than every 48 hours.

This study is limited by several factors. First, it is confined to a 48 hour time period. Second, to analyze quantitative bacterial changes in series of paired cultures which vary widely in the numbers and types of organisms initially present is quite difficult. Third, "wound appearance" is poorly defined, though it remains the most frequently used means of determining wound treatment and whether or not a wound is graftable. Fourth, a retrospective classification of wounds into two categories based on the performance of allograft was made. It can be argued that this does not classify the wound but merely selects those wounds on which allograft performed well. Our results are discussed within the framework of these limitations.

For wounds on which allograft "took," treatment with allograft resulted in a wound appearance which was statistically indistinguishable from that of wounds treated with the synthetic material or coarse-mesh gauze. Wound appearance following autograft "take" was therefore as good as that following any form of treatment. Allograft "take" significantly reduced bacterial colonization; no other form of treatment did so. Wounds which "take" allograft are therefore best treated with allograft, provided there is a reason not to use the patient's own skin. Our data suggest that the synthetic dressing and coarse-mesh gauze were "next best" treatments: both resulted in a wound appearance which was statistically indistinguishable from that of allograft, but neither caused a significant change in the surface bacterial counts. Treatment with porcine cutaneous xenograft was worst in terms of wound appearance and, again, not associated with a quantitative change in the wound microbial flora.

For wounds on which allograft did not "take," there was no significant quantitative change in wound microbiology following treatment with any of the four dressings although, for each dressing, the bacterial counts increased more frequently than they decreased. Wound appearance, which probably reflects debridement, was the only feature distinguishing between the performance of the four dressings. The appearance of wounds treated with either the synthetic dressing or with coarse-mesh gauze was best and these were statistically indistinguishable. The appearance of areas treated with allograft was ranked significantly worse than that of areas treated with either gauze or the synthetic dressing. The appearance of areas treated with porcine cutaneous xenograft was worse than that of areas treated with the other three dressings.

Although the performance of porcine cutaneous xenograft was worst in terms of wound appearance, it is not the intention of this study to condemn its use. The xenograft used in this study was obtained weekly from a commercial source, and no formal attempt was made to study either the viability or the sterility of this material.

Recent reports of microbial contamination in commercial xenograft suggest that this may be one factor which limited its efficacy. There appeared to be considerable variation in the performance of the pigskin and, on some occasions, its performance was equal to that of cutaneous allograft. Our results, therefore, are confined to specific batches of porcine xenograft obtained from a commercial source during a five-month period.

A 48 hour treatment with any of the four dressings did not significantly alter wound microbiology in wounds where allograft did not "take." However, the combined results of all four treatment modalities (Fig. 2) indicate that a 48 hour treatment of such wounds results in a significant increase of colonizing bacteria. This suggests that, on wounds which were not graftable, dressings should be changed more frequently than once every 48 hours.

It is reasonable to assume that wounds which "take" allograft will also "take" autograft. Indeed, this is the basis for using allograft as a test of graft "take." The data suggest that the use of allograft is the treatment of choice for patients with wounds which are ready for autograft acceptance but in whom there is reason to use a material other than the patient's own skin: patients with very large burns and limited donor sites, patients undergoing massive surgical excisions, patients with graftable tissue adjacent to nongraftable wounds in whom autografting in a single operative procedure is desired, and patients with an intercurrent illness, which makes a formal grafting procedure and an anesthetic hazardous.

The data also indicate, however, that wounds which are not graftable are better treated with either mesh gauze or the synthetic dressing. The use of either human cadaver cutaneous allograft or porcine cutaneous xenograft to prepare a wound for grafting is not supported by our data in this and other reports.

In a report which outlined the development of this synthetic dressing, both the membrane and wound closure effects of the dressing were considered and tested in experimental animals. It is important to emphasize that this formal study deals only with wound appearance and surface microbiology. The membrane effects of the four treatment modalities were not studied. Although these data indicate that there was no significant difference between the performance of the synthetic laminate and coarse-mesh gauze in terms of surface colonization or wound appearance, a laboratory evaluation indicated that animals subjected to 60% body surface area skin excisions were kept alive by coverage with the synthetic dressing but not by coarse-mesh gauze.

Clinically, when large area wounds are treated with coarse-mesh gauze, frequent soakings with saline or antimicrobial solutions are used to prevent wound desiccation. The preliminary observations in patients with large-area wounds treated with the synthetic material suggest that frequent soaking is not necessary when the synthetic dressing is used.

The development of this synthetic dressing follows many reports of other synthetic materials used for temporary wound coverage within the past 10 years. Ivalon sponges, nylon velours, collagen films, polyurethane sponges, and polyaminoacid films have been evaluated by many different investigators. The clinical success of these materials has been limited by poor adherence of the dressings to the underlying tissue, stiffness, suppuration beneath the dressings, or retention of the dressing materials in the wound. The new synthetic dressing eliminates many but not all of these problems.

The initial adherence of the dressing was not as good as it was on experimental wounds in the rat, although no external support was necessary if the wound could be immobilized. Patient immobilization was considered undesirable; therefore, the use of a simple Surgiflex^{R} covering to secure the dressing in place for several hours was necessary. The dressing was quite flexible and somewhat stretchable in two dimensions. It conformed well to irregular wound surfaces and permitted active motion of the extremities to which it was applied. The dressing was manufactured with a seven-inch width and an indefinite length, which simplified its application. An entire upper extremity, for example, could be covered with two strips of the synthetic material.

There was no fragmentation or retention of the synthetic dressing in the wound even when the dressing was left in place for three days or longer. The coarse-mesh gauze dressings, in comparison, were loosely woven and fragmentation and retention of the gauze filaments was frequently noted.

The use of any opaque material to cover a wound involves the risk that, should suppuration occur underneath the dressing, it might not be evident until the dressing is removed. For cutaneous allograft and xenograft, this is rarely a problem, as infection under the graft is easily recognized by failure of graft take or the formation of blisters or small abscesses between the graft and the underlying tissue, which may be incised and drained. With gauze dressings, suppuration may be obscured, and frequent dressing changes are necessary for wound inspection. Infection underneath the synthetic dressing was easily recognized because the dressing is quite thin and the expanded teflon membrane surface is semitransparent; any collection of blood or purulent material within the nylon matrix of the dressing caused staining of the expanded teflon surface. In practice, this staining often appeared to

reflect debris within the dressing, which was removed from the wound when the dressing was changed. On wounds which were thought to be ungraftable, such staining was usually present within 24 hours after application of the dressing, and the dressing was changed on a daily basis. On wounds which were thought to be graftable, the staining sometimes did not occur for as long as three to four days. That the staining may not always indicate serious infection is suggested by the observation that many wounds were grafted immediately following removal of stained dressings with excellent "take" of the autograft.

Because our formal study failed to reveal any consistent decontamination of the wound following treatment with the synthetic dressing, the dressing was soaked in 5% mafenide acetate solution before use on larger surface area wounds. The efficacy of topical chemotherapeutic agents on open wounds has been demonstrated by a number of investigators, and the use of such agents is safe, provided that the total dose of the anti-infective agent does not exceed the recommended limits for parenteral use of the drug. Used in this fashion, daily changes of the dressing were effective in debriding wounds and reducing wound colonization, i.e., preparing wounds for graft acceptance.

This study suggests that the synthetic dressing may have a place in the treatment of burned patients. On graftable wounds, where an autograft substitute is desired, the synthetic dressing may be used as a temporary cover, if fresh human cadaver allograft is not available. On granulation tissue which requires preparation for grafting (i.e., debridement and microbial control), either the synthetic dressing or coarse-mesh gauze appear to be the most effective treatment modalities. Within the framework of the formal study, there was no difference between the performance of gauze or the synthetic laminate. However, clinical experience with the synthetic dressing and its laboratory evaluation suggest that this material may have several advantages over the coarse-mesh gauze because the dressing provides membrane function, does not fragment and is not retained in the wound, permits joint motion, and allows prompt recognition of purulent material within the dressing.

BIBLIOGRAPHY

1. Abbenhaus JI: Collagen sheets as a dressing for large excised burns. Surg Forum 16:477, 1965.

2. Abbenhaus JI, Donald P: The use of collagen grafts for replacement of major skin loss. Laryngoscope 81:1650-1651, 1971.

3. Alexander JW, MacMillan BG, et al: Clinical evaluation of Integard, a new synthetic dressing for autografted and heterografted skin. Surg Gynecol Obstet 136:274, 1973.

4. Allen HE, Edgerton MT, Rodeheaver GT, Prusak MP, Kurtz LD, Edlich RG: Skin dressings in the treatment of contaminated wounds. Am J Surg 126:45, 1973.

5. Blocker TG, Dukes CD: Studies on the survival of skin homografts. Plast Reconstr Surg 10:243, 1952.

6. Bromberg BE, Song IC, Mohn MP: The use of pig skin as a temporary biological dressing. Plast Reconstr Surg 36:80, 1965.

7. Burke JF: The use of skin transplantation and immunosuppression in the treatment of extensive full thickness thermal burns. Presented at the Fifth Annual Meeting of the American Burn Association, Dallas, Texas, April 1973.

8. Burke JF: Skin transplantation and immunosuppression in the treatment of extensive full thickness thermal burns. Presented at the American Society of Plastic & Reconstructive Surgeons Meeting, October 1974.

9. Burleson R, Eiseman B: Mechanisms of antibacterial effect of biologic dressings. Ann Surg 177:181, 1973.

10. Chardack WM, Brueske AD, Santomauro AP, Fazekas G: Experimental studies on synthetic substitutes for skin and their use in the treatment of burns. Ann Surg 155:127, 1962.

11. Chardack WM, Day CE, Fazekas G, Minsley N: Synthetic skin: An experimental study. J Trauma 1:54, 1961.

12. Chardack WM: The use of synthetic substitute for skin in the treatment of burns. Milit Med 127:335, 1962.

13. Condict C: Bacterial contamination of skin dressings - how great a hazard? Presented at the Seventh Annual Meeting of the American Burn Association, Denver, Colorado, March 1975.

14. Converse JM, Rappaport FT: The vascularization of skin autografts and homografts - an experimental study in man. Ann Surg 143:306, 1956.

15. Dressler DP, Rozin RR, Skornik WA, Harrison HN, Soroff HS: A new prosthetic skin for the control of experimental burn wound sepsis. Ann NY Acad Sci 150:980, 1968.

16. Eade CT: The relationship between granulation tissue, bacteria, and skin grafts in burned patients. Plast Reconstr Surg 22:42, 1958.

17. Gay EC: Skin dressings in treatment of debrided wounds. Am J Surg 72:212, 1946.

18. German JC, Wooley TE, Achauer B, Furnas DW, Bartlett RH: A critical reappraisal. Arch Surg 104:806, 1972.

19. Guidalian J, Jelenko C, Callaway D, McKnight JT: A comparative study of synthetic and biological materials for wound dressings. J Trauma 13:31, 1973.

20. Hait M, Stark R, Battista OA, McCord CW: Microcrystalline collagen as a biological dressing, vascular prosthesis, and hemostatic agent. Surg Forum 20:51, 1969.

21. Hall WC, Liotta D, DeBakey ME: Artificial skin. Trans Am Soc Artif Intern Organs 12:340, 1966.

22. Harrison HN, Lindberg RB, Mason AD: Management of the burn wound: bacterial and histologic responses to excised rat wounds to synthetic skin grafts. In Research in Burns, A. B. Wallace and A. W. Wilkinson, Editors, London, Livingstone, Ltd., 1966, pp. 327-343.

23. Hinshaw JR, Miller ER: Histology of healing split-thickness and full-thickness autogenous skin grafts and donor sites. Arch Surg 91:658, 1965.

24. Jackson D: A clinical study of the use of skin homografts for burns. Br J Plast Surg 7:26, 1954.

25. Jewett TC, Chardack WM: Temporary closure of a granulating burn wound with a synthetic sponge. Am J Surg 106:24, 1963.

26. Krizek TJ, Robson MC, Kho F: Bacterial growth and skin graft survival. Surg Forum 18:518, 1967.

27. Larson DL: Closure of the burn wound. J Trauma 5:254, 1965.

28. Levine NS, Lindberg RB, Mason AD Jr, Pruitt BA Jr: A simple technique for quantifying microorganisms in open wounds. (In preparation.)

29. Levine NS, Lindberg RB, Salisbury RE, Mason AD Jr, Pruitt BA Jr: A comparison of coarse mesh gauze versus biologic dressings on granulating wounds. In press, Am J Surg.

30. Levine NS, Salisbury RE, Mason AD Jr, Peterson HD, Pruitt, BA Jr: Development and laboratory evaluation of a synthetic skin substitute. Presented at the Plastic Surgery Research Council, 20th Annual Meeting, April 1975.

31. Miller TA, Switzer WE, Foley FD, Moncrief JA: Early homografting of second degree burns. Plast Reconstr Surg 40:117, 1967.

32. Perry VP: A review of skin preservation. Cryobiology 3:109, 1966.

33. Pappas AM, Hyatt GW: The evaluation of collagen film applied to skin defects in mice. Surg Forum 10:844, 1959.

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

35. Rappaport I, Pepino AT, Dietrick W: Early use of xenografts as a biological dressing in burn trauma. Amer J Surg 120:144, 1970.

36. Robson MC, Krizek TJ: The effect of human amniotic membranes on the bacterial population of infected rat burns. Ann Surg 177:144, 1973.

37. Robson MC, Krizek TJ: Predicting skin graft survival. J Trauma 13:213, 1973.

38. Siegel S: Nonparametric Statistics for the Behavioral Sciences. McGraw Hill Book Co., New York, 1956, pp. 75-83.

39. Siegel S: Nonparametric Statistics for the Behavioral Sciences. McGraw Hill Book Co., New York, 1956, pp. 166-172.

40. Shuck JM: The use of homografts in burn therapy. Surg Clin N Amer 50:1325, 1970.

41. Skornik WA, Dressler AP, Rhicard, JK: Adherence of prosthetic skin. J Biomed Mater Res 2:447, 1968.

42. Song IC: Pigskin and bovine collagen as possible temporary dressings for skin wounds. Surg Forum 14:464, 1963.

43. Song IC, Bromberg BE, et al: Heterografts as biological dressings for large skin wounds. Surgery 59:576, 1966.

44. Spira M, Fissette J, Hall W, Hardy SB, Gerow FJ: Evaluation of synthetic fabrics as artificial skin grafts to experimental burn wounds. J Biomed Material Res 3:213, 1969.

45. Switzer WE, Moncrief JA, et al: The use of canine heterografts in the therapy of thermal injury. J Trauma 6:391, 1966.

46. Switzer WE: Sixth National Burn Seminar: Use of Homografts. J Trauma 7:79, 1967.

47. Taylor PH, Moncrief JA, Switzer WE, Rose LR, Pugsley LQ: Ivalon as a cover for excised burn wounds. Arch Surg 86:250, 1963.

48. Walder AI, May PD, Bingham CP, Wright JR: Evaluation of synthetic films as wound covers. Trans Amer Soc Artif Int Organs 15: 29, 1969.

49. Wheeler LM: Medical-surgical dressing for burns and the like. U.S. Patent No. 3, 648, 692. March 14, 1972.

PRESENTATIONS

Levine NS. Development of a temporary skin substitute. Research Council, American Society of Plastic & Reconstructive Surgeons, New Haven, Connecticut, April 1975.

PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA OE 6962	75 07 01	CSD-DRA&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
74 07 01	K. COMP.	U	U	NA	NL	[X] YES [ ] NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A161102B71P	02	070
b. CONTRIBUTING	61102A	3A161102B71P	01	
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)* (U) Use of Hypoxia-Induced Polycythemic Mouse in The Assay of Erythropoietin in Burned Soldiers (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
73 02	75 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: 75	.6	17
FISCAL YEAR CURRENT:		

19. RESPONSIBLE DOD ORGANIZATION

NAME:* US Army Institute of Surgical Research

ADDRESS:* Fort Sam Houston, Texas 78234

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

20. PERFORMING ORGANIZATION

NAME:* US Army Institute of Surgical Research
Metabolic Branch

ADDRESS:* Fort Sam Houston, Texas 78234

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME:* Willard A. Andes, MAJ, MC
TELEPHONE: 512-221-3411
SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS
NAME: John Beason
NAME: Philip W. Rogers, MD DA

21. GENERAL USE

FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Erythropoietin; (U) Anemia; (U) Burned soldiers; (U) Renin-angiotensin

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

23. (U) To set up an assay to measure erythropoietin excretion in patients with thermal injury or trauma. This assay procedure will be used in the evaluation of the anemia of the thermally injured patient and the relationship of erythropoietin to renin in the traumatized soldier.

24. (U) Polycythemia is induced by placing Swiss-Webster mice in a 0.4 atmosphere chamber for approximately 100 hours. Concentrated urine is injected intraperitoneally; then radioiron given. The percent iron utilization by red cells is calculated and compared to Erythropoietin B standard obtained from the World Health Organization.

25. (U) 74 07 - 75 06 The use of the hypoxia induced polycythemic mouse in the bioassay of erythropoietin has been very successful. A dose response curve comparable to that of other investigators has been obtained and data collected from normal personnel has been within the normal range, consistent and reproducible. Five patients with thermal injury ranging from 15-65% total body surface burns have been studied thus far with concurrent evaluation of the renin angiotensin system, glomerular filtration rate, sodium excretion, bone marrow morphology, red cell mass, plasma volume, and total blood volume. Erythropoiesis in the burned patient was poorly responsive to increased amount of erythropoietin as evidenced by low reticulocyte counts and elevated myeloid: erythroid ratios in the bone marrow.

*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 MAR 68 FOR ARMY USE ARE OBSOLETE

FINAL REPORT

PROJECT NO. 3A161102B71P-02, BASIC RESEARCH IN SUPPORT OF MILITARY MEDICINE - SURGICAL PATHOLOGY

REPORT TITLE: USE OF HYPOXIA-INDUCED POLYCYTHEMIC MOUSE IN THE ASSAY OF ERYTHROPOIETIN IN BURNED SOLDIERS

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

Investigators

W. Abe Andes, M.D., Major, MC
Philip W. Rogers, M.D.
John Beason

Reports Control Symbol MEDDH 288 (R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A16112, BASIC RESEARCH IN SUPPORT OF MILITARY MEDICINE - SURGICAL PATHOLOGY

REPORT TITLE: USE OF HYPOXIA-INDUCED POLYCYTHEMIC MOUSE IN THE ASSAY OF ERYTHROPOIETIN IN BURNED SOLDIERS

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

Period covered in this report: 1 July 1974 - 30 June 1975

Investigators: W. Abe Andes, MD, Major, MC
Arthur D. Mason, Jr., MD
James Murray

Reports Control Symbol MEDDH-288 (R1)

Erythropoietin excretion as measured in polycythemic mice was persistently increased following major thermal injury in 4 of 5 patients. A good correlation was found between erythropoietin excretion and red cell mass but not between erythropoietin excretion and hematocrit.

In spite of the elevated erythropoietin, erythropoiesis in these thermally injured patients was inadequate to compensate for erythrocyte deficits as judged by bone marrow morphology, reticulocyte counts, and transfusion requirements.

Erythropoietin
Anemia
Burned Soldiers
Renin-angiotensin

USE OF HYPOXIA-INDUCED POLYCTHEMIC MOUSE IN THE ASSAY OF ERYTHROPOIETIN IN BURNED SOLDIERS

Previous studies have documented prompt responses in the renin-angiotensin system or erythropoietin (ESF) activation following phlebotomy (1,2). Stimuli such as salt-depletion and extracellular fluid volume (ECFV) expansion have been applied to normal man to cause renin (PRA) changes but their effects on ESF have not been evaluated (3,4). A significant correlation between PRA and ESF following thermal injury prompted this study of the two hormones in normal volunteers. Our observations indicate that although PRA was closely linked to ECFV changes, ESF excretion varied independently of these factors and increased only after an acute reduction in red cell mass.

METHODS

Thermally Injured Patients. Five adult male burn patients were studied from 1 to 56 days postburn (5). The total body surface area burned ranged from 15-64% (mean = 44%). The patient with a 65% burn expired 10 days postburn while 4 patients survived with minimal complications. Certain portions of their hospital courses have been described before (5). Most blood and urine samples were obtained during the first month postburn at 4-8 day intervals. Urine was collected during 12-hour periods without preservatives and then frozen during and after collection.

Normal Subjects. Sequential 24-hour urine samples were collected and blood was taken from the normal subjects each morning at 8:00 A.M. corresponding to the previous day's collection. Daily weights were obtained throughout the study. A total of 240 ml blood was taken for diagnostic studies from each subject during the study. The study was divided into five periods as follows: Period I-Days 1-3 (Control). An uncontrolled diet was taken for the first 3 days. Period II- Days 4-6 (ECFV depletion). Eighty mg of furosemide was taken by mouth by each subject on days 4 and 5. A 50 mEq/day Na^+ diet was begun on day 4 and

---

1. Adamson, J.W. 1968. The erythropoietin/hematocrit relationship in normal and polycythemic man: Implications of marrow regulation. Blood 32: 597-609.

2. Weber, M.A., J.E. Thornell, and C.S. Stokes. 1973. Effect of hemorrhage with and without fluid replacement on plasma renin activity. Amer. J. Physiol. 225: 1161-1164.

3. Huber, E., T. Koerner, L.B. [illegible], B. Klimka, and A. Purnode. 1969. Application of [illegible] to the physiologic measurement of [illegible] in normal human subjects. J. Clin. Endocrinol. 29: 384-395.

[illegible], and R.H. Underwood. 1972. Studies of [illegible] concentration in normal man. [illegible]

[illegible] and E.A. Pruitt, Jr. The [illegible] injury. Submitted for publi-[illegible]

continued until day 10. Period III- Days 7-9 (Phlebotomy). On the morning of day 7, phlebotomies of 500 ml from subjects A (84 kg) and B (67 kg), and 750 ml from subject C (97 kg) were taken over one hour without fluid replacement into individual donor bags and refrigerated. The 50 mEq/day Na^+ diet was continued. Each subject's usual laboratory activities were continued. Period IV - Days 10-13 (Salt repletion, anemia). A diet of approximately 200 mEq Na^+ was begun on day 10 and continued until the study was terminated on the morning of day 13. Period V - Days 14-15 (Autotransfusion). Each subject's previously phlebotomized blood was infused on the morning of day 14.

Analytical Methods. Patients and normal subjects were supine at rest for at least 30 minutes after at least an 8-hour fast prior to sampling for PRA. Seven ml of blood was drawn into ice-cold Vacutainer(R) tubes with EDTA. These tubes were immediately centrifuged in a refrigerated centrifuge (4°C) and the plasma frozen for later analysis of PRA. PRA was assayed using the Squibb Angiotension I immutope kit based on the radioimmunoassay of Haber, Koerner, Page, Kliman, and Purnode (3). Samples were counted in a Packard Auto-Gamma Spectrometer, Model 3002, for 10 minutes. Urine for ESF was kept frozen until thawed for dialysis and assay by the method of Adamson, Alexanian, Martinez, and Finch (6). Erythropoietin excretion was based on the ^{59}Fe incorporation of exhypoxic polycythemic mice (5). Serum for determination of electrolytes and creatinine was obtained from blood clotted in glass tubes. Serum and urine sodium and potassium were measured by flame photometry. Hematocrits were measured in heparinized microhematocrit tubes.

RESULTS

Thermal Injury. ESF and PRA were at their highest levels soon after burning (5,7). Both fell to nearly normal levels within approximately a month postburn. All patients became anemic and only the patient with a 15% burn did not require blood transfusion. No patient manifested hypertension regardless of the degree of hyperreninemia. The greatest increases in PRA were noted early in the course of the larger burns (Fig. 1). A similar trend was seen in ESF excretion. ESF was correlated with PRA as seen in Fig. 2.

Normal Subjects. Mild headaches were noted by each subject during Periods II and III. ESF excretion is shown in Fig. 3. ESF was significantly greater in the phlebotomy period (III) than in the other periods

---

6. Adamson, J.W., R. Alexanian, C. Martinez, and C.A. Finch. 1966 Erythropoietin excretion in normal man. Blood 28: 354-365.

7. Rogers, P.W., and N.A. Kurtzman. 1972 A study of the renin angiotensin system in the thermally injured patient. 5th International Congress of Nephrology 118: 659.

($p < 0.01$). ESF excretion and hematocrits fell in Period IV. Renal function as judged by creatinine clearances remained normal in each subject throughout the study. ESF excretion bore no relation to the corresponding urine volume with variations between 500 and 2750 ml/day ($r=0.06$).

PRA varied with the conditions of the study as shown in Fig. 4. All subjects had a prompt response to furosemide and Na^+ restriction with weight loss and elevated PRA (normal = 1-3 ng/ml/hour; mean during control periods = 1.2 ng/ml/hour). During Period IV, PRA fell in each subject coincident with weight gain. Weight changes and PRA were related as estimated by analysis of variance with a regression as follows:

$$\ln Y = 4.6046 - 0.0585 \text{ (body weight)} + 1.1477 \text{ (weight loss)}$$

(Y=PRA; body weight in kg). and $r^2 = 0.833$.

PRA was also closely related to the excretion of Na^+ as shown if Fig. 5.

ESF and PRA were responsive to different stimuli inasmuch as ESF excretion did not increase until after phlebotomy at a time when PRA had previously reached its peak. PRA fell while ESF was increasing in Period III. ESF fell gradually during Period IV while PRA fell dramatically with increased salt intake. The correlation coefficient between corresponding ESF and PRA measurements overall was 0.49 and during Periods II and III, it was 0.33.

Serum sodium fell slightly but significantly with salt depletion during Periods II and III as compared with the salt-repleted periods, the mean falling from 141 to 140 mEq/liter ($p < 0.01$). Serum potassium fell from 4.1 to 3.9 mEq/liter ($p < 0.01$).

DISCUSSION

Plasma renin levels have been found to be elevated for up to two months following burn injury (7,8). This may occur in spite of normal blood volume, blood pressure, cardiac output, and pulmonary wedge pressure (7). Massive potassium excretion and prolonged renal sodium conservation were found in the same patients. PRA was greatest soon after burns and related to burn size in this and previous studies. Experimental animals studies have also described situations favoring the simultaneous elaboration of both PRA and ESF. Maneuvers such as microsphere injection resulting in renal infarction, renal artery clamping and hypoxia

---

8. Dolecek, R., M. Zavada, M. Adamkova, and K. Leikep. 1973. Plasma renin like activity (RLA) and angiotensin II levels after major burns. Acta. Chirurgiae Plast. 15 166-169.

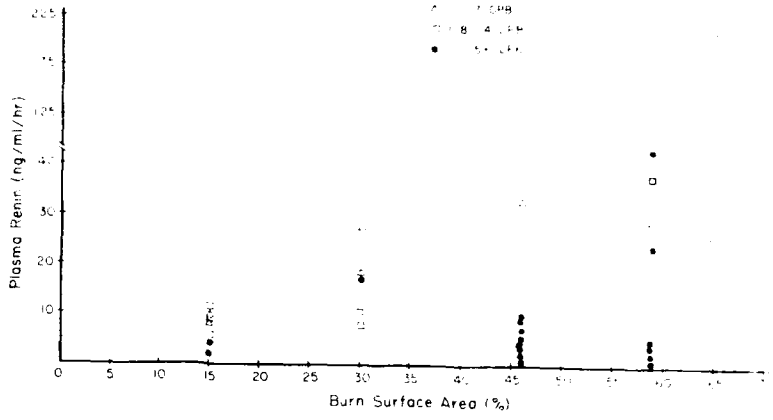


Figure 1. Plasma renin activity in 5 thermally injured patients. The distribution of samples is represented as follows: Δ = Days 1-7 postburn; □ = Days 8-14; ● = Days 15-60.

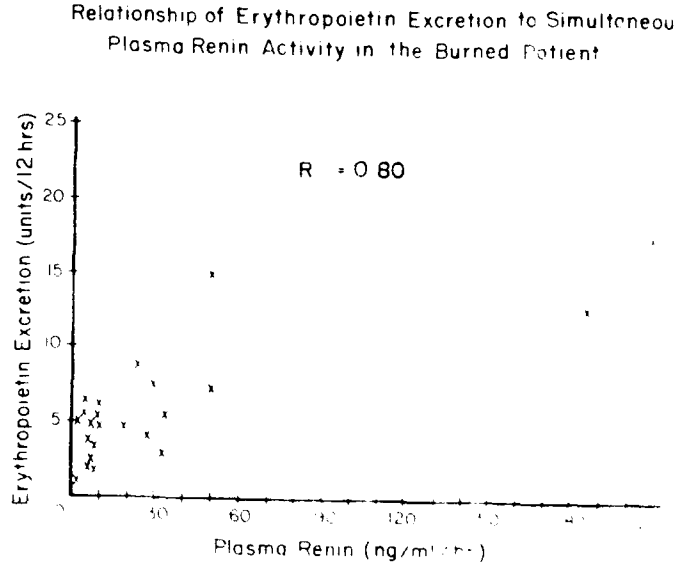


Figure 2. The relationship of erythropoietin excretion to plasma renin activity in 5 thermally injured patients. Erythropoietin was collected during 12-hour periods and supine renin samples were drawn during such collections.

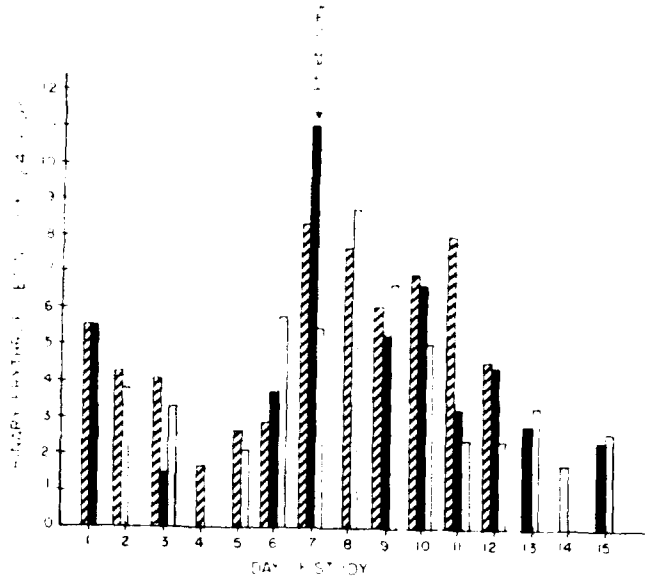


Figure 3. The 24-hour urinary erythropoietin excretion of 3 healthy subjects during 5 study periods: Period I- Control; Period II - Salt Depletion; Period III - Phlebotomy and salt-depletion; Period IV - Anemia; Period V - Autotransfusion. Each subject is represented by a different bar.

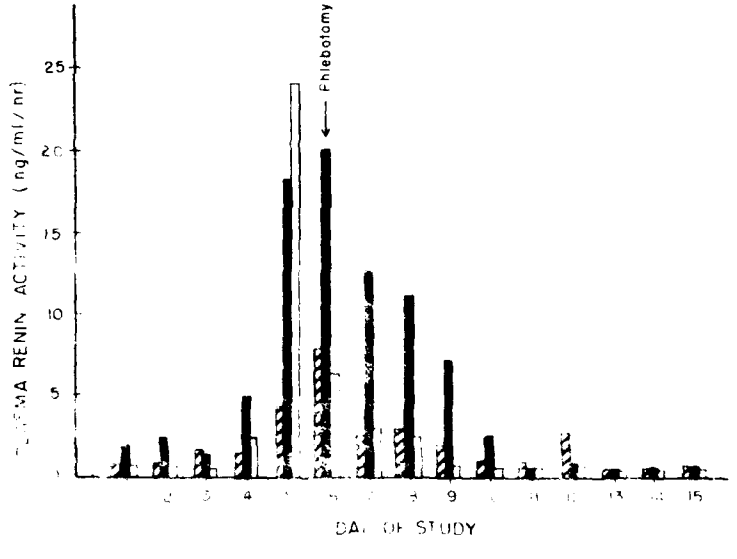


Figure 4. Plasma renin activity of subjects during 5 study periods. See Figure 3 and text for details.

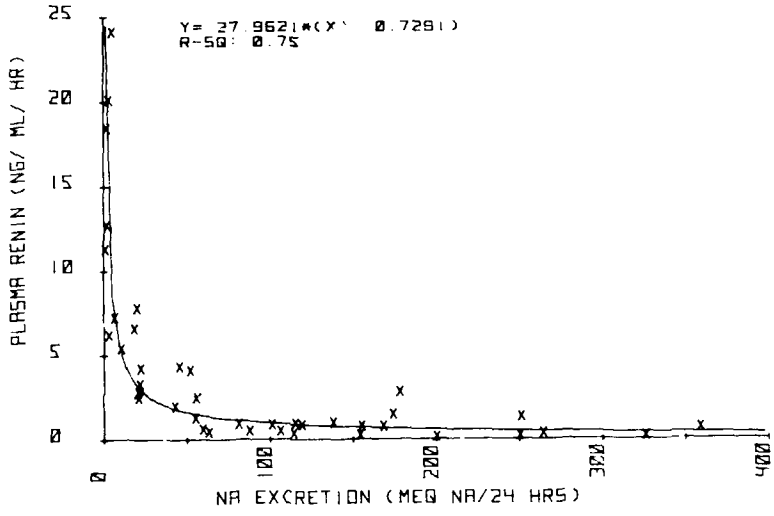


Figure 5. The relationship between urinary sodium excretion and plasma renin activity of healthy subjects during 5 study periods. See Figure 3 and text.

have caused increases in both (9-11). Human conditions such as Bartter's syndrome, hypertension, and renal artery stenosis have been recorded with both increased ESF and PRA (12-14). These observations, together with the almost invariable anemia and possible similarity of activation mechanisms of ESF and PRA led us to investigate the availability of the two hormones in the same patients or subjects at the same time (15-16).

A combination of anemia and ECFV depletion led to the elevations of both ESF and PRA after phlebotomy in Period III in normal subjects, but only PRA rose during ECFV depletion (Period II) and ESF excretion increased after red cell mass reduction at a time when PRA was falling (Period III). The lack of ESF elevation after ECFV depletion in normal subjects was not unexpected since we did not achieve the reduction of renal blood flow necessary in experimental animals to cause ESF changes. The fall in PRA after phlebotomy was almost certainly caused by concurrent expansion of ECFV as evidenced by a weight gain in each subject. The magnitude of red cell mass reduction was more evident in Period IV following ECFV restoration than immediately post-phlebotomy as evidenced by hematocrit changes (Table I). A dissociation between ESF and PRA was evident in Period IV with a rapid fall in PRA as ECFV repletion occurred while ESF remained slightly above control values. The gradual fall in ESF from peak levels after phlebotomy was more likely due to unknown causes than to ECFV repletion. Similar findings have been noted previously (1).

---

9. Abbrecht, P.H., R.L. Malvin, and A.J. Vander. 1966. Renal production of erythropoietin and renin after experimental kidney infarction. Nature 211: 1318-1319.

10. Fisher, J.W., A. Samuels, and J. Langston. 1967. Effects of angiotensin, norepinephrine and renal artery constriction on erythropoietin production. J. Pharmacol. Exper. Therap. 157: 618-625.

11. Gould, A.B., and S.A. Goodman. 1970. The effect of hypoxia on the renin-angiotensin system. Lab. Invest. 22: 443-447.

12. Erkelens, D.W., and L.W.S. van Eps. 1973. Bartter's syndrome and erythrocytosis. Amer. J. Med. 55: 711-719.

13. Jepson, J., and E.E. McGarry. 1968. Polycythemia and increased erythropoietin production in a patient with hypertrophy of the juxtaglomerular apparatus. Blood 32: 370-375.

14. Bourgoignie, J.J., N.I. Gallagher, H.M. Perry, Jr., L. Kurz, M.A. Warnecke, and R.M. Donati. 1968. Renin and erythropoietin in normotensive and hypertensive patients. J Lab Clin Med 71: 523-536.

15. Oparil, S., and E. Haber. 1974. The renin-angiotensin system. N. Engl. J. Med. 291: 389-401.

16. Zanjani, E.D., J.F. Contrera, G.W. Cooper, A.S. Gordon, and K. K. Wong. 1967. Renal erythropoietic factor: Role of ions and vasoactive agents in erythropoietin formation. Science 156: 1367-1368.

TABLE I

Thermally Injured Patients Not Requiring Transfusion

Number	83+
Mean Burn Size*	16%
Largest Burn*	45%
HOSPITAL STAY (Mean Days)	36
Children (12 years)	13
Mean Age	26 years

+ of 279 consecutive admissions

* of total body surface area burned

Dissociation of PRA and ESF with responsiveness to separate stimuli implies that the kidney is able to discriminate between multiple stimuli, which, unless studied separately, might appear to be a single factor (17,18). Such findings provide further impetus for the study of the complex endocrine functions of the kidneys.

---

17. Krantz, S.B., and L.O. Jacobson. 1970. Erythropoietin and the Regulation of Erythropoiesis. Univ. of Chicago Press, Chicago 39-40.

18. Donati, R.M., J.J. Bourgoignie, C. Kuhn, N.I. Gallagher, and H.M. Perry, Jr., 1968. Dissociation of circulating renin and erythropoietin in rats. Circ. Res. 22: 91-95.

REFERENCES

1. Adamson,J.W. 1968. The erythropoietin/hematocrit relationship in normal and polycythemic man: Implications of marrow regulation. Blood 32: 597-609.

2. Weber,M.A., I.R.Thornell, and C.S. Stokes. 1973. Effect of hemorrhage with and without fluid replacement on plasma renin activity. Amer. J. Physiol. 225: 1161-1164.

3. Haber,E.,T. Koerner,L.B. Page, B. Kliman, and A. Purnode. 1969. Application of a radioimmunoassay for angiotensin I to the physiologic measurements of plasma renin activity in normal human subjects. J Clin Endocrinol. 29: 1349-1355.

4. Williams, G.H., J.P. Cain, R.G. Diuhy, and R.H. Underwood. 1972. Studies of the control of plasma aldosterone concentration in normal man. J. Clin. Invest. 51: 1731-1742.

5. Andes, W.A., P.W Rogers, J. Beason, and B.A. Pruitt, Jr., The erythropoietin response to the anemia of thermal injury. Submitted for publication.

6. Adamson,J.W., R. Alexanian, C. Martinez, and C.A. Finch.1966. Erythropoietin excretion in normal man. Blood 28: 354-365.

7. Rogers,P.W., and N.A. Kurtzman. 1972. A study of the renin-angiotensin system in the thermally injured patient. 5th International Congress of Nephrology 118: 659.

8. Dolecek,R.,M. Zavada, M. Adamkova, and K. Leikep. 1973. Plasma renin like activity (RLA) and antiotensin II levels after major burns. Acta. Chirurgiae Plast. 15: 166-169.

9. Abbrecht,P.H.,R.L. Malvin, and A.J. Vander. 1966. Renal production of erythropoietin and renin after experimental kidney infarction. Nature 211: 1318-1319.

10. Fisher,J.W., A. Samuels, and J. Langston, 1967. Effects of angiotensin, norepinephrine and renal artery constriction on erythropoietin production. J. Pharmacol Exper. Therap. 157: 618-625.

11. Gould,AB.B., and S.A. Goodman. 1970: The effect of hypoxia on the renin-angiotensin system. Lab. Invest. 22: 443-447.

12. Erkelens,D.W., and L.W.S. van Eps. 1973. Bartter's syndrome and erythrocytosis. Amer. J. Med 55: 711-719.

13. Jepson,J., and E.E. McGarry. 1968. Polycythemia and increased erythropoietin production in a patient with hypertrophy of the juxtaglomerular apparatus. Blood 32: 370-375.

14. Bourgoignie,J.J., N.I. Gallagher,H.M. Perry,Jr., L. Kurz, M.A. Warnecke, and R. M. Donati. 1968. Renin and erythropoietin in normotensive and hypertensive patients. J. Lab. Clin. Med. 71: 523-536.

15. Oparil,S., and E. Haber. 1974. The renin-angiotensin system. N Engl. J. Med. 291: 389-401.

16. Zanjani,E.D., J.F. Contrera, G.W. Cooper,A.S. Gordon, and K. K. Wong. 1967. Renal erythropoietic factor: Role of ions and vasoactive agents in erythropoietin formation. Science 156: 1367-1368.

17. Krantz,S.B., and L.O. Jacobson. 1970. Erythropoietin and the Regulation of Erythropoiesis. Univ of Chicago Press, Chicago 39-40.

18. Donati, R.M., J.J. Bourgoignie, C. Kuhn, N.I. Gallagher, and H. M. Perry, Jr., 1968. Dissociation of circulating renin and erythropoietin in rats. Circ. Res. 22: 91-95.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OF 6388	75 07 01	DD DR&E/AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8b. DISP'N INSTR'N	8c. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
75 07 01	D. CHANGE	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO. CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A161102B71P	02	069
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code) (U) To Determine The Role of The Prostaglandins (PG) in The Response to Volume Expansion in The Dog--A Model of Changes in Injured Troops (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
75 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 75	.5	13
	CURRENT 76	.5	14

19. RESPONSIBLE DOD ORGANIZATION

NAME: US Army Institute of Surgical Research

ADDRESS: Fort Sam Houston, Texas 78234

RESPONSIBLE INDIVIDUAL

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

TELEPHONE: 512-221-2720

20. PERFORMING ORGANIZATION

NAME: US Army Institute of Surgical Research
Metabolic Branch, Nephrology Svc

ADDRESS: Fort Sam Houston, Texas 78234

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

NAME: David B. Olin, MAJ, MC

TELEPHONE: 512-221-4698

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Richard H. Merrill, LTC, MC

NAME: DA

21. GENERAL USE

FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Animal; (U) Renal; (U) Autoregulation; (U) Dogs; (U) Prostaglandins; (U) Volume Expansion

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

25. (U) In the established dog model of volume expansion, responses in adaptation and autoregulation occur. Renal prostaglandins are thought to play a role in this autoregulation. The objective in this study is to observe response to volume expansion in the absence of prostaglandins as a model of changes in injured troops.

24. (U) Prostaglandin inhibition will be accomplished with parenteral Indomethacin or mecofenamate 30 to 60 minutes prior to study. Changes in renal blood flow will be measured using clearance of PAH and radiolabelled microsphere distribution. Other parameters followed will be fractional excretion of sodium, water reabsorption and water clearance, osmolar clearance and blood pressure.

25. (U) 75 02 - 75 06 To date only preliminary studies have been completed. Dogs have been studied in hypropenic state with GFR, RBF and fractional excretion of sodium. In addition C_{OSM} and C_{H_2O} have been determined. Five percent body weight expansion is accomplished over 45 minutes and the animals were restudied. Indomethacin was then administered to block prostaglandins. There has been no change observed in fractional excretion of sodium or C_{H_2O} when prostaglandins are inhibited. Microsphere distribution have not been done in these early studies. A finding of interest has been a rise in blood pressure noted in all animals 15-30 minutes after Indomethacin. Further studies are necessary to enable statistical analysis.

*Available to contractors upon originator's approval

DD FORM 1 MAR 66 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 66 AND 1498-1, 1 MAR 65 FOR ARMY USE) ARE OBSOLETE

ANNUAL PROGRESS REPORT

PROJECT NO. 3A161102B71P -02, BASIC RESEARCH IN SUPPORT OF MILITARY MEDICINE - SURGICAL PATHOLOGY

REPORT TITLE: TO DETERMINE THE ROLE OF THE PROSTAGLANDINS (PG) IN THE RESPONSE TO VOLUME EXPANSION IN THE DOG- A MODEL OF CHANGES IN INJURED TROOPS

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

Investigators

David B. Olin, MD, Major, MC
Richard H. Merrill, MD, LTC, MC

Reports Control Symbol MEDDH-288 (R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A161102B71R-02, BASIC RESEARCH IN SUPPORT OF MILITARY MEDICINE - SURGICAL PATHOLOGY

REPORT TITLE: TO DETERMINE THE ROLE OF THE PROSTAGLANDINS IN THE RESPONSE TO VOLUME EXPANSION IN THE DOG - A MODEL OF CHANGES IN INJURED TROOPS

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

Period covered in this report: 1 July 1974 - 30 June 1975

Investigators: David B. Olin, MD, Major, MC
Richard H. Merrill, MD, LTC, MC

The renal responses to volume expansion include an increase in glomerular filtration rate (GFR) and renal blood flow (RBF), and in increase in fractional excretion of sodium. Many theories for the natriuresis have been proposed invoking vascular, physical and osmotic factors. The finding that prostaglandins are renal medullary substances which can cause a naturesis when injected into a renal artery, led to the hypothesis that renal autoregulation might be due to the actions of medullary prostaglandins. The ability to block prostaglandin synthesis with indomethacin or meclofenamic acid enables the study of the renal response to volume expansion in the presence and absence of prostaglandins.

Anesthetized dogs have been studied in the hydropenic state and following 5% body weight volume expansion with Lactated Ringers Solution. After observing a urine volume response, and completion of appropriate studies, either indomethacin or meclofenamic acid are given intravenously and all clearance studies are repeated thirty minutes after the dose. In addition to fractional excretion of sodium, C_{OSM} and C_{H_2O} are being determined to examine distal tuble delivery of sodium. Additional studies with prostaglandin inhibition prior to volume expansion and studies in unaesthetized dogs are planned.

To date insufficient data is available for statistical analysis or interpretation.

Animal
Renal
Autoregulation
Prostaglandins
Volume Expansion Dogs
Dogs

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OF 6387	75 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
75 07 01	D. CHANGE	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO. CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A161102B71P	02	068
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code) (U) To Determine Whether The Prostaglandins are Important in Development of Acute Renal Failure--A Rabbit Model of Renal Failure in Soldiers (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS
003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
75 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 75	.5	13
FISCAL YEAR CURRENT 76	.5	14

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

20. PERFORMING ORGANIZATION
NAME: US Army Institute of Surgical Research
ADDRESS: Metabolic Branch
Fort Sam Houston, Texas 78234
PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME: David B. Olin, MAJ, MC
TELEPHONE: 512-221-4698
SOCIAL SECURITY ACCOUNT NUMBER:
ASSOCIATE INVESTIGATORS
NAME: Richard H. Merrill, LTC, MC
NAME: DA

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Renal failure; (U) Gentamicin; (U) Animal; (U) Prostaglandins; (U) Rabbits

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

23. (U) In an established model of Gentamicin nephrotoxicity in the rabbit, animals treated with Indomethacin or mecofenamate will be observed for differences in course of disease compared to control rabbits to develop improved treatment for injured soldiers with renal failure.

24. (U) Rabbits will be challenged with daily doses of Gentamicin of varying dosage with a dose established which will give predictable nephrotoxicity. At the dose of predictable nephrotoxicity control animals and Indomethacin treated animals will be followed observing changes in development of disease, severity of clinical disease, and differences in pathology at the time of sacrifice or death.

25. (U) 74 07 - 75 06 The preliminary studies in this protocol were designed to establish a model of gentamicin nephrotoxicity. The initial two groups receiving 40 mg/kg and 80 mg/kg per day for two weeks showed no biochemical evidence of renal disease. On light microscopy no abnormalities were noted. An additional group placed on 1% NH_4Cl drinking water and treated with 40 mg/kg for two weeks showed mild metabolic acidosis and slight rise in BUN although light microscopy failed to show significant pathology. Further studies looking at rabbit handling of gentamicin will be performed.

*Available to contractors upon originator's approval

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

ANNUAL PROGRESS REPORT

PROJECT NO. 3A161102B71P-02, BASIC RESEARCH IN SUPPORT OF MILITARY MEDICINE - SURGICAL PATHOLOGY

REPORT TITLE: TO DETERMINE WHETHER THE PROSTAGLANDINS ARE IMPORTANT IN DEVELOPMENT OF ACUTE RENAL FAILURE- A RABBIT MODEL OF RENAL FAILURE IN SOLDIERS

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

Investigators

David B. Olin, MD, Major, MC
Richard H. Merrill, MD, LTC, MC

Reports Control Symbol MEDDH-288 (R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A161L2B71R-02, BASIC RESEARCH IN SUPPORT OF MILITARY MEDICINE - SURGICAL PATHOLOGY

REPORT TITLE: TO DETERMINE WHETHER THE PROSTAGLANDINS ARE IMPORTANT IN DEVELOPEMENT OF ACUTE RENAL FAILURE A RABBIT MODEL OF RENAL FAILURE IN SOLDIERS

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

Period covered in this report: 1 July 1974 - 30 June 1975

Investigators: David B. Olin, MD, Major, MC
Richard H. Merrill, LTC, MC

Reports Control Symbol MEDDH-288 (R1)

Acute reversible renal failure remains an important complication in the burned patient. The use of aminoglycoside antibiotics in the burned also is associated with a risk of nephrotoxicity. The present study is designed to develop a working model of gentamicin nephrotoxicity in the rabbit. The pathophysiology of acute renal failure and in particular nephrotoxic renal failure is not well understood. The role of vasoactive agents (i.e. renin, angiotensin, prostaglandins) have been postulated and investigated. In the model of gentamacin nephrotoxicity we have investigated the effect of prostaglandin inhibition in the development of nephrotoxicity.

Preliminary studies utilizing 2-4 kilogram rabbits receiving 40 mg/kg and 80 mg/kg per day gentamicin for 2 weeks failed to show either biochemical change in renal function or anatomic changes as studied by lgiht microscopy of the kidney. Another group of rabbits given 1% NH_4Cl in their drinking water and 40 mg/kg gentamicin for 2 weeks showed a slight increase in BUN but; light microscopy failed to show any anatomical changes.

Further work is in progress at this time and is necessary before final conclusions can be reached.

Renal Failure
Gentamicin
Animal
Prostaglandins
Rabbits

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OE 6389	75 07 01	DD-DR&E(AR)636

3. DATE PREV SMRY	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
75 07 01	D. CHANGE	U	U	NA	NL	X YES / NO	A. WORK UNIT

10. NO. USES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A161102B71P	02	070
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)

(U) Renal Function in The Burned Soldier. I. Histology (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
75 01	Cont	DA	C. In-House

17. CONTRACT/GRANT: Not Applicable

a. DATES EFFECTIVE — EXPIRATION
b. NUMBER
c. TYPE — d. AMOUNT
e. NO OF AWARD — f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
FISCAL YEAR — PRECEDING 75	.8	24
FISCAL YEAR — CURRENT 76	.9	27

19. RESPONSIBLE DOD ORGANIZATION

NAME: US Army Institute of Surgical Research
ADDRESS: Fort Sam Houston, Texas 78234

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

20. PERFORMING ORGANIZATION

NAME: US Army Institute of Surgical Research
ADDRESS: Renal Section, Metabolic Branch
Fort Sam Houston, Texas 78234

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME: William D. Myers, LTC, MC
TELEPHONE: 512-221-6532
SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS
NAME: Thomas Rizzo, Jr., MAJ, MC
NAME: Richard H. Merrill, LTC, MC DA

21. GENERAL USE

FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Electronmicroscopy immunofluorescence; (U) Azotemia, (U) Burn; (U) Human; (U) Histology; (U) Glomerulus

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

23. (U) To identify electronmicroscopic glomerular pathology post burn and relate it to renal function changes in burned soldiers.

24. (U) All patients in the burn unit with immediate postmortem authorization will undergo within one hour postmortem percutaneous needle biopsy of kidney. Tissue will be examined by light, electron, and immunofluorescent microscopy. Premortem renal function will be monitored by urinalysis, BUN, serum creatinine, urine creatinine, urine sodium, and urine potassium. Patients will be grouped by serum creatinine and pattern of function (6p.I:Cr less than 1.2; 6pII:Cr more than 1.2; A:prerenal; B:renal) Clinical course noting type of burn, crush injury, resuscitation, coagulopathy, shock, sepsis, hypoxia, and medications will be recorded for future correlation.

25. (U) 75 01 - 75 06 As of 1 Apr 75, 12 patients have undergone postmortem percutaneous needle biopsy of the kidney within two hours of death. Five patients had tissue adequate for study by electronmicroscopy and three patients had tissue adequate for immunofluorescent microscopy. Three patients with normal renal function had normal EM. One patient with renal azotemia had normal glomeruli by EM and the remaining patient had pre-renal azotemia with EM revealing sclerosis from preburn disease. All immunofluorescent studies were negative for IgG, IgM, IgA, fibrin, and complement.

*Available to contractors upon originator's approval

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

ANNUAL PROGRESS REPORT

PROJECT NO. 3A161102B71P-02, BASIC RESEARCH IN SUPPORT OF MILITARY MEDICINE

REPORT TITLE: RENAL FUNCTION IN THE BURNED SOLDIER. I. HISTOLOGY

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

1 January 1975 - 30 June 1975

Investigators:

William D. Myers, MD, Lieutenant Colonel, MC
Thomas Rizzo, Jr., MD, Major, MC
Richard H. Merrill, MD, Major, MC
John McPhaul, MD, Colonel, MC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A161102B71P-Q2, BASIC RESEARCH IN SUPPORT OF MILITARY MEDICINE

REPORT TITLE: RENAL FUNCTION IN THE BURNED SOLDIER. I. HISTOLOGY

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

Period covered in this report: 1 January 1975 - 30 June 1975

Investigators: William D. Myers, MD, Lieutenant Colonel, MC
Thomas Rizzo, Jr., MD, Major, MC
Richard H. Merrill, MD, Major, MC
John McPhaul, MD, Colonel, MC

Reports Control Symbol MEDDH-288(R1)

Twelve patients have undergone postmortem percutaneous needle biopsy of the kidney within two hours of death. Five patients had tissue adequate for study by electronmicroscopy, and three patients had tissue adequate for immunofluorescent microscopy. Three patients with normal renal function had normal electronmicroscopy. One patient with renal azotemia had normal glomeruli by electronmicroscopy and the remaining patient had prerenal azotemia with electronmicroscopy revealing sclerosis from preburn disease. All immunofluorescent studies were negative for IgG, IgM, IgA, fibrin, and complement.

Too little data is available at this time for adequate interpretation.

Electronmicroscopy immunofluorescence
Azotemia
Burn
Human
Histology
Glomerulus

RENAL FUNCTION IN THE BURNED SOLDIER. I. HISTOLOGY

The incidence of renal insufficiency following thermal injury has decreased with improved initial fluid resuscitation.[1] However, even with adequate resuscitation, azotemia, which progresses to marked renal insufficiency, is occasionally seen. The typical pattern is that of a "prerenal" insufficiency manifested by a BUN/creatinine ratio greater than 10, urine urea nitrogen/serum urea nitrogen or urine creatinine/serum creatinine ratio greater than 20, urine specific gravity greater than 1.015, and a low excretion of sodium with a high excretion of potassium. The tubules continue to perform maximally as glomerular filtration progressively declines, revealing a glomerulotubular imbalance.

Graber and Sevitt[2] observed occasional lipid deposits in glomeruli and areas of tubular necrosis in the thermally injured patient. However, in spite of microscopic pathology, the tubules were able to reabsorb sodium and excrete potassium normally. It may be that the reduction in urine flow allows the tubules to reclaim most sodium from the slowly moving filtrate. A block in plasma flow from the glomerulus to the tubule could explain the clinical findings. Graber and Sevitt described multiple fine droplets of fat within the glomerular tufts and possibly within the endothelium and epithelium. However, no electron microscopy was performed. Tissue submitted from ISR autopsy specimens in the past for electron microscopy and immunofluorescent microscopy have been unsatisfactory for detailed study. Tissue obtained within one hour of death will hopefully provide interpretable study material.

This study will attempt to identify any electronmicroscopic glomerular pathology associated with normal and abnormal renal function in the thermally injured patient. All patients with immediate postmortem authorization will undergo within one hour postmortem percutaneous needle biopsy of the kidney. Tissue will be examined by light and electron microscopy at the ISR Lab. Renal tissue will also be rapidly frozen and sent to Dr. McPhaul at Wilford Hall Air Force Hospital for immunofluorescent microscopy to determine the presence of IgG, IgA, fibrin, and complement. Renal function will be monitored by urinalysis, BUN, serum creatinine, urine creatinine, urine sodium, and urine potassium. Patients will be grouped by serum creatinine and pattern of function (Group I: Cr less than 1.2; Group II: Cr greater than 1.2; A: prerenal; B: renal). Clinical course, noting time of burn,

---

1. Moncrief JA: Burns. New Eng J Med 288:444, 1973.
2. Graber IG, Sevitt S: Renal function in burned patients and its relationship to morphological changes. J Clin Path 12:25, 1959.

crush injury, resuscitation, coagulopathy, shock, sepsis, hypoxia, and medications will be recorded for future correlation.

Conclusion

Too little data is available at this time for adequate interpretation.

PUBLICATIONS AND/OR PRESENTATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OE 6980	75 07 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
74 07 01	D. CHANGE	U	U	NA	NL	[X] YES [ ] NO	A. WORK UNIT

10. NO. CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A161102B71P	02	067
b. CONTRIBUTING	61102A	3A161102B71P	01	
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) The Effects of Calcium on The Renin-Angiotensin System-Use of an Animal Model of Hypertension 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
74 01	Cont	DA		C. In-House

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

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 75	.5	14
FISCAL YEAR CURRENT 76	.5	15

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

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

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

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME: Richard H. Merrill, LTC, MC
TELEPHONE: 512-221-5416
SOCIAL SECURITY ACCOUNT NUMBER

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

ASSOCIATE INVESTIGATORS
NAME:
NAME: DA

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Calcium; (U) Renin; (U) Angiotensin; (U) Hypertension; (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 assess the role of calcium in the stimulation of the renin angiotensin system as related to hypertension in military personnel.

24. (U) A series of dogs will be studied as the experimental model. Various concentrations of calcium will be infused directly into the renal artery, and the production of renin angiotensin will be measured by direct cannulization of the renal vein. The contralateral kidney will remain uninfused and will serve as a control.

25. (U) 74 07 - 75 06 The preliminary aim of this protocol was to perform calcium infusion in the Awake Intact Dog. We have experienced considerable difficulty in keeping the catheters required patent for a prolonged period of time and therefore we switched to the lightly anesthetized animal model. We have done approximately six, experimental animals, the results thus far are inconclusive due to difficulty with laboratory analysis of the samples and technical problems with the surgical model. Early results indicate that calcium does have a stimulatory affect on the Renin-Angiotensin-System and the study will be pursued.

*Available to contractors upon originator's approval

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

ANNUAL PROGRESS REPORT

PROJECT NO. 3A161102B71P-02, BASIC RESEARCH IN SUPPORT OF MILITARY MEDICINE - SURGICAL PATHOLOGY

REPORT TITLE: THE EFFECTS OF CALCIUM ON THE RENIN-ANGIOTENSIN SYSTEM - USE OF AN ANIMAL MODEL OF HYPERTENSION

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

Investigators

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

Reports Control Symbol MEDDH-288 (R1)

UNCLASSIFIED

ABSTRACT

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

REPORT TITLE: THE EFFECTS OF CALCIUM ON THE RENIN-ANGIOTENSIN SYSTEM - USE OF AN ANIMAL MODEL OF HYPERTENSION

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

Period covered in this report: 1 July 1974 - 30 June 1975

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

Reports Control Symbol MEDDH-288 (R1)

The role of calcium in the production of hypertension was evaluated. Calcium was infused in low concentration into the renal artery of a lightly anesthetized dog. The data is incomplete due to technical problems, but preliminary observations indicate that plasma renin activity (PRA) does rise during calcium infusions. The study will be continued.

Calcium
Renin
Angiotensin
Hypertension
Dogs

THE EFFECTS OF CALCIUM ON THE RENIN-ANGIOTENSIN SYSTEM - USE OF AN ANIMAL MODEL OF HYPERTENSION

The association of hypercalcemia and hypertension is well recognized but the pathophysiology is obscure. Studies in dogs and other experimental preparations have shown that hypercalcemia alone or in combination with hyperkalemia, alkalosis, and hypomagnesemia is capable of inducing vasoconstriction. This seems to be true in peripheral vessels as well as in renal vasculature. It is also well known that calcium has a positive ionotrophic effect on cardiac contractility. Hypertension can be aggravated by states of increased cardiac contractility, combined with increased peripheral resistance, but it is uncertain whether calcium produces the observed hypertension via these mechanisms or whether it has a direct humoral effect, perhaps acting by way of the renin angiotensin system. Weidmann, et al (1) showed that in normals, as well as in patients with renal failure, infusion of calcium caused an elevated blood pressure, and the correlation between the blood pressure and the increment in serum calcium was significant. These investigators failed to show a correlation between the blood pressure and plasma renin, but other investigators have demonstrated an increased renin production in kidney slices incubated with increasing calcium concentrations. If calcium concentration plays a role in regulating output of renin from the kidney, the exact manner in which this occurs is not known. Calcium may produce local vasoconstriction of the renal artery, leading to ischemia, with the expected rise in plasma renin levels as in unilateral renovascular hypertension. Also, the calcium may act by altering the renal handling of sodium, magnesium, phosphorus, etc., and thus indirectly affect the output of aldosterone via the renin-angiotensin system.

METHODS

A series of dogs were studied, each dog serving as his own control. A flank incision was made and a renal artery and vein cannulated. During the control period, plasma renin levels in the renal artery and vein were measured. At the same time, PAH and inulin clearances were determined in each kidney, and a peripheral arterial pressure was recorded. Calcium (total and ionized), phosphorus, potassium, and magnesium levels, were determined during the test period in the renal artery, vein, and urine. Various concentrations of calcium were infused directly into the renal artery during which time the above measurements were repeated. All attempts were made to keep renal artery pressure constant as well as peripheral serum sodium, potassium, and magnesium. Although the dogs' vital signs were measured,

---

Weidmann, Peter; Massry, Shaul G; Coburn, Jack W; Maxwell, Morton H.; Atleson, Joyce; Kleeman Charles R: Blood pressure effects of acute hypercalcemia: studies in patients with chronic renal failure. Annals of Internal Medicine 76: 741-745, 1972.

no attempt was made to monitor the cardiac output and peripheral resistance, at least in the preliminary phase of the study. During each of the collection periods, plasma was collected and frozen for aldosterone determinations.

RESULTS

The preliminary aim of this protocol was to perform calcium infusions in the Awake Intact Dog. We have experienced considerable difficulty in keeping the number of catheters required patent for a prolonged period of time and therefore we switched to the lightly anesthetized animal model. We have done approximately six experimental animals, the results thus far are inconclusive due to difficulty with laboratory analysis of the samples and technical problems with the surgical model.

DISCUSSION

Early results indicate that calcium does have a stimulatory effect on the Renin-Angiotensin-System and the study will be pursued.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OE 6977	75 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
74 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	3A161102B71P	02	066
b. CONTRIBUTING	61101A	3A16110A91C	00	
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)

(U) Evaluation of Calcium Metabolism 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
74 01	Cont	DA	C. In-House

17. CONTRACT/GRANT: Not Applicable

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 75	.2	7
	CURRENT 76	.3	9

19. RESPONSIBLE DOD ORGANIZATION

NAME: US Army Institute of Surgical Research

ADDRESS: Fort Sam Houston, Texas 78234

RESPONSIBLE INDIVIDUAL

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

TELEPHONE: 512-221-2720

20. PERFORMING ORGANIZATION

NAME: US Army Institute of Surgical Research
Metabolic Branch

ADDRESS: Fort Sam Houston, Texas 78234

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

NAME: Richard H. Merrill, LTC, MC

TELEPHONE: 512-221-5416

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: James Long, LTC, MC

NAME: William D. Meyers, LTC, MC DA

21. GENERAL USE

FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Calcium metabolism; (U) Burns; (U) Renal failure; (U) Hypocalcemia; (U) Humans

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

23. (U) The objective of this study is to determine the mechanism of hypocalcemia and hypocalciuria in thermally injured patients. In addition the role of calcium in the production of renal impairment in thermally injured troops is to be assessed.

24. (U) Calcium balance studies will be performed on burn patients, with all calcium intake and output measured. If, as we expect, the patient is in positive calcium balance, we will investigate the skin as the site of calcium deposition. Skin biopsies and eschar biopsies will be performed and analyzed for calcium. In addition, parathyroid hormone levels will be determined, and the response to the development of hypocalcemia will be documented. This will be correlated with any functional change in renal metabolism.

25. (U) 74 07 - 75 06 The main impediment for the completion of this protocol has been the inability to obtain reproducible results with the Ionized Calcium Electrode; however, in the past month Captain Webb from the Laboratory Branch has taken a personal interest in this project and has succeeded in standardizing the electrode and in obtaining consistent results. Preliminary data indicate that indeed the burned patient has a significant early hypocalcemia both total and ionized. Firm conclusions cannot be drawn from this preliminary data.

*Available to contractors upon originator's approval

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

ANNUAL PROGRESS REPORT

PROJECT NO. 3A161102B71P-02, BASIC RESEARCH IN SUPPORT OF MILITARY MEDICINE, SURGICAL PATHOLOGY

REPORT TITLE: EVALUATION OF CALCIUM METABOLISM IN BURNED PATIENTS

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

Investigators

Richard H. Merrill, M.D., LTC, MC
Philip W. Rogers, M.D.

Reports Control Symbol MEDDH-288 (R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A161102B71P-02, BASIC RESEARCH IN SUPPORT OF MILITARY MEDICINE, SURGICAL PATHOLOGY

REPORT TITLE: EVALUATION OF CALCIUM METABOLISM IN BURNED PATIENTS

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

Period covered in this report: 1 July 1974 - 30 June 1975

Investigators: Richard H. Merrill, M.D., LTC, MC
James M. Lentz, M.D., LTC, MC
Philip W. Rogers, M.D.
Calvin Kennedy, BS
Ralph Goldsmith, M.D.

Reports Control Symbol MEDDH-299 (R1)

Hypocalcemia is a common event in the burned patient, as is reflected by the low total serum calciums and electrocardiographic changes of hypocalcemia. To evaluate the etiology of this hypocalcemia approximately 20 patients have been studied during this report period. The small number of patients and the data do not permit any firm conclusion, however certain observations may be made. Hypocalcemia is an early event being observed immediately post injury. The data on ionized calcium is incomplete because of difficulty with equipment, however the initial impression is that immediately after burn the ionized calcium is normal, but drops to low levels by the second and third week post injury. These changes are reflected by electrocardiograph abnormalities. The urinary excretion of calcium is low initially and slowly rises throughout the convalescent course. The skin calcium appears to be unmeasurbaly low in both the burned and unburned skin. The parathormone levels and the stool calcium levels have not yet been analyzed.

Calcium Metabolism
Burns
Renal Failure
Hypocalcemia
Humans

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OE 6967	75 07 01	DD DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISS'N INSTR'N	8b. SPECIFIC DATA CONTRACTOR ACCESS	9. LEVEL OF SUM
74 07 01	D. CHANGE	U	U	NA	NL	[X] YES [ ] NO	A. WORK UNIT

10. NO. CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62110A	3A162110A821	00	108
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code) (U) Evaluation of Steroids in the Management of Inhalation Injury of Military Personnel (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS
003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
73 03	Cont	DA	C. In-House

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

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 75	.5	12
	CURRENT 76	.5	12

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

20. PERFORMING ORGANIZATION
NAME: US Army Institute of Surgical Research
Clinical Division
ADDRESS: Fort Sam Houston, Texas 78234
PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME: Peter A Petroff, MD, MAJ, MC
TELEPHONE: 512-221-4224
SOCIAL SECURITY ACCOUNT NUMBER
ASSOCIATE INVESTIGATORS
NAME: James M Long, MD, LTC, MC
NAME: DA

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Burns; (U) Inhalation Injury; (U) Steroids; (U) Burn Patients

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

23. (U) Pulmonary injury due to the inhalation of products of incomplete combustion or toxic fumes may be quite lethal by itself and, when this injury occurs in association with a cutaneous thermal injury, the mortality is very high. The incidence of inhalation injury in a large series of burns by standard clinical criteria has been reported to be about 3%. The diagnosis of inhalation injury with the use of the 133Xenon lung scan has proven to be a far more accurate diagnostic tool than if clinical criteria are used alone. The objective of this study is to evaluate the use of systemically administered steroids as a means of treating inhalation injury in military personnel.

24. (U) All patients between the ages of 15-40 years who have sustained burns within 48 hours of admission will be randomized for double-blind administration of steroids or a placebo as a means to evaluate them in the treatment of inhalation injury. The 133Xenon lung scan will be used to detect the presence or absence of an inhalation injury.

25. (U) 74 07 - 75 06 Twenty-five patients have begun the study. Though the drug code has not been broken as yet, some information is available to the effect that 1) complications appear to be related to burn size and infection rather than to the use of the agent, i.e., two treatment groups cannot be distinguished; 2) flow rates improve with time whether or not the patient is getting the drug or placebo.

*Available to contractors upon originator's approval

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

ANNUAL PROGRESS REPORT

PROJECT NO. 3A162110A821-00, COMBAT SURGERY

REPORT TITLE: EVALUATION OF STEROIDS IN THE MANAGEMENT OF INHALATION INJURY OF MILITARY PERSONNEL

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

1 July 1974 - 30 June 1975

Investigators:

Peter A. Petroff, MD, Major, MC
James M. Long, MD, Lieutenant Colonel, MC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A162110A821-00, COMBAT SURGERY

REPORT TITLE: EVALUATION OF STEROIDS IN THE MANAGEMENT OF INHALATION INJURY OF MILITARY PERSONNEL

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

Period covered in this report: 1 July 1974 - 30 June 1975

Investigators: Peter A. Petroff, MD, Major, MC
James M. Long, MD, Lieutenant Colonel, MC

Reports Control Symbol MEDDH-288(R1)

In order to evaluate the effect of a three-day course of high-dose steroids, 30 patients were studied in a double blind manner (15 receiving steroids, 15 receiving a placebo). No difference was found in the complication rate, time of complications, or ultimate outcome of the patient. In addition, 12 of the patients had serial pulmonary function measurements (seven in the steroid group, five in the non-steroid group), and again no differences were noted between the two groups as far as improvement in volume or flow measurements.

Burns
Inhalation injury
Steroids
Burn patients

EVALUATION OF STEROIDS IN THE MANAGEMENT OF INHALATION INJURY OF MILITARY PERSONNEL

For many years, steroids in high dosages have been advocated for the treatment of inhalation injury. In addition, De la Pena, et al,[1] have shown in an animal model that very high doses (equivalent to 7.0 g of methylprednisolone for human beings) given one hour after inhalation of smoke will improve mortality. However, in a referral situation, patients are rarely seen that early in their course. For these reasons, we have been evaluating the effectiveness of large doses (0.5 g, q.i.d. for 3 days) of Decadron in patients with a positive ^{133}Xenon lung scan.

METHOD

Thirty patients, without clinical evidence of chronic pulmonary disease and with positive ^{133}Xenon lung scans, were begun on three days of high dosage (20 g/day) Decadron or placebo in a double blind manner within 72 hours of injury. Pulmonary function tests were done prior to the first dose and at 48 hours after the start of therapy where possible. These studies included maximum expiratory flow-volume curves and measurement of dynamic compliance and pulmonary resistance. Volume and flow were measured using an Ohio Model 840 dry spirometer and pressure with a 12 cm esophageal balloon, the tip placed 42 cm from the nares, connected to a Statham pressure transducer. A Tetronix oscilloscope was used for the recording of MEFV, and an Electronics for Medicine strip recorder was used for recording volume, flow, and pressure signals for the dynamic compliance and pulmonary resistance.

In addition, following completion of the study, electrocardiograms and x-rays were reviewed, with the investigators noting if any pulmonary complications had occurred and when they occurred. Lastly, pathology reports were reviewed as to the cause of death and the presence of pulmonary lesions.

RESULTS

Table 1 shows the clinical findings in the two groups of patients: The groups were similar in age, per cent total body surface burn and

---

1. De la Pena L, Skornik WA Dressler DP: Methylprednisolone in the treatment of smoke inhalation. Presented at the American Burn Association Meeting, 1975.

TABLE 1

Group	No.	Age	Per Cent Total Body Surface Burn	Per Cent Death (Total)	Per Cent Death (1st 10 Days)	Per Cent Pulmonary Complications (Total)	Per Cent Pulmonary Complications (1st 10 Days)	Per Cent Dying of Pulmonary Complications
Steroid treated	15	33.3	51.2	60.0	20.0	73.3	46.7	26.7
Placebo treated	15	29.7	56.0	73.3	20.0	86.7	53.3	46.7

per cent third degree burn. In addition, the mortality and complication rates were similar.

Table 2 shows the results of the pulmonary function studies for the two groups. As can be seen, both groups showed a slight decrease in vital capacity and peak flow, with little change in flow rates at 50 per cent and 25 per cent of the vital capacity. Interestingly, the pulmonary resistance decreased in the steroid-treated group. Only one patient in the control group had tests done at both 0 and 48 hours and no comparison could be made.

CONCLUSION

Analysis of the data is continuing, but, at the present time, it appears that steroids given in this manner are not beneficial. This does not exclude the use of steroids either immediately after the injury is sustained or at time of development of a pulmonary complication.

PRESENTATIONS AND/OR PUBLICATIONS

None.

TABLE 2

Group	No.	Vital Capacity (l)		Peak Flow (l/sec)		Flow at 50 Per Cent Vital Capacity (l/sec)		Flow at 25 Per Cent Vital Capacity (l/sec)		Pulmonary Resistance	
		Day 0	48 Hours	Day 0	48 Hours	Day 0	48 Hours	Day 0	48 Hours	Day 0	48 Hours
Steroid treated	7	4.08	3.65	6.45	6.26	2.85	2.84	1.09	1.23	4.64	3.76
Placebo treated	5	4.50	4.24	8.62	8.49	5.10	5.30	2.12	2.13	—	—

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OF 6399	75 07 01	DD DR&E AR(6)6

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
75 07 01	D. CHANGE	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO. CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62110A	3A162110A821	00	106
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)

(U) Nebulized Gentamicin in Soldiers with Severe Burns (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 Clincial Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
74 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	75	.4	13
FISCAL YEAR — CURRENT	76	.2	6

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

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

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

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

ASSOCIATE INVESTIGATORS
NAME: Peter Petroff, MAJ, MC
NAME: DA

21. GENERAL USE

FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Gentamicin serum levels; (U) Gentamicin; (U) Nebulized antibiotics; (U) Pneumonia; (U) Burn therapy; (U) Humans

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

23. (U) Determine if nebulized Gentamicin is an effective therapeutic modality in the treatment of bronchopneumonia in burned soldiers.

24. (U) A double-blinded administration of normal saline or Gentamicin 1 mg/kg via a nebulizer every 8 hours after the diagnosis of bronchopneumonia has been made. Quantitative and qualitative bacteriologic sputum analysis and serum levels of Gentamicin are serially made. Comprehensive pathologic description and analysis are made on the lungs of patients who die who were included in the study.

25. (U) 74 07 - 75 06 Eight of nine Gentamicin treated patients radiographically cleared their bronchopneumonia; whereas, only two of the 8 placebo treated patients cleared their pneumonia. Seven patients in each group died. The two groups are statistically different at the 0.02 level using Fisher's exact probability test. At autopsy none of the Gentamicin treated patients had evidence of pneumonia while 3 of the placebo group died of bronchopneumonia. Serum Gentamicin levels have not been done on the submitted serum samples. The quantitative and qualitative bacteriologic data from sputum specimens was not different in the two groups. There were no complications attributable to this treatment modality.

*Available to contractors upon originator's approval

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

ANNUAL PROGRESS REPORT

PROJECT NO. 3A162110A821-00, COMBAT SURGERY

REPORT TITLE: NEBULIZED GENTAMICIN IN SOLDIERS WITH SEVERE BURNS

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

1 July 1974 - 30 June 1975

Investigators:

Daryl R. Erickson, MD, Major, MC
Peter A. Petroff, MD, Major, MC
Thomas A. Rizzo, Jr, MD, Major, MC
Robert B. Lindberg, PhD
Hugh D. Peterson, MD, DDS, Colonel, MC
Basil A. Pruitt, Jr, MD, Colonel, MC

Report Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A162110A821-00, COMBAT SURGERY

REPORT TITLE: NEBULIZED GENTAMICIN IN SOLDIERS WITH SEVERE BURNS

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

Period covered in this report: 1 July 1974 - 30 June 1975

Investigators: Daryl R. Erickson, MD, Major, MC
Peter A. Petroff, MD, Major, MC
Thomas A. Rizzo, Jr, MD, Major, MC
Robert B. Lindberg, PhD
Hugh D. Peterson, MD,DDS, Colonel, MC
Basil A. Pruitt, Jr, MD, Colonel, MC

Report Control Symbol MEDDH-288(R1)

Nebulized gentamicin or normal saline was used as an adjunctive therapeutic modality in 19 burn patients with bronchopneumonia diagnosed roentgenographically in this prospective, double-blind study. Eleven patients were in the gentamicin group and 8 were in the placebo group.

Eight of 11 patients (73%) in the gentamicin group had roentgenologic clearing of their bronchopneumonia compared with 3 of the 8 patients (38%) in the placebo group. None of the 10 patients who ultimately died in the gentamicin group had bronchopneumonia at autopsy; whereas, 3 of 7 (43%) in the placebo group died of bronchopneumonia.

There was no morbidity associated with nebulized gentamicin therapy. No resistant bacterial organisms developed during the study. Therefore, nebulized gentamicin appears to be a safe, effective adjunctive therapeutic modality in the treatment of bronchopneumonia in severely burned soldiers; however, more patients are required to make a statistically valid comparison.

Gentamicin serum levels
Gentamicin
Nebulized antibiotic
Pneumonia
Burn therapy
Humans

NEBULIZED GENTAMICIN IN SOLDIERS WITH SEVERE BURNS

Bronchopneumonia was diagnosed in 186 of 563 (33.1%) of the thermally injured patients admitted to the United States Army Institute of Surgical Research from 1 January 1972 through 31 December 1973. During this same period, bronchopneumonia was a cause of death in 101 of the 215 patients (47.0%) who died. Therefore, 18% of all admissions died with bronchopneumonia. The most alarming aspect of these figures is that in spite of intensive medical care with highly trained personnel and the most modern technics, equipment and drugs, 54% of all burned patients who developed bronchopneumonia died as a result of that process.

High concentrations of an effective antibiotic on the exposed surface of burn wounds has been demonstrated to control the bacterial proliferation and invasion formerly seen in such wounds. Using the same concept we theorized that bronchopneumonia might be better controlled by topically applying antibiotic to the epithelial surface of small airways.

Topical application of the antibiotic down into the alveoli can be achieved by nebulization of its liquid form into 5 micron or smaller droplets. The Bard-Parker nebulizer which provides fluid particles between 0.25 and 6.0 micra in size can be used on volume as well as pressure cycle respirators. Consequently, the mechanism for effecting the proposed therapy was readily available and easy to use. Theoretically the antibacterial agent should meet the following criteria: (a) not injure healthy or diseased tissues, (b) be effective against most gram-negative pathogens, (c) have a high level in tracheobronchial secretions after administration, and (d) be minimally absorbed into the systemic circulation. Gentamicin seemed to meet all these specifications. There was no change in pulmonary function studies done in healthy volunteers (1). Gentamicin is widely recognized as an effective antibacterial agent for most gram-negative organisms and certain coagulase positive staphylococci. Significantly higher levels of the drug can be obtained in sputum when it is instilled intratracheally than when it is given parenterally (2). The lipoid solubility of antibiotics is directly related to their systemic absorption from the tracheobronchial tree in rats (3). Gentamicin, which is water soluble and almost completely lipoid insoluble, produced low serum levels (1.9 ug/ml) when instilled in the infected tracheobronchial tree of humans. In the same study, parenteral administration of the same daily dose (240 mg) resulted in significantly higher serum levels (6.4 ug/ml) (4). Therefore, even if gentamicin is given parenterally and via nebulization the total amount in the blood should be less than the toxic level of

---

1. Petroff PA, Erickson DR: Unpublished data.
2. Klastersky J, Geuning C, Mouawad E, et al: Endotracheal gentamicin in bronchial infections in patients with tracheostomy. Chest 61: 117-120, 1972.
3. Burton JA, Schonker LS: Absorption of antibiotics from the rat lung. Proc Soc Exp Biol Med 145: 752-756, 1974.
4. Klastersky J, Geuning C, Mouawad E, et al: ibid.

12 ug/ml (5).

A pilot study revealed that nebulized gentamicin in the treatment of bronchopneumonia appeared to be efficacious. However, a double-blind, prospective study was initiated to control investigator bias regarding interpretation of the pulmonary roentgenograms, morphologic findings and patient selection.

METHODS

In the pilot study 8 adult burn patients who required intubation for respiratory support were given 240 mg gentamicin nebulized via their endotracheal tube daily in doses divided as follows: 40 mg every 4 hours or 80 mg every 8 hours. The gentamicin was continued until the patient died or no longer required respiratory support. The comparison group of 13 patients was retrospectively selected to include all of the other patients in our intensive care unit requiring endotracheal intubation during the time the nebulized gentamicin was being used.

After evaluating the results of the pilot study, a formal protocol was initiated to study the problem prospectively. Initially, 50 patients were to be randomly assigned to one of 6 groups (Table 1). However, after 19 patients had been studied the prinicipal investigator was separated from the Army and prepared this evaluation of the therapy.

Table 1. Nebulized Gentamicin Study

Assignment Groups

1. Placebo with less than 40% BSA burn
2. Nebulized gentamicin with less than 40% BSA burn
3. Placebo with 40% to 65% BSA burn
4. Nebulized gentamicin with 40% to 65% BSA burn
5. Control with greater than 65% burn
6. Nebulized gentamicin with greater than 65% BSA burn

Patients were considered for inclusion in the study when a pulmonary infiltrate was detected on a portable chest roentgenogram. If the radiologist and the pulmonologist agreed that the infiltrate was unequivocally a pneumonia, the patient entered the study protocol. When the radiologist and/or pulmonologist felt that the infiltrate was atelectasis, intensive pulmonary toilet was instituted for 24 hours. If, at the end of 24 hours, the infiltrate was still present, the patient was considered to have pneumonia and entered into the study protocol. If the radiologist and/or pulmonologist could not eliminate pulmonary edema as causing the infiltrate, all appropriate diagnostic and therapeutic modalities were

---

5. Goodman LS, Gilman A (eds): The Pharmacologic Basis of Therapeutics. The MacMillan Co., London, 1970, p. 1298.

instituted to determine the underlying pathophysiologic mechanism. A gram stain of endotracheal secretions was done in those patients with possible pulmonary edema. If the smear revealed polymorphonuclear cells and bacteria, the patient was considered to have pneumonia and entered the study. When the gram stain was negative, the patient was reevaluated in 24 hours. If at the end of that time the infiltrate had cleared or was clearing, the gram stain was still negative and the basilar rales had cleared or were clearing, the patient was considered to have pulmonary edema and was not entered into the study. Those patients who were reevaluated at the end of 24 hours and found to have no change in the pulmonary infiltrate, gram stain positive endotracheal secretions and localized rales were considered to have pneumonia and entered into the study.

Control patients received 4 cc of sterile normal saline while the other patients received gentamicin 1.0 mg/kg in enough sterile normal saline to make a total of 4 cc administered every 8 hours via a Bard-Parker nebulizer. This nebulizer in conjunction with a Bird IPPB apparatus was used for non-intubated patients. Intubated patients received the saline or gentamicin solution via a Bard-Parker nebulizer attached an MA-1 respirator. Nebulization therapy was continued until the infiltrate cleared radiographically or the patient died. None of the personnel actively involved in the patient's care knew which drug was being used. All aspects of management, including parenteral antibiotic administration, was the responsibility of the primary surgeon. The patient, or next of kin, was required to give consent after a full explanation of the study before instituting the protocol in each case.

Quantitative and qualitative endotracheal secretion cultures were obtained before instituting therapy and repeated at one hour, 2 hours, 4 hours, 8 hours and daily after the therapy was started. The daily sputum specimens were collected 4 hours after a treatment with nebulized gentamicin and at least one hour after the last tracheal suction. The predominant organism was reported on most specimens.

Serum samples for gentamicin assay were drawn before therapy began and then again at one hour, 4 hours and 8 hours after therapy was initiated. Serum samples were also drawn just prior to a treatment every other day. Samples from 24 hour urine collections were submitted every other day beginning on the fifth day of the study.

In those patients who died and came to autopsy, postmortem lung specimens were processed as usual. A detailed gross and microscopic description of pulmonary tissues was done. Routine postmortem bacteriologic studies were done.

RESULTS

Pilot Study. There was no statistically significant difference in age or burn size between the two groups. Pulmonary infiltrates seen on chest roentgenograms were initially similar in the two groups. All patients in both groups ultimately died and autopsies were done in all cases. Bronchopneumonia was listed as a cause of death by the pathologist in one of the 8 patients (13%) receiving nebulized

gentamicin; whereas, 9 of the 13 patients (69%) not receiving nebulized gentamicin died with pneumonia.

Prospective Double-blind Study (Tables 2,3). Nineteen patients were randomly assigned to one of the two groups; 11 were in the gentamicin group and 8 in the placebo group.

A. Age and Burn Size (Table 4). Both groups are similar with respect to patient age. The group receiving nebulized gentamicin has 5 patients in the over 65% BSA burn classification, while the placebo group has only one patient in that category.

B. Onset of Pulmonary Infiltrate (Table 5). The gentamicin group had an average postburn day (PBD) onset of bronchopneumonia of 14; whereas the placebo groups average PBD is 23. The gentamicin group includes 6 patients with definitely diagnosed inhalation injury while the placebo group has 3. When PBD onset of bronchopneumonia is related to the diagnosis of inhalation injury in these patients, those with inhalation injuries on the average developed their bronchopneumonia on PBD 11 compared with PBD 21 for those without inhalation injury (Table 6). One patient who had an inhalation injury and developed a bronchopneumonia on PBD 40 was excluded from the figures in Table 6 because inhalation injuries are healed by that time and this patient was felt to have developed a bronchopneumonia unrelated to his inhalation injury.

C. Roentgenologic Evidence That Bronchopneumonia Had Cleared (Table 7). Eight of 11 patients in the group treated with nebulized gentamicin cleared their pulmonary infiltrate. Three of 8 patients in the placebo group had roentgenologic evidence of clearing of their pneumonic process.

D. Nasotracheal Intubation and Tracheostomy (Table 8). In the gentamicin group, 3 patients were nasotracheally intubated and 3 had a tracheostomy at the start of their study period. By the time their pulmonary roentgenograms had cleared one patient was intubated and 4 had tracheostomies.

In the placebo group, 4 patients were nasotracheally intubated and 2 had tracheostomies at the start of their study period. By the time these patients died or cleared their bronchopneumonia, 2 required nasotracheal intubation and 4 required tracheostomies. All of these patients required assisted ventilation because of their pulmonary parenchymal lesion. In addition, one patient who ultimately cleared his pneumonia required nasotracheal intubation after he was started in the placebo therapy group and was extubated prior to complete resolution of the pneumonia. No patients who received the gentamicin therapy required intubation after the therapy began.

E. Morphologic Analysis (Table 9). None of the gentamicin treated patients died with bronchopneumonia; whereas, 2 of 5 patients died with bronchopneumonia while receiving nebulized normal saline. One patient in each group ultimately survived his thermal injury. Five of the 8 placebo treated patients (63%) died while in the study as compared to 3 of 11 (27%) of the patients

Table 2. Nebulized Gentamicin Study

Gentamicin Group

Patient	Age	Burn Size % BSA	Inhalation Injury	Days in Study	CXR Cleared	Days to Death After Study	Cause of Death
G-1	21	14	+	7	+	Lived	--
G-2	43	36	0	8	+	11	G.I. hemorrhage
G-3	46	47	0	6	0	0	Sepsis; hyaline membrane disease
G-4	33	55	+	3	+	4	Unexplained cardiac arrest. No autopsy
G-5	48	61	+	13	+	13	Invasive burn wound sepsis; severe hematogenous pneumonia
G-6	18	63	0	7	+	13	Acute bacterial endocarditis
G-7	18	67	+	7	+	9	Gram negative sepsis
G-8	26	68	+	16	0	0	Invasive burn wound sepsis; hematogenous pneumonia
G-9	46	79	+	15	0	0	Sepsis; hyaline membrane disease; no bronchopneumonia
G-10	26	84	0	5	+	7	Gram negative sepsis
G-11	46	93	0	3	+	4	Gram negative sepsis
Ave.	34	61	6/11	8	8/11	6	
Range	18-48	14-93		3-15		0-13	

Table 3. Nebulized Gentamicin Study

Placebo Group

Patient	Age	Burn Size % BSA	Inhalation Injury	Days in Study	CXR Cleared	Days to Death After Study	Cause of Death
P-1	50	25	0	24	0	0	Severe bronchopneumonia
P-2	26	41	0	31	0	0	Sepsis; Lungs never cleared. No autopsy
P-3	25	43	0	3	+	49	Acute bacterial endocarditis
P-4	45	51	0	6	0	0	Severe bronchopneumonia
P-5	45	55	+	16	+	24	Moderate bronchopneumonia
P-6	33	55	+	17	0	0	Hyaline membrane disease No bronchopneumonia
P-7	40	63	+	21	+	Lived	---
P-8	26	68	0	7	0	0	Invasive burn wound sepsis; Severe hematogenous pneumonia
Ave.	36	50	3/8	16	3/8	10	
Range	25-50	25-68		3-31		0-49	

Table 4. Nebulized Gentamicin Study

Number of Patients in Each Treatment Group

Treatment Group	0-39% BSA	40-65% BSA	66-100% BSA
Gentamicin	2	4	5
Placebo	1	6	1

Table 5. Nebulized Gentamicin Study

Inhalation Injury Related to PBD Onset Pneumonia

Gentamicin		Placebo	
Inhalation Injury	PBD	Inhalation Injury	PBD
+	9	0	21
+	5	0	24
0	28	+	5
0	16	0	29
+	12	+	40
0	7	0	37
+	7	0	17
0	10	+	11
+	26		
+	15		
0	23		
Ave. 6/11	14	3/8	23
Range	5-28		5-40

Table 6. Nebulized Gentamicin Study

Onset of Pneumonia Related to Inhalation Injury

With inhalation injury*	(n = 8)
Average	11 PBD
Range	5-26 PBD
Without inhalation injury	(n = 10)
Average	21 PBD
Range	7-37 PBD

* one patient excluded who developed bronchopneumonia on PBD 40

Table 7. Nebulized Gentamicin Study

Roentgenologic Evidence of Clearing

Group	Cleared	Percent
Gentamicin (n = 11)	8	73
Placebo (n = 8)	3	38

Table 8. Nebulized Gentamicin Study

Nasotracheal Intubation and Tracheostomy

Group	Start		Finish	
Gentamicin (n = 11)	NT- 3 Trach-3	6	NT- 1 Trach- 4	5*
Placebo (n = 8)	NT- 4 Trach-2	6	NT- 2 Trach-4	6**

* 2 patients had severe adult hyaline membrane disease; 1 patient had severe hematogenous pneumonia

** 1 patient had severe adult hyaline membrane disease; 1 patient had severe hematogenous pneumonia

Table 9. Nebulized Gentamicin Study

Bronchopneumonia as Cause of Death

Group	Number	Percent
Gentamicin (n = 10)	0	0
Placebo (n = 7)	3	43

receiving nebulized gentamicin. The same pathologist, who was blinded as to study group, examined all gross and microscopic lung specimens. Bronchopneumonia, atelectasis, interstitial infiltrates, edema, hemorrhage, inhalation injury, hyaline membrane disease, abscesses, infarcts and emboli were described, located and graded as to severity. Tracheitis, bronchitis, and tracheal ulceration were described, located and graded as to severity. Cause of death was determined by his study of the clinical record and autopsy material.

Analysis of all pathologic data revealed no difference between the two groups, except for the aforementioned bronchopneumonia. One patient in each treatment group had hematogenous pneumonia secondary to invasive burn wound sepsis. Two patients in the gentamicin group and one patient in the placebo group had severe adult hyaline membrane disease (shock lung) with no bronchopneumonia.

F. Bacteriologic Analysis. There was no difference in the predominant organisms in the pretreatment endotracheal secretion cultures between the two groups. Pseudmonas aeruginosa, Klebsiella, Enterobacter aerogenes, Providencia stuartii and non-hemolytic streptococcus were found. There was no consistent change in the number of bacteria cultured from secretion in either group during the study period.

No appearance of resistant organisms was found in the patients treated with gentamicin. Both groups had similar, but not consistent, changes in the predominant organism grown from endotracheal secretions during treatment.

G. Gentamicin Levels. Serum and urine specimens were collected at the specified intervals. Due to technical difficulties, measurement of gentamicin levels have not yet been made.

DISCUSSION

Airborne pneumonia is the most common cause of sepsis and death in the severely burned patients in our Institute. The precise pathophysiology of this problem is incompletely defined and presently accepted treatment modalities are inadequate.

Throughout this study all patients received parenteral antibiotics at the discretion of their attending physician. There was no difference between the two groups as to parenteral antibiotic administration. All patients were treated topically with silver sulfadiazene. The only apparent discrepancy between the two groups is the day postburn on which they were diagnosed as having bronchopneumonia. This variation can best be explained by the increased incidence of inhalation injury in the gentamicin treated group. Patients with inhalation injuries typically get pneumonia in the first two weeks postburn; whereas, those burn patients who do not have inhalation injuries most frequently get bronchopneumonia after the third week (Table 6). The bronchopneumonia related to inhalation injuries has the same clinical and pathologic characteristics as that which is found in burned patients who have not had an inhalation injury. Therefore, the fact that there were more patients with inhalation injury in the

gentamicin treated group than in the placebo group does not detract from the validity of our analysis.

Thermally injured patients normally have elevated temperatures and frequently have sepsis from sites other than the lung. Therefore, the criterion used to define bronchopneumonia in these patients was a pulmonary infiltrate on a chest roentgenogram which was not related to atelectasis or pulmonary edema. Because it is frequently impossible to exclude hematogenous pneumonia and always impossible to exclude adult hyaline membrane disease from bronchopneumonia by radiographic technics, patients with these processes were found among the patients in the study.

Among those patients who did not clear their pneumonic infiltrate one patient in each group (G-8,P-8) had severe hematogenous pneumonia at autopsy secondary to invasive burn wound sepsis. There were two patients (G-3,G-9) in the gentamicin therapy group and one patient (P-6) in the placebo group who had severe adult hyaline membrane disease at autopsy. None of these 5 patients had morphologic evidence of bronchopneumonia.

In the placebo group 3 of 8 patients (38%) cleared their pulmonary infiltrate. Among those patients who did not clear, 2 patients (P-1,P-4) definitely died because of severe bronchopneumonia.One other patient (P-2) may well have died of bronchopneumonia as his pulmonary infiltrate never cleared and clinically there was no other source of sepsis. No definitive pulmonary diagnosis could be made in this patient as autopsy permission was denied. One patient (P-8) died of invasive burn wound sepsis with resultant severe hematogenous pneumonia. The fifth patient (P-6) who did not clear his pulmonary infiltrate while in the study died of severe adult hyaline membrane disease without any evidence of bronchopneumonia. Therefore, 2 of the 5 patients who died while in the study did so of bronchopneumonia while a third may well have died of bronchopneumonia. Only one patient in this group (P-7) ultimately survived his thermal injury and it took 21 days to clear his infiltrative process. Two other patients (P-3,P-5) cleared their pneumonic processes while in the study and one (P-5) died as a result of moderately severe bronchopneumonia and severe adult hyaline membrane disease 24 days after being in the study.

In the gentamicin group 8 of 11 patients (73%) cleared their pulmonary infiltrate. None of the patients who failed to clear their pneumonic process had any evidence of bronchopneumonia at autopsy. Two patients (G-3,G-9) died with severe adult hyaline membrane disease and one (G-8) died with severe hematogenous pneumonia secondary to invasive Pseudomonas burn wound sepsis. This group also had one patient (G-1) who ultimately survived his thermal injury. The remaining 7 patients died from 4 to 13 days (average 9 days) after clearing their pulmonary infiltrate and had no evidence of bronchopneumonia at autopsy.

Clearing the pneumonic infiltrates took from 3 to 13 days (average 7 days) in the gentamicin group as compared with 3 to 31 days (average 15 days) in the placebo group. If this trend continues as more patients are studied, treatment of assumed bronchopneumonia with nebulized gentamicin may significantly

decrease the time it takes bronchopneumonia to resolve. In addition, if the observed mortality rate due to bronchopneumonia, zero in the gentamicin group and 67 to 100% in the placebo group, remains the same this adjunctive therapeutic modality will be of definite value in the treatment of bronchopneumonia.

The finding that 10 of 11 (91%) of the patients in the gentamicin group and 7 of 8 (88%) of those in the placebo group died suggests that even a useful adjunctive therapy for bronchopneumonia in severely burned patients is not enough to prevent their ultimate death. These patients continue to be very susceptible to sepsis from other sites, primarily their open burn wound.

In this limited number of patients there is no evidence that suggests a toxic effect of the nebulized gentamicin. Supporting evidence for this is that there were no consistent morphologic changes other than the absence of bronchopneumonia in the gentamicin treated group compared to those in the placebo group. Additional evidence is that once the bronchopneumonia cleared and if the patients with adult hyaline membrane disease and hematogenous pneumonia are excluded, most of the patients were able to ventilate without respirator support.

Since 50% of the patients in the gentamicin group had documented premortem inhalation injuries, and since they all cleared their early onset bronchopneumonia, a prospective study using nebulized gentamicin on a prophylactic basis should be considered.

In theory there should have been a decrease in the colony count of endotracheal secretion cultures in the gentamicin group but such was not observed. Rather than detracting from the use of nebulized gentamicin as an adjunctive therapy in the treatment of bronchopneumonia, this finding reinforces the opinion that qualitative sputum cultures are helpful in the selection of the antibiotic, but quantitative cultures are not of great usefulness.

The following experimental data suggest, at least in theory, the physiologic rationale for the therapeutic efficacy of nebulized gentamicin. Burned, wound infected rats have a markedly decreased ability to clear inhaled bacteria from their lungs (6). When burned rats have their wounds seeded with Pseudomonas and are treated with systemic gentamicin or polymyxin E, they recover the ability to clear bacteria from their lungs (7). Unburned rats with chemically induced acute hemorrhagic pulmonary edema are also unable to clear bacteria from their lungs and, in fact, have a proliferation of bacteria; both phenomena can be corrected by treatment with parenteral tetracycline (8).

---

6. Skornik WA, Dressler DP: Lung bacterial clearance in the burned rat. Ann Surg. 172: 837-843, 1970.

7. Dressler DP, Skornik WA: Pathophysiology of thermal respiratory injury. In Research in Burns, Matter P, Barclay TL, Lunickova Z (eds), 1970, p. 457-459.

8. Johnson WG, Jay SJ, Pierce AK: Bacterial growth in vivo. J Clin Invest 53: 1320-1325, 1974.

The alveolar macrophage's ability to kill bacteria is a major mechanism of early resistance to infection in the lung (9). A decreased ability of leukocytes from burned patients to kill bacteria despite normal phagocytosis has been demonstrated (10). Therefore, gentamicin, polymyxin E and tetracycline apparently increase the susceptibility of the bacteria to the bactericidal action of the alveolar macrophages and thereby prevent the development or hasten the resolution of bronchopneumonia in the face of decreased host resistance to bacterial invasion.

While there is no information in this study that explains how nebulized gentamicin along with parenteral antibiotics helps the alveolar macrophage clear bronchopneumonia, there is definite evidence that it does help clear bronchopneumonia more quickly than when only parenteral antibiotics are used. If macrophages can phagocytize bacteria but are unable to kill them when host "resistance" is depressed, then it appears that nebulized gentamicin helps kill the bacteria. Theoretically, it seems probable that a higher level of antibiotic on the epithelial surface of the airway, the bailiwick of the alveolar macrophage, acts synergistically with the macrophage to kill the topically invading bacteria permitting earlier resolution of the bronchopneumonia.

CONCLUSIONS

Seventy-three percent of burn patients treated with nebulized gentamicin cleared their pulmonary infiltrate and none of these patients who died had any evidence of bronchopneumonia at autopsy. In contrast, only 38% of the patients who received the placebo cleared their infiltrate and 43% of these patients who died had severe bronchopneumonia at autopsy.

Nebulized gentamicin appears to be without significant side effects.

Nebulized gentamicin appears to be a useful adjunctive therapeutic modality that requires further study to document its efficacy.

PUBLICATIONS AND/OR PRESENTATIONS

None

---

9. Green GM, Kass EH: The role of the alveolar macrophage in the clearance of bacteria from the lung. J Exp Med 119: 167-176, 1964.

10. Alexander JW, Hegg M, Altemeier WA: Neutrophil function in selected surgical disorders. Ann Surg 168: 447-457, 1968.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA 0E 6968	75 07 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISP'N INSTR'N	8b. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
74 07 01	K. COMP	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO. CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62110A	3A162110A821	00	110
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)* (U) Fibrinogen-Fibrin Degradation Products in the Thermally Injured Animals: A Model of the Burned Soldier (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*
003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
72 10	75 05	DA	C. In-House

17. CONTRACT/GRANT
a. DATES/EFFECTIVE: Not Applicable 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 75	.6	17
FISCAL YEAR CURRENT		

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

20. PERFORMING ORGANIZATION
NAME:* US Army Institute of Surgical Research Renal Section
ADDRESS:* Fort Sam Houston, Texas 78234
PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME:* Willard A Andes, MD, MAJ, MC
TELEPHONE: 512-221-3411
SOCIAL SECURITY ACCOUNT NUMBER:
ASSOCIATE INVESTIGATORS
NAME: Robert B Lindberg, PhD
NAME: DA

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Fibrinogen; (U) Antiplasmins; (U) Rats; (U) Thermal Injury

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

23. (U) To study the responses in fibrinogen, fibrin-degradation products, fibrinolytic activity, and other coagulation parameters in the thermally injured rat model of the burned soldier.

24. (U) Rats are given a 30% scald burn followed by resuscitation. Half of the animals' burn wounds are then seeded with Pseudomonas aeruginosa, ISR strain 8-28-3. They are sacrificed at one and four hours, one, two, three, six, and ten days postburn for study.

25. (U) 74 07 - 75 05 Infected animals were sacrificed or died within six days postburn while the burned uninfected animals survived. Mean fibrinogen levels in the burned-infected animals were higher than in the burned animals after day 1 postburn. Plasminogen fell dramatically three days postburn in the infected animals but remained normal in the burned animals. Antiplasmin, but not antiactivator, activity was extremely elevated in the infected animals 6 days postburn. Crossed immunoelectrophoresis reveals that alpha-2 - acute phase globulin levels were increased 100-300 times normal in the infected animals. These studies indicate that fibrinolysis was probably impaired by burn wound infection in the rat and was closely related to their demise.

*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. 3A162110A821-00, COMBAT SURGERY

REPORT TITLE: FIBRINOGEN-FIBRIN DEGRADATION PRODUCTS IN THE THERMALLY INJURED ANIMALS: A MODEL OF THE BURNED SOLDIER

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

Investigators

W. Abe Andes, MD, Major, MC
Robert B. Lindberg, PhD
D.D. McEuen
J. P. Baron

Reports Control Symbol MEDDH-288 (R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A162110A821-00, COMBAT SURGERY

REPORT TITLE: FIBRINOGEN-FIBRIN DEGRADATION PRODUCTS IN THE THERMALLY INJURED ANIMALS: A MODEL OF THE BURNED SOLDIER

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

Period covered in this report: 1 July 1974 - 30 June 1975

Investigators: W. Abe Andes, MD, Major, MC
Robert B. Lindberg, PhD
D. D. McEuen
J. P. Baron

Reports Control Symbol MEDDH-288(R1)

Infection is the leading cause of death in the burned patient. To study the effects of uncomplicated and lethally-infected burn wounds on certain hematologic indices, a dorsal scald burn was administered to 250 rats. One group was then infected with Pseudomonas aeruginosa. These rats expired within 10 days. A burned, uninfected, group survived. Sequential determinations were made of fibrinogen, fibrinolysis, antiplasmins, plasminogen anti-activators, cultures, and autopsy findings.

Fibrinogen concentrations were higher in the infected animals within 2 days postburn and at day 3 ($p < 0.05$). Plasminogen levels fell precipitously 3 days postburn in the burned-infected group. Acid neutralization of antiplasmins was not entirely effective. Serum from infected animals markedly prolonged the lysis time of clots in a standard test system, lowered plasmin activity in the caseinolytic assay, and transiently prolonged the euglobulin lysis time.

The cause of these findings remains unknown. The marked fall in plasminogen in the infected animals may be entirely due to unneutralized antiplasmin activity and was coincident with a decline in fibrin-related antigens. These antiplasmins may exert deleterious effects on microcirculatory dynamics and were closely linked to the animals' demise.

Fibrinogen
Antiplasmins
Rats
Thermal Injury

FIBRINOGEN-FIBRIN DEGRADATION PRODUCTS IN THE THERMALLY INJURED ANIMALS: A MODEL OF THE BURNED SOLDIER

Infection remains the leading cause of death in the bruned patient despite advances in topical therapy, knowledge of pathogenesis, and newer antibiotics. The infected-burned rat model has been a reliable model of the severely burned patient, displaying similar gross and microscopic changes and reproducible bacteriology (1).

The importance of fibrinolytic disturbances in the burned or burned-infected animal is unknown at present. This study was designed to compare certain fibrinolytic measurements in burned and burned-infected rats in the hope that such information will permit improved therapy in patients with thermal injury.

MATERIALS AND METHODS

Animal Infection

Two-hundred and fifty male, Sprague-Dawley, Holtzman strain rats weighing 175-200 grams were anesthetized with pentobarbital (0.04 mg/gm body weight) administered intraperitoneally. A 30%, 3rd degree, dorsal scald burn was administered by previously described techniques (2). Ten ml of isotonic saline were administered intraperitoneally to each rat as a resuscitative measure. Animals were burned in groups of 50 at approximately one month intervals. Normal, unburned controls of the same strain and weight range were treated in the same manner to establish normal values. Within one hour postburn each group of 50 rats was randomly divided into two subgroups. In one subgroup the burn surface was seeded with one ml of a broth culture of Pseudomonas aeruginosa containing 10^8 organisms per ml (USAISR strain 8-28-3-63) isolated from a patient with burn wound sepsis. The animals were then housed in individual cages and offered food and water ad libitum.

Blood samples were taken by cardiac puncture in plastic syringes during ether anesthesia at one and four hours, and one, two, three, six, and ten days postburn. Ten to 20 animals from each subgroup were studied at each interval of sampling from at least three animals in each group. All blood studies were done on freshly drawn specimens with the exception of fibrinogen-fibrin related antigens (FR-antigen) and plasminogen determinations for which blood was stored at 4^oC and -66^oC respectively until analyzed. Serum was prepared by drawing blood in separate plastic syringes and allowing it to clot in glass tubes for two hours at room temperature. The tubes were then centrifuged and the serum pooled such that each sample tested represented

---

1. Teplitz,C. (1965): Pathogenesis of Pseudomonas vasculitis and septic lesions, Archives of Pathology 80, 297-307.

2. Walker,HL, and Mason,AD,Jr. (1968): A standard animal burn Journal of Trauma 8,1049-1051.

equal volumes from 3-4 normals or rats from each group of burned animals. Pseudomonas was inoculated into such sera and into TSB broth in certain experiments and tested after a 30 minute or 15 hour incubation at 37° C. Portions of such pooled serums were then added in the assays to be described using pooled serum from normal healthy animals as controls.

Laboratory Procedures

Fibrinogen levels were measured by a modified turbidimetric method using 9 volumes of blood collected in 1 volume of 3.2% (w/v) sodium citrate, (3). Platelets were counted in this blood using phase microscopy. Fr-antigen titers were measured by the staphylococcal-clumping method of Leavelle,, using 2 ml of whole blood collected with 1.0 mg soybean trypsin inhibitor and clotted with by neutralization by a modification of the Remmert and Cohen caseinolytic method using streptokinase as an activator. Blood was collected in 0.5% (w/v) epsilonaminocaproic acid (EACA) in 3.2% sodium citrate, 1 part per 9 parts blood (5,6). These assays were run in groups of 4 (Table I). Serial thrombin times were performed in 80 animals by the method of Brodsky and Lewis (1966) (7) one and 4 hours postburn and daily thereafter.

Studies of fibrinolysis inhibition using a timed clot lysis technique were performed with minor modifications of the method of von Kaulla and the same terminology will be employed (8,9). Antiplasmin activity was assayed using streptokinase-activated, human fibrinolysin obtained as Thrombolysi.(R) from Merck, Sharp & Dohme (Lot 1443). Equal amounts of fibrinogen (333) obtained from Sigma (Fraction 1, type 1, 72% clottable protein) were used in each clot lysis tube. The amount of fibrinolysin was doubled to 2.4 mg/ml (final concentration) because of marked prolongation noted with the original

---

3. Perfentjev,IA, Johnson,M, and Clifton,EE. (1953): The determination of plasma fibrinogen by turbidity with ammonium sulfate. Archives of Biochemistry and Biophysics 47:470-480.

4. Leavelle,DE, Mertens,BF, Bowie,EJW, and Owens,CA. (1971): Staphylococcal clumping on microtiter plates: A rapid, simple method for measuring fibrinogen split products. American Journal of Clinical Pathology 55,452-457.

5. Remmert,LF, and Cohen,P(1949): Partial purification and properties of a proteolytic enzyme of human serum. Journal of biological Chemistry. 181: 431-448.

6. Sherry,S.,Fletcher,AP, and Alkjaersig,N. (1959): Studies on enhanced fibrinolytic activity in man. Journal of Clinical Investigation 38:810-822.

7. Brodsky,I. and Lewis,HD (1966): Evaluation of fibrinolysis in hepatic cirrhosis, relation of serial thrombin time and euglobulin lysis time. American Journal of Clinical Pathology, 45,61-69.

8. Von Kaulla,KN, and Schultz,RL. (1958): Methods for the evaluation of human fibrinolysis:STudies with two combined techniques. American Journal of Clinical Pathology 29: 104-112, 1958.

(1.2 mg/ml) concentration (9). Fifty or 100 ul of pooled serum from normal burned, or burned-infected animals was added to the fibrinolysin solution before clotting with thrombin. Normal saline was used as a blank control. EACA was added in other experiments. EACA or serum was mixed and incubated for one-half hour at room temperature prior to clotting in the antiplasmin assay. The clots were then incubated at 27°C and observed for complete lysis. The euglobulin lysis time test was prepared and tested within one hour from the time of blood withdrawal. Duplicate or triplicate euglobulin tests were done on several animals on each day in each group. Antiactivator activity by the method of Aoki and von Kaulla, (1969) was studied utilizing half volumes (0.5 ml plasma + 7.5 ml deionized water) of the same reagents. Thrombin (from Parke Davis Co, Bovine origin) was dissolved in deionized water instead of glycerol and used within one-half hour of preparation. In later experiments, serum from normal, burned, or burned-infected rats was incubated for 3.5 hours with 12 mM (final concentration) 3,5-diiodosalicylic acid (Eastman chemicals) and then dialyzed for 16 hours as in Aoki and von Kaulla (1969). Such treated serum was then added to the antiplasmin and antiactivator assays.

RESULTS

Effects of Infection

Less than 2% of the rats died as a direct result of burning. There were no deaths among the uninfected burned animals after the day of burning. All burned-infected animals which were not sacrificed died within ten days of burning. Within two days of burning, the burned-infected animals developed signs of illness with decreased activity, poor feeding and petechial or ecchymotic lesions of the eschar. A few of these animals bled from the eschar, most commonly at the burned-unburned skin junction. Infected animals had invariable gross findings of infection including pulmonary lesions, invasion of the eschar, and abscess formation in the kidneys, peritoneal cavity, or mesentery. Burned animals healed their eschars within several weeks. The spleens and livers in the two groups were similar in appearance. Spleen cultures were positive for Pseudomonas aeruginosa only in infected animals. Such cultures occurred three or more days postburn. No apparent relation between the severity of gross findings in the infected animals and in vitro test was noted.

Laboratory Finding

Mean fibrinogen levels fell slightly during the first four hours postburn but rose subsequently to high levels (Fig. 1). The mean in the burned-infected animals remained higher than that in the burned animals after day 1 and the levels were significantly higher three days postburn ($P < 0.05$). FR-antigens were usually elevated as early as one hour postburn (Fig. 2) and peak levels occurred two days after burning. Platelet counts were higher in the burned animals than in the burned-infected animals but at no time significantly so. A return to normal was delayed in some way by infection (Fig. 3). Plasminogen levels in both groups of animals remained

9. Aoki N., and Von Kaulla KN. (1969): Inactivation of human serum plasminogen and antiactivator by synthetic fibrinolysis inducers. Thrombosis et Diathesis Haemorrhagica, 22: 251-262.

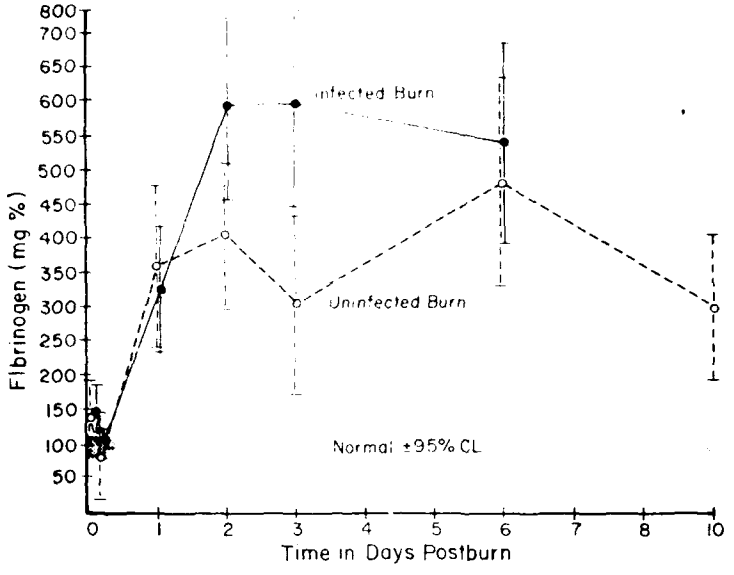


Figure 1. Fibrinogen concentration (mg%) in burned rats with and without Pseudomonas infection of the eschar. Results are expressed with 95% confidence limits (CL) of the mean indicated.

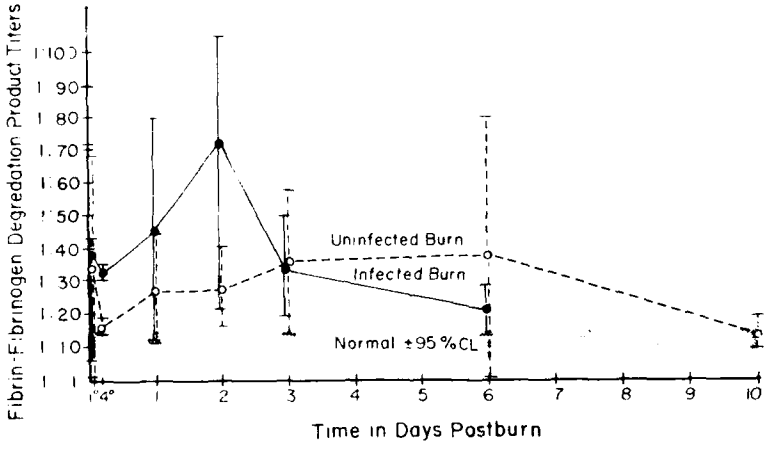


Figure 2. Fibrin-fibrinogen related antigen titer in burned rats with and without Pseudomonas infection. Results are expressed with 95% CL of the mean.

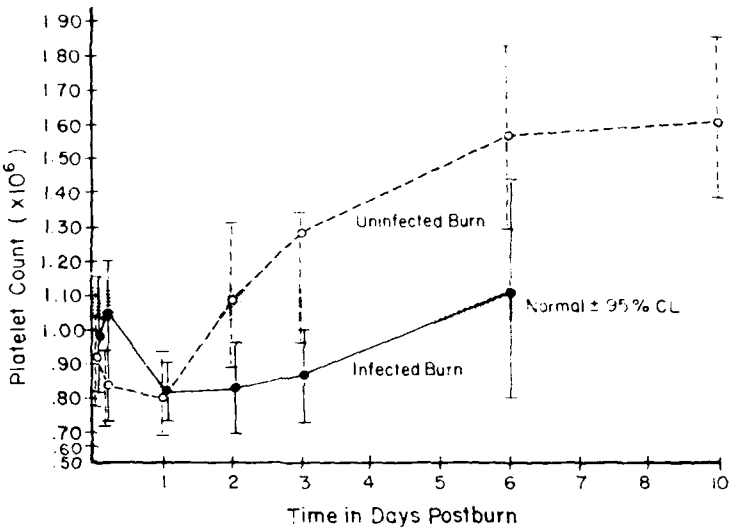


Figure 3. Platelet counts (X $10^6/mm^3$) in burned rats with and without infection during the postburn period. Results are expressed with 95% CL of the mean.

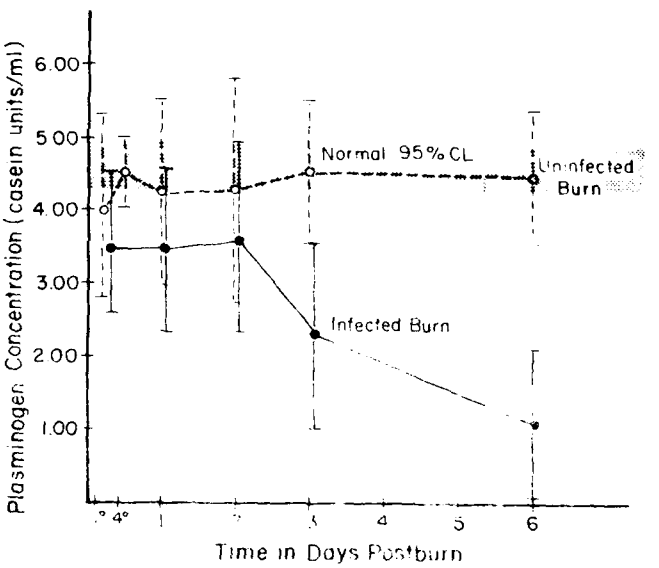


Figure 4. Plasminogen concentration (casein u/ml) in burned rats [illegible] and without infection. Results are expressed with [illegible] CL of the mean.

near normal for two days postburn. Such levels persisted in the non-infected animals, but by day three postburn values in the infected rats had dropped and by day 6 were markedly lower (Fig.4) than in the uninfected animals.

When 0.05 ml serum (1% of the total assay volume) from the infected animals 6 days postburn was added to the plasminogen assay of normal rats, caseinolysis was reduced as shown in Table 1. This occurrence in spite of heating the serum or treating the plasma with 1/6 N HCl and subsequent neutralization with 1/6 N NaOH or 1/3 N HCl and neutralization. Serum from normal or uninfected rats induced no reduction. Thus, acid neutralization of the antiplasmin activity was inadequate to abolish such effects (10).

Antiplasmin activity rose dramatically six days postburn in the infected animals. Euglobulin lysis times were prolonged in both groups on days 1 and 3 but even more markedly six days postburn in the infected animals. Antiactivator activity was increased three days postburn in the infected animals but had returned to normal by six days postburn. Inoculation of normal serum or broth with our strain of pseudomonas followed by 30 minute or 15 hour incubations caused no increase in antiplasmin or antiactivator activity as compared with the same serum or broth alone. The synthetic compound, 3-5-diiodosalicylic acid reduced the time for complete clot lysis in the antiactivator assay from 73 to 40 minutes when added to normal rat serum and from 290 to 42 minutes with the 3 days postburn infected serum. Serum antiplasmin activity was entirely abolished in all specimens tested (normal and infected rats) with the same 12mM concentration of the compound. The antiplasmin and antiactivator (not shown) activity of EACA was also eliminated. Alpha$_2$ - acute phase globulin but no α_1 macroglobulin levels were markedly elevated six days postburn in the infected animals (Table II).

No consistent pattern of serial thrombin times prolongation was seen at any sampling period postburn in any group. The mean prolongation of the thrombin time after a one hour incubation at 37^0 was 162% of the initial value in the entire group of burned, non-infected animals and 171% in the infected animals (mean in 10 normal rats was 155%). None of these differences was significant.

DISCUSSION

Classical Pseudomonas burn wound infection in the severely burned patient is usually a lethal complication (11). Rats, rabbits, guinea pigs, domestic pigs, primates, gerbils, goats, dogs, and mice have been used as experimentally burned models. The rat was chosen for this study because it is frequently used as a model in experimental studies of fibrinolysis.

---

10. Sherry,S.,Lindemeyer,RI, Fletcher, AP, and Alkjaersig,N. (1959) Studies on enhanced fibrinolytic activity in man. Journal of Clinical Investigation 38 810-822.

11. Curreri,PW, Lindberg,RB, and Divincenti,FC, Pruitt,BA, Jr., (1976). Intravenous administration of carbenicillin for septicemia due to Pseudomonas aeruginosa following thermal injury. Journal of Infectious Disease 122 Supplement, 540 547

TABLE I

Plasminogen Assays Performed 30 Minutes After Various Additions

Addition	Antiplasmin Neutralization		Value
50λ Normal serum	1/6 N HCl	1/6 N NaOH	6.0
50λ Normal serum	1/6 N HCl	1/6 N NaOH	6.0
50λ Infected serum*	1/6 N HCl	1/6 N NaOH	5.2
50λ Infected serum	1/6 N HCl	1/6 N NaOH	4.8
100λ Phosphate buffer	1/6 N HCl	1/6 N NaOH	3.1
100λ Normal serum	1/6 N HCl	1/6 N NaOH	3.4
100λ Infected serum	1/6 N HCl	1/6 N NaOH	2.5
100λ Infected serum+	1/6 N HCl	1/6 N NaOH	2.6
100λ Phosphate buffer	1/6 N HCl	1/6 N NaOH	5.4
100λ Pseudomonas inoculated, Infected serum≠	1/6 N HCl	1/6 N NaOH	3.1
100λ Pseudomonas inoculated Infected serum°	1/6 N HCl	1/6 N HaOH	3.2
100λ Infected serum	1/3 N HCl	1/3 N HaOH	3.1

*Equal volumes of sera pooled from

+Serum heated to 56°C for 30 minutes before addition

≠ 30 minute incubation at 37°C

°15 hour incubation at 37°C

TABLE 2

Alpha$_1$-macroglobulin and α_2-acute phase globulin levels six days postburn in the burned and burned-infected rat*.

	α_1-Macroglobulin	α_2-Acute Phase Globulin
Burned 1	7720	287
Burned 2	7720	306
Burned-Infected 1	6690	5372
Burned-Infected 2	6860	5443
Normal	7100-11800 mg/l	15-50 mg/l

*Determined by Ganrot (1973) and reported here with his kind permission. Both 1 and 2 refer to pooled sera from four animals in each burned or burned-infected group.

Pseudomonas must be specifically seeded onto the eschar of the rat to incite infection in the burned tissue. Twenty to thirty percent dorsal burns alone cause insignificant mortality in such a rat model whereas seeding the same wound with certain strains will cause virtually 100% mortality within a few days. Counts of 10^8 to 10^9 organisms per gram of tissue are attained in the burn wound by the time the animals expire (1). The present study confirmed these observations with similar gross manifestations and death of the animal within ten days.

Hyperfibrinogenemia is not unusual after burns or other trauma (12,13). An unexpected finding was the greater elevation in the infected animals. Such hyperfibrinogenemia has been explored experimentally but its causes are uncertain (14,15). Interestingly, changes in FR-antigen titers seemed to precede changes in fibrinogen concentration. Although such precedences have not been published regarding burned animals, increases in fibrinogen have been induced in rabbits by injecting homologous FR-antigen (14). Furthermore, FR-antigen titers are elevated as soon as they have been measured following human burns with tremendous fibrinogen elevations 24 or more hours postburn (16). The insignificant in-vitro fibrinolytic activity generated in each group at any stage postburn as measured by the serial thrombin time seems to relegate intravascular fibrinogenolysis to a minor role. Localized wound fibrinolysis remains as a possible cause of the elevation in FR-antigen (17,18).

Disseminated intravascular clotting (DIC) might account for elevated FR-antigen titers and be considered likely in animals suffering a gram negative infection. However, markedly elevated and fairly stable fibrinogen levels, little bleeding, insignificant platelet count changes, and the lack of postmortem evidence seem strong evidence against significant DIC. This is not surprising inasmuch as rather extreme conditions are usually required

---

12. Campbell,DA, Gabriel,LT, Van Hoek,DW. (1950): A study of the clotting mechanism in thermal burns. Surgical Forum 11, 515-518.

13. Yegge,J. (1970): Changes in blood coagulation and fibrinolysis during the postoperative period. American Journal of Surgery,119,225-232.

14. Bocci,V. and Pacini,A. (1973): Factors regulating plasma protein synthesis II. Influences of fibrinogenolytic products on plasma fibrinogen concentration. Thrombosis et Diathesis Haemorrhagica, 29, 63-65.

15. Kropatkin,ML, and Izak G. (1968): Studies of the hypercoagulable state: the regulation of fibrinogen production in experimental hyper and hypofibrinogenemia. Thrombosis et Diathesis Haemorrhagica, 19: 547-555.

16. Andes, WA (1974): Acute hematologic changes in the severely burned patient. Abstract and Presentation, Program, American Burn Assn., Dallas, Texas.

17.Meyers,A. (1972): Fibrin split products in the severely burned patient. Archives of Surgery, 105, 404-407.

18. Merskey, C. (1973): Editorial: Definbrination syndrome or . . . ? Blood 41: 599-603.

to induce DIC in the rat. Such maneuvers as administration of epsilonamino-caproic acid, use of pregnant animals, or prolonged high dose endotoxin infusion are usually employed for such induction (19,20).

The fall in plasminogen that we observed during the postburn period in the infected animals has apparently not been reported under such conditions before. Other investigators have noted similar changes, but under other conditions employing staphylococcal infection (21). In selected experiments it has beensuggested that depletion of plasminogen activator or inhibition of its release by humoral factors causes reduced fibrinolytic activity (22). Adsorption of the enzyme onto fibrin as it forms has also been suggested as one possible mechanism of depletion (11). Diffusion of plasminogen and activator into thrombi in excess of their usual inhibitors probably best explains effective in-vivo fibrinolytic activity and possible lowered systemic levels of the proenzyme in other circumstances (23).

Inasmuch as lowered levels of plasminogen may represent a serious handicap to proper remodeling of the microcirculation, studies were undertaken to evaluate possible mechanisms of its fall. Decreases due to excessive utilization or inhibition seemed the most likely possibilities. Plasminogen fall and antiplasmin activity without infection have been noted by other investigators but the mechanisms and importance of the active compounds are unclear. In the studies by Horne et al (1973) (24), a transient, small decrease in α_1- globulin levels was noted after streptokinase (a plasminogen activator) or plasmin (human or rat) injection implying that consumption of the globulin occurred as a response to the formation or introduction of plasmin in the rat. Inhibition of fibrin-plate lysis was also noted in these studies. Ganrot (25) noted that α_2-acute

---

19. Schoendorf,TH, Rosenberg M, and Beller,FK (1971): Endotoxin-*induced desseminated* intravascular coagulation in nonpregnant rats. American Journal of Pathology, 65: 51-58.

20. Margaretten,W., Zunker,HE, and McKay,DG (1964): Production of the generalized Schwartzman reaction in pregnant rats by intravenous infusion of thrombin. Laboratory Investigation, 13, 552-559.

21. Lipinski,B., Lybulska,J.,Worowski,K, and Jeljaszewicz,J (1969): Blood clotting and fibrinolysis in experimental staphylococcal infection. Pathologia *et Microbiologia* 34: 295-304.

22. Bergstein,JM, and Michael,AF (1973): Renal cortical fibrinolytic activity in the rabbit following one or two doses of endotoxin. Thrombosis et Diathesis Haemorrhagica. 29: 27-29.

23. Wolf,P. (1968): Fibrinolytic activity and coronary artery disease. Lancet. 1: 1312.

24. Horne,CHW, Forbes,CD, and Prentice,CRM (1973): Antiplasmin activity of *rat serum slow globulins*. British Journal of Haematology. 24: 115-121.

25. Ganrot K. (1973): Rat -acute phase globulin, a human macroglobulin homologue: Interaction with plasmin and trypsin. Biochimica et Biophysica Acta 322: 62-67.

phase globulin was bound to plasminogen using crossed immunoelectrophoresis and autogradiography. That this α_2 - globulin is causing some of the changes in fibrinolysis noted here is likely, but unproven until the pure protein can be used in the assays.

The activity of slow α_1 and α_2 globulins in the rat has not been otherwise defined to our knowledge (26). The tremendous elevation in antiplasmin activity in this study may have been related to inflammatory processes occurring in the infected animals. Such elevations may be similar to those of other, nonspecific, "acute phase" reactants such as haptoglobin or C-reactive protein. Whether the spectrum of action noted here is limited to antiplasmin activity is not absolutely clear on the basis of these experiments. That there is limited antiactivator activity present seems likely in view of the differences noted in our antiactivator (transient activity) and antiplasmin (progressively greater activity) tests. Also, inhibition of proteolysis in the plasminogen assay (employing excess streptokinase as activator in addition to any endogenous activator) when small amounts of infected serum are added, would seem to more closely define the serum activity as an antiplasmin. The prolongation in euglobulin lysis time might not help differentiate between antiactivator and antiplasmin activity, but the pattern of prolongation seems to parallel more closely the latter since marked inhibition of both clot lysis and casein proteolysis occurred 6 days postburn in the infected animals.

Thus, marked antiplasmin(s) activity has been found and studied in a burned rat model infected with a lethal Pseudomonas aeruginosa burn wound infection. Such antiplasmin activity may account for inordinate fibrin deposits in complications involving accelerated or even physiologic coagulation after human burns preliminary studies from this laboratory or other trauma. Deposition of fibrin with imparied mechnaisms for its removal may impair microcirculatory flow. Antiplasmins may affect results of several commonly employed clinical tests such as the euglobulin lysis time and plasminogen assay but are not commonly studied in interpreting these tests. The outcome of other clinical conditions associated with infection or trauma, may also be influenced by such antiplasmin activity and warrants similar investigations as those described here.

PUBLICATIONS AND/OR PRESENTATIONS:

None

---

26. Weimer, HE and Benjamin, DC (1965): Immunochemical detection of an acute phase protein in rat serum. American Journal of Physiology 209: 736-744.

REFERENCES

1. Teplitz,C. (1965): Pathogenesis of Pseudomonas vasculitis and septic lesions. Archives of Pathology 80: 297-307.

2. Walker,HL, and Mason,AD,Jr.(1968): A standard animal burn. Journal of Trauma 8: 1049-1051.

3. Perfentev,IA, Johnson, M, and Clifton, EE.(1953): The determination of plasma fibrinogen by turbidity with ammonium sulfate. Archives of Biochemistry and Biophysics 47: 470-480.

4. Leavelle,DE, Mertens,BF, Bowie,EJW, and Owens, CA. (1971): Staphylococcal clumping on microtiter plates: A rapid, simple method for measuring fibrinogen split products. American Journal of Clinical Pathology 55: 452-457.

5. Remmert,LF, and Cohen,P (1949): Partial purification and properties of a proteolytic enzyme of human serum. Journal of Biological Chemistry. 181: 431-448.

6. Sherry,S., Fletcher,AP, and Alkjaersig,N. (1959): Studies on enhanced fibrinolytic activity in man. Journal of Clinical Investigation 38: 810-822.

7. Brodsky,I. and Lewis, HD (1966): Evaluation of fibrinolysis in hepatic cirrhosis, relation of serial thrombin time and euglobulin lysis time American Journal of Clinical Pathology 45: 61-69.

8. Von Kaulla,KN, and Schultz,RL. (1958): Methods for the evaluation of human fibrinolysis: Studies with two combined techniques. American Journal of Clinical Pathology 29: 104-112, 1958.

9. Aoki N., and Von Kaulla KN. (1969): Inactivation of human serum plasminogen and antiactivator by synthetic fibrinolysis inducers. Thrombosis et Diathesis Haemorrhagica,22: 251-262.

10. Sherry, S., Lindemeyer, RI, Fletcher, AP, and Alkjaersig,N. (1959): Studies on enhanced fibrinolytic activity in man. Journal of Clinical Investigation. 38: 810-822.

11. Curreri,PW,Lindberg,RB, Divincenti, FC, Pruitt, BA, Jr., (1970): Intravenous administration of carbenicillin for septicemia due to Pseudomonas aeruginosa following thermal injury. Journal of Infectious Disease. 122, Supplement, 540-547.

12. Campbell,DA, Gabriel,LT, Van Hoek,DW. (1950): A study of the clotting mechanism in thermal burns. Surgical Forum 11: 515-518.

13. Yegge,J. (1970): Changes in blood coagulation and fibrinolysis during the postoperative period. American Journal of Surgery 119: 225-232.

14. Bocci,W. and Pacini,A. (1973): Factors regulating plasma protein synthesis II. Influences of fibrinogenolytic products on plasma fibrinogen concentration. Thrombosis et Diatheisis Haemorrhagica, 29: 63-65.

15. Kropatkin,ML, and Izak G. (1968): Studies on the hypercoagulable state: the regulation of fibrinogen production in experimental hyper and hypofibrinogenemia. Thrombosis et Diathesis Haemorrhagica. 19: 547-555.

16. Andes,WA (1974): Acute hematologic changes in the severly burned patient. Abstract and Presentation, Program American Burn Assn., Dallas, Texas.

17. Meyers,A. (1972): Fibrin split products in the severely burned patient. Archives of Surgery, 105: 404-407.

18. Merskey,C. (1973): Editorial: Definbrination syndrome or...? Blood 41: 599-603.

19. Schoendorf,TH, Rosenberg M, and Beller,FK (1971): Endotoxin-induced desseminated intravascular coagulation in nonpregnant rats. American Journal of Pathology, 65: 51-58.

20. Margaretten,W., Zunker,HE, and McKay,DG (1964): Production of the generalized Schwartzman reaction in pregnant rats by intravenous infusion of thrombin. Laboratory Investigation 13: 552-559.

21. Lipinski,B., Lybulska,J., Worowski,K, and Jeljaszewicz, J (1969): Blood clotting and fibrinolysis in experimental staphylococcal infection. Pathologia et Microbiologia 34: 295-304.

22. Bergstein, JM, and Michael, AF (1973): Renal cortical fibrinolytic activity in the rabbit following one or two doses of endotoxin. Thrombosis et Diathesis Haemorrhagica. 29: 27-29.

23. Wolf, P. (1968): Fibrinolytic activity and coronary artery disease. Lancet. 1: 1312.

24. Horne, CHW, Forbes, CD, and Prentice, CRM (1973): Antiplasmin activity of rat serum slow globulins. British Journal of Haematology. 24: 115-121.

25. Ganrot K. (1973): Rat - acute phase globulin, a human, macroglobulin homologue: Interaction with plasmin and trypsin. Biochimica et Biophysica Acta 322: 62-67.

26. Weimer,HE, and Benjamin, DC (1965): Immunochemical detection of an actue-phase protein in rat serum. American Journal of Physiology 209: 736-744.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0F 6392	75 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
75 07 01	K. COMP	U	U	NA	NL	[X] YES [ ] NO	A. WORK UNIT

10. NO. CODES.	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62110A	3A162110A821	00	112
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) Platelet Behavior and Postsurgical Hemorrhage in Dogs Anticoagulated with Ancrod: A Model of Changes Influencing Surgery in Injured Troops(44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
75 01	75 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. K DOLS (In thousands)
FISCAL YEAR PRECEDING 75	.9	26
FISCAL YEAR CURRENT		

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

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

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

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME: Clement L Slade, MD, CPT, MC
TELEPHONE: 512-221-3411
SOCIAL SECURITY ACCOUNT NUMBER:

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

ASSOCIATE INVESTIGATORS
NAME:
NAME: DA

22. KEYWORDS (Precede EACH with Security Classification Code): (U) Ancrod; (U) Platelets; (U) Anticoagulation; (U) Defibrination; (U) Aggregation; (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 study platelet behavior following anticoagulation with the defibrinating enzyme Ancrod. Platelet aggregation is the most important factor influencing hemostasis following defibrination with Ancrod. Ancrod is a very potent anticoagulant with few undesirable side effects. Vein reconstructions following the penetrating or crush injuries which occur frequently in modern warfare have not been possible with conventional anticoagulants. With Ancrod it may be possible to attempt vascular reconstruction in cases which were previously regarded as inoperable.

24. (U) Adult mongrel dogs are defibrinated with an infusion of Ancrod 2 u/kg administered over a two-hour period. Platelet aggregation is studied immediately before defibrination, immediately after defibrination, and daily thereafter. Defibrination is maintained with daily intramuscular doses of Ancrod, 1 u/kg. In addition to platelet aggregation, fibrinogen levels, fibrin-split product levels, prothrombin times, partial thromboplastin times, and thrombin clotting times are also measured.

25. (U) 75 01 - 75 06 There is significant inhibition of platelet aggregation following defibrination with Ancrod. The decrease is most pronounced for at least 48 hours following defibrination. Platelet aggregation returns toward normal, but is still significantly inhibited 96 hours after defibrination. There is a negative correlation between platelet aggregation and fibrin degradation product level.

*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. 3A162110A821-00, COMBAT SURGERY

REPORT TITLE: PLATELET BEHAVIOR AND POSTSURGICAL HEMORRHAGE IN DOGS ANTICOAGULATED WITH ANCROD--A MODEL OF CHANGES INFLUENCING SURGERY IN INJURED TROOPS

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

1 July 1974 - 30 June 1975

Investigator:

Clement L. Slade, M.D., Captain, MC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: PLATELET BEHAVIOR AND POSTSURGICAL HEMORRHAGE IN DOGS ANTICOAGULATED WITH ANCROD--A MODEL OF CHANGES INFLUENCING SURGERY IN INJURED TROOPS

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

Period covered in this report: 1 July 1974 - 30 June 1975

Investigators: Clement L. Slade, M.D., Captain, MC

Reports Control Symbol MEDDH-288(R1)

Ancrod is a thrombin like enzyme derived from the venom of the Malayan pit vipor. This agent is a potent anticoagulant with few undesirable side effects. When Ancrod is administered parenterally, there is a rapid lowering of plasma fibrinogen and the other clotting factors are not affected. Early investigators reported no changes in platelet aggregation following administration of the drug, while a recent report suggested a transient defect in platelet aggregation following defibrination with Ancrod. The purpose of this study was to study platelet aggregation over a 96 hour period following defibrination with Ancrod and to attempt to correlate any changes in platelet aggregation with fibrin degradation following administration of Ancrod.

Eleven dogs were treated with an intravenous infusion of Ancrod and maintained in a fibrinogenopenic state for 96 hours with supplementary intramuscular doses of Ancrod. There was a dramatic inhibition of platelet aggregation for 48 hours following defibrination with a gradual return towards normal at 72 and 96 hours. Inhibition of aggregation was maximum at the time of the highest fibrin degradation product levels. These results suggest that vascular surgical procedures on patients defibrinated with Ancrod should be delayed until after fibrin degradation product levels have returned to a steady state.

Ancrod
Platelets
Anticoagulation
Defibrination
Aggregation
Dogs

PLATELET BEHAVIOR AND POSTSURGICAL HEMORRHAGE IN DOGS ANTICOAGULATED WITH ANCROD--A MODEL OF CHANGES INFLUENCING SURGERY IN INJURED TROOPS

Ancrod is a thrombin like enzyme derived from the venom of the Malayan pit vipor Ancistrodon Rhodostoma. When this substance is injected parenterally it produces a rapid lowering of the plasma fibrinogen level and there is little effect on the other coagulation factors. Platelet counts are similarly unaffected. Reports of the in vitro aggregation of platelets from animals and man following defibrination with Ancrod have been conflicting. The purpose of this study was to measure platelet aggregation induced with adenesine diphosphate before and after defibrination with Ancrod.

A group of eleven adult, heart worm free, mongrel dogs was used in this study. Blood samples were drawn by venipuncture of the jugular vein using plastic syringes and immediately citrated. Samples were drawn prior to defibrination with Ancrod, and at one, twenty-four, forty-eight, and ninety-six hours following defibrination. The Ancrod used in these studies was supplied by Dr. Joseph Donahoe of Abbott Laboratories and had an activity of 100 NIH units per ml. Defibrination was achieved with an intravenous infusion of Ancrod, 2 units per kilogram of body weight diluted in 250cc of normal saline and administered over two hours. Defibrination was maintained throughout the 96 hour period with an intramuscular injection of Ancrod 1 unit per kilogram given every 24 hours. Defibrination was monitored with determinations of fibrinogen levels by a thrombin clottable protein technique. Fibrin degradation product levels were measured on each blood sample with a tube dilution modification of the Thrombowellco test. The platelet aggregation studies were done one hour after venipuncture on a Cronolog aggregometer using platelet rich plasma diluted to a count of 300,000 with platelet poor plasma derived from the same sample. Aggregation was initiated with ADP added to achieve a final concentration of 5 micromolar.

Figure one shows aggregation patterns from one dog in the study group. A is the pattern prior to defibrination, B the pattern at one hour after defibrination, C at 24 hours after defibrination, D at 48 hours after defibrination and E at 96 hours after defibrination. There is profound inhibition of aggregation immediately after defibrination with a gradual return toward normal over the 96 hour period. Aggregation was quantified by measuring the slope of the initial deflection on each curve.

The data for all eleven dogs is tabulated in Figure 2. In Figure 2 the Y axis is the slope of the initial deflection and the X axis is time. The dots are the values for each dog at a point in time. The open circles are the mean for the group at each point in time. For the entire group, as for the individual dog in Figure 1, there is profound inhibition of aggregation following defibrination with a return toward normal after 48 hours.

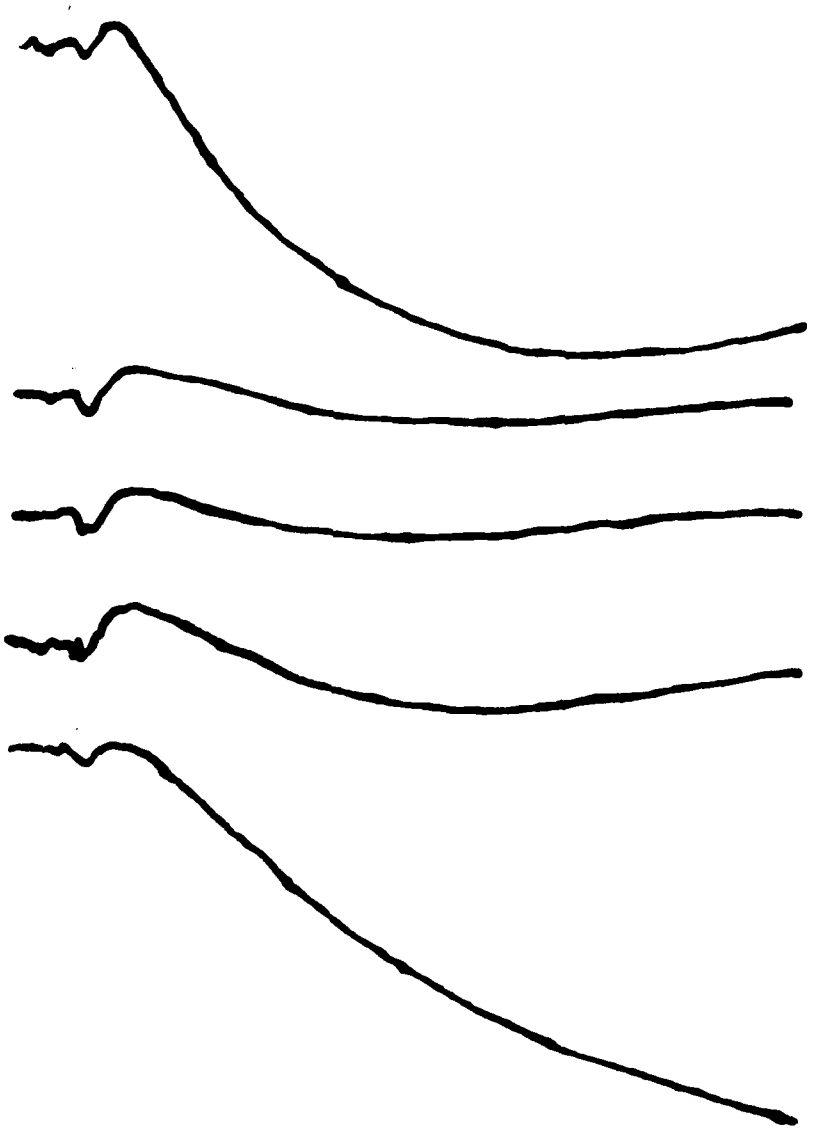


Figure 1. Platelet aggregation following defibrination with Ancrod. The vertical axis is optical density. The horizontal is time. A) Pre infusion B) One hour post infusion C) 24 hours D) 48 hours E) 96 hours.

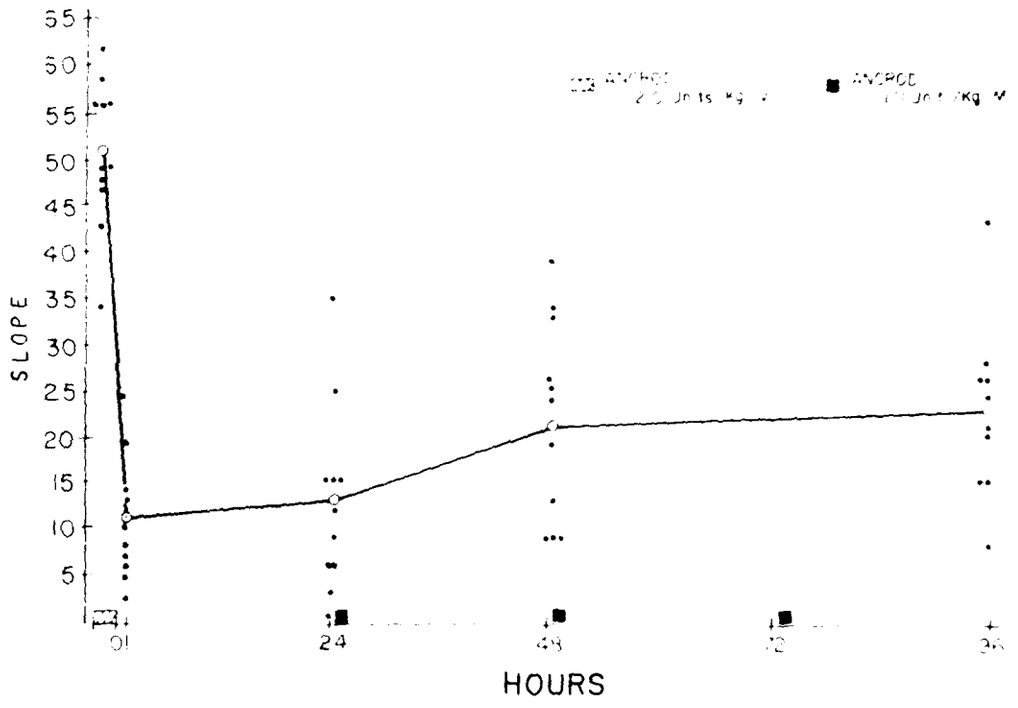


Figure 2. Platelet aggregation of the study group following defibrination with Ancrod. The vertical axis is the slope of the initial deflection of the aggregation curve. The horizontal axis is time after infusion. The cross hatched rectangle indicates the initial infusion. The darkened squares indicate the supplemental injections. The open circles are the means for the group at each point.

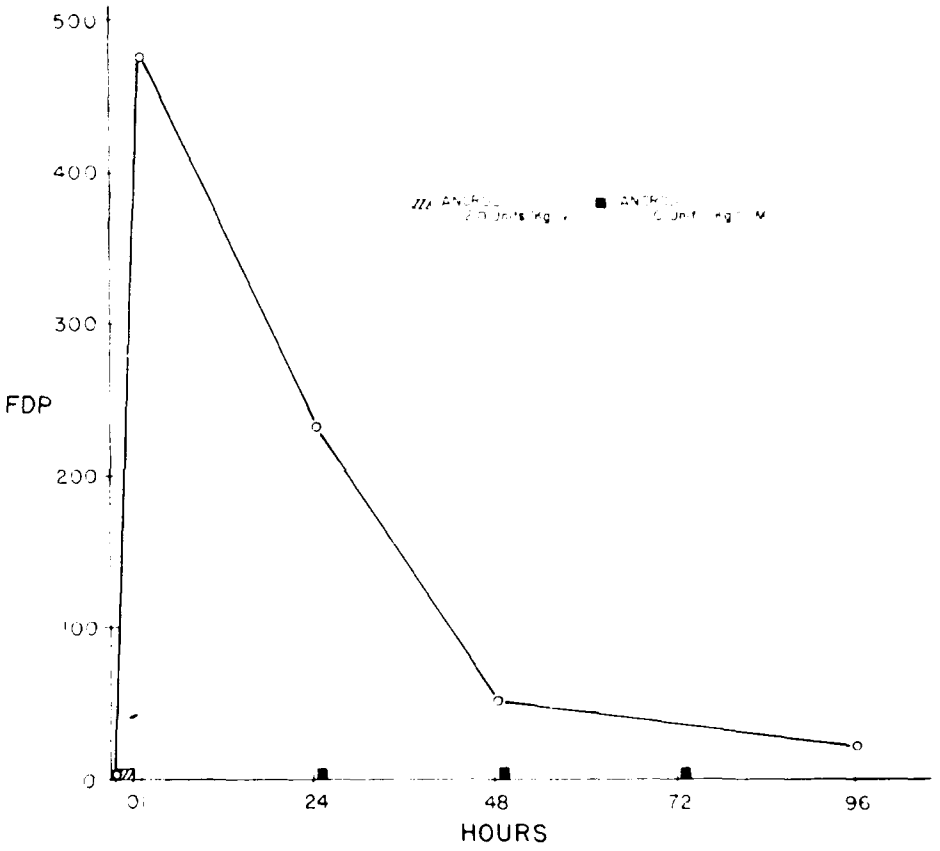


Figure 3. Fibrin degredation product levels following defibrination with Ancrod expressed in micrograms per milliliter.

When the data from Figure 2 is submitted to a Scheffe Test for multiple contrast we can say with 95% confidence that A $>$ D,E, $>$ B,C, but that there is not a significant difference between D and E or B and C. Further, we can say with 99% confidence that A $>$ B,C. Thus, we can say that the differences in aggregation between the pre-defibrination value and the values for 1 and 24 hours after aggregation are real as is the return toward normal aggregation shown by the group at 48 and 96 hours. Further there is still a real difference between aggregation at 96 hours and the pre-infusion value.

Figure 3 shows fibrin degradation product levels after administration of Ancrod. Open circles indicate the means of the group at each point in time and are expressed in microliters per milliliter. As expected there is a dramatic rise in the FDP level following the initial Ancrod infusion with a fall over the 96 hours despite the subsequent administration of Ancrod. When fibrinogen levels were measured by the Thrombin clottable protein technique on each sample, there was no measurable fibrinogen at any time following defibrination. There appears to be a negative correlation between platelet aggregation and fibrin degradation product level but the results await statistical testing.

SUMMARY

There is a significant inhibition of platelet aggregation following defibrination with Ancrod. The decrease is most pronounced for at least 48 hours following defibrination. Platelet aggregation returns to normal but is still significantly inhibited 96 hours after defibrination. There is a negative correlation between platelet aggregation and fibrin degradation product level.

REFERENCES: None

PUBLICATIONS AND/OR PRESENTATIONS

Slade CL. "Platelet aggregation in dogs anticoagulated with Ancrod" presented at the V Congress of the International Society of Thrombosis and Hemostasis, Paris, France July 21-26 1975.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OF 6393	75 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
75 07 01	D. CHANGE	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO. / CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62110A	3A162110A821	00	110
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) Low Flow Vein Anastomosis in Dogs Anticoagulated with Ancrod: A Model for Blood Vessel Repair in Injured Troops (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS:
003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
75 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: 75	.2	6
FISCAL YEAR CURRENT: 76	.8	22

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

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

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

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME: Clement L Slade, MD, CPT, MC
TELEPHONE: 512-221-3411
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: Willard A Andes, MD, MAJ, MC
NAME: John W Sagartz, CPT, VC DA

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Coagulation; (U) Ancrod; (U) Vascular Surgery; (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) Ancrod is a thrombin like enzyme derived from the venom of the Malayan pit viper. It is a very potent anticoagulant with few undesirable side effects. Vein reconstructions following the penetrating or crush injuries which occur frequently in modern warfare have not been possible with conventional anticoagulants. With Ancrod it may be possible to perform vascular reconstruction in cases which were previously regarded as inoperable. The purpose of this study is to develop a vascular surgical model which is highly predisposed to thrombosis and to utilize this model in the study of Ancrod as a surgical anticoagulant.

24. (U) The femoral vein was surgically exposed bilaterally in a group of six adult Mongrel dogs. The vein was then cut transversely and reanastomosed. Venograms were performed at two and four weeks post operatively.

25. (U) 75 01 - 75 06 Most of the vein anastomoses remained patent at two weeks post operatively. Veins which were thrombosed at two weeks had recanalized and were patent by four weeks postoperatively. The dog is thus not an adequate model for these studies. It may be necessary to continue this work in primates.

*Available to contractors upon originator's approval

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

ANNUAL PROGRESS REPORT

PROJECT NO. 3A162110A821-00, COMBAT SURGERY

REPORT TITLE: LOW FLOW VEIN ANASTOMOSIS IN DOGS ANTICOAGULATED WITH ANCROD--A MODEL FOR BLOOD VESSEL REPAIR IN INJURED TROOPS

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

1 July 1974- 30 June 1975

Investigators:

Clement L. Slade, M.D., Captain, MC
Willard A. Andes, M.D., Major, MC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A162110A821-00, COMBAT SURGERY

REPORT TITLE: LOW FLOW VEIN ANASTOMOSIS IN DOGS ANTICOAGULATED WITH ANCROD--A MODEL FOR BLOOD VESSEL REPAIR IN INJURED TROOPS

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

Period covered in this report: 1 July 1974 - 30 June 1975

Investigators: Clement L. Slade, M.D., Captain, MC
Willard A. Andes, M.D., Major, MC

Reports Control Symbol MEDDH-288(R1)

Ancrod is a thrombin like enzyme derived from the venom of the Malayan pit vipor. This agent is a very potent anticoagulant with few undesirable side effects. It is possible this anticoagulant could be used as an adjunct in vascular surgical procedures which are predisposed to thrombosis. The purpose of this study is to develop a model vascular surgical procedure which is highly predisposed to thrombosis and to use this model in the study of Ancrod as a surgical anticoagulant.

Femoral vein anastomoses were performed bilaterally on six adult mongrel dogs who were not anticoagulated. Follow up venograms at two and four weeks postoperatively, revealed a high rate of patency of the anastomosis. Veins which had clotted at two weeks postoperatively, were found to have recanalized by four weeks postoperatively. The dog is thus not an adequate model for these studies. It may be necessary to use primates to continue this work.

Coagulation
Ancrod
Vascular surgery
Dogs

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA OE 6983	75 07 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
74 07 01	K. COMP	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62110A	3A162110A821	00	113
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)* (U) Evaluation of Enzymatic Debridement in Burned Hands of Soldiers with Thermal Injury (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*
003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
74 01	74 10	DA	C. In-House

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

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR — PRECEDING	75	.3	10
FISCAL YEAR — CURRENT			

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

20. PERFORMING ORGANIZATION
NAME:* US Army Institute of Surgical Research
ADDRESS:* Surgical Study Branch
Fort Sam Houston, Texas 78234
PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME:* Hugh D. Peterson, MD, COL, MC
TELEPHONE: 512-221-3301
SOCIAL SECURITY ACCOUNT NUMBER
ASSOCIATE INVESTIGATORS
NAME:
NAME: DA

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Travase; (U) Sutilains; (U) Burned hands; (U) Grafting; (U) Humans

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

23. (U) To evaluate the efficacy of enzymatic debridement of burned hands with an assessment of grafting, early active motion, and final functional results in soldiers with thermal injury.

24. (U) Comparable hand burns, seen within the first 72 hours, will be treated with b.i.d. dressing changes and an active range of motion program with the dressing kept continually moist with saline. The enzyme-treated hands will be covered b.i.d. with Travase. The nonenzyme-treated hands will be covered with either the base of the Travase minus the enzyme or saline soaks. No topical chemotherapy will be used. The wound will be monitored for burn wound sepsis with both biopsy and surface culture. Any evidence of deterioration of the wound will result in cessation of enzymatic debridement and application of a chemotherapeutic agent.

25. (U) 74 07 - 74 10 All burned hands studied in Travase resulted in rapid dissolution of the surface of the eschar resulting in a soft wet wound allowing earlier range of motion but most importantly not resulting in a graftable base any sooner than the saline soaked hands. The results of this study suggest that although the eschar starts separating earlier with enzymatic debridement a graftable base is not achieved any earlier and perhaps the only role of enzymatic debridement is to soften the eschar which may result in the need for less escharotomies and allow for retention of more phalanges in severely burned hands. This will await further investigation.

*Available to contractors upon originator's approval

DD FORM 1 MAR 68 1498 PREVIOUS EDITIONS OF [illegible] FORM ARE OBSOLETE DD FORMS 1498A 1 NOV 68 AND 1498 1 MAR 68 FOR ARMY USE) ARE [illegible]

FINAL REPORT

PROJECT NO. 3A162110A821-00, COMBAT SURGERY

REPORT TITLE: EVALUATION OF ENZYMATIC DEBRIDEMENT IN BURNED HANDS OF SOLDIERS WITH THERMAL INJURY

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

1 July 1974 - 30 October 1974

Investigator:

Hugh D. Peterson, D.D.S., M.D., Colonel, MC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A162110A821-00, COMBAT SURGERY

REPORT TITLE: EVALUATION OF ENZYMATIC DEBRIDEMENT IN BURNED HANDS OF SOLDIERS WITH THERMAL INJURY

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

Period covered in this report: 1 July 1974 - 30 October 1974

Investigator: Hugh D. Peterson, D.D.S., M.D., Colonel, MC

Reports Control Symbol MEDDH-288(R1)

The goal of this protocol was to evaluate the efficacy of early enzymatic debridement of burned hands as far as early grafting, earlier motion and final function. Six patients were studied and in each it was found that on the hand treated with Travase, the enzyme to be evaluated, rapid dissolution of the eschar occurred. However the enzyme failed to yield a graftable wound base any more rapidly than the saline soaked hand. The hands treated with enzymes did not soften appreciably more than the saline soaked hands and both saline soaked and enzyme treated hands softened more rapidly than hands treated without soaks. The rapid dissolution of the eschar per se with enzymes can be considered of little clinical value since its failure to yield a graftable base either because of necrotic fat, which is not debrided by the proteolytic enzyme, or slimy deep dermis which resists enzymatic activity, makes it inapplicable as a therapeutic modality for burned hands.

The only possible application that can be seen for Travase in the burned hand is to soften the eschar rapidly which perhaps would substitute for digital escharotomies in terms of phalangeal survival. It can be stated unequivocally that in this study enzymatic debridement did not lead to more rapid grafting nor better early motion. Its evaluation as a replacement for digital escharotomies would require another study which is not anticipated at this time. This protocol is therefore terminated as we have been unable to discern any real benefit from enzymatic debridement of burned hands no matter what the depth of the burn.

Humans
Grafting
Travase
Sutilains
Burned hands

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA 0E 6984	75 07 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISP'N INSTR'N	8b. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
74 07 01	D. CHANGE	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62110A	3A162110A821	00	114
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)* (U) A Prospective Comparison Study of Sulfamylon and Silver Sulfadiazine in the Treatment of Burned Troops (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
73 12	Cont	DA	C. In-House

17. CONTRACT/GRANT Not Applicable

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:*

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 75	.5	13
	CURRENT 76	.5	16

19. RESPONSIBLE DOD ORGANIZATION

NAME:* US Army Institute of Surgical Research

ADDRESS:* Fort Sam Houston, Texas 78234

RESPONSIBLE INDIVIDUAL

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

TELEPHONE: 512-221-2720

20. PERFORMING ORGANIZATION

NAME:* US Army Institute of Surgical Research
Surgical Study Branch

ADDRESS:* Fort Sam Houston, Texas 78234

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

NAME:* Hugh D Peterson, MD, COL, MC

TELEPHONE: 512-221-4253

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Arthur D Mason, Jr, MD

NAME: DA

21. GENERAL USE

FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Topical Therapy; (U) Burn Injury; (U) Sulfamylon (mafenide); (U) Silvadene (silver sulfadiazine); (U) Humans

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

23. (U) To compare silver sulfadiazine with Sulfamylon in six groups of burned soldiers two groups by age 16 to 40, and greater than 40, and three by burn - size 30 to 50 per cent, to 70 per cent, and greater than 70 per cent. The parameters to be evaluated are survival, acid base balance, pain of application, bacteriology of the burn wound, pulmonary function, and clinical status of the injured troops.

24. (U) Burns greater than 30 per cent, seen within the first 72 hours, will be randomized by pairs in the aforementioned groups and placed in one of the two agents. The pulmonary functions, acid base balance, and wound bacteriology will then be followed meticulously until the eschar is entirely separated or until the patient has expired.

25. (U) 74 07 - 75 06 Through June 1974, 80 patients were studied in the comparison study. At that time it seemed that the patients in silver sulfadiazine were doing better. Sulfamylon was discontinued and only silver sulfadiazine was used from June until October at which time 104 patients with burns greater than 30% had been studied with a LA 50 on the probit of 64.5% compared to a historical probit of the last 5 years of Sulfamylon with an LA 50, age 15 to 40 of approximately 50%. Since that time, silver sulfadiazine has been used as an initial agent with Sulfamylon held in reserve for wound complications and used as soaks. Further evaluation of the burn wound in silver sulfadiazine treated patients is continuing with thought towards a protocol using no topical agent to compare with silver sulfadiazine.

*Available to contractors upon originator's approval

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

ANNUAL PROGRESS REPORT

PROJECT NO. 3A162110A821-00, COMBAT SURGERY

REPORT TITLE: A PROSPECTIVE COMPARISON STUDY OF SULFAMYLON AND SILVER SULFADIAZINE IN THE TREATMENT OF BURNED TROOPS

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

1 July 1974 - 30 June 1975

Investigators:

Hugh D. Peterson, DDS, MD, Colonel, MC
Arthur D. Mason, Jr., MD
Basil A. Pruitt, Jr., MD, Colonel, MC

Reports Control Symbol MEDDH-283(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A162110A821-00, COMBAT SURGERY

REPORT TITLE: A PROSPECTIVE COMPARISON STUDY OF SULFAMYLON AND SILVER SULFADIAZINE IN THE TREATMENT OF BURNED TROOPS

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

Period covered in this report: 1 July 1974 - 30 June 1975

Investigators: Hugh D. Peterson, D.D.S., M.D., Colonel, MC
Arthur D. Mason, Jr., M.D.
Basil A. Pruitt, Jr., M.D., Colonel, MC

Reports Control Symbol MEDDH-288(R1)

Through June 1974 80 patients had been studied in the comparison group and it appeared that the silver sulfadiazine patients fared better both by gross survival, fewer early pulmonary complications, less pain on application and a better state of well being in the early post burn period. It was elected at that time to discontinue the use of Sulfamylon and use only silver sulfadiazine as the initial topical wound care agent. This was done from June through October of 1974. In November three patients were treated with Sulfamylon burn cream as their initial topical agent. The agent required removal from two of these patients because of hyperventilation, they were then placed in Silvadene and were eventual survivors. The other patient expired while receiving a combination of Sulfamylon burn cream and Sulfamylon soaks to excised areas. Subsequent statistical comparison revealed an LA50 with silver sulfadiazine in the 15 to 40 age group of 64% and using Sulfamylon of less than 50%. Use of Sulfamylon burn cream as the initial agent was again discontinued and that agent has since been used only for the treatment of wound complications and in the form of soaks for debridement or protection of mesh graft.

In May 1975, after using Silvadene as the initial topical agent since November 1974, it appeared as if the mean time of death was decreasing. It was elected to subject all patients treated with silver sulfadiazine to statistical analysis in order to discern any change in the pattern of survival. For this purpose all silver sulfadiazine treated patients were divided into three groups. The first group were the initial 40 patients in the comparison study which ran from December 1973 through June 1974. The second group were those patients from June 1974 through October 1974, when silver sulfadiazine was the only initial agent. When groups 1 and 2 were added together they formed the 104 patients which were compared for statistical significance. The third group were those patients from

November 1974 to the present where again silver sulfadiazine was the only initial agent and there appeared to be a decrease in the mean time to death. Statistical analysis of all three groups revealed that the LA50 in the 15 to 40 age group had remained the same and that all three groups were essentially identical with no difference in survival.

The basic tenets outlined in the last annual report continue to be true. The overall survival is better in the silver sulfadiazine treated patients. The early pulmonary complications appear to be less frequent in silver sulfadiazine treated patients and easier to manage. There is no pain with the application of silver sulfadizine. The general well being of the patients judged by time of removal of the nasogastric tube, tolerance of a regular diet, ability to ambulate, and orientation all continue to be better in the silver sulfadiazine treated patients. There is again no doubt that Sulfamylon controls the gram negative organisms in the wound better than silver sulfadiazine. It has in fact, during the last period of examination, been used in macerated wounds, in wounds that appear to be degenerating, and in patients with positive blood cultures and normal appearing wounds. In several of these cases, the clinical course has been reversed by Sulfamylon. Blood cultures have reverted to negative, however this is also related to antibiotic manipulation and varied wound management, such as mesh beds for the drying of macerated wounds, tangential excision for removing of eschar, and subeschar infusion. In the majority of cases the patients developed a respiratory and cerebral intolerance to Sulfamylon requiring its removal. In those patients salvaged by these various maneuvers and developing an intolerance to Sulfamylon, there cannot be any thought that sepsis was the intervening cause of the cerebral and pulmonary problems, because sepsis was not further documented and the patient survived.

At present silver sulfadiazine burn cream is used as the initial primary agent at this unit with use of Sulfamylon burn cream reserved for the special indications noted above. Extensive use of Sulfamylon in the form of soaks later in the post-burn course and following separation of the bulk of the eschar have yielded excellent results with almost no toxicity.

Topical therapy
Burn injury
Sulfamylon (mafenide)
Silvadene (silver sulfadiazine)
Humans

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA OF 6391	75 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
75 07 01	D. CHANGE	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO. CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62110A	3A162110A821	00	105
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)* (U) Evaluation of the Effect of Fresh-Frozen Plasma on Leukocyte Chemotaxis in Burned Soldiers (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*
003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
74 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	PRECEDING	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	75	.8	23
	CURRENT 76	1.0	28

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

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

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

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

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME:* James W. Taylor, MAJ, MC
TELEPHONE: 512-221-2943
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: James M. Long, LTC, MC
NAME: Arthur D. Mason, Jr, MD DA

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Burns; (U) Leukocytes; (U) Chemotaxis; (U) Humans; (U) Plasma

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

23. (U) Evaluation of the in vivo effect of fresh-frozen plasma on leukocyte chemotaxis in thermally injured military personnel.

24. (U) Patients with burns over 55% of the total body surface are divided on admission into two groups. One receives the routine resuscitation, the other receives fresh-frozen plasma on colloid during their resuscitation. The functional chemotactic index is determined serially in these patients according to the Warden modification of the Boyden technique namely the ability of leukocytes to migrate through nucleopore filter toward a complement dependent chemotactic agent, CASEN-SERUM. Other patients who have a low chemotactic index but who had received no fresh-frozen plasma prior to determination, are treated with three units of fresh-frozen plasma per day and the functional chemotactic index determined during the period of treatment with fresh-frozen plasma.

25. (U) 74 07 - 75 06 Nine patients were divided into two groups. The first received no fresh-frozen plasma in their resuscitation, the second received fresh-frozen plasma. The small size of the groups prevented the results from being statistically significant. The results in the patients who first were shown to have a low functional chemotactic index and subsequently were treated with fresh-frozen plasma indicated that fresh-frozen plasma could in vivo increase the functional chemotactic index (mean FCI pre-treatment 37.7 plus/minus 4.2; mean FCI post-treatment 56 plus/minus 4.6, (p less than .01). Impairment of chemotaxis in direct relation to burn size was confirmed as was the relation of chemotactic suppression to subsequent septic complications.

*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. 3A162110A821-00, COMBAT SURGERY

REPORT TITLE: EVALUATION OF THE EFFECT OF FRESH-FROZEN PLASMA ON LEUKOCYTE CHEMOTAXIS IN BURNED SOLDIERS

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

1 September 1974 - 30 June 1975

Investigators:

James W. Taylor, M.D., Major, MC
James M. Long, III, M.D., Lieutenant Colonel, MC
Arthur D. Mason, JR., M.D.
Basil A. Pruitt, Jr., M.D., Colonel, MC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A162110A821-00, COMBAT SURGERY

REPORT TITLE: EVALUATION OF THE EFFECT OF FRESH-FROZEN PLASMA ON LEUKOCYTE CHEMOTAXIS IN BURNED SOLDIERS

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

Period covered in this report: 1 September 1974 - 30 June 1975

Investigators: James W. Taylor, M.D., Major, MC
James M. Long, III, M.D., Lieutenant Colonel, MC
Arthur D. Mason, Jr., M.D.
Basil A. Pruitt, Jr., M.D., Colonel, MC

Reports Control Symbol MEDDH-288(R1)

It has previously been reported that leukocytes from burn patients showed decreased chemotaxis and that after 72 hours post burn impairment of leukocyte chemotaxis is directly correlated with the clinical status of the patient and is highly predictive for ultimate mortality. Furthermore, it has been shown previously that in vitro normal serum can restore chemotaxis to normal in the suppressed granulocytes from burn patients. This study assesses the in vivo effect of fresh frozen plasma on the functional chemotacric index of thermally injured patients.

Nine patients with burn greater than 55% of the total body surface were divided on admission into two groups. Four of the patients received a routine resuscitation with plasmanate as colloid. The other five patients received some fresh-frozen plasma during their resuscitation. Subsequently, serial functional chemotactic indices were determined. The size of the study group at this time precludes statistical [illegible]. In the second portion of the study, patients with a demonstrated low functional chemotactic index received three units of fresh-frozen plasma per day and serial studies were done on the functional chemotactic index. The [illegible] in the post-treatment group was significantly [illegible] [illegible].

This data suggests that the functional chemotactic index can be altered in vivo by the administration of fresh-frozen plasma.

EVALUATION OF THE EFFECT OF FRESH-FROZEN PLASMA ON LEUKOCYTE CHEMOTAXIS IN BURNED SOLDIERS

Warden et al have reported that burn patients show decreased leukocyte chemotaxis and that at 72 hours suppression of leukoctye chemotaxis is directly correlated with the clinical status of the patient and is highly predictive for ultimate mortality. In addition, Warden et al have demonstrated in vitro that normal serum can restore chemotaxis to normal in the suppressed granulocytes from burn patients. Furthermore, Warden demonstrated that the serum factor responsible for the restoration was inactivated by an exposure to a temperature of 56°C for 20 minutes.[1,2]

Patients with extensive thermal burns at the USA ISR routinely receive large volumes of plasmanate on the 2nd and occasionally the 3rd post burn day. Since 10 hours of heating at 60°C are required to manufacture plasmanate, this colloid should contain little of the serum factor which restores chemotaxis. In this study, we substituted fresh frozen plasma for plasmanate in a series of large burns and compared the leukocyte chemotaxis in those patients to the leukocyte chemotaxis in a paired series of burn patients who received standard therapy with plasmanate. In a subsequent series of patients we established that the patient had decreased leukocyte chemotaxis in the period 72 hours post burning and then treated the patient with three units of fresh-frozen plasma per day and serially followed the leukocyte chemotaxis in order to detect any possible changes that the fresh-frozen plasma might produce.

MATERIALS AND METHODS

Evaluation of In Vitro Effect of Fresh-frozen Plasma and Plasmanate on Leukocyte Chemotaxis

Evaluation of the leukocyte chemotaxis was carried out according to the method of Boyden as modified by Warden et al.[1] After separation of the leukocyte rich supernatant, 2 ml of the supernatant was incubated in a 1:1 ratio with either fresh-frozen plasma from an AB donor or with plasmanate at 37° for 20 minutes. After dilution with Hank's solution 2 ml of the mixture, containing approximately 4×10^6 cells were placed in the upper compartment of the chemotactic chamber for evaluation of leukocyte chemotaxis. Each sample of blood from a burn patient was divided into three aliquots. One was treated with fresh-frozen plasma, the second with plasmanate and the third was incubated with Hanks solution.

---

1. Warden GD, Mason AD Jr., Pruitt BA Jr.: Suppression of Leukocyte Chemotaxis in vitro by chemotherapeutic agents used in the management of thermal injuries. Ann Surg 181:363-369, 1975.
2. Warden GD, Mason AD Jr., Pruitt BA Jr.: Evaluation of leukocyte chemotaxis in vitro in thermally injured patients. J of Clin Invest 54:1001-1004, 1974.

Evaluation of the In Vivo Effect of Fresh-Frozen Plasma on Leukocyte Chemotaxis

The patients with burn wounds greater than 55% of their total body surface who were admitted to the ISR within the first 24 hours after thermal injury were randomly divided into two groups. One group received the standard resuscitation which included plasmanate as the colloid and the second group received some fresh-frozen plasma during their resuscitation. Initially it was desired that the second group would receive fresh-frozen plasma as the only colloid during their resuscitation but in practice this proved to be impossible. Blood samples were drawn serially during these patients' hospital courses and the chemotactic index was determined according to the method of Warden et al with the only alterations being that each determination was performed in triplicate and that the physician doing the assay was blinded as to the size and nature of the burn which was associated with a given blood sample. This blinding was done by sending the physician multiple samples of blood from both patients on this study and from burn patients who for various reasons did not fit into this study. These blood samples were numbered but were not identified with a patients name. This greater number of patient determinations were done in order to see if previous findings were reproducible.

Evaluation In Vivo of the Effect of Fresh-Frozen Plasma on Leukocyte Chemotaxis in Patients who Previously have been shown to have Decreased Chemotaxis

One group of patients received approximately 3 units of fresh-frozen plasma per day after they were shown to have decreased leukocyte chemotaxis. Subsequently chemotactic function was studied serially. This group of patients was compared to a group of patients who had serial determinations of the chemotactic index and who received no fresh-frozen plasma.

RESULTS

In Vitro Effect of Fresh-Frozen Plasma and Plasmanate on Leukocyte Chemotaxis

Four blood samples from thermally injured patients were studied. The results are shown in table I. These confirmed what we expected from earlier studies showing that the factor present in fresh serum is also present in fresh-frozen plasma and that plasmanate appeared to lack the factor.

Comparison of the present study to an earlier study

Table 2 shows a comparison of the control values of the chemotactic index obtained in earlier study to the control values obtained in the present study. It should be noted that in the present study the same subject was used for all the control values.

Table 1

IN VITRO EFFECT OF FRESH FROZEN PLASMA AND PLASMANATE ON LEUKOCYTE CHEMOTAXIS OF THERMALLY INJURED PATIENTS

PATIENT	BASELINE	INCUBATED WITH FRESH FROZEN PLASMA	INCUBATED WITH PLASMANATE
S	38.4	323.3	37.9
T	14.0	152.9	46.3
M	80.5	143.6	62.5
H	45.9	68.6	50.4

Table 2

CONTROL VALUES

	WARDEN'S STUDY 44 NORMAL VOLUNTEERS	PRESENT STUDY 1 SUBJECT 31 TIMES
MEAN C.I.	764.4	966.6
95% CON. LIMITS	750-780	933 - 1000

*Warden GD, Mason AD, Jr., Pruitt, BA, Jr. J. Clin. Inves. 54:1001, 1974.

Table 3 shows a comparison of the functional chemotactic indices after 72 hours post burn. It should be noted that the present study supports the contention that the survivors have a higher functional chemotactic index than the nonsurvivors. Some notable differences from the earlier study were present. Earlier results sharply divided the survivors from the nonsurvivors on the basis of chemotactic index. The present study shows that the mean functional chemotactic index is significantly higher for the survivors than for the nonsurvivors but that individual survivors are not distinctly and predictably separated from the nonsurvivors by the functional chemotactic index. In other words there is an overlap of values. Furthermore the two studies differ in that the mean burn sizes for both nonsurvivors and the survivors are larger in the present study.

Effect of Fresh-Frozen Plasma on the Functional Chemotactic Index in burns greater than 55% of the total body surface

Nine patients were divided into two groups as shown in Table 4. Four patients received no fresh-frozen plasma during their resuscitation and five patients received varying amounts of fresh-frozen plasma during their resuscitation. The number of units of fresh-frozen plasma which the treated group received during the first week post burn and the mean of the functional chemotactic indices during the first post burn week are also shown in Table 4. This data is interesting and possible suggestive but the small number of patients makes statistical assay unwarranted. The group that received no fresh-frozen plasma had lower functional chemotactic indices than the treated group but this is partially explained by the fact that the mean burn size of the group that did not get fresh-frozen plasma was slightly larger than the mean for the group that did.

The In Vivo Effect of Fresh-Frozen Plasma on the Functional Chemotactic Index in patients who have been demonstrated to have a low functional Chemotactic Index

Twelve patients were demonstrated to have a low functional chemotactic index and were subsequently given three units of fresh frozen plasma per day with serial functional chemotactic indices determined during the period of therapy. Table 5 shows the results of these determinations and shows the mean of the functional chemotactic indices which were determined after therapy with fresh-frozen plasma had begun. The first three patients on table 5 were studied late in their burn course. Patient 4 was first studied nine days post burn. The other patients were first studied on their 4th post burn day. The functional chemotactic indices after treatment had begun were significantly different than the pre-treatment chemotactic indices.

A further comparison was made with this data in order to study what difference time might make in altering the chemotactic index of a group of patients who were not treated with fresh frozen plasma. This was done by selecting those patients from the previous study who had received no fresh-frozen plasma and who had a series of chemotactic indices

Table 3

FUNCTIONAL CHEMOTACTIC INDICES AFTER 72H POST-BURN

	WARDEN'S STUDY*		PRESENT STUDY THROUGH POST BURN DAY 21	
	SURVIVORS	NON-SURVIVORS	SURVIVORS	NON-SURVIVORS
NUMBER OF PATIENTS	12	23	9	15
NUMBER OF DETERMINATIONS	36	34	39	45
F.C.I.	97.7	39.9	61.4	44.7
S.E.	3.2	2.3	3.8	3.3
RANGE	61.2 - 130	14.6 - 75	19.7 - 118	11 - 100.7
SIGNIFICANCE	p = 0.01		p = 0.01	
BURN SIZE	44.6	57.9	54	65
RANGE	25.5 - 70.5	31.5 - 92.0	34 - 67	40 - 83
MEAN DAY OF TEST	Not Reported	Not Reported	9.42 + 0.79	8.87 + 0.73

*Warden GD, Mason AD, Jr, Pruitt BA, Jr. J. Clin. Invest. 54:1001, 1974.

Table 4

EFFECT OF FFP ON FCI IN BURNS GREATER THAN 55% TBS

PATIENT		BURN SIZE	# UNIT FFP IN 1ST WEEK		MEAN FCI
A		66%	0		53.6%
B		72%	0		34.9%
C		83%	0		17.8%
D		75%	0		23.6%
	MEAN	74%		MEAN	32.3%
E		58%	12		85.0%
F		79%	7		33.1%
G		75%	9		47.0%
H		60%	3		99.1%
I		67%	2		60.2%
	MEAN	67.8%		MEAN	64.9%

Table 5

FUNCTIONAL CHEMOTACTIC INDICES IN PATIENTS TREATED WITH THREE UNITS OF FRESH FROZEN PLASMA PER DAY

PATIENT	% TBS/% 3°	DAY 0	DAY 1	DAY 2	DAY 3	DAY 4	DAY 5	DAY 6	DAY 7	MEAN AFTER TREATMENT
1	46.5/20.5	37			59			86		72.5
2	69/36.5	68.4	93.9							93.9
3	46.5/3	30.8	43.9							43.9
4	53/35	48.6		48.8			56.9	51.7	54.0	52.9
5	53/19	35.9		35.5	54.4		57.7			49.2
6	78/49	15.4		30.1	35.2		46.0			37.1
7	61.5/24.5	40.0		38.8	49.2		53.3			47.1
8	67/39.5	15.8		18.4	37.1		53.2			36.2
9	73/41	39.8		42.7	75.0		78.8			65.5
10	61/0	50.8	57.3	57.6						57.4
11	44/0	33.9	56.0	57.7						56.9
12	62.5/14	36.1		59.1						59.1
MEAN		37.7*								56.0*

* $p < .01$

Table 6

IN VIVO EFFECT OF FRESH FROZEN PLASMA ON THE FUNCTIONAL CHEMOTACTIC INDEX OF PATIENTS
WHO HAVE BEEN DEMONSTRATED TO HAVE A LOW FUNCTIONAL CHEMOTACTIC INDEX

	PATIENT	TBS/+ 3°	PRE-TREATMENT (=POST BURN DAY) 4 to 6	DAYS POST TREATMENT	1	2	3	4	5	MEAN OF POST TREATMENT VALUES
CONTROLS - No FFP	A	66/37.5	61.3				33.9		65.6	49.8
	B	72/31	29.3			29.7		21.1	45.6	32.8
		53/43.5	16.4			11.0		26.1		18.6
		75/5	23.6						19.2	19.2
		46/10	73.1			47.0				34.0
		45.5/1	74.0			56.8			42.1	49.5
		39/23	22.7			41.5		12.2		26.6
	MEAN TBS BURN 30.8		MEAN 42.9							MEAN 33.6
PATIENTS WITH FFP		55/39	35.9			35.5	54.4		57.7	49.2
		45/9	15.4			30.1	35.2		46.0	37.1
		51.5/24	40.3			38.8	49.2		53.3	47.1
		57/39.5	15.8			18.4	37.1		53.2	36.2
		73/41	39.8			42.7	75.0		76.8	65.5
		57/3	53.6		57.8	57.6				57.7
		60/3	33.9		56.1	57.7				56.9
		62.5/14	38.1			59.1				59.1
	MEAN TBS 39.5		MEAN 33.5							MEAN 51.1

done. The functional chemotactic index which was compared to the pre-treatment group was the first chemotactic index that was done on these patients. This index was measured after 72 hours post burn and up to the 6th day. Subsequent chemotactic indices were tabulated according to the date that they followed the initial chemotactic index. Table 6 shows this data. The group treated with fresh-frozen plasma contains only those patients whose first chemotactic index was done on the 4th post burn day and who were treated with fresh-frozen plasma subsequently. The pre-treatment values for the functional chemotactic index in the control group were not statistically different from the mean post treatment values of the control group. Likewise the pre-treatment values for the control group was not different statistically from the pre-treatment values of the fresh-frozen plasma group. The post treatment values for the fresh-frozen plasma group were however, very significantly different from the pre-treatment values ($p < .01$). Likewise the mean post treatment values of the fresh-frozen plasma group were significantly differnet from the mean post treatment values of the control group ($p < .05$).

DISCUSSION

An attempt has been made to study the in vivo effect of fresh-frozen plasma on the functional chemotactic index of burn patients. In the initial portion of this evaluation a number of burn patients were studied and the data supported the previous findings of Warden et al.[1] The present study differed however, from the earlier study in that although survivors had lower functional chemotactic indices than nonsurvivors, the results in this study were not absolutely predictive since some patients with lower functional chemotactic indices survived and many patients with high functional chemotactic indices ultimately died. Possibly some of this variation was caused by our giving fresh-frozen plasma to some of the patients during their resuscitation. It is interesting to note that the three patients in the series who had the highest initial functional chemotactic indices had all received fresh-frozen plasma during their resuscitation.

The attempt to determine whether functional chemotactic index could be altered by substituting fresh-frozen plasma for plasmanate during the resuscitation was inconclusive because the sample size is too small for statistical significance. Despite this failing this small series is suggestive that fresh-frozen plasma may have an effect. The study in which burn patients were first demonstrated to have a low chemotactic index and then were given fresh-frozen plasma produced more statistically satisfying results. The series is relatively small but appears to be statistically significant. We believe that this study indicated that fresh-frozen plasma can favorably alter in vivo the functional chemotactic

---

1. Warden GD, Mason AD Jr., Pruitt BA Jr.: Suppression of Leukocyte Chemotaxis in vitro by chemotherapeutic agents used in the management of thermal injuries. Ann Surg 181:363-369, 1975.

index in thermally injured patients. It is hoped that such an elevation in functional chemotactic index can diminish the susceptibility to opportunistic infection in burn patients. Further studies will be required to ascertain whether fresh-frozen plasma enhances the chances of survival in the thermally injured patient.

REFERENCES

1. Warden GD, Mason AD Jr., Pruitt BA Jr.: Suppression of Leukocyte Chemotaxis in vitro by chemotherapeutic agents used in the management of thermal injuries. Ann Surg 181:363-369, 1975.

2. Warden GD, Mason AD Jr., Pruitt BA Jr.: Evaluation of leukocyte chemotaxis in vitro in thermally injured patients. J of Clin Invest 54:1001-1004, 1974.

PRESENTATIONS and/or PUBLICATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA 0E 6979	75 07 01	DD DR&E/AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY*	6. WORK SECURITY*	7. REGRADING*	8a. DISP'N INSTR'N	8b. SPECIFIC DATA: CONTRACTOR ACCESS	9. LEVEL OF SUM
74 07 01	K.TERM	U	U	NA	NL	[X] YES [ ] NO	A. WORK UNIT

10. NO CODES*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62110A	3A162110A821	00	111
b. CONTRIBUTING	61101A	3A161110A91C	00	
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)* (U) Assessment of Thermal Conductivity for the Measurement of Gastric Mucosal Blood Flow in a Model of Stress Ulcer as it Occurs in Burned Soldiers(44

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*
003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
73 11	75 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 PRECEDING 75	.1	2
FISCAL YEAR CURRENT		

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

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

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

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

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME:* Joseph C McAlhany, Jr, MD, MAJ, MC
TELEPHONE: 512-221-2943
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME:
NAME: DA

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Thermal Conductivity; (U) Stomach; (U) Mucosal Blood Flow; (U) Dogs

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

23. (U) To evaluate the possibility of utilizing thermal conductivity as a means of estimating regional gastric mucosal blood flow in the canine stomach as a laboratory simulation of the burned soldier.

24. (U) The study is to be performed on mongrel dogs weighing between 10 and 15 kgs prepared with deinnervated gastric pouches. Aminopyrine clearances will be utilized to obtain reference gastric blood flow measurement. A flexible catheter with a tip thermistor will be obtained and modified to enable self heating. The thermistor tip will be heated above that of "core gastric temperature" and then placed on the gastric mucosa to obtain a temperature record. The temperature records will then be correlated with documented aminopyrine clearances to determine if the degree of cooling of the tip thermistor correlates with mucosal blood flow.

25. (U) 74 07 - 75 01 This study has been terminated due to the inability to obtain adequate thermal conductivity changes in animals utilizing currently available thermistor probes.

*Available to contractors upon originator's approval

FINAL REPORT

PROJECT NO. 3A162110A821-00, COMBAT SURGERY

REPORT TITLE: ASSESSMENT OF THERMAL CONDUCTIVITY FOR MEASUREMENT OF GASTRIC MUCOSAL BLOOD FLOW IN A MODEL OF STRESS ULCER AS IT OCCURS IN BURNED SOLDIERS

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

1 July 1974 - 28 February 1975

Investigators:

Joseph C. McAlhany, Jr., M.D., Major, MC
Albert J. Czaja, M.D., Major, MC
Arthur D. Mason, Jr., M.D.
Travis S. Masterson, Jr., M.S.

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A162110A821-00, COMBAT SURGERY

REPORT TITLE: ASSESSMENT OF THERMAL CONDUCTIVITY FOR MEASUREMENT OF GASTRIC MUCOSAL BLOOD FLOW IN A MODEL OF STRESS ULCER AS IT OCCURS IN BURNED SOLDIERS

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

Period covered in this report: 1 July 1974 - 28 February 1975

Investigators: Joseph C. McAlhany, Jr., M.D., Major, MC
Albert J. Czaja, M.D., Major, MC
Arthur D. Mason, Jr., M.D.
Travis S. Masterson, Jr., M.S.

Reports Control Symbol MEDDH-288(R1)

Thermal conductivity of the gastric mucosa in mongrel dogs was unable to be evaluated because of technical difficulties. A commercially available thermistor probe proved unsuccessful due to rigidity with difficulty in passing the probe through the fiberoptic gastroscope. After passage of the commercially available probe accurate localization on the gastric mucosa was unable to be obtained. Subsequent construction of a thermistor probe which was readily passed through the gastroscope and allowed accurate positioning on the gastric mucosa was accomplished. The technical difficulties encountered included heating of the sensitive thermistor tip by the light source from the gastroscope and difficulty in obtaining reproducible conductivity measurements of the gastric mucosa.

Stomach
Thermal conductivity
Dogs
Mucosal Blood flow

ASSESSMENT OF THERMAL CONDUCTIVITY FOR MEASUREMENT OF GASTRIC MUCOSAL BLOOD FLOW

Presently, no methods exist for the quantitative or qualitative measurement of gastric mucosal blood flow in the human. Since a technique for the estimation of gastric mucosal blood flow in the human is desireable this study was designed to evaluate the possibility of utilizing thermal conductivity as a means of estimating regional gastric mucosal blood flow. A commercially available catheter with a tip thermistor was obtained. In principle the thermistor tip was to be heated above that of "core gastric temperature" and then placed on the gastric mucosa to obtain a temperature record. The temperature records were to be correlated with documented aminopyrine clearances to determine if the degree of thermal conductivity correlated with mucosal blood flow. The commercially available thermistor probe was unsuccessful because of extreme rigidity and difficulty in passage through the fiberoptic panendoscope. Moreover, after passage the probe was unable to be accurately placed on the gastric mucosa for conductivity measurements.

A thermistor probe was constructed which was more flexible and easily passed through the fiberoptic panendoscope. The thermistor tip proved to be extremely sensitive to the light source from the fiberoptic panendoscope and accurate and reproducible measurements of thermal conductivity of the gastric mucosa were unable to be obtained. Several mongrel dogs were given intravenous medications to influence gastric mucosal blood flow but due to the technical difficulties of localization on the gastric mucosa and temperature sensitivity, no significant changes were recorded. Technical difficulties made further experimentation impossible.

SUMMARY

Thermistor probes are at present unsatisfactory in design and sensitivity for obtaining thermal conductivity measurements of the gastric mucosa.

REFERENCES: None

PUBLICATIONS AND/OR PRESENTATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA 0E 6953	75 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
74 07 01	D. CHANGE	U	U	NA	NL	[X] YES [ ] NO	A. WORK UNIT

10. NO. CODES*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62110A	3A162110A821	00	107
b. CONTRIBUTING	61101A	3A161101A91C	00	
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)* (U) Evaluation of Gastric Physiologic Disturbances Associated with Thermal Injury in a Military Population (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*
003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
72 01	Cont	DA	C. In-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 75	.9	25
	CURRENT 76	.7	18

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

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

20. PERFORMING ORGANIZATION
NAME* US Army Institute of Surgical Research
Burn Study Branch
ADDRESS* Fort Sam Houston, Texas 78234

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME* Joseph C McAlhany, Jr, MD, MAJ, MC
TELEPHONE 512-221-3411
SOCIAL SECURITY ACCOUNT NUMBER

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

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

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Thermal Injury; (U) Burn Patients; (U) Evaluation; (U) Gastric Physiologic Disturbances

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

23. (U) To study the gastric pathophysiology of the thermally injured soldier, so as to better define etiologic factors responsible for Curling's ulcer.

24. (U) Evaluation to be carried out on thermally injured soldiers with greater than 30% TBS area injury admitted to the USAISR. Study will be stratified so that a group of patients in the 30 to 50% TBS area injury and the second group in the 50 to 70% TBS area injury will be included. Investigative procedures will be performed within 24 hours if possible and at 72 hours postburn. Burns of greater than 50% body surface area will also be studied at 5 to 7 days post injury and all patients will then be studied between the 9th and 12th day post injury and at 30 days or discharge. Studies will encompass: (1) gastric endoscopy with photography and biopsy for semiquantitative mucous determination, (2) ion flux across the gastric mucosa, (3) coagulation studies, (4) measurements of gastric clearance of radioactive isotopes, and (5) evaluation of the role of bacteremia.

25. (U) 74 07 - 75 06 Studies have now been performed on 77 burn patients admitted to the USAISR. Endoscopic findings document the existence of definite gastric mucosal abnormalities occurring early post burn which are persistent. The true incidence of acute gastroduodenal lesions or Curling's ulcer would appear to be much higher than previously determined by clinical course, operative and autopsy findings. Measurements of mucous substance, ion flux, coagulation and gastric clearances of radioactive isotopes have been evaluated.

*Available to contractors upon originator's approval

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

ANNUAL PROGRESS REPORT

PROJECT NO. 3A162110A821-00, COMBAT SURGERY

REPORT TITLE: EVALUATION OF GASTRIC PHYSIOLOGIC DISTURBANCES ASSOCIATED WITH THERMAL INJURY IN A MILITARY POPULATION

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

1 July 1974 - 30 June 1975

Investigators:

Joseph C. McAlhany, Jr , M.D., Major, MC
Albert J. Czaja, M.D. Major, MC
Basil A. Pruitt, Jr., M.D., Colonel, MC
Robert Lull, M.D., Lieutenant Colonel, MC
Willard A. Andes, M.D., Major, MC
*Samuel S. Spicer, M.D.

*Department of Pathology
Medical University
Charleston, S. Carolina 29401

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A162110A821-00, COMBAT SURGERY

REPORT TITLE: EVALUATION OF GASTRIC PHYSIOLOGIC DISTURBANCES ASSOCIATED WITH THERMAL INJURY IN A MILITARY POPULATION

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

Period covered in this report: 1 July 1974 - 30 June 1975

Investigators: Joseph C. McAlhany, Jr., M.D., Major, MC
Albert J. Czaja, M.D., Major, MC
Basil A. Pruitt, Jr., M.D., Colonel, MC
Robert Lull, M.D., Lieutenant Colonel, MC
Willard A. Andes, M.D., Major, MC
*Samuel S. Spicer, M.D.

Reports Control Symbol MEDDH-288(RI)

Gastric pathophysiology in the thermally injured soldier was studied in order to describe factors which may be important in the etiology of Curling's ulcer. The methods for clinical study encompassed 1) gastric endoscopy with photography and biopsy for semiquantitative mucous determinations, 2) ion flux across the gastric mucosa, 3) coagulation studies 4) measurements of gastric clearance of a radioactive isotope.

Seventy-seven adult burned soldiers were evaluated with burns of greater than 25% total body surface (TBS) sustained within one week of admission to the US Army Institute of Surgical Research. Initial studies were performed within 72 hours post burn and were repeated during the second and third week post injury.

Gastroduodenoscopy demonstrated that superficial gastric and duodenal mucosal lesions occurred soon after thermal injury in most patients (86.8%) with burns involving more than 35% of their total body surface. Deeper, ulcerative lesions developed later in areas of intense early mucosal injury. Gastric and duodenal abnormalities frequently coexisted without clinical signs or symptoms. Twenty-one gastric biopsies in 9 patients demonstrated normal quantities of superficial and deep cellular mucosubstances as determined by mucous histochemical evaluation. Diffuse gastric mucosal disease was present in 78% of the patients with normal cellular mucosubstance. Permeability of the gastric mucosal barrier to hydrogen back diffusion was studied by a lithium flux technique. Diffuse gastric mucosal lesions were present in 7 of 10 patients with a normal mucosal barrier suggesting that an increased back diffusion of hydrogen ions was not an etiologic factor in the development of these early

gastric lesions. The disruption of the gastric mucosal barrier in eight patients correlated with endoscopic and clinical progression of mucosal disease. Gastric clearance of radioactive technectium was too variable for analysis and this aspect of the study was discontinued. To evaluate the role of microvascular thrombosis in the etiology of acute gastritis after burns, 29 burn patients had serial coagulation studies and mucosal biopsies stained for fibrin thrombi performed. Erosive gastritis was present in 22 patients or 76% of these patients and duodenitis was demonstrated on biopsy even though 5 patients had consumption coagulopathy. There was similarly no correlation between serial coagulation studies and the endoscopic manifestations of gastroduodenal mucosal disease.

Evaluation
Gastric physiologic disturbances
Thermal injury
Burn patients

*Present address: Department of Pathology, Medical University, Charleston, S. Carolina 29401

EVALUATION OF GASTRIC PHYSIOLOGIC DISTURBANCES ASSOCIATED WITH THERMAL INJURY IN A MILITARY POPULATION

Gastric pathophysiology of the thermally injured soldier has been studied to better define the etiologic factors responsible for Curling's ulcer. This clinical study encompassed 1) gastric endoscopy with photography and biopsy for semiquantitative mucous determinations, 2) measurement of ion flux across the gastric mucosa, and 3) measurement of coagulation indices.

Thermally injured patients with greater than 25% total body surface area injury were evaluated within one week of admission to the U.S. Army Institute of Surgical Research. Written informed consent was obtained from all patients prior to study. A minimum age limit of 15 years was established for the study. Clinical investigative procedures were performed within 24 hours, if possible, and at 72 hours post burn. Further evaluation was carried out during the second and third week post injury.

Gastroduodenal Endoscopy

To determine the true incidence, morphology and behavior of acute gastroduodenal disease following thermal injury, early and serial fiberoptic gastroduodenoscopies were performed in 77 adult burn patients. A history compatible with chronic peptic ulcer disease or gastritis, evidence of previous gastrointestinal surgery, or a history suggestive of excessive alcohol, aspirin, or steroid consumption eliminated the patient from consideration. Resuscitation fluids, systemic and topical antibiotics, vitamins, antacids, analgesics, anesthetics, and nutritional support were managed independently by each attending physician. Gastroduodenoscopy was performed with the Olympus GIF-D fiberoptic panendoscope. Premedication consisted of sufficient intravenous diazepam to induce drowsiness (up to 20 mgs). This was occasionally supplemented by intravenous meperidine (up to 25 mg). Each endoscopic procedure thoroughly evaluated the distal two-thirds of the esophagus, the entire stomach, and the first portion of the duodenum. Photographs were taken of each area examined and representative mucosal biopsies were procured. In patients with nasogastric tubes in place, superficial gastric lesions that were in a linear distribution or that were localized to a discrete area of the stomach were attributed to the effects of mechanical irritation and were discounted from the study.

During the current period of study, 77 thermally injured patients were evaluated by early gastroduodenoscopy. Ages ranged from 16 to 74 years (average age 34.8 years) and the burn size varied from 23% to 96% total body surface (average burn size 56.6%). One hundred and nine gastroduodenoscopies were performed without complication in the 77 patients. Twenty-two patients were examined serially. Fifty-nine of the 77 patients were studied within 72 hours after injury. Four of these patients were examined within 24 hours of injury (one as early as 12 hours post burn). The remaining patients were evaluated 4 to 20

days post injury.

Thirty-five biopsy specimens (32 gastric and 3 duodenal) were obtained from 19 of the patients evaluated within the initial 72 hour period. Postmortem examinations confirmed the findings described at gastroduodenoscopy.

Superficial Gastric Mucosal Injury

Superficial mucosal abnormalities were recognized at gastroscopy in 59 patients (86.8) as early as five hours post burn. Three patients with burns involving less than 30% of their total body surface had repeatedly normal examinations. The remainder of the patients had burns involving more than 35% of their total body surface. In the group of larger burns gastric mucosal abnormalities were recognized soon after thermal injury. Three types of superficial gastric mucosal lesions were recognized. Most commonly, small punctate erythematous lesions, a few millimeters in diameter, were diffusely scattered over the rugal crests of the fundus and body. Areas of central pallor were frequently present within these small circumscribed areas of erythema. A second variety of lesions suggested a conglommeration of the smaller lesions, appearing as a large (often greater than 2 cm in diameter), irregularly shaped, confluent area of erythema and mucosal hemorrhage. Discrete erosions with circumferential erythema represented the third type of lesion usually encountered. All three varieties of lesions could be present within the same stomach as early as 24 hours after injury. The histology of these lesions revealed microvascular congestion, mucosal hemorrhage with mild inflammation, and cellular disruption above the muscularis mucosae.

The gastric lesions were always distrubuted over the fundus and body of the stomach. While disease was never isolated to the antrum, in approximately one-third of the patients the antrum was also abnormal. Serial evaluations indicated that the antrum was usually less extensively involved than the more proximal areas.

Gastric Ulceration

Eighteen patients in the study group (26.5) had gastric ulcers by endoscopy. Six of these patients had concomitant duodenal ulcers. All 18 patients had superficial gastric mucosal disease and the ulcerations were located in areas of diffuse mucosal abnormalities. The earliest detection of a discrete gastric ulcer was at 96 hours post burn.

Superficial Duodenal Inflammation

Forty-eight of the 77 patients (81.4) had diffuse mucosal abnormalities of the proximal duodenum at the time of their initial endoscopy. All patients with duodenal involvement had burns of greater than 35% total body surface, and in all but two cases acute gastric mucosal disease was also present. Erythema, edema, increased mucosal friabili-

ty, and erosions in the bulb and proximal duodenum characterized the endoscopic picture of "duodenitis" in these patients. In some cases, there appeared to be actual mucosal sloughs within the duodenal cap. The histology of representative areas demonstrated microvascular congestion with mucosal hemorrhage, increased round cell infiltration, and occasionally, cystic dilatation of Brunner's glands.

Duodenal Ulceration

Twenty of the 77 patients had duodenal ulcerations (26%). Six of these patients also had multiple gastric ulcers. Duodenal ulcerations were not observed within 72 hours after injury, the earliest lesion being detected on the 4th post burn day. All duodenal ulcer patients had an accompanying "duodenitis"; in two of these patients an ulceration was actually observed to evolve in the area of an early erosive disease. With one exception, all patients with duodenal ulcers also had acute gastric mucosal disease.

Gastric Mucosubstance Histochemistry

A change in the mucous protective barrier has been suggested as a basis for acute gastric ulcerations. An alteration in this barrier might result from decreased mucus production or a change in the character of the mucus produced, thereby rendering the gastric mucosal membrane more susceptible to damage. Histochemical techniques provide a means of studying directly the cells of the gastric mucosa that produce the gastric mucosubstance. The purpose of this aspect of the study was to evaluate with histochemical methods the mucosubstance present in the various gastric epithelial cells and to correlate the content of mucosubstance with acute ulcerative disease of the gastric mucosa. Endoscopic evaluation of the stomach and duodenum was performed without complication in nine male patients. Twenty-one gastric biopsies were taken from areas of intact mucosa in the body of the stomach. Sections of each biopsy were prepared for routine examination with hemotoxlyn and eosin stains. Special histochemical techniques were utilized for visualizing and differentiating the carbohydrate secretions. Histochemical methods included the alcian blue (AB) - periodic acid Schiff (PAS) method and a sequence of aldehyde fuchsin (AF) followed by alcian blue (AB) at pH 2.5 and azure A at pH 4.5. The surface epithelium and mucous neck cells were evaluated specifically with the AB - PAS sequence which colors neutral mucosubstance red and acidic mucosubstance turquoise. The chief cells were appraised mainly with the AF - AB sequence which stains sulfated mucosubstance purple. Specimens were coded and the quantity of mucosubstance in the cells of each specimen was graded (0-4+) without knowledge of the endoscopic findings.

All nine patients, irrespective of mucosal disease, had a 3-4+ amount of neutral mucosubstance in the surface epithelial cells as evidenced by intense red coloration with the AB - PAS sequence. The

mucous neck cells stained a moderately strong (3+) red indicative of neutral mucosubstance with the AB - PAS sequence but occasionally were colored the turquoise indicative of acidic mucosubstance. The chief cells were depleted of sulfated mucosubstance in all specimens. The diminished affinity of the chief cells for AF varied from very weak staining (1+) to absence of reactivity (0). Specimens from 14 chronic duodenal ulcer patients and two canine stomachs were evaluated by the same histochemical techniques used in the staining of sulfated mucosubstances of the chief cells. These specimens demonstrated an abundance of sulfated mucosubstance by staining strongly purple with the AF - AB sequence. Mucosubstance of the normal human stomach could not be assessed because of post mortem autolysis; surgical specimens were not available from unburned patients with normal stomachs.

This aspect of the study indicated that gastric mucus production, as judged by direct cellular histochemical evaluation, is normal following thermal injury even in the presence of acute gastroduodenal disease. The role of sulfated mucosubstance, present in human chief cells, has not been established. It is interesting to contrast this depletion of the sulfated mucosubstance in all patients after thermal injury to the chief cells in chronic duodenal ulcer patients which contain an abundance of sulfated mucosubstance. Depletion of this anti-peptic sulfated mucosubstance may reflect a cellular response to increased pepsin neutralization after thermal injury. Conversely, this depletion could enhance the potential for peptic injury to an already damaged mucosa.

A decreased production of gastric mucus does not appear to be an etiologic factor for the development of acute gastroduodenal lesions after thermal injury since acute gastric mucosal disease was encountered in most patients despite normal quantities of cellular mucosubstances.

Ion Flux Across the Gastric Mucosa

A protective gastric mucosal barrier has been described and documented by Davenport.[1] The epithelial cells of the gastric mucosa have been described as the true barrier. Disruption of this gastric mucosal barrier (GMB) has been documented in critically ill patients and is believed to reflect poor vascular perfusion with resultant mucosal injury.[2] This is said to result in an increased permeability of the gastric mucosa to hydrogen ion which in turn may lead to progressive

---

1. Davenport HW: Physiology of the Digestive Tract. Chicago, Year Book Publishers, Inc., 1966.
2. Skillman JJ, Gould SA, Chung RSK and Silen W: The Gastric Mucosal Barrier: Clinical and Experimental Studies in Critically Ill and Normal Man, and in the Rabbit. Ann. Surgery, 172:564-584, 1970.

mucosal damage and the development of "stress" ulcerations. In order to define the possible pathogenic influences for the development of gastroduodenal lesions after thermal injury, this aspect of the study was designed to determine the status of the GMB after thermal injury using a lithium flux technique to determine the integrity of the GMB.[3,4]

Lithium flux measurements and initial endoscopy were performed within 72 hours post burn on 18 adult patients. Ten of the 18 patients demonstrated no disruption of the GMB, although in 7 of the 10 endoscopic examination disclosed acute gastric mucosal lesions. Eight patients were documented to have disruption of the GMB within 72 hours post burn. Six of these 8 patients also demonstrated acute gastric mucosal lesions at initial endoscopy. In 7 of these 8 patients who manifested disruption of the GMB within 72 hours post burn, gastric bleeding, gastric ulcer perforation or endoscopic progression of the mucosal disease was documented. Endoscopic progression of mucosal disease was documented in only two of the 10 patients with a normal GMB within 72 hours post burn.

This data documented that an increased back diffusion of hydrogen (H+) ion was not an etiologic factor in the development of early gastric lesions after thermal injury. However, GMB disruption did correlate with endoscopic and clinical progression of mucosal disease, which suggests that back diffusion of hydrogen (H+) ion plays a contributory role in the progression of this disease and could be a useful prognostic index.

SUMMARY AND CONCLUSIONS

Gastroduodenoscopy indicates that damage to the gastric and duodenal mucosa occurs soon after thermal injury in most patients (87%) with greater than 35% total body surface burn. The early occurrence, morphology and histology of the lesions suggest that mucosal ischemia is a primary etiologic factor. The proximal duodenum is involved almost as frequently as the gastric mucosa. Deeper, ulcerative lesions develop later in areas of intense, early mucosal injury. Severe gastric and duodenal abnormalities frequently coexist without clinical signs or symptoms.

Gastric mucosal disease was encountered despite normal quantities of superficial and deep cellular mucosubstance suggesting that loss of the gastric mucosal protective barrier is not an important etiologic

---

3. Chung RSK, Field M and Silen W: Gastric Mucosal Permeability to Hydrogen and Lithium: A New Method for Quantitation of the Gastric Mucosal Barrier. Surg. Forum, 21:297, 1970.
4. Smith BM, Skillman JJ, Edwards BG and Silen W: Permeability of the Human Gastric Mucosa. N Engl J Med 285:716, 1971.

factor in the development of acute gastric lesions after thermal injury.

The presence of acute gastric mucosal lesions in patients with a normal gastric mucosal barrier suggested an increased back diffusion of hydrogen ion was not an etiologic factor in the development of early gastric mucosal disease. GMB defects did correlate with progression of mucosal disease, suggesting that disruption of the gastric mucosal barrier may play a contributory role in the progression of the acute mucosal disease.

REFERENCES

1. Davenport, HW: Physiology of the Digestive Tract. Chicago, Year Book Publishers, Inc., 1966.

2. Skillman, JJ, Gould, SA, Chung, RSK and Silen, W: The Gastric Mucosal Barrier: Clinical and Experimental Studies in Critically Ill and Normal Man, and in the Rabbit. Ann. Surgery, 172:564-584, 1970.

3. Chung, RSK, Field, M and Silen, W: Gastric Mucosal Permeability to Hydrogen and Lithium: A New Method for Quantitation of the Gastric Mucosal Barrier. Surg. Forum, 21:297, 1970.

4. Smith, BM, Skillman, JJ, Edwards, BC and Silen, W: Permeability of the Human Gastric Mucosa. N Engl J Med 285:716, 1971.

PRESENTATIONS:

1. Histochemical study of gastric mucosubstances after thermal injury: correlation with gastroduodenal endoscopic findings. McAlhany JC, Jr., Czaja AJ, Cathcart RS III, Spicer SS, Foley FD. Presented at American Burn Assoc, Cincinnati, Ohio, Apr 1974.

2. Serial endoscopic evaluation of acute gastroduodenal disease following thermal injury. Czaja AJ, McAlhany JC, Jr., Pruitt BA, Jr. Presented at the American College of Physicians, New York City, New York, 1 Apr 1974.

3. Acute duodenal disease after thermal injury: assessment by early and serial endoscopy. Czaja AJ, McAlhany JC, Jr., and Pruitt BA, Jr. Presented at American Gastroenterological Assn. San Francisco, Calif., 22 May 1974.

4. Acute gastric disease after cutaneous thermal injury: Czaja AJ, McAlhany JC, Jr., Andes W, Pruitt BA, Jr. Presented at Western Surgical Assn. November 1974.

5. Gastric mucosal barrier in thermally injured patients: correlation with gastroduodenal endoscopy. McAlhany JC, Czaja AJ, Mason AD, Villareal Y. Presented at Surgical Forum, American College of Surgeons, Oct, 1974.

6. Perforated Curling's ulcer: Incidence, Diagnosis and Treatment. McAlhany JC, Jr., Czaja AJ Pruitt BA, Jr. Presented at American Burn Assn., Denver Colo. Mar 1975.

7. The Evolution and the Natural History of Curling's Ulceration. Czaja AJ, McAlhany JC Jr. and Pruitt, BA Jr. Presented at American Society for Gastrointestinal Endoscopy. San Francisco, Calif. May 1974.

PUBLICATIONS

1. Czaja AJ, McAlhany JC, Jr., Pruitt BA, Jr: Acute gastroduodenal disease following thermal injury: an endoscopic evaluation of incidence and natural history. New England Journal of Medicine 291:925-929, 1974.

2. McAlhany JC, Jr, Czaja AJ, Cathcart RS III, Spicer SS, Foley FD: Histochemical study of gastric mucosubstances after thermal injury: correlation with endoscopic evidence of acute gastroduodenal disease. In press.

3. McAlhany JC, Jr., Czaja AJ, Villareal Y, Mason AD Jr., and Pruitt BA Jr.: The Gastric Mucosal Barrier in Thermally Injured Patients Correlation with Gastroduodenal Endoscopy. Surg. Forum 25:414-416, 1974.

4. Czaja,AJ, McAlhany JC, Jr., Andes WA and Pruitt BA Jr.: Acute Gastric Disease after Cutaneous Thermal Injury. Arch Surg 110:600-605, 1975.

5. Czaja AJ, McAlhany JC Jr., and Pruitt BA Jr.: Pathogenesis of Acute Duodenal Disease after Burns: Role of Acid Secretion. Gastroenterology 68:1023, 1975.

EXHIBITS

Serial endoscopic evaluation of acute gastroduodenal disease following thermal injury. American College of Physicians, N.Y. Apr 74, American Gastroenterological Association, San Francisco May 74, and American College of Surgeons Oct 74.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1 AGENCY ACCESSION	2 DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0E 6964	75 07 01	DD DR&E AR(6)(n

3 DATE PREV SUM'RY	4 KIND OF SUMMARY	5 SUMMARY SCTY	6 WORK SECURITY	7 REGRADING	8a DISP'N INSTR'N	8b SPECIFIC DATA-CONTRACTOR ACCESS	9 LEVEL OF SUM
74 07 01	K. COMP	U	U	NA	NL	[X] YES [ ] NO	A. WORK UNIT

10 NO CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62110A	3A162110A821	00	106
b. CONTRIBUTING				
c. CONTRIBUTING				

11 TITLE (Precede with Security Classification Code) (U) The Efficacy of Parenteral Fat Emulsion in Thermally Injured Soldiers (44)

12 SCIENTIFIC AND TECHNOLOGICAL AREAS
003500 Clinical Medicine

13 START DATE	14 ESTIMATED COMPLETION DATE	15 FUNDING AGENCY	16 PERFORMANCE METHOD
73 02	75 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 75	.5	16
FISCAL YEAR CURRENT		

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

20 PERFORMING ORGANIZATION
NAME: US Army Institute of Surgical Research
Burn Study Branch
ADDRESS: Fort Sam Houston, Texas 78234
PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME: James M Long, MD, LTC, MC
TELEPHONE: 512-221-3301
SOCIAL SECURITY ACCOUNT NUMBER
ASSOCIATE INVESTIGATORS
NAME: Douglas W Wilmore, MD
NAME: Basil A Pruitt, Jr, COL, MC DA

21 GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22 KEYWORDS (Precede EACH with Security Classification Code) (U) Intralipid; (U) Soybean Oil Emulsion; (U) Crystalline Amino Acid Solution; (U) Hypertonic Glucose; (U) Intravenous Feeding; (U) Burn Injury

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

23. (U) To determine the efficacy of intravenous 10% soybean oil emulsion (Intralipid) when compared to intravenous glucose as a source of calories for total intravenous feeding in thermally injured soldiers.

24. (U) Isonitrogenous intravenous diets containing different compositions of fat and carbohydrate were evaluated in 5 patients to determine the relative protein-sparing effect of intravenously administered fat emulsion and glucose.

25. (U) 74 07 - 75 06 Isonitrogenous intravenous diets containing 1458, 875, 350, or 110 kcal/m2/day as glucose and 1108, 583, or zero kcal/m2/day as 10% modified soybean oil emulsion were infused into 5 patients during 29 three-day studies. Urinary urea nitrogen, blood urea nitrogen, blood sugar, and serum insulin levels were measured daily. Metabolic rate was measured or estimated from previous observations. The intravenous fat emulsion (Intralipid) was found to be safe and free of untoward side effects during a previous report period. Urea nitrogen excretion was inversely related to carbohydrate intake (p less than 0.01), directly related to metabolic rate (p less than 0.01), and not affected by fat intake. Although this intravenous fat emulsion is an excellent source of essential fatty acids, it did not effect any protein sparing in these soldiers.

*Available [illegible] 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. 3A162110A821-00, COMBAT SURGERY

REPORT TITLE: THE EFFICACY OF PARENTERAL FAT EMULSION IN THERMALLY INJURED SOLDIERS

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

1 July 1974 - 30 June 1975

Investigators:

James M. Long, III, Lieutenant Colonel, MC
Douglas W. Wilmore, M.D.
Basil A. Pruitt, Jr., Colonel, MC
Arthur D. Mason, Jr., M.D.

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A162110A821-00, COMBAT SURGERY

REPORT TITLE: THE EFFICACY OF PARENTERAL FAT EMULSION IN THERMALLY INJURED SOLDIERS

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

Period covered in this report: 1 July 1974 - 30 June 1975

Investigators: James M. Long, III, Lieutenant Colonel, MC
Douglas W. Wilmore, M.D.
Basil A. Pruitt, Jr., Colonel, MC
Arthur D. Mason, Jr., M.D.

Reports Control Symbol MEDDH-288(R1)

Clinical evaluation of a 10 per cent modified soy bean oil emulsion (Intralipid^{R}, Vitrum, Stockholm) was undertaken (1) to determine safety and tolerance in thermally injured soldiers and (2) to help clarify the nutritional efficacy of fat as an energy source. As noted in a previous report, this soy bean oil emulsion was associated with few, if any, toxic side effects, and it should be considered safe for use in severely injured man.

To determine the efficacy of fat as an energy source, isonitrogenous intravenous diets containing 11.7 grams nitrogen/m^2/day and 13 different combinations of carbohydrate (110-2360 kilocalories/m^2/day) and fat (0-1108 kilocalories/m^2/day) were fed to 5 patients during 34 studies of three days or longer. Urea nitrogen excretion was inversely related to carbohydrate intake ($p < 0.01$) and directly related to resting metabolic rate ($p < 0.01$). Fat infusion did not affect nitrogen excretion at any level of carbohydrate intake. Urea nitrogen excretion decreased toward its lowest values as carbohydrate calorie intake approached metabolic rate. Insulin levels were directly related to carbohydrate intake and to body size. These data indicate that the primary determinants of nitrogen excretion during isonitrogenous intravenous feeding are the amount of carbohydrate infused and the resting metabolic rate. We conclude that, when a primary clinical goal is nitrogen conservation, carbohydrate calories should be given in amounts approximating the metabolic rate. Additional calories and essential fatty acids can be safely given as intravenous fat emulsion, but fat did not affect nitrogen sparing in these patients.

Intravenous Fat Emulsion	Intralipid^{R}
Crystalline Amino Acid Solution	Hypertonic Glucose
Parenteral Nutrition	Burn Patients

THE EFFICACY OF PARENTERAL FAT EMULSION IN THERMALLY INJURED SOLDIERS

The metabolic response to severe trauma is characterized by protein wasting, loss of body weight, and increased energy expenditure. This response to stress, which is a basic neurohormonal reflex, is a graded response: when injury, or stress, is severe, the response is pronounced, and the erosion of body mass may be life-threatening. The consequences of this post-traumatic catabolic response are most readily apparent in patients who have sustained major thermal injuries. Burn injuries of more than 40% of total body surface area cause the most profound increase of metabolic rate and loss of protoplasmic mass associated with any disease process. Failure to provide adequate metabolic and nutritional support to these patients may result in rapid erosion of energy and protein stores that are essential for maintaining integrated body function and may predispose the patient to potentially lethal complications.

Vigorous nutritional support, preferably using enteral feeding but, when necessary, using supplemental or total intravenous feeding, can reduce body wasting and achieve weight stablization. In most patients who have a maximal catabolic response to thermal injury, caloric and nitrogen equilibrium can be achieved by providing at least 2000 kilocalories and 15 grams of nitrogen per square meter body surface area each day.[1] When the burn patient does require intravenous nutrition support, an increased incidence of complications, such as catheter-related septicemia, central venous thrombosis, hyperglycemia, and hyperosmolar dehydration may occur. For this reason, the concept of an intravenous fat emulsion is quite appealing, if total nutritional support of critically injured patients can, in fact, be accomplished by peripheral venous infusion without introducing significant new hazards and without sacrificing the nutritional efficacy of the feeding program. Therefore, clinical evaluation of a 10% modified soy bean oil emulsion (Intralipid^{R}, Vitrum, Stockholm) was undertaken 1) to determine safety and tolerance in severely injured soldiers and 2) to help clarify the nutritional efficacy of fat as an energy source.

Studies To Determine Safety

An extensive clinical evaluation of fat emulsion in thermally injured patients has been published by Wilmore and associates from this Institute,[2] and those results are briefly summarized here. Single-unit

---

1. Soroff HS, Pearson E, Artz CP: An estimation of the nitrogen requirements for equilibrium in burned patients. Surg Gynecol Obstet 132:159-172, 1961.
2. Wilmore DW, Moylan JA, Helmcamp GM, Pruitt BA Jr: Clinical evaluation of a 10% intravenous fat emulsion for parenteral nutrition in thermally injured patients. Ann Surg 178:503-513, 1973.

infusions of soy bean oil emulsion were evaluated in 12 hypermetabolic burn patients and 15 convalescing patients who were essentially normal. No significant hyperpyrexia occurred in either group in response to fat infusion. Vital signs, complete blood counts, and liver function studies remained unchanged. Fat clearance curves demonstrated an accelerated disappearance of the emulsion from the plasma of acutely burned patients (Figure 1).

^{133}Xenon perfusion-ventilation lung scans remained normal, and pulmonary diffusion capacity using a carbon monoxide rebreathing technique was not altered after fat infusion. Blood gas determinations were done after single and multiple unit infusions of Intralipid^{R} in 20 patients, none of whom developed any significant alterations of ventilation or gas exchange. These tests verified the safety of this fat emulsion in critically ill patients who had potentially marginal pulmonary function.

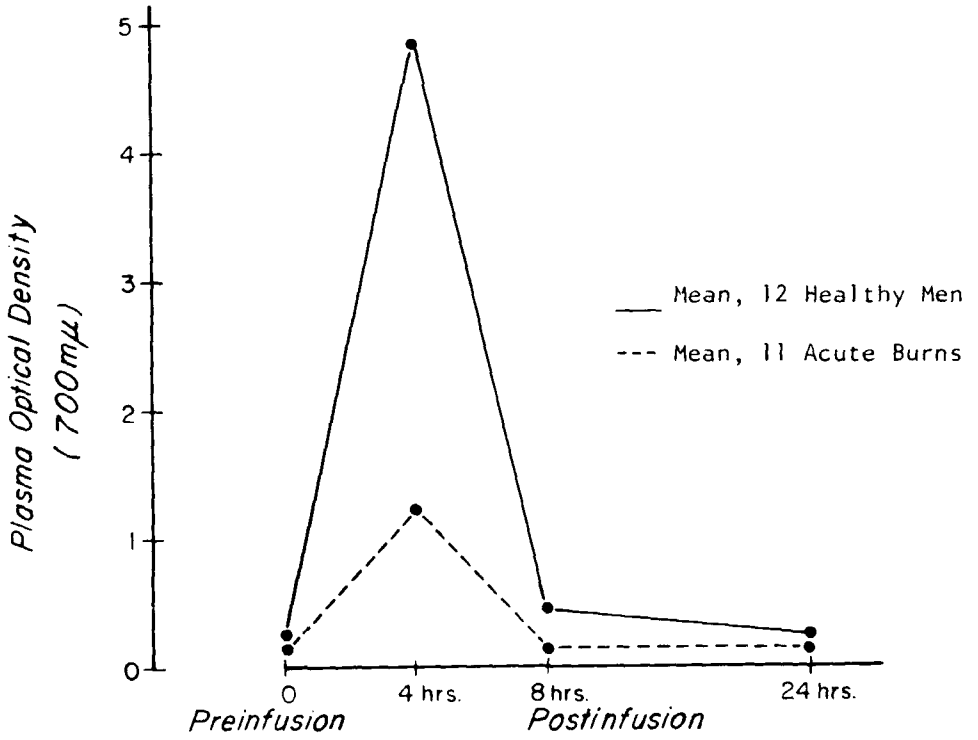


Figure 1. Fat clearance after 500 ml infusions of soy bean oil emulsion was accelerated in hypermetabolic, non-septic burn patients.

Multiple units of soy bean oil emulsion were infused in 10 patients to assess long-term effects. Although the average dose of fat emulsion administered did not exceed 3.3 grams/kg body weight in 24 hours, daily variations did occur, and several patients received as much as 5 grams/kg body weight on one or more days during the test periods. There were no untoward effects that could be related to the fat emulsion. Thermogenic reactions did not occur in response to the infusions; liver, renal and pulmonary function were unchanged from pre-infusion levels. Four of these critically ill patients died from unassociated causes, and at autopsies they did not have excess accumulation of fat in the lung, liver or other organs.

We conclude on the basis of these observations and the reports of other investigators that this soy bean oil emulsion is associated with few, if any, toxic side effects and that it is safe for use in severely injured man.

Studies to Clarify Nutritional Efficacy

Although clearance of fat emulsion from the plasma can not be equated with fat utilization, numerous studies suggest that infused fat emulsion is, in fact, utilized. Of particular note is the report by Geyer[3] that respiratory quotient does shift during fat infusion to reflect fat utilization. As previously published by Wilmore[2] and by Helmcamp and Wilmore,[4] biochemical evidence of essential fatty acid deficiency does occur in severely injured man, and this nutritional inadequacy can be successfully treated by inclusion of polyunsaturated fatty acids in the diet, intravenously or orally.

The following study[5] was undertaken to specifically determine the relative effects of fat and carbohydrate on nitrogen conservation during total intravenous feeding. Isonitrogenous intravenous diets containing 11.7 grams nitrogen/m^2/day as a crystalline amino acid solution (FreAmine, McGaw, Irvine, CA) were given to 5 patients during 29 three-day studies.

---

2. Wilmore DW, Moylan JA, Helmcamp GM, Pruitt BA Jr: Clinical evaluation of a 10% intravenous fat emulsion for parenteral nutrition in thermally injured patients. Ann Surg 178:503-513, 1973.
3. Geyer RP: Parenteral emulsions -- Formulations, preparation, use in animals. In Parenteral Nutrition, ed. HC Meng and DH Law. Charles C. Thomas, Springfield, p 339.
4. Helmcamp GM, Wilmore DW, Johnson AA, Pruitt BA Jr: Essential fatty acid deficiency in red cells after thermal injury: Correction with intravenous fat therapy. Amer J Clin Nutr 26:1331-1338, 1973.
5. Long JM, Wilmore DW, Mason AD Jr, Pruitt BA Jr: Effect of carbohydrate and fat intake on nitrogen excretion during total intravenous feeding. In press, 1975.

Each diet contained 1458, 875, 350, or 110 kilocalories/m^2/day as carbohydrate and 1108, 583, or zero kilocalories/m^2/day as fat emulsion. The numbers of three-day studies done with each diet are given in Figure 2. Each patient, stable after injury or operation, received at least three different diets for three days each in a randomly selected sequence. Validity of three-day study periods was confirmed by infusion of constant diets for 6 days or longer in 2 patients, neither of whom showed any significant adaptation after the second day of a particular intravenous regimen. The patient group included a fistula patient, a victim of chronic malnutrition after near-total gastrectomy for malignancy, a patient after amputation for electrical injury, and two moderately hypermetabolic burn patients, one of whom had chronic infection.

NUMBER OF THREE-DAY STUDIES WITH VARIED CALORIC SOURCE

Nitrogen Intake: 11.7 g/m^2/day

Fat Intake (kcal/m^2/day) \ Carbohydrate Intake (kcal/m^2/day)	110	350	875	1458
0	1	2	2	6
583	0	0	5	1
1108	1	6	2	3

Figure 2. The number of three-day studies are shown in the square corresponding to each combination of fat and carbohydrate.

Essential electrolytes were infused with each diet to maintain steady normal serum values during the study periods. Vitamin dosages were the same for each diet, and exogenous insulin was not required. Blood glucose, blood urea nitrogen, plasma insulin, and urine urea nitrogen were measured daily and resting metabolic rates were measured or predicted from previous observations.

Urea nitrogen excretion was inversely related to carbohydrate intake ($p < 0.01$), and addition of intravenous fat did not significantly influence urea nitrogen excretion at any level of carbohydrate intake (Figure 3). Furthermore, urea nitrogen excretion was directly related to resting metabolic rate ($p < 0.01$). Multiple regression analysis

NITROGEN EXCRETION WITH VARIED CALORIC SOURCE

Nitrogen Intake: 11.7 $g/m^2/day$

Fat Intake ($kcal/m^2/day$) \ Carbohydrate Intake ($kcal/m^2/day$)	110	350	875	1458
0	12.0	9.3	8.0	7.0
583	-	-	7.2	5.4
1108	11.6	9.5	8.0	6.7

Figure 3. Mean urea nitrogen excretion is shown for each combination of fat and carbohydrate. ($g/m^2/day$)

yielded this mathematical prediction of nitrogen excretion for these patients on isonitrogenous intravenous diets:

$$N_e = 17.44 - 1.997 \log_e C + 0.0752\ MR$$

$$r^2 = 0.89, \quad p < 0.05$$

N_e = nitrogen excretion ($g/m^2/day$)

C = carbohydrate intake ($kcal/m^2/day$)

MR = metabolic rate ($kcal/m^2/day$)

When expressed as a function of the ratio of carbohydrate intake to resting metabolic rate, urea nitrogen excretion decreased to its lowest values as carbohydrate calorie intake approached metabolic rate, the point at which the ratio reached 1.0 (Figure 4). Increasing carbohydrate intake

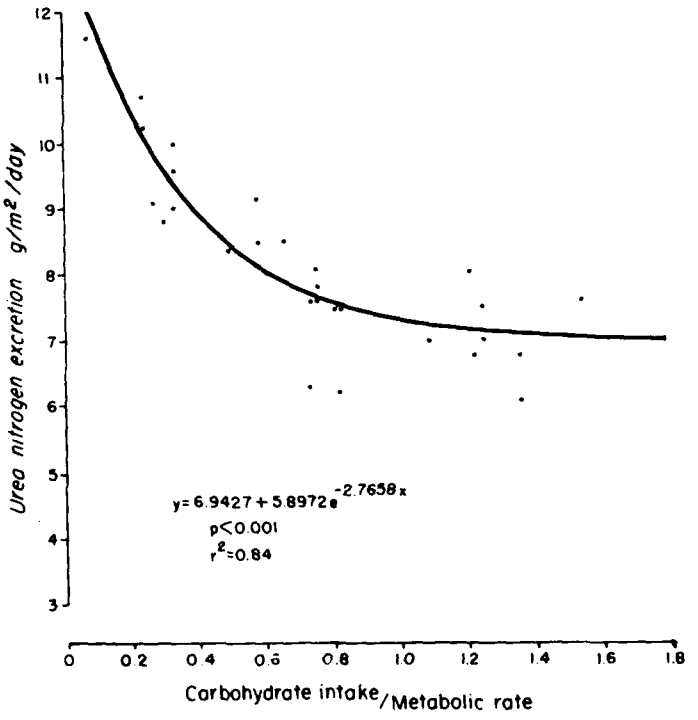


Figure 4. Urea nitrogen excretion reached a plateau as carbohydrate caloric intake approached metabolic rate, the point represented by 1.0 on the horizontal axis.

above metabolic rate did not effect any further decrease of nitrogen excretion.

Plasma insulin concentrations increased as carbohydrate dosage increased and as body size increased. Blood urea nitrogen levels were not altered by changing caloric source. Blood glucose remained normal, as did serum electrolytes, during the 29 three-day studies. In two patients who received supranormal carbohydrate dosages during 5 additional studies, adding exogenous insulin to control hyperglycemia caused further decrease of nitrogen excretion to a mean of 4.4 g/m^2/day, well below their predicted minimum of 6.8 g/m^2/day.

This study indicates that the determinants of nitrogen excretion during isonitrogenous total intravenous feeding of critically ill or injured man are, primarily, the amount of carbohydrate infused, and, secondarily, the metabolic rate. Nitrogen excretion decreased steadily as carbohydrate intake increased up to the level of the resting metabolic rate. When carbohydrate dosages exceeded metabolic rate, nitrogen retention was not further enhanced except when exogenous insulin was administered to control hyperglycemia. The relationship demonstrated by these data remained strikingly consistent despite the heterogenous group of patients who represented a broad range of ages (11 to 59 years) and metabolic rates (30 to 64 kcal/m^2/hour).

Since the introduction of intravenous fat emulsion, several authors have concluded that nitrogen conservation occurred with either cottonseed oil emulsion or soy bean oil emulsion.[6-11] Careful analysis of available data from those studies, however, has shown either that the patient groups were too small to derive meaningful conclusions or that the conclusions were not necessarily justified by the data. Where positive nitrogen balance was reported during fat infusion, other factors which are known to influence protein metabolism, such as nitrogen or carbohydrate intake, were also being changed at the same time fat was

---

6. Gorens SW, Geyer RP, Matthews LW. Stare FW: Parenteral nutrition. Observations on the use of fat emulsions for intravenous nutrition in man. J Lab Clin Med 34:1627-1633, 1949.
7. Van Itallie TB, Moore FD, Geyer RP, Stare FJ: Will fat emulsions given intravenously promote protein synthesis: Metabolic studies on normal subjects and surgical patients. Surgery 36:720-731, 1954.
8. Artz CP, Williams, TK: The protein-sparing effect of intravenous fat emulsions. Metabolism 6:682-690, 1957.
9. Wadstrom LB, Wiklund PE: Effect of fat emulsions on nitrogen balance in the postoperative period. Acta Chir Scan, Suppl 325:50-54, 1964.
10. Hallberg D. Schuberth O, Wretlind A: Experimental and clinical studies with fat emulsion for intravenous nutrition. Nutr Dieta (Basel) 8:245-281, 1966.
11. Zohrab WJ, McHattie JD, Jeejeebhoy KN: Total parenteral alimentation with lipid. Gastroenterology 64:583-592, 1973.

being added to or subtracted from the diet. None of the studies considered the possible impact of metabolic rate on nitrogen excretion.

The findings of the present study are corroborated by the report of Brennan and Moore[12] who showed that the nitrogen sparing effect of the soy bean oil emulsion was accounted for solely by the glycerol contained in the emulsion to provide isotonicity. Any further nitrogen conservation could be related to adaptation to starvation. Further supportive data has been presented by Professor Heller,[13] who observed progressive deterioration of nitrogen balance as fat emulsion comprised larger percentages of intravenously administered diets fed patients undergoing prolonged radiotherapy for metastatic malignancy.

We believe that the observations made during the present study become clinically important to severely stressed patients in whom a primary therapeutic goal is to minimize protein wasting. Such patients include those who are severely traumatized or burned. The first priority for energy support of these patients should be to provide carbohydrate calories in dosages amounting to more than 80% of resting metabolic rate. Intravenous fat could then be used as a satisfactory source of additional calories to meet additional energy requirements and promote weight gain or to provide a source of essential fatty acids.

Conclusion

Intralipid^{R} has been shown to be a safe and convenient source of essential fatty acids to correct biochemical evidence of essential fatty acid deficiency in critically injured soldiers. Intravenous fat emulsion is also an excellent source of additional calories, particularly when a primary nutritional goal is weight gain. We would caution, however, that severely stressed patients in whom the primary nutritional goal is nitrogen sparing should receive carbohydrate calories in doses approximating metabolic rate. Although intravenous fat emulsion increases our flexibility in the area of nutritional support for the severely injured soldier, fat did not appear to exert any protein sparing effect in these patients.

---

12. Brennan MF, Moore FD: An intravenous fat emulsion as a nitrogen sparer: comparison with glucose. J Surg Res 14:501-504, 1973.
13. Heller KL: The effect of fat in complete parenteral nutrition on the nitrogen balance. Presented at Ninth International Congress of Nutrition, Mexico City, 1972.

REFERENCES:

1. Soroff HS, Pearson E, Artz CP: An estimation of the nitrogen requirements for equilibrium in burned patients. Surg Gynecol Obstet 132:159-172, 1961.

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

3. Geyer RP: Parenteral emulsions -- Formulations, preparation, and use in animals. In Parenteral Nutrition, ed. HC Meng and DH Law, Charles C. Thomas, Springfield, p 339.

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

5. Long JM, Wilmore DW, Mason AD Jr, Pruitt BA Jr: Effect of carbohydrate and fat intake on nitrogen excretion during total intravenous feeding. In press, 1975.

6. Gorens SW, Geyer RP, Matthews LW, Stare FW: Parenteral nutrition. Observations on the use of fat emulsions for intravenous nutrition in man. J Lab Clin Med 34:1627-1633, 1949.

7. Van Itallie TB, Moore FD, Geyer RP, Stare FJ: Will fat emulsions given intravenously promote protein synthesis: Metabolic studies on normal subjects and surgical patients. Surgery 36:720-731, 1954.

8. Artz CP, Williams TK: The protein-sparing effect of intravenous fat emulsions. Metabolism 6:682-690, 1957.

9. Wadstrom LB, Wiklund PE: Effect of fat emulsions on nitrogen balance in the postoperative period. Acta Chir Scan, Suppl 325:50-54, 1964.

10. Hallberg D, Schuberth O, Wretlind A: Experimental and clinical studies with fat emulsion for intravenous nutrition. Nutr Dieta (Basel) 8:245-281, 1966.

11. Zohrab WJ, McHattie JD, Jeejeebhoy KN: Total parenteral alimentation with lipid. Gastroenterology 64:583-592, 1973.

12. Brennan MF, Moore FD: An intravenous fat emulsion as a nitrogen sparer: comparison with glucose. J Surg Res 14:501-504, 1973.

13. Heller KL: The effect of fat in complete parenteral nutrition on the nitrogen balance. Presented at Ninth International Congress of Nutrition, Mexico City, 1972.

PRESENTATIONS

Wilmore DW: Clinical Evaluation of a 10% Intravenous Fat Emulsion for Parenteral Nutrition in Thermally Injured Patients. American Surgical Association, Los Angeles, CA, 27 April 1973.

Long JM: Fat-Carbohydrate Interaction: Nitrogen Sparing Effect of Varying Caloric Sources for Total Intravenous Feeding. Surgical Forum, American College of Surgeons Clinical Congress, Miami, FL, 22 October 1974.

Long JM: Effect of Carbohydrate and Fat on Nitrogen Excretion during Total Intravenous Feeding. International Congress of Parenteral Nutrition, Montpellier, France, 13 September 1974.

Long JM: Comparison of Carbohydrate and Fat as Caloric Sources for Total Intravenous Feeding. South Texas Chapter, American College of Surgeons, Galveston, TX, 31 January 1975.

Long, JM: Effect of Carbohydrate and Fat Intake on Nitrogen Excretion during Total Intravenous Feeding. American Burn Association, Denver, CO, 21 March 1975.

Wilmore DW: The Efficacy of Parenteral Nutrition after Major Injury. International School of Medical Sciences, Erice, Trapani, Sicily, 27 May 1975.

Long, JM: Use of Intravenous Fat Emulsion in Trauma and Burn Patients. American Medical Association Symposium on Intravenous Fat Emulsion, Chicago, IL, 6 June 1975.

Long JM: Comparison of Carbohydrate and Fat as Caloric Sources. To be presented at the Surgical Forum, American College of Surgeons Clinical Congress, San Francisco, CA, 13 October 1975.

PUBLICATIONS:

Wilmore DW, Moylan JA, Helmcamp GM, Pruitt BA Jr: Clinical Evaluation of a 10% Intravenous Fat Emulsion for Parenteral Nutrition in Thermally Injured Patients. Ann Surg 178:503-513, 1973.

Helmcamp GM, Wilmore DW, Johnson AA, Pruitt BA Jr: Essential Fatty Acid Deficiency in Red Cells after Thermal Injury: Correction with Intravenous Fat Therapy. Amer J Clin Nutr 26:1331-1338, 1973.

Long JM, Wilmore DW, Mason AD Jr, Pruitt BA Jr: Fat-Carbohydrate Interaction: Nitrogen Sparing Effect of Varying Caloric Sources for Total Intravenous Feeding. Surg Forum 25:61-62, 1974.

Long JM, Wilmore DW, Mason AD Jr, Pruitt BA Jr: Effect of Carbohydrate and Fat on Nitrogen Excretion during Total Intravenous Feeding. Proceed-

ings of International Congress of Parenteral Nutrition, Montpellier, France, 1974.

Long JM, Wilmore DW, Mason AD Jr, Pruitt BA Jr: Effect of Carbohydrate and Fat Intake on Nitrogen Excretion during Total Intravenous Feeding. Ann Surg, to be published, 1975.

Long JM: Use of Intravenous Fat Emulsion after Trauma and Burns. Proceedings of the American Medical Association Symposium on Intravenous Fat Emulsions, Chicago, 1975.

Wilmore DW, Long JM, Mason AD Jr, Pruitt BA Jr: The Efficacy of Enteral and Parenteral Nutrition after Major Injury. A chapter in Surgical Metabolism, ed. D. Cuthbertson, to be published, 1975.

Long JM, Wilmore DW, Mason AD Jr, Pruitt BA Jr: Comparison of Carbohydrate and Fat as Caloric Sources. Surg Forum, to be published, 1975.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0E 6981	75 07 01	DD-DR&E/AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA: CONTRACTOR ACCESS	9. LEVEL OF SUM
74 07 01	D. CHANGE	U	U	NA	NL	[X] YES [ ] NO	A. WORK UNIT

10. NO. CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62110A	3A162110A821	00	109
b. CONTRIBUTING	61101A	3A161101A91C	00	
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code) (U) The Zinc Requirements of the Burned Rat and the Influence of Zinc on LDH Activity, Growth Rate and Wound Healing: A Model 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
73 11	Cont	DA	C. In-House

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

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
FISCAL YEAR PRECEDING 75	.3	9
FISCAL YEAR CURRENT 76	.3	10

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

20. PERFORMING ORGANIZATION
NAME: US Army Institute of Surgical Research
Laboratory Animal Branch
ADDRESS: Fort Sam Houston, Texas 78234
PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME: Donald J Johnson, MAJ, VC
TELEPHONE: 512-221-4951
SOCIAL SECURITY ACCOUNT NUMBER
ASSOCIATE INVESTIGATORS
NAME: Ysidro Villarreal, BS
NAME: DA

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Zinc Requirements; (U) Burns; (U) Wound Healing; (U) Lactate Dehydrogenase; (U) Rats

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

23. (U) To determine the effect of zinc dosage on plasma and tissue zinc levels and on lactate dehydrogenase (LDH) total activity and isoenzyme patterns during the postburn period. To determine if there are any gross changes in the healing process of the burn wound as a function of zinc intake as a guide to the care of burned soldiers. An elevated zinc requirement in burned rats may indicate a need for increasing the zinc intake of burned soldiers.

24. (U) Blood and tissue samples will be obtained at 24 hours, 48 hours, 72 hours, one week, and two weeks postburn for zinc and LDH total activity and isoenzyme pattern. Animals will be observed for signs of zinc deficiency as related to burn wound healing.

25. (U) 74 07 - 75 06 Serum zinc levels are decreased in burned animals which do not receive zinc supplementation. The administration of 100 micrograms of zinc intraperitoneally maintains the serum zinc level within normal limits during the post burn period. Alteration of the zinc and copper levels in the liver have been found in some of the burned and control animals on a zinc free diet.

DD FORM 1498 ... PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE.

ANNUAL PROGRESS REPORT

PROJECT NO. 3A162110A821-00, COMBAT SURGERY

REPORT TITLE: THE ZINC REQUIREMENTS OF THE BURNED RAT AND THE INFLUENCE OF ZINC ON LDH ACTIVITY, GROWTH RATE AND WOUND HEALING: A MODEL OF BURNED SOLDIERS

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

1 July 1974 - 30 June 1975

Investigators:

Donald J. Johnson, DVM, Major, VC
Ysidro Villarreal, BS
Harrel L. Walker, MS
Arthur D. Mason, Jr, MD

Report Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A162110A821-00, COMBAT SURGERY

REPORT TITLE: THE ZINC REQUIREMENTS OF THE BURNED RAT AND THE INFLUENCE OF ZINC ON LDH ACTIVITY, GROWTH RATE AND WOUND HEALING: A MODEL 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 1974 - 30 June 1975

Investigators: Donald J. Johnson, DVM, Major, VC
Ysidro Villarreal, BS
Harrel L. Walker, MS
Arthur D. Mason, Jr., MD

Report Control Symbol MEDDH-288(R1)

The purpose of this study is to determine if rats with large scald burns have an increased zinc requirement. Rats were scald burned over 60% of the total body surface and administered parenteral zinc at different dosages. Rats were also fed a zinc free diet to minimize the intake of exogenous zinc.

The low dose of zinc administered (50 micrograms daily) resulted in a slower repletion of plasma zinc levels and a slower growth rate than the high dose (100 micrograms daily). Significant alteration in the tissue zinc levels and LDH isoenzyme patterns have not been demonstrated.

Further studies will not be conducted using the present sampling frequency due to the lack of evidence to support marked alteration in tissue zinc levels in the postburn period.

Burns
Rat
Zinc requirments
Wound healing
Lactate dehydrogenase

THE ZINC REQUIREMENTS OF THE BURNED RAT AND THE INFLUENCE OF ZINC ON LDH ACTIVITY, GROWTH RATE AND WOUND HEALING: A MODEL OF BURNED SOLDIERS

A considerable loss of zinc is thought to occur in the postburn period. Approximately 20% of the total body zinc is present in the skin of man and the zinc in the burned areas may be lost with the eschar. Additional zinc losses may result from serum protein loss from the burn wound and in the urine. These losses may elevate the requirement for zinc in the burned man or animal. In this study burned rats were used to determine if an increased zinc requirement is needed for optimal wound healing.

METHODS

Male Sprague-Dawley rats weighing between 160-180 grams were used in this study. All animals were anesthetized with pentobarbital sodium and shaved over the area to be burned. Animals in the burn groups were 3^{O} scald burned over 60% of the total body surface by the method of Walker and Mason (1). All animals were fed a zinc-free diet (Nutritional Biochemical Co.) and zinc was administered daily as zinc acetate solution intraperitoneally. Animals not receiving zinc were administered normal saline intraperitoneally. The zinc content of tissues was determined by atomic absorbtion spectrophotometry. LDH isoenzyme patterns were determined by split-gel electrophoresis. Animals were obsrved daily for signs of zinc deficiency.

RESULTS

The growth rate of the control and burned animals is shown in Table 1. Five animals were studied in each group. The rate of weight gain in the burned animals appears to be dose related with the animals receiving the 100 ug zinc daily having the fastest growth response.

Table 1. Growth Rate

	Group	Treatment ug/zinc	Gm. Weight Gain/Days Postburn 15	30
I	Control	50	100	160
II	Control	None	47	94
III	60% Burn	None	5	21
IV	60% Burn	50 (daily)	14	36
V	60% Burn	100 (daily)	17	54

1. Walker HL, Mason AD, Jr: A standard animal burn. J Trauma 8: 1049-1051, 1968.

The plasma zinc levels are shown in Table 2. Three animals per group were studied at each time interval. The plasma zinc level is low from 24 hours postburn until one week postburn in all the burn groups. At two weeks postburn the animals receiving 100 ug zinc daily had plasma zincs in the normal range of 100 - 180 ug/100 ml. The unburned animals without zinc administration had a gradual decline in plasma zinc over the two week period.

Table 2. Mean Plasma Zinc Levels

	Group	Treatment ug/zinc	Time Postburn 24 hrs	48 hrs	96 hrs	7 days	14 days
I	Control	None	162	158	138	90	67
II	60% Burn	None	75	87	77	102	40
III	60% Burn	50 (daily)	77	70	100	129	62
IV	60% Burn	100 (daily)	64	93	105	120	145

* All values are micrograms per 100 ml plasma

Sixty-three liver samples were analyzed for zinc content. The zinc levels ranged from 40-93 ug zinc/gm of wet tissue. The zinc content of 35 testicles was 28-78 ug/gm of wet tissue. The level of zinc in the tissues varied greatly in each group of animals and no apparent relationship of the tissue zinc levels with time postburn or amount of zinc administered was seen. LDH isoenzyme patterns had altered patterns during the first 48 hours postburn with a return to normal patterns at 96 hours postburn. No signs of zinc deficiency were found in animals studied.

SUMMARY

The growth rate of the burned animal is the simplest means of determining the adequacy of parenteral zinc administration. 50 ug of zinc daily has been reported to rapidly replete a dietary induced zinc deficiency in the rat (2). In our study this dose does not result in rapid restoration of plasma zinc levels or optimum growth rate following burn injury.

The absence of signs of overt zinc deficiency may be due to the short period of time the animals were studied and the age of the animals. Neonatal rats are normally used in dietary zinc depletion studies. The rats used in this study were from 6-7 weeks of age when burned and therefore the animals may have sufficient zinc reserves to prevent the signs of a zinc deficiency.

2. Prasad AS (editor): Zince Metabolism. Chas. C. Thomas, Springfield, Ill., 1961.

The lack of evidence to indicate a marked alteration of tissue zinc levels and the rapid restoration of normal LDH isoenzyme patterns indicates further investigation of zinc levels at frequent intervals is not warranted. The analysis of the skin zinc levels on the rats studied so far has not been completed. No additional studies are planned until completion of this analysis and a re-design of the study.

PUBLICATIONS AND/OR PRESENTATIONS

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OE 6957	75 07 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
74 07 01	K. COMP	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO. CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	62110A	3A162110A821	00	112
b. CONTRIBUTING	61101A	3A161101A91C	00	
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code) (U) Immunity in Burned Animals - A Laboratory Model of Changes Occurring 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
73 04	75 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	75	.5	14
FISCAL YEAR — CURRENT			

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

20. PERFORMING ORGANIZATION
NAME: US Army Institute of Surgical Research
Burn Study Branch
ADDRESS: Fort Sam Houston, Texas 78234
PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME: Norman S. Levine, MD, LTC, MC
TELEPHONE: 512-221-2943
SOCIAL SECURITY ACCOUNT NUMBER
ASSOCIATE INVESTIGATORS
NAME: Harrel L. Walker, MS
NAME: Arthur D. Mason, Jr, MD DA

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Burns; (U) Bacterial infection; (U) Viral infection; (U) Rats; (U) Mice

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

23. (U) The objectives of this study were modified to test the interaction between wound coverage and survival on large clean excisions and smaller infected excisions as a model of changes in burned troops.

24. (U) Different wound dressings were tested on their ability to promote survival following 60% BSA rat excision and 15% infected excisions.

25. (U) 74 07 - 75 06 Rat cutaneous allograft, human xenograft and a synthetic dressing all promoted survival following 15% excision and seeding with Pseudomonas aeruginosa. Animals with open infected wounds died. No reproducible sterilization of the wound was encountered with any of these dressings in this model. Allograft and the synthetic dressing promoted survival following 60% BSA rat skin excisions. Steri-drape and gauze coverage did not.

*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 68 (FOR ARMY USE) ARE OBSOLETE

FINAL REPORT

PROJECT NO. 3A162110A821-00, COMBAT SURGERY

REPORT TITLE: IMMUNITY IN BURNED ANIMALS-A LABORATORY MODEL OF CHANGES OCCURRING IN BURNED TROOPS

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

1 July 1974 - 30 June 1975

Investigators:

Norman S. Levine, MD, Lieutenant Colonel, MC
Harrel L. Walker, MS
Arthur D. Mason, Jr., MD

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A162110A821-00, COMBAT SURGERY

REPORT TITLE: IMMUNITY IN BURNED ANIMALS-A LABORATORY MODEL OF CHANGES OCCURRING 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 1974 - 30 June 1975

Investigators: Norman S. Levine, MD, Lieutenant Colonel, MC
Harrel L. Walker, MS
Arthur D. Mason, Jr., MD

Reports Control Symbol MEDDH-288(R1)

Rat cutaneous allograft, human xenograft, and a synthetic dressing (consisting of a nylon matrix and a teflon membrane) were compared for effectiveness in promoting survival in laboratory animals. One group of Sprague-Dawley rats was subjected to excision of 15% of the total body surface area and seeding of the wounds with Pseudomonas aeruginosa; 60% total body surface area excision was performed on a second group with no seeding of the wounds. Rat cutaneous allograft, human xenograft, and the synthetic dressing all promoted survival following 15% excision and seeding with Pseudomonas aeruginosa. Animals with open-infected wounds died. No reproducible sterilization of the wound was encountered with any of the dressings. There was no survival in the group with 60% body surface area excision if the wounds were left uncovered. If allograft coverage was used, 10 day survival was 90%, with 75% survival for animals whose wounds were covered with the synthetic dressing. Coverage with Steri-drape or coarse mesh gauze resulted in no survivors. This study failed to support any consistent differences between the performance of allograft, xenograft, or synthetic dressing. The data suggests that the synthetic dressing may be useful as a temporary skin substitute.

Burns
Bacterial infection
Viral infection
Rats
Mice

IMMUNITY IN BURNED ANIMALS-A LABORATORY MODEL OF CHANGES OCCURRING IN BURNED TROOPS

A previous report indicated that at least some of the beneficial effects of cutaneous allograft could be achieved with a nonviable wound cover such as formalin-fixed cutaneous allograft. This report explores the possibility that some of the functions of skin may be replicated by a synthetic model of skin which is made entirely from commercially available materials.

METHODS

A synthetic dressing consisting of a nylon matrix and a teflon membrane was used.[1] Comparison of the synthetic dressing to rat cutaneous allograft and other wound covers was done on Sprague-Dawley rats of 180-200 g weight.

To study the behavior of the dressing on contaminated wounds, dorsal skin excisions, comprising 15% of the total body surface area of the rat, were performed and seeded with 1 cc of a known dilution of Pseudomonas aeruginosa, Strain 12-44. The inoculum was left on the open wounds for 10 minutes. Then, either rat allograft (from closely related Sprague-Dawley rats), human xenograft, synthetic dressing, or no dressing was applied. Nine animals were included in each group. One of these was biopsied for quantitative bacterial analysis after 10 minutes of inoculation. Three animals from each group were biopsied at specified days after inoculation and coverage (Tables 1 and 2). In the first experiment, granulation tissue was allowed to develop by excising the skin four days before inoculation, covering the fascial surface with sterile gauze, and stripping the gauze from the underlying tissue immediately prior to inoculation. In the second experiment, the skin excision was performed immediately before seeding of the wound.

The effect of the dressing was then studied on rats subjected to 60% body surface area skin excisions which were not seeded. Truncal skin was removed circumferentially en bloc from 5 mm distal to the axillary crease to 5 mm proximal to the skin folds of the hips. This area was either left open (20 animals), covered with multiple strips of freshly harvested rat cutaneous split-thickness allograft (20 animals), covered with synthetic dressing (20 animals), Steri-drape (10 animals), or four layers of coarse mesh gauze (10 animals).

---

1. Levine NS, Peterson HD, Mason AD Jr: Use of a synthetic dressing on denuded wounds in burned patients. USAISR Annual Research Report, 30 June 1975.

TABLE 1. EFFECT OF NO TREATMENT, RAT ALLOGRAFT, HUMAN XENOGRAFT, AND SYNTHETIC DRESSING ON 15% BSA* RAT GRANULATION TISSUE SEEDED WITH PSEUDOMONAS AERUGINOSA**

		Biopsy Culture (Day 0)	Biopsy Culture (Day 2)	Biopsy Culture (Day 4)
No Cover		2×10^3	4.1×10^5 (7 others died)	
Rat Allograft	(A-1)	1×10^3	1.2×10^4	5×10^3
	(A-2)		1.6×10^3	4×10^4
	(A-3)		3.7×10^3	5×10^4
Human Xenograft	(X-1)	5×10^3	3×10^4	7×10^1
	(X-2)		8.5×10^3	10
	(X-3)		1.5×10^3	3×10^2
Synthetic	(S-1)	2×10^4	1×10^4	8×10^5
	(S-2)		1.2×10^3	2×10^6
	(S-3)		9×10^2	7×10^4

*BSA = body surface area

**Wounds were seeded with a 1 cc inoculum of Pseudomonas aeruginosa (2×10^3) in saline.

TABLE 2. EFFECT OF NO TREATMENT, RAT ALLOGRAFT, HUMAN XENOGRAFT, AND SYNTHETIC DRESSING ON 15% BSA* RAT FASCIAL WOUNDS SEEDED WITH PSEUDOMONAS AERUGINOSA**

		Biopsy Culture (Day 0)	Biopsy Culture (Day 4)	Biopsy Culture (Day 8)
No Cover		5×10^3	10 animals dead	
Rat Allograft	(A-1)	1.2×10^4	1.7×10^4	No growth
	(A-2)		8×10^3	2×10^4
	(A-3)		2.6×10^2	7.1×10^2
Human Xenograft	(X-1)	7×10^3	6×10^3	5×10^1
	(X-2)		7.3×10^3	6.2×10^3
	(X-3)		1×10^3	4×10^6
Synthetic	(S-1)	8.5×10^3	2×10^4	2.6×10^3
	(S-2)		No growth	No growth
	(S-3)		5.6×10^3	1.1×10^2

*BSA = body surface area

**Wounds were seeded with a 1 cc inoculum of Pseudomonas aeruginosa (5×10^4) in saline.

Dressings were secured to the wound edges with metal clips. Rat cutaneous allograft was harvested from donor rats of the same nonisogenic strain with the electric dermatome. Usually, four or five strips of allograft were needed to cover the 60% excision. Survival of the animals was observed for 10 days.

RESULTS

In the experimentally seeded group of animals, there were some inconsistencies noted in the performance of the different wound covers. In one experiment, those wounds treated with xenograft appeared to demonstrate the lowest number of organisms in the underlying tissue at four days following application of either one of the three dressings or no treatment. This variation between the colony counts in biopsies of wounds treated with different dressings was not verified by the next experiment (Table 2), in which the lowest bacterial counts appeared in the animals treated with the synthetic dressing. Further repetition of this type of experiment failed to support any consistent differences between the performance of allograft, xenograft, or synthetic dressing in this model. The only consistent finding was that all three dressings appeared to prevent the death from systemic pseudomonas infection which occurred in the seeded, uncovered models.

The 60% body surface area excision was uniformly lethal if no form of wound coverage was employed. If allograft coverage was used, 10 day survival was 90%. If synthetic coverage was used, 10 day survival was 75%. Coverage with Steri-drape or coarse mesh gauze resulted in no survivors. After 10 days, there were occasional deaths in the synthetic-covered group (from infection arising in the margins of the dressing) and in the allograft-covered group (if allograft rejection occurred).

CONCLUSIONS

In two models of skin graft function, the beneficial effects of allograft could be duplicated in part by a syntnetic dressing. The data suggest that a synthetic dressing of this or similar construction may be useful as a temporary skin substitute.

PRESENTATIONS AND/OR PUBLICATIONS

None

PUBLICATIONS

1 July 1974 - 30 June 1975

Salisbury RE, Mason AD, Jr, Levine NS, Pruitt BA, Jr, Wade CWR: Artificial tendons: Design, application, and results. Journal of Trauma 14:580-586, July 1974.

Czaja AJ, McAlhany JC, Pruitt BA, Jr: Acute gastroduodenal disease after thermal injury. An endoscopic evaluation of incidence and natural history. New England Journal of Medicine 291:925-929, October 31, 1974.

Czaja AJ, McAlhany JC, Pruitt BA, Jr: Acute duodenitis and duodenal ulceration after burns. Clinical and pathological characteristics. Journal of the American Medical Association 232:621-624, May 12, 1975.

Wilmore DW, Long JM, Mason AD, Jr, Skreen BW, Pruitt BA, Jr: Catecholamines: Mediator of the hypermetabolic response to thermal injury. Annals of Surgery 180:653-669, October 1974.

Warden GD, Mason AD, Jr, Pruitt BA, Jr: Evaluation of leukocyte chemotaxis in vitro in thermally injured patients. Journal of Clinical Investigation 54:1001-1004, October 1974.

Wilmore DW, Mason AD, Jr, Johnson DW, Pruitt, BA, Jr: Effect of ambient temperature on heat production and heat loss in burn patients. Journal of Applied Physiology 38:593-597, April 1975.

Pruitt, BA, Jr: Infections caused by pseudomonas species in patients with burns and in other surgical patients. Journal of Infectious Diseases 130, Suppl:S8-S13, November 1974.

Salisbury RE, McKeel DW, Mason AD, Jr: Ischemic necrosis of the hand after thermal injuries. Journal of Bone and Joint Surgery 56A:1701-1707. December 1974.

Salisbury RE, McKeel DW, Pruitt BA, Jr, Mason AD, Jr, Palermo N, Wade CWR: Morphologic observations of neosheath development of undifferentiated connective tissue development around artificial tendons. Journal of Biomedical Material Research Symposium No.5, Part I: 175-184, 1974.

Wilmore DW: Nutrition and metabolism following thermal injury. Clinics in Plastic Surgery 1:603-619, October 1974.

Lindberg RB, Latta RL: Phage typing of pseudomonas aeruginosa: Clinical and epidemiologic considerations. Journal of Infectious Diseases 130. Suppl:S33-S42, November 1974.

Slogoff SS, Allen GW: The role of baroreceptors in the cardiovascular response to ketamine. Anesthesia and Analgesia - Current Researches 53:704-707, Sept-Oct 1974.

Warden GD, Mason AD, Jr, Pruitt BA, Jr: Suppression of leukocyte chemotaxis in vitro by chemotherapeutic agents used in the management of thermal injuries. Annals of Surgery 181:363-369, March 1975

Salisbury RE, Silverstein P, Goodwin MN, Jr: Upper extremity fungal invasions secondary to large burns. Plastic and Reconstructive Surgery 54:654-659, December 1974.

DISTRIBUTION LIST

5 copies to:

US Army Medical Research and
Development Command
Washington, DC 20314

12 copies to:

Defense Documentation Center
ATTN: DDC-TCA
Alexandria, Virginia 22314

1 copy to:

Superintendent
Academy of Health Sciences, US Army
ATTN: AHS-COM
Fort Sam Houston, Texas 78234

1 copy to:

Dir of Biol & Med Sciences Div
Office of Naval Research
800 N. Quincy Street
Arlington, VA 22217

1 copy to:

CO, Naval Medical R&D Command
National Naval Medical Center
Bethesda, MD 20014

1 copy to:

Dir of Prof Svcs
Office of the Surgeon General
Department of the Air Force
Washington, DC 20314

1 copy to:

Director of Defense Research and Engineering
ATTN: Assistant Director (Environmental and Life Sciences)
Washington, DC 20301

510 * U.S. GOVERNMENT PRINTING OFFICE: 1976-675-381/280