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

Research Report

ANNUAL RESEARCH PROGRESS REPORT

REPORTS CONTROL SYMBOL MEDDH-288(R1) 30 June 1971

Project Nos.
3A061102B71R-01, RES. IN BIOMED. SCIENCES
3A061101A91C-00, IN-HOUSE LAB. INDEPENDENT RES.
3A062110A821-00, COMBAT SURGERY
3A061102B71P-08, BASIC RES.IN SUPPORT OF MIL.MED.

UNCLASSIFIED

Security Classification

DOCUMENT CONTROL DATA - R & D

(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)

1. ORIGINATING ACTIVITY (Corporate author)
US Army Institute of Surgical Research
Brooke Army Medical Center
Fort Sam Houston, Texas 78234

2a. REPORT SECURITY CLASSIFICATION
NONE

2b. GROUP
NONE

3. REPORT TITLE

ANNUAL RESEARCH PROGRESS REPORT, 31 June 1971

4. DESCRIPTIVE NOTES (Type of report and inclusive dates)
ISR Annual Research Report for Period 1 July 1970 - 30 June 1971

5. AUTHOR(S) (First name, middle initial, last name)

See Individual Reports

6. REPORT DATE
30 June 1971

7a. TOTAL NO OF PAGES

7b. NO OF REFS
NA

8a. CONTRACT OR GRANT NO
NONE

b. PROJECT NO
61102A 3A061102B71R

c.

d.

9a. ORIGINATOR'S REPORT NUMBER(S)
ISR DCD-19-71

9b. OTHER REPORT NO(S) (Any other numbers that may be assigned this report)
NA

10. DISTRIBUTION STATEMENT

Approved for public release. Distribution unlimited.

11. SUPPLEMENTARY NOTES
NONE

12. SPONSORING MILITARY ACTIVITY
Hq, US Army Med Res & Dev Command
DA, Washington, DC 20314

13. ABSTRACT

This report documents the clinical and laboratory activities of the USAISR during fiscal 1971. These activities include patient care, clinical investigation and laboratory research in the areas of (1) burn injury, (2) acute renal failure and (3) general trauma. Special emphasis is placed on the clinical managment of burned patients and on studies related to the prevention and treatment of burn wound infection. (U)

DD FORM 1 NOV 68 1473 REPLACES DD FORM 1473, 1 JAN 66, WHICH IS OBSOLETE FOR ARMY USE.

UNCLASSIFIED

Security Classification

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.

14 KEY WORDS	LINK A		LINK B		LINK C	
	ROLE	WT	ROLE	WT	ROLE	WT
See individual reports.						

AD _____

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

ANNUAL RESEARCH PROGRESS REPORT

30 June 1971

UNCLASSIFIED

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

MEDEW-RS

30 July 1971

SUBJECT: Annual Research Report FY 1971

TO: See Distribution

Annual reports of the U.S. Army Institute of Surgical Research for FY 1970 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., M.D.
Lieutenant Colonel, MC
Commander & Director

FOREWORD

The clinical care and research progress reported herein illustrate again the value of the multidisciplinary team approach to specific military medical problems. The contributions of our veterinarians, microbiologists, biochemists, dietitians, nurses, physical and occupational therapists as well as those of all our variously trained physicians have been essential to the advances in burn care and to the development of this Institute as a whole.

Within the past year prognostications of scientific progress and achievement as well as budget needs covering the next five to ten years have been requested and supplied. The assumption that research is a predictably ordered process which can be routinely programmed in this manner has in part contributed to the current disaffection with science in general, since such predictions, once issuing from the investigator, are taken as promises of effortless solutions with no allowance made for unforeseen problems, frequently making realization less than expectation. Review of this Institute's "track record" reveals that advances in burn care have been more episodic than smoothly progressive and that serendipity and perceptive insight, both unprogrammed, have been of striking importance. Another limiting consideration in terms of assessing payoff is the "lag" period, again illustrated by the history of this Institute. Only after 17 years had the necessary professional team been assembled and the meticulous, tedious, descriptive groundwork been laid which resulted in the development of effective topical chemotherapy.

The foresight of the Medical Department in establishing this burn unit has only recently been recognized. The quality, economy and advances of burn care promoted by this Institute are now being transmitted to the country at large by organization of other burn centers, 40 per cent of which are directed by physicians who have served at this Institute. The exponential nature of the accomplishments, once the proper background was created, has more than justified the Medical Department's investment and recommends similar multidisciplinary units capable of effective synthesis of clinical and investigative activities for any planned attack on other areas of interest in military medicine.

Basil A. Pruitt Jr.

BASIL A. PRUITT, JR., M.D.
LTC, 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	71 07 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8. DISP'N INSTR'N	8a. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
70 07 01	D. CHANGE	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO. CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A061102B71R	01	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

	PRECEDING FY	a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
FISCAL YEAR	71	40.3	434.2
CURRENT FY		40.3	456.0

19. RESPONSIBLE DOD ORGANIZATION

NAME: US Army Institute of Surgical Research

ADDRESS: Ft Sam Houston, Texas 78234

RESPONSIBLE INDIVIDUAL

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

TELEPHONE: 512-221-2720

20. PERFORMING ORGANIZATION

NAME: US Army Institute of Surgical Research
Clinical Division

ADDRESS: Ft Sam Houston, Texas 78234

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

NAME: P.W. Curreri, LTC, MC

TELEPHONE: 512-221-3301

SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS

NAME: B.A. Pruitt, Jr, LTC, MC

NAME: DA

21. GENERAL USE

FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede each with Security Classification Code)

(U) Thermal injury; (U) Topical therapy; (U) Autograft; (U) Homograft; (U) Heterograft; (U) Resuscitation; (U) Air evacuation; (U) Mortality

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

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

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

25. (U) 70 01 - 71 06 During 1970, 325 patients were admitted to the Institute; 184 patients were evacuated from Vietnam via Japan. Pulmonary infection with gram negative bacteria continued to be the most frequently observed complication of thermal injury, and intensive investigation of methods to prevent and more adequately treat this complication are well under way. Principles of management previously developed at this Institute remain unchanged. Several new clinical approaches to the treatment of severe thermal injury and its complications have been evaluated and adopted.

*Available to contractors upon originator's approval

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

ANNUAL PROGRESS REPORT

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

REPORT TITLE: CLINICAL OPERATION, BURN CENTER

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

1 January - 31 December 1970

Investigators:

P. William Curreri, M.D., LTC, MC
Basil A. Pruitt, Jr., M.D., LTC, MC
Harold M. Bruck, M.D., LTC, MC
Morris J. Asch, M.D., LTC, MC
Andrew M. Munster, M.D., LTC, MC
Wellford W. Inge, Jr., M.D., LTC, MC
Thomas W. Newsome, M.D., MAJ, MC
Joseph A. Moylan, Jr., M.D., MAJ, MC
William F. McManus, M.D., MAJ, MC
Jon M. Reckler, M.D., MAJ, MC
Paul Silverstein, M.D., MAJ, MC
Alan H. Morris, M.D., MAJ, MC
Kenneth W. Spitzer, CPT, MSC
Martin G. White, M.D., MAJ, MC
F. Daniel Foley, M.D.
Gerald Nash, M.D., MAJ, MC
Malcolm N. Goodwin, Jr., M.D., MAJ, MC
Joseph M. Garfield, M.D., MAJ, MC
J. Gilbert Stone, M.D., MAJ, MC
Gary W. Allen, M.D., MAJ, MC
Katherine F. Galloway LTC, ANC
Lois A. Johns, LTC, ANC
Wilma F. Hall, MAJ, AMSC
Elaine M. Kirkman, CPT, AMSC
Kilulu M.P. Von Prince, MAJ, AMSC
Leah Palm, CPT, AMSC
Robert L. Remig, CPT, AMSC
Mary E. Spitzer, CPT, AMSC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

BLANK PAGE

ABSTRACT

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

REPORT TITLE: CLINICAL OPERATION, BURN CENTER

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

Period covered in this report: 1 January - 31 December 1970

Investigators: P. William Curreri, M.D., LTC, MC
Basil A. Pruitt, Jr., M.D., LTC, MC
Harold M. Bruck, M.D., LTC, MC
Morris J. Asch, M.D., LTC, MC
Andrew M. Munster, M.D., LTC, MC
Wellford W. Inge, Jr., M.D., LTC, MC
Thomas W. Newsome, M.D., MAJ, MC
Joseph A. Moylan, Jr., M.D., MAJ, MC
William F. McManus, M.D., MAJ, MC
Jon M. Reckler, M.D., MAJ, MC
Paul Silverstein, M.D., MAJ, MC
Alan H. Morris, M.D., MAJ, MC
Kenneth W. Spitzer, CPT, MSC
Martin G. White, M.D., MAJ, MC
F. Daniel Foley, M.D.
Gerald Nash, M.D., MAJ, MC
Malcolm N. Goodwin, Jr., M.D., MAJ, MC
Joseph M. Garfield, M.D., MAJ, MC
J. Gilbert Stone, M.D., MAJ, MC
Gary W. Allen, M.D., MAJ, MC
Katherine F. Galloway, LTC, ANC
Lois A. Johns, LTC, ANC
Wilma F. Hall, MAJ, AMSC
Elaine M. Kirkman, CPT, AMSC
Kilulu M.P. Von Prince, MAJ, AMSC
Leah Palm, CPT, AMSC
Robert L. Remig, CPT, AMSC
Mary E. Spitzer, CPT, AMSC

Reports Control Symbol MEDDH-288(R1)

The Clinical Division of the US Army Institute of Surgical Research has continued to place a major emphasis on providing optimal clinical care for military personnel with major thermal injury during the past year. At the same time, considerable

effort has been expended to pursue clinical investigation of the complex physiological, biochemical, and bacteriological alterations noted in burn patients. In addition, almost every request for participation of Institute personnel in educational programs related to the treatment of trauma has been fulfilled. This report summarizes the activities of the Burn Center in 1970 and notes the few adopted departures from previous therapeutic modalities. It also cites the recognizable current complications of burn injury which most frequently contribute to a fatal outcome.

The clinical experience with porcine heterograft, ketamine anesthesia, ultrasonic flowmeter measurements, serial pulmonary function evaluation, and treatment of hypertrophic scar is outlined. In addition, a detailed statistical summary is included, in order to evaluate changing trends in the incidence of complications and mortality. Pulmonary infection remains the commonest complication of thermal injury, and for this reason analysis of bacterial cultures from sputum and lung tissue is included. Providencia stuartii, a gram-negative bacteria, has been recovered from sputum with increasing frequency during the past two years, while the incidence of other bacteria in sputum has decreased or remained unchanged. Mortality rates in the 30%-60% burn group appeard to increase during the same time period. However, more extensive investigation will be required to establish any definite relationship between the above observations.

Thermal Injury
Topical Therapy
Autograft
Homograft
Heterograft
Resuscitation
Air Evacuation
Mortality

CLINICAL OPERATION, BURN CENTER

Personnel responsible for direction of clinical research, education, and medical management at the US Army Institute of Surgical Research have remained relatively unchanged since publication of the last Annual Report. Therefore, this report will focus only on recent changes in medical therapy, current trends in clinical research, and statistical analysis of clinical results during calendar year 1970. Readers interested in admission requirements and evacuation procedures are referred to the last Annual Report, published 30 June 1970.

The total number of admissions to the Burn Center increased from 301 in 1969 to 325 in 1970, primarily as a result of a slight increase in the number of casualties evacuated from the Republic of Vietnam. In April 1970, the 106th General Hospital, at Kishine Barracks, Yokohama, Japan, was closed, and a new burn holding unit was established at the US Army Hospital, Camp Zama, Japan. Burn teams from the Institute were again sent on request to Japan for preflight evaluation and in-flight care of thermally injured patients evacuated from the Far East. These teams made 20 flights from Yokota Air Force Base, Japan, to Kelly Air Force Base, Texas, in 1970, and 172 patients were escorted to the Burn Center.

In addition, 66 emergency air evacuation missions were accomplished within the Continental United States, as well as two to El Salvador, one to Nicaragua, one to the Canal Zone, and two to Alaska. Following preflight evaluation and treatment, 93 patients were safely returned to the Institute for definitive care.

Clinical Management

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

Extensive studies of circulatory changes in circumferentially burned extremities utilizing an ultrasonic flowmeter have been rewarding, and ultrasonic evaluation of extremity circulation is now routinely used as an adjunct to clinical signs in assessing the need for escharotomy. The addition of this clinical tool has allowed conservative therapy consisting of extremity elevation and exercise in approximately 50% of limbs which would have required

escharotomy on the basis of clinical signs alone. The importance of hourly active exercise in preventing venous stasis in the distal extremity was demonstrated.

Since pulmonary complications with respiratory failure still remain the leading cause of death in the vast majority of patients who expire, greater efforts are now being exerted to diagnose pulmonary complications as early as possible. In addition to serial chest radiographs and arterial blood gas studies, sequential measurements of tidal volume, respiratory rate, minute volume, and concentration of inspired oxygen are made several times each day during the early postburn period. Increasing minute volume with tachypnea and labored respirations has been the most reliable sign of impending respiratory complications and usually precedes by several days changes in blood gases, chest X-ray and physical findings. Careful observation for signs of physical exhaustion as a result of increased respiratory effort is required in order that mechanical ventilatory assistance can be instituted at the optimal time. The addition of a cryogenic air compressor to the intensive care unit has been of great value in allowing ventilatory assistance by pressure sensitive respirators without danger of oxygen toxicity.

We have now had more than two years' experience with the utilization of split-thickness porcine cutaneous heterograft as a substitute for fresh human homograft in the preparation of granulating wounds for application of autograft. Both fresh procine heterograft and frozen, electron-beam sterilized porcine heterograft appear to be satisfactory substitutes. The latter has the advantage of indefinite storage but the disadvantage of slightly increased purchase cost. Both animal and clinical studies indicate that the application of porcine heterograft elicits very little, if any, clinically significant immune response. Because it never vascularizes, the graft is lost as a result of primary ischemia rather than immunologic rejection.

During the previous 18 months, controlled clinical trials were completed with ketamine anesthesia, and subsequently this anesthetic has been extensively utilized for minor reconstructive operative procedures. There exists a high incidence of perceptual alterations following administration of ketamine as compared to control groups receiving halothane, regardless of the extent of preoperative informative preparation. However, almost all patients receiving ketamine found it acceptable and were amenable to its use repetitively.

More experience has been gained in the treatment of hypertrophic scar formation. The application of pressure devices in the form of stockings and gloves, the early application of isoprene splints to the neck and extremities, and the intradermal injection of steroids have all had limited and varied success in the treatment of hypertrophic scar formation and contractures, although each is associated with some clinical improvement. Of prime importance is early initiation of therapy in patients with deep second-degree burns.

Clinical Research Projects

Most of the current clinical investigations are reported in detail in the subsequent pages of this report. They encompass many disciplines and include experimental studies in nutrition, intracellular metabolism, platelet function and coagulation dynamics, soft tissue calcification, pulmonary and renal function, and gastric physiology. In addition, greater efforts have been expended in the following areas: the development of artificial skin substitutes and alternative topical antibiotics, preparation for the evaluation of Pseudomonas hyperimmune gamma globulin, and the experimental study of an *in vitro* assay to detect circulating endotoxin.

Education

One of the prime missions of the Institute embodies the dissemination of current methods of burn treatment to both the military and civilian medical community. This is accomplished through in-house training for surgical residents, foreign physicians, and paramedical personnel, as well as extensive publication and lecture commitments fulfilled by the clinical staff each year.

During the report period, eight surgical residents from Brooke General Hospital, Wilford Hall USAF Hospital, and Fitzsimons General Hospital each participated as an active member of the medical staff for 2- to 3-month periods as part of their surgical training. Also, a senior medical officer from the Public Health Service Indian Medical Center in Phoenix, Arizona, participated in a 2-1/2 month training period, and three medical students from the University of Texas Medical School at San Antonio and Tufts University School of Medicine had 4- to 6-week assignments with the Institute. In addition, 11 physicians representing the US Army, the US Air Force, the US Army Reserve, the US Navy Reserve, and civilian burn units each spent one to two weeks at the Institute during the calender year for postgraduate training. Finally, approximately 150 civilians and 125 military physicians, students and paramedical personnel visited

the Institute in 1970. Thirty foreign physicians from Australia, Canada, England, Ghana, India, Israel, Japan, Okinawa, Pakistan, Portugal, Republic of Germany, Sweden, Thailand, and Venezuela received extensive briefings regarding the development and maintenance of burn units in general and this Institute in particular.

More than 90 formal presentations were given by members of the Clinical Division during the report period. The staff continued to actively participate in teaching programs for physicians, nurses, and physical therapists at the Medical Field Service School, and in addition supported the Brooke General Hospital Symposium on Surgical and Orthopedic Aspects of Trauma, the School of Aerospace Medicine at Brooks Air Force Base, Texas, the Military Medicine and Allied Sciences Course at Walter Reed Army Medical Center, the Army Chaplain Training Program at Brooke Army Medical Center, as well as numerous national and regional civilian medical organizations. Formal scientific presentations were made to the American Burn Association, the American Medical Association, the Third International Congress for Research in Burns, the International Joint Conference on Burn Therapy, the US Civil Defense Council, the American Association for the Surgery of Trauma, the American College of Surgeons, and the American Surgical Association.

Statistical Resume

During calendar year 1970, 325 thermally injured patients were admitted to the US Army Institute of Surgical Research, of which 184 (57%) were evacuated from the Republic of Vietnam. There were 321 dispositions during the year, and all subsequent statistics refer to these dispositions.

The patients ranged in age from 6 months to 81 years and included 292 males and 29 females. The average patient was 23.9 years of age, with a 30.3% total body surface burn (11.9% third-degree burn). Of the 321 dispositions, 250 (78%) had some full-thickness burn.

Thirty-six patients (11%) were in the pediatric age group (0-14 years). The average child was 6.4 years of age, with 29.4% total body surface burn (18.6% third-degree burn). Seventy-eight per cent of the children had full-thickness thermal injury.

There were 70 deaths among the 321 dispositions, resulting in an over-all mortality rate of 21.8%, which approximates the mortality rate in 1969. The average patient dying was 29 years of age, with a 51.9% total body surface burn, of which 32.6% was third-

degree burn. Sixty-two (89%) of these patients had postmortem examinations. Only 11 patients evacuated from the Republic of Vietnam expired, as compared to 59 patients admitted from the Continental United States, in part reflecting the selection process which occurs during the chain of evacuation from the Far East as well as the longstanding policy of evacuating only the more severely burned patients (greater than 20%) referred from within the Continental United States. Thirteen of the 70 patients were in the pediatric age group. The average postburn day of death was 19.

The source of admission of patients during 1970 is tabulated in Table 1. Again, the majority of patients were evacuated from the Republic of Vietnam, a total of 184 (54%). Active duty or re-retired military personnel accounted for 235 dispositions. In addition, 44 dependents of military personnel and 17 Beneficiaries of the Veterans Administration required in-patient therapy.

Table 2 summarizes the mode of injury in patients evacuated from the Far East from 1965-1970. Most striking is the marked rise in aircraft (primarily helicopter) accidents during the past year. Simultaneously, there appears to be a modest decrease in the number of men injured in armed personnel carriers struck by rocket-propelled grenades.

Table 3 illustrates the effect of age and total body surface burn on mortality. It is important to note that only one patient with greater than 60% burn survived--again reinforcing our previous reports that topical bacterial suppression has not significantly reduced mortality in patients with extensive thermal injury. Likewise, there was only one survivor less than 2 years of age and one survivor greater than 60 years of age, reconfirming the increased mortality in the pediatric and geriatric age groups.

The mortality rates in increments of 10% total body surface burn for 1968-1970 are tabulated in Table 4. Most striking is the increased mortality rate noted in 1969 and 1970 in the 30-60% total body surface burn group as compared to similar data from 1968.

In Table 5, the survival and mortality data for patients with greater than 30% burns (1955-1970) are presented. No striking change was noted in 1970.

Table 6 compares mortality in the years 1962 and 1963 prior to the use of topical Sulfamylon with the cumulative experience

Table 1. Source of Admission, 1970

Area	A	AD	AF	AFD	N	ND	VAB	Other	TOTAL
1st Army	1	3	0	0	0	0	0	0	4
3rd Army	11	5	0	1	5	2	2	4	30
4th Army	10	11	4	5	4	1	13	12	60
5th Army	2	3	2	1	0	1	0	1	10
6th Army	1	3	3	2	0	0	1	2	12
MDW	1	0	1	0	0	0	0	0	2
Germany	2	0	0	0	0	0	0	0	2
Viet Nam	153	0	6	0	24	0	0	1	184
Alaska	0	0	0	0	0	0	1	0	1
Korea	3	0	0	0	0	0	0	0	3
Hawaii	0	1	0	0	0	0	0	0	1
Hong Kong	0	0	0	0	1	0	0	0	1
Canal Zone	0	1	0	0	0	0	0	1	2
Okinawa	0	3	0	1	0	0	0	0	5
San Salvador	0	0	0	0	0	0	0	4	4
Nicaragua	1	0	0	0	0	0	0	0	1
Japan	0	0	0	1	0	0	0	0	1
	185	29	16	11	34	4	17	25	321

A - Army
AF - Air Force
N - Navy & Marine Corps
D - Dependent
VAB - Veterans Administration Beneficiary
MDW - Military District of Washington

Other: Civilian Emergency (14)
Designee of Secretary of Army (5)
US Public Health Service Beneficiary (4)
Bureau of Employees' Compensation Beneficiary (1)
Job Corps (1)

Table 2. Mode of Injury - Patients from Viet Nam, 1965-1970

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

Table 3. Age, Body Surface Involvement & Mortality, 1970

Age (Yrs)	Per Cent Burn										Total Cases	Total Deaths	% Mortality
	0-10	10-20	20-30	30-40	40-50	50-60	60-70	70-80	80-90	90-100			
0-1	0	0	2 (2)	0	0	1 (1)	0	0	0	0	3	3	100
1-2	0	0	1 (1)	0	1	0	0	0	0	0	2	1	50
2-3	3	1	0	1 (1)	0	0	0	0	0	0	5	1	20
3-4	0	0	1	1 (1)	1	0	0	0	0	0	3	1	33 3
4-5	0	1	0	1	2	0	0	0	0	0	4	0	0
5-10	2	2	0	1 (1)	3 (2)	0	1 (1)	0	0	0	9	4	44.4
10-15	1	3	2 (1)	2	1 (1)	0	0	0	0	1 (1)	10	3	33.3
15-20	7	9	9	5 (1)	3	0	2 (2)	0	0	1 (1)	36	4	11.1
20-30	25	34	40 (2)	39 (2)	31 (6)	10 (4)	4 (4)	6 (6)	3 (3)	0	192	27	14.1
30-40	5	2	5 (1)	4 (1)	3 (2)	3 (3)	0	1 (1)	0	0	23	8	34.8
40-50	1	3 (1)	2	4 (1)	1 (1)	2 (1)	3 (3)	1 (1)	0	0	17	8	47.1
50-60	1	3	1 (1)	2 (1)	0	1 (1)	3 (3)	1	0	0	12	6	50
60-70	0	1	1 (1)	0	1 (1)	0	0	0	0	0	3	2	66.7
70-80	0	1 (1)	0	0	0	0	0	0	0	0	1	1	100
80-90	0	0	1 (1)	0	0	0	0	0	0	0	1	1	100
Total	45	60	65	60	47	17	13	9	3	2	321		
Deaths	0	2	10	9	13	10	13	8	3	2		70	
Mortality	0	3.3	15.4	15.0	27.7	58.8	100	88.9	100	100			21.8

Note: Deaths shown in parentheses.

Table 4. Per Cent Body Surface Involvement and Mortality, 1968 - 1970

% Burn	0-10	10-20	20-30	30-40	40-50	50-60	60-70	70-80	80-90	90-100	Total
					(1968)						
No. Burned	71	68	84	66	47	35	19	9	2	0	401
Deaths	0	0	3	6	6	6	8	8	1	0	38
% Mortality	0	0	3.6	9.1	12.8	17.1	42.1	88.9	50	0	9.5
					(1969)						
No. Burned	27	45	56	55	56	26	20	14	6	4	309
Deaths	0	1	1	10	11	14	11	12	6	4	70
% Mortality	0	2.2	1.8	18.2	19.6	53.8	55	85.7	100	100	22.7
					(1970)						
No. Burned	45	60	65	60	47	17	13	9	3	2	321
Deaths	0	2	10	9	13	10	13	8	3	2	70
% Mortality	0	3.3	15.4	15	27.7	58.8	100	88.9	100	100	21.8

Table 5. Per Cent Burn Versus Survival, 1955-1970

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

Table 6. Comparison of Burn Mortality Rates, 1962-63 and 1964-70

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
1964-70	1087	22	2.0	324	41	12.6	251	58	23.1	144	59	41.0	224	178	79.5

between 1964 and 1970 during which time topical Sulfamylon was almost universally utilized to obtain bacterial suppression. Improved survival figures in the latter group are evident in burns up to 60% of the body surface.

The average required hospitalization for all patients was 54.7 days. The average postburn day of admission to the Institute was 11.2 days. The latter figure is somewhat prejudiced toward later admission by a number of patients with relatively small burns evacuated from the Republic of Vietnam via Japan at 20 to 30 days postburn in the second half of the year.

During the year, 2,357 operations were performed on 282 patients, an average of 8.4 per patient. Ward procedures without general anesthesia accounted for 1,789 of these operations. General anesthesia was administered to 202 patients on 530 occasions, an average of 2.6 per patient. Autograft was applied at 487 operations to 192 patients (average--2.5 per patient). Homograft application was utilized 1,169 times in 209 patients, and porcine heterograft was employed on 304 occasions in 110 patients.

Escharotomy was required in 79 patients (24.6%). Thirty-six patients (11.2%) required an amputation of at least a distal portion of one or more extremities. Of these, 24 had major amputations because of extensive soft tissue destruction from thermal injury, electrical injury, or invasive fungal infection.

Tracheostomies were performed in 69 patients (21.5%) with respiratory failure or upper airway obstruction. Fifty-two of these patients eventually expired, indicating the severity and progressive nature of their respiratory dysfunction despite vigorous ventilatory support.

As in previous years, pulmonary infection was again the most frequent complication following thermal injuries. Almost one-third of all patients had a clinical diagnosis of pneumonia during their hospital course. Thirty-seven cases of severe inhalation injury were seen. Pneumothorax occurred in 19 patients, usually as a result of suppurative pneumonitis with abscess formation.

Of 1,679 blood cultures obtained, 254 (15%) yielded microorganisms in 104 patients (32.4%). Intravenous catheters were placed through cutdowns in 53.3% of patients. Percutaneous catheters were utilized in the remaining patient population requiring the administration of intravenous fluids. Forty-nine

patients underwent exploration of previous cutdown sites for suspected suppurative thrombophlebitis, of which 28 subsequently proved to be positive by clinical, bacteriological and pathological criteria. A total of 753,948 ml of blood was administered to 166 patients (average--4,542 ml/patient).

All but four patients were treated topically with Sulfamylon. Skin reactions were noted in only 14 patients (4.4%), and, after treatment with antihistamines, most tolerated continued Sulfamylon application.

Associated injuries were noted in 113 patients (35.2%). Forty patients (12.5%) had chondritis of the ears requiring operative excision of infected cartilage. Curling's ulcer was diagnosed in 48 patients (15%) of which seven required operative intervention to control hemorrhage. An intraperitoneal operative procedure was necessary in 17 additional patients for a variety of traumatic or infectious complications.

In Table 7, mortality rates of patients admitted from the Continental United States are considered apart from the fatalities occurring in patients evacuated from the Republic of Vietnam. Such a comparison tends to eliminate any bias resulting from a delay in admission to this Institute of the Vietnam casualty group, during which time the more severely injured patients with significant complications of thermal injury may have expired. Estimated mortality is derived from probit analysis of survival data at this institution between 1964-1967. The increased mortality in the CONUS patients, first observed last year, remains apparent. Predicted mortality in 1968 was 19.4% as compared to 22.7% in 1970, indicating patient admissions with a similar range of estimated extent of burn. However, actual mortality of CONUS patients was only 17.1% in 1968 as compared to 42% in 1969 and 40.2% in 1970. This increased mortality rate occurred primarily in the patients with 20-60% total body surface burn, the group which had previously benefited most from the use of topical antibacterial therapy. Most of these patients died with pulmonary complications. Of 42 fatalities with burns of 20-60%, 32 expired with pneumonia. In four others, pulmonary edema and/or inhalation injury contributed significantly to the fatal outcome. Providencia stuartii, a gram-negative bacteria, was recovered from postmortem cultures of lung tissue in 21 of the 32 patients dying with pneumonia (Table 8). Pseudomonas was recovered from 11 patients and Aerobacter from 10.

Pneumonia was thought to be a significant cause of death in 45 of the 70 patients (all burn sizes) who expired in 1970, and

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

Per Cent Burn	1968						1969						1970					
	CONUS Patients			Viet Nam Patients			CONUS Patients			Viet Nam Patients			CONUS Patients			Viet Nam Patients		
	No	Deaths	Predicted Deaths	No.	Deaths	Predicted Deaths	No.	Deaths	Predicted Deaths	No.	Deaths	Predicted Deaths	No.	Deaths	Predicted Deaths	No.	Deaths	Predicted Deaths
0-10	16	0	0	45	0	0	6	0	0	11	0	0	11	0	0	26	0	0
10-20	10	0	0	49	0	0	13	0	0	24	1	0	9	0	0	37	0	0
20-30	11	1	0	58	1	0	9	0	0	37	0	0	10	2	0	47	1	0
30-40	8	2	0.2	52	2	1.4	10	3	0.3	37	2	1.0	14	3	0.4	38	2	1.0
40-50	4	0	0.6	37	4	5.8	9	4	1.4	40	4	6.2	14	5	2.2	26	4	4.1
50-60	10	2	4.0	18	2	7.1	8	5	3.2	13	4	5.2	8	7	3.2	6	1	2.4
60-70	2	0	1.3	13	6	8.3	8	6	5.1	7	1	4.5	4	4	2.6	3	3	1.9
70-80	7	6	5.7	2	2	1.6	10	8	8.1	1	1	0.8	8	8	6.5	0	0	-
80-90	2	1	1.8	0	0	-	4	4	3.7	0	0	-	3	3	2.7	0	0	-
90-100	0	0	-	0	0	-	4	4	3.8	0	0	-	1	1	1.0	0	0	-
TOTAL	70	12	13.6	274	17	24.2	81	34	25.6	170	13	17.7	82	33	18.6	183	11	9.4
Mortality		17.1%			6.2%			42%			7.6%			40.2%			6.0%	
Predicted		19.4%			8.8%			32%			10.4%			22.7%			5.1%	

Table 8. Causes of Death, 1970

Patient	Age	Sex	% Burn Total	% Burn 3°	PBD Death	Cause of Death
1.	14	M	92	92	2	Acute renal failure (hemoglobinuric nephrosis)
2.	19	M	92	81	1	Severe inhalation injury; septicemia (E. coli)
3.	22	M	87	76½	15	Invasive mycotic burn wound sepsis (Candida); pneumonia (Candida, Pseudomonas, E. coli)
4.	21	M	85	80	6	Severe inhalation injury; pneumonia (Providence, Staph.-coag. pos., Aerobacter); suppurative thrombophlebitis (Providence, Staph.-coag. pos., Aerobacter); septicemia (Providence)
5.	22	M	83	60	4	Hyperkalemia
6.	21	M	79	48½	26	Invasive bacterial and mycotic burn wound sepsis (Providence)
7.	29	M	75½	68	8	Bronchopneumonia (Providence, Pseudomonas); hyperkalemia
8.	41	M	75	59	19	Bronchopneumonia (Providence, Staph. - coag. pos., Proteus); gastric ulcers with moderate hemorrhage; invasive bacterial and mycotic burn wound sepsis (Providence, Staph.-coag. pos., Proteus)
9.	22	M	74	71½	16	Invasive bacterial and mycotic burn wound sepsis (Providence, Pseudomonas, Staph.-coag. pos.); suppurative thrombophlebitis; septicemia (Providence, Proteus)
10.	25	M	73	48	1	Severe inhalation injury with hypoxic encephalopathy
11.	29	M	73	39½	15	Interstitial pulmonary edema; bronchopneumonia with abscess formation (Pseudomonas, Staph.-coag. neg., Providence, E. coli)
12.	37	F	72½	66	34	*Invasive mycotic burn wound sepsis (Aspergillus, Candida); bronchopneumonia (Providence)
13.	21	M	72½	34½	2	*Cause of death uncertain
14.	9	M	68½	64	17	Bronchopneumonia (Providence, Staph.-coag. neg., Candida); pulmonary embolus
15.	20	M	68½	12	6	Invasive bacterial burn wound sepsis (Pseudomonas, Staph.-coag. neg.); pulmonary hemorrhage and edema
16.	53	F	68	55½	3	Pulmonary edema; bronchopneumonia (Aerobacter); pancreatitis
17.	49	M	68	30	2	Severe inhalation injury; burn shock (irreversible)
18.	52	M	68	27	5	Cause of death uncertain

* Autopsy not performed.

Table 8--page 2

Patient	Age	Sex	% Burn Total	% Burn 3°	PBD Death	Cause of Death
19.	41	F	67	65	7	Pulmonary edema; interstitial pulmonary edema; chronic bronchitis and emphysema
20.	18	M	66½	37½	34	Breakdown of gastroduodenal anastomosis with peritonitis and hemorrhage; Curling's ulcer
21	50	M	66	56	12	Pneumonia (Providence, Staph.-coag. neg., Aerobacter); septicemia (Aerobacter, Staph.-coag. pos.); suppurative thrombophlebitis (Aerobacter, Providence, Serratia, Staph.-coag. pos.)
22.	48	F	65½	60	15	Pulmonary edema; interstitial pulmonary edema; bronchopneumonia (Staph.-coag. pos.); bilateral adrenal hemorrhage
23	20	M	64½	41½	56	Pulmonary edema; pyelonephritis (Candida)
24	20	M	62½	24	32	Bronchopneumonia (Providence)
25.	21	M	61	0	36	Septicemia (Staph.-coag. pos., Providence); bacterial endocarditis; bronchopneumonia (Providence, Staph.-coag. pos.)
26.	18	M	60	15	6	Diffuse intravascular coagulation; pulmonary edema; hypoxic encephalopathy
27.	58	F	58½	56	5	Pulmonary edema
28.	35	M	58½	42½	8	Pulmonary edema; interstitial pulmonary edema
29.	39	M	58½	34½	4	Inhalation injury; bacterial laryngotracheobronchitis and bronchopneumonia (Pseudomonas)
30	43	F	58	55	4	*Bronchopneumonia (E. coli); delirium tremens; cirrhosis, Laennec's
31.	24	M	58	39½	23	Pneumonia with abscess formation (Providence, Staph.-coag. pos.); suppurative thrombophlebitis
32.	6/12	F	55½	55½	2	Acute renal tubular necrosis
33.	25	M	51	20½	14	Pneumonia with abscess formation (Staph.-coag. pos., Aerobacter)
34.	22	M	51	12½	9	Pulmonary edema; bronchopneumonia (Providence); pancytopenia
35.	21	M	50½	22	24	Bronchopneumonia (Pseudomonas, Staph.-coag. neg.)
36.	34	M	50	26	8	Bronchopneumonia (Providence); septicemia (Providence); suppurative thrombophlebitis (E. coli, Staph.-coag. neg.)

* Autopsy not performed.

Table 8--page 3

Patient	Age	Sex	% Body Surface Burned	Burn Index	PBD Death	Cause of Death
37.	42	M	48	43	2	Burn shock; intoxication (ethyl alcohol)
38.	23	F	48	38	8	[a]Severe inhalation injury; bronchopneumonia (Staph.-coag. pos., Aerobacter)
39.	24	M	47½	18	42	Bronchopneumonia (Providence, Staph.-coag. pos., E. coli)
40.	35	M	47	10	36	Pneumonia with abscess formation (Providence, Aerobacter)
41.	24	M	46½	33	55	[a]Bronchopneumonia with abscess formation (Staph.-coag. neg., Providence, Streptococcus, Serratia)
42.	6	M	46½	31½	4	Pulmonary edema; bronchopneumonia; cerebral edema
43.	14	M	46	24½	25	Bronchopneumonia (Providence); septicemia (Providence)
44.	29	M	45	0	22	Pneumonia (Herpes, Pseudomonas, Aerobacter, Providence, Staph.-coag. neg.)
45.	67	M	44½	30	34	Lobar pneumonia (Pseudomonas, Providence, Staph.-coag. neg.); pulmonary emphysema; atherosclerotic heart disease
46.	37	M	43	34	48	Bronchopneumonia with abscess formation (Providence, Aerobacter, Proteus, Staph.-coag. neg., Aspergillus); septicemia (Providence, Aerobacter, Pseudomonas); bacterial burn wound sepsis (Providence, Pseudomonas); otitis and mastoiditis (Aerobacter)
47.	21	M	41	17	22	Interstitial edema; bronchopneumonia (Aerobacter, Providence); invasive mycotic burn wound sepsis (Fonsecaea)
48.	6	M	40	17½	6	Bronchopneumonia
49.	20	M	40	3	4	Fat embolization; open fracture, right femur
50.	19	M	39½	23	39	Pneumonia with abscess formation (Providence, Pseudomonas)
51.	54	F	39	25	53	Septicemia (Candida), pyelonephritis (Pseudomonas, Candida, Providence), bronchopneumonia (Aerobacter)
52.	8	F	38	22	6	Pneumonia (Staph.-coag. pos., Aerobacter), septicemia (Aerobacter)
53.	37	M	38	20½	7	Curling's ulcer with hemorrhage; septicemia (Providence)
54.	45	M	37½	0	35	Pneumonia with empyema (Providence, Proteus); interstitial pulmonary edema)

[a] Autopsy not performed

Table 8--page 4

Patient	Age	Sex	% Burn Total	% Burn 3°	PBD Death	Cause of Death
55.	2	M	36½	27	15	Septicemia (Staph.-coag. neg.); suppurative thrombophlebitis (Staph.-coag. neg.)
56	21	M	35½	0	42	Bronchopneumonia (Providence, Pseudomonas); interstitial pulmonary edema
57.	3-6/12	F	33	21½	4	Interstitial pulmonary edema
58	25	M	32	11	13	Tracheobronchitis and bronchopneumonia (Pseudomonas, Providence, Staph.-coag. pos.); interstitial pulmonary edema; invasive mycotic and bacterial burn wound infection (Staph.-coag. pos.); suppurative thrombophlebitis (Staph.-coag. pos.)
59.	81	F	28	22	16	*Bronchopneumonia (Providence, Aerobacter)
60.	61	M	28	20½	22	Pneumonia (Providence, Pseudomonas)
61	21	M	28	16½	63	Pneumonia (Providence, Staph.-coag. neg., Herpes); disseminated Herpesvirus hominis infection
62	1-3/12	M	28	9½	34	Pneumonia (Providence, Pseudomonas, Candida); invasive bacterial and mycotic burn wound sepsis (Providence); septicemia (Providence)
63.	10/12	F	26½	22½	35	Septicemia (Staph.-coag. pos., Providence); invasive purulent sinusitis and nasopharyngitis; pneumonia (Staph.-coag. pos., Providence, Pseudomonas)
64.	55	M	26	15	5	Anoxic encephalopathy
65.	38	M	26	6½	15	Acute renal tubular necrosis; pneumonia with abscess formation (Providence, Pseudomonas, Staph.-coag. pos.); inhalation injury
66.	13	M	22½	8	57	Pneumonia (Aerobacter, Staph.-coag. neg.); tracheo-esophageal fistula
67.	28	M	20½	14½	6	Bronchopneumonia (Providence, Proteus, Staph.-coag. pos. and neg.)
68.	7/12	F	20	16½	5	Acute renal failure; pulmonary edema
69.	72	M	15½	4	4	*Congestive heart failure with pulmonary edema; bronchopneumonia (Proteus, Aerobacter); chronic renal failure
70.	49	M	14	5½	27	Pulmonary emboli; bilateral femoral vein thrombosis

* Autopsy not performed.

providence was recovered from the lungs in 29 of these (Table 8). In addition, postmortem blood cultures grew out Providence on nine occasions as compared to three positive cultures each of Aerobacter and Staphylococcus (coagulase positive), and one each of E. coli, Proteus, Pseudomonas, Candida, and Staphylococcus (coagulase-negative).

Careful analysis of premortem sputum cultures has revealed no significant change in incidence of bacterial strains with the exception of Providencia stuartii, which has shown a tenfold increase in incidence between 1967 and 1970. Isolation of this organism from the sputum was associated with a 61.8% patient mortality. The above data suggest the possibility that this gram-negative bacterial strain may subsequently prove to be a significant pathogen associated with infectious complications of thermal injury. It appears to attack primarily via the respiratory tract rather than through the burn wound itself. At present, no satisfactory antibacterial agent is available for treatment of more than 90% of recovered Providence strains.

Bacteriological surveys have failed to identify a source of bacterial seeding on the ward. In fact, many patients are found to harbor Providence on their burn wounds at the time of admission, before exposure to the ward environment has occurred. Independent review of treatment methods has not revealed any significant departures from the clinical procedures practiced in 1967 or 1968. Therefore, we suspect, at present, that the increased incidence of Providence pneumonia represents a de novo burn complication and have intensified our efforts to perfect an animal model for future experimental investigation.

Summary

During calendar year 1970, 325 patients were admitted to the US Army Institute of Surgical Research. Previously reported therapeutic modalities developed at the Institute over the past five years were maintained. In addition, the clinical utilization of the ultrasonic flowmeter, more sophisticated pulmonary function measurements, split-thickness porcine heterograft, and ketamine anesthesia has allowed for improved therapeutic management of patients with thermal injury.

Recent clinical research activities include investigations into a wide variety of physiological and biochemical abnormalities which are frequently observed in patients following severe trauma. Also, a major effort has been made to share the Institute's extensive clinical experience in management of thermal injury

with both the military and civilian medical and paramedical community by active participation in a large number of training programs and medical meetings. A list of publications, presentations and exhibits follows this text.

Pulmonary infection remains the most frequent complication of burn injury. Mortality data suggest that fatalities in the 20% to 60% burn patient have increased during the past two years as a result of septic complications. Providencia stuartii has appeared in sputum cultures with greater frequency during this same time period. Unfortunately, most strains of this gram-negative bacteria are resistant to presently available antibiotics. These results have inspired a more intensive experimental search for methods which might be implemented in the prevention and treatment of pneumonia.

The senior author wishes to express his deep gratitude to all the staff members of the Clinical Division, each of whom has contributed substantially to the care and rehabilitation of a large number of severely injured patients during the past two years. Their enthusiasm and scientific curiosity are truly commendable, and their unselfish devotion to delivering the best possible medical care assures the continued success of the Institute in developing new and improved treatment.

Publications

Pruitt BA Jr, Flemma RJ, DiVincenti FC, Foley FD, Mason AD Jr: Pulmonary complications in burn patients. J Thoracic & Cardiovasc Surg 59:7-20, 1970.

O'Neill JA Jr: The influence of thermal burns on gastric acid secretion. Surgery 67:267-271, 1970.

Zawacki BE, Switzer WE, Mason AD Jr, Johns LA: Does increased evaporative water loss cause hypermetabolism in burned patients. Ann Surg 171:236-240, 1970.

Pruitt BA Jr, Stein JM, Foley FD, Moncrief JA, O'Neill JA Jr: Intravenous therapy in burn patients: Suppurative thrombophlebitis and other life-threatening complications. Arch Surg 100:399-404, 1970.

Stein JM, Pruitt BA Jr: Suppurative thrombophlebitis--A lethal iatrogenic disease. New Engl J Med 282:1452-1455, 1970.

Pruitt BA Jr, DiVincenti FC, Mason AD Jr, Foley FD, Flemma RJ: The occurrence and significance of pneumonia and other pulmonary complications in burned patjents: Comparison of conventional and topical treatments. J Trauma 10:519-531, 1970.

Curreri PW, Katz AJ, Dotin LN, Pruitt BA Jr: Coagulation abnormalities in the thermally injured patients. In, Current Topics in Surgical Research, Vol 2, DB Skinner and PA Ebert, Eds, New York, Academic Press Inc, 1970, pp 401-411.

Bruck HM, Asch MJ, Pruitt BA Jr: Burns in children: A 10-year experience with 412 patients. J Trauma 10:658-662, 1970.

Curreri PW, Asch MJ, Pruitt BA Jr: The treatment of chemical burns: Specialized diagnostic, therapeutic and prognostic considerations. J Trauma 10:634-642, 1970.

Curreri PW, Lindberg RB, DiVincenti FC, Pruitt BA Jr: Intravenous administration of carbenicillin for septicemia due to Pseudomonas aeruginosa following thermal injury. J Infect Dis 122:S40-43, 1970.

Zawacki BE Jr: Recent advances in burn treatment. Surgery 68: 412-418, 1970.

Moncrief JA, Pruitt BA Jr: Electric injury. Postgrad Med 48: 189-194, 1970.

Pruitt BA Jr, Foley FD, Moncrief JA: Curling's ulcer: A clinical-pathologic study of 323 cases. Ann Surg 172:523-539, 1970.

Pruitt BA Jr: Management of burns in the multiple injury patient. Surg Clin N Amer 550:1283-1300, 1970.

Curreri PW, Pruitt BA Jr: Evaluation and treatment of the burned patient. Amer J Occup Ther 24:475-480, 1970.

Von Prince KMP, Curreri PW, Pruitt BA Jr: Application of fingernail hooks in splinting of burned hands. Amer J Occup Ther, 24:556-559, 1970.

Asch MJ, White MG, Pruitt BA Jr: Acid base changes associated with topical Sulfamylon therapy: Retrospective study of 100 burn patients. Ann Surg: 172:946-950, 1970.

Presentations

Pruitt BA Jr: Management of Thermal Injury, Randolph AFB Hosp Prof Mtg, Randolph AFB, Texas.

The following presentations were made to MFSS Physical Therapy Students, MFSS, BAMC, FSHT, 23 Jan 1970:

Galloway KF: Nursing Care for the Burned Patient.
Von Prince KMP: Occupational Therapy for the Burned Patient.
Hall WF, Kirkman EM: Physical Therapy for the Burned Patient.
Reckler JM: Physiology and Treatment of the Burned Patient.

Curreri PW: Management of Burns. AMEDD Off Basic Course, MFSS, BAMC, FSHT, 28 Jan 1970.

Galloway KF: Nursing Care for the Burned Patient. Clin Spec Course, BGH, BAMC, FSHT, 30 Jan 1970.

Pruitt BA Jr: ACS Sect Mtg, Portland,Ore, 2-4 Feb 70.

Curreri PW: Coagulation Abnormalities in Burns: Carbenicillin in Treatment of Pseudomonas Infections; and, Fungus Infections in Burns. Surg Staff Conf, Univ of Texas Med Sch, San Antonio, Texas, 6 Feb 70.

Galloway KF: Nursing Care of the Burned Patient. Flight Nurses, Sch of Aerospace Med, Brooks AFB,Tex, 6 Feb 1970.

Curreri PW: Treatment of Burns. San Antonio Explorer Scouts, BGH, BAMC, FSHT, 7 Feb 1970.

Pruitt BA Jr: Univ of So Cal Conf on Emergency Med, Los Angeles, Cal, 10-12 Feb 70.

The following presentations were made to the Brooke Gen Hosp Symp on Surgical & Orthopaedic Aspects of Trauma, BGH, BAMC, FSHT 2-5 Mar 1970.
Curreri PW: Resuscitation, Initial Treatment and Transportion of the Burn Patient.
Pruitt BA Jr: Septic Complications--Pulmonary and Vascular.
Silverstein,P: Coverage of the Burn Wound.
Inge WW Jr: Electrical Injury.
Moylan JA Jr: Chemical Injury.
Bruck HM: Nonsentic Complications of Thermal Injury.

Galloway KF: Concepts of Intensive Care Nursing. Adv Nursing Career Course, MFSS, BAMC, FSHT, 11 Mar 1970.

Moylan JA Jr: Treatment of Burns. Allied Med Officers Course, MFSS, BAMC, FSHT, 12 Mar 1970.

Galloway KF: Nursing Care of the Burned Patient. Flight Nurses and Technicians, Sch of Aerospace Med, Brooks AFB, Texas, 16 Mar 1970.

Galloway KF: Nursing Care of the Burned Patient. RN Club, BAMC, FSHT, 17 Mar 1970.

Pruitt BA Jr, Curreri PW: Management of Burns and Current Research. Mil Med and Allied Sciences Course, Walter Reed Army Med Ctr, Washington DC, 17,18 Mar 1970.

Curreri PW: Coagulation Factor Changes in Burns, Carbenicillin Therapy and Opportunistic Infections. San Antonio Surg Soc, San Antonio, Texas, 24 Mar 1970.

Galloway,KF: Concepts of Intensive Care Nursing: Adv Nursing Career Course, MFSS, BAMC, FSHT, 24 Mar 1970.

Inge WW Jr: Treatment of Burns. Allied Med Officers Course, Sch of Aerospace Med, Brooks AFB, Tex, on Ward 14A, BAMC, FSHT, 25 Mar 1970.

Silverstein P: Care of the Burned Patient. Boston City Hosp, Boston, Mass, 2 Apr 1970.

The following presentations were made to the Second Anl Mtg of the Amer Bur Assoc, Boston, Mass, 10-11 Apr 1970.

Reckler JM: A Critical Evaluation of Fluid Resuscitation in the Burned Patient.
Bruck HM: Invasive Phycomycosis: A Highly Lethal Complication of Thermal Injury.
Galloway KF: Orientation and Education of New Nursing Service Personnel to a Burn Unit.
Moylan JA Jr: Ocular Complications of Thermal Injury: A Review of a Five-Year Experience Including 112 Patients.
Curreri PW: A Study of Coagulation Factors in the Thermally Injured Patient.
Von Prince KMP: The Use of Special Devices in Burn Wound Care.

Curreri PW: Burns. Dept of Surgery Grand Rounds, Univ of Penn. Sch of Med, Phila, Penn, 14 Apr 1970.

Bruck HM: A 10-Year Experience with Burns in Children. Dept of Pediatrics, Columbia Coll of Physicians & Surgeons, New York, N Y, 14 Apr 1970.

Galloway KF: Panel on the Therapeutic Team. Army Chaplain Tng Program, BGH, BAMC, FSHT, 14 Apr 1970.

Bruck HM: Current Problems in Burn Therapy. Surg Staff, Columbia Coll of Physicians & Surgeons, New York, N Y, 16 Apr 1970.

The following presentations were made to the Tennessee Chap of the Amer Physical Therapy Assoc, Knoxville, Tenn 25 Apr 1970:

Bruck HM: Initial Care, Infection, Role of Sulfamylon and wound Coverage; Burns of the Hand; Burns and Fractures.
Kirkman EM: Role of the Physical Therapist in the Acute Burn: Maintenance of Function and Prevention of Contractures: The Use of Splinting Devices in Burns.

The following presentations were made to the Nebraska Chap of the Amer Physical Therapy Assoc, Scottsbluff, Neb, 25 Apr 1970:

Curreri PW: Evaluation and Therapy of the Thermally Injured Patient: Diagnosis and Treatment of Common Complications in Burned Patients.
Miller AV: Intensive Care of the Patient with Major Burns: Specialized Knowledge and Techniques of the Burn Unit Nurse.
Hall WF: Physical Therapy Principles and Practice Prior to Eschar Separation; Functional Results Following Intensive Physical Therapy Programs in the Convalescent Patient.
Von Prince KMP: Principles of Splinting and Special Devices Prior to Definitive Grafting; Manufacture of Splints and Special Devices.

Galloway KF: Nursing Care of Burns. Lackland AFB RN Club, Lackland AFB, Texas, 28 Apr 1970.

Pruitt BA Jr: Curling's Ulcer: A Clinical-Pathologic Study of 321 Cases. Amer Surg Assoc Mtg, White Sulphur Springs, W Va, 27-29 Apr 1970.

Pruitt BA Jr: Pulmonary Effects of Thoracic and Nonthoracic Trauma; and, Temporary and Permanent Burn Wound Coverage. Symp on Trauma, and Symp on Burns, Sch of Med Univ of N Mex, Albuquerque, N Mex, 1,2 May 1970.

Pruitt BA Jr: The Management of Infections in Seriously Burned Patients. Symp on Changing Patterns of Bacterial Infections and Antibiotic Therapy, San Francisco, Cal, 7,8 May 1970.

Galloway KF: Nursing Care of the Burned Patient. Flight Nurses and Technicians, School of Aerospace Med, Brooks AFB, Tex, 11 May 1970.

Pruitt BA Jr: Emergency Measures in the Treatment of Burns; and, The Surgical Treatment and Complications of Penetrating Wounds of the Colon and Rectum. ACS 14th Anl Postgrad Course on Fractures and Other Trauma, Chicago, Ill, 13-16 May 1970.

Galloway KF: Goals of Intensive Care Nursing. BGH and ISR Nursing Staff and Corpsmen, BGH, BAMC, FSHT, 15 May 1970.

Pruitt BA Jr: Participant in Workshop on Burn Injury; Participant in Workshop on Gastrointestinal Response to Trauma. Inst of Gen Med Sci Trauma Symp, Washington, DC, 18-20 May 1970.

Curreri PW: Treatment of Burns. Camp Lejeune and Cherry Point Hosp Staff, Camp Lejeune, N Car, 21 May 1970.

Pruitt BA Jr: Experiences with Hyperalimentation in Burn Patients. Shock Comm of Natl Res Council, Wash DC, 21 May 1970.

Moylan JA Jr: Treatment of Burns. AMEDD Off Adv Course, MFSS, BAMC, FSHT, 12 Jun 1970.

Curreri PW: Supranormal Dietary Intake In Thermally Injured Hypermetabolic Patients. AMA 19th Anl Convention, Chicago, Ill, 24 Jun 1970.

Bruck HM: Institute of Surgical Research Burn Ward. Intern Orientation Program, BGH, BAMC, FSHT, 30 Jun 1970.

Galloway KF: Nursing Care of Burned Patients. 91C Clinical Specialist Course, BGH, BAMC, FSHT, 1 Jul 1970.

Newsome TW: Treatment of Burns. AMEDD Off Basic Course, MFSS, BAMC, FSHT, 23 Jul 1970.

Pruitt BA Jr: Burns in Children. House Staff, San Antonio Children's Hosp, San Antonio, Texas, 25 Jul 1970.

Galloway KF: Nursing Care of Burned Patients. Baptist Hosp Sch of Nursing, San Antonio, Texas, 6 Aug 1970.

Silverstein P: Treatment of Burns. AMEDD Off Basic Course, MFSS, BAMC, FSHT, 7 Aug 1970.

Curreri PW: Treatment of Burns. Interns, BGH, BAMC, FSHT, 8 Aug 1970.

Curreri PW: Treatment of Burns. AMEDD Off Basic Course, MFSS, BAMC, FSHT, 21 Aug 1970.

Inge WW Jr: Treatment of Burns. AMEDD Off Basic Course, MFSS, BAMC, FSHT, 2 Sep 1970.

Galloway KF: Nursing Care of the Burned Patient. Nursing In-Service Program, Randolph AFB Hosp, Randolph AFB, Texas, 2 Sep 1970.

Pruitt BA Jr: Care of the Extensively Burned Patient. US Army Hosp, Frankfurt, Germany, 9 Sep 71.

Galloway KF: Concepts of Intensive Care Nursing. Adv Nursing Career Course, MFSS, BAMC, FSHT, 10 Sep 1970.

Pruitt BA Jr: Pathophysiology of Early Postburn Period;and, Treatment of the Extensively Burned Patient. US Army Hosp, Nuremberg, Germany, 10 Sep 1970.

Pruitt BA Jr: Management of Complications of Burns, and, Management of the Extensively Burned Patient. US Army Hosp, Landstuhl, Germany, 11 Sep 1970.

Pruitt BA Jr: Current Management of the Severely Burned Patient. Med-Surg Staff of US Army Hosp, Heidelberg, Germany, 14 Sep 1970.

Newsome TW: Complications of Burns. Med Staff, Randolph AFB Hosp, Randolph AFB, Texas, 15 Sep 1970.

Pruitt BA Jr: Early Hemodynamic Changes in the Burned Patient; and, Coverage of the Burn Wound. Staff of the Oslo City Hosp, Oslo, Norway, 18 Sep 1970.

Palm L: Panel on Perceptual Motor Dysfunction. Continuing Med Edu Program of Texas Occup Therapy Assoc, Austin, Tex, 21 Sep 1970.

The following presentations were made at the meeting of the Third International Congress for Research in Burns, Prague, Czech, 21-25 Sep 1970:

Pruitt BA Jr: Hemodynamic Studies of Burned Patients During Resuscitation; and, Diagnosis and Treatment of Curling's Ulcer.
Curreri PW: A Study of Coagulation Factors in the Thermally Injured Patient.

Galloway KF: Nursing Care of the Burned Patient. Flight Nurses & Med Technicians, Sch of Aerospace Med, Brooks AFB, Texas, 23 Sep 1970.

Silverstein P: Treatment of Burns. AMEDD Off Basic Course, MFSS, BAMC, FSHT, 2 Oct 1970.

The following presentations were made to the International Joint Conf on Burn Therapy, Seattle, Wash, 3,4 Oct 1970:

Moylan JA Jr: Initial Care and Resuscitation; and, Complications of Burns.
Hall WF: Use of Physical Therapy and Hydrotherapy in the Treatment of Burn Patients.
McGee SA: Specialized Nursing Care for the Burn Patient
Remig RL: Occupational Therapy for the Burn Patient, with Emphasis on Splint Making and Special Apparatus.

Curreri PW: Management of Mass Burn Casualties in Industrial Accidents. US Civil Defense Council, Fort Worth, Texas, 7 Oct 1970.

Pruitt BA Jr: Discussion of Two Papers. Amer Assoc for Surgery of Trauma Meeting, Chicago, Ill, 8-10 Oct 1970.

The following presentations were made to the American Assoc for the Surgery of Trauma, Chicago, Ill, 9 Oct 1970:

Curreri PW: Intracellular Cation Alterations Following Major Trauma: Effect of Supranormal Caloric Intake.
Moylan JA Jr: Circulatory Effects of Thermal Injury on Extremities with and Without Escharotomy.

The following presentations were made at the American Coll of Surgeons Mtg, Chicao, Ill, 12-16 Oct 1970:

Pruitt BA Jr: Member, Gen Surg Panel, "Total Management of the Burn Patient".
Surgical Dressings and Drapes. Pre- and Postop Care Panel, ACS Physiologic Response to Injury; and, Gastrointestinal Problems, Postgrad Course--Trauma

Pruitt BA Jr: Hemodynamics of the Early Postburn Period; Control of Sepsis; and, Treatment of Common Complications of Thermal Injury. Surg House Staff, Upstate Med Ctr, Syracuse, N Y, 16 Oct 1970.

Pruitt BA Jr: Use of Sulfamylon in the Treatment of Extensive Burns; and, Use of Homografts and Heterografts. Burn Teaching Day, St Joseph's Hosp and Regional Med Program, Syracuse, N Y, 17 Oct 1970.

The following presentations were made at the Pastoral Care Symposium, BGH, BAMC, FSHT, 19 Oct 1970:

Pruitt BA Jr: Introduction to Institute of Surgical Research.
Galloway KF, Curreri PW, and Hall WF: Panel on, "The Burn Team that Works Together.

Curreri PW: Intracellular Cation Alterations Following Major Trauma: Effectof Supranormal Caloric Intake. San Antonio Research Club, San Antonio, Texas, 21 Oct 1970.

Newsome TW: Treatment of Burns. AMEDD Off Basic Course, MFSS, BAMC, FSHT, 22 Oct 1970.

Pruitt BA Jr: Management of Burn Patients with Fractures. Burns and Fractures Conf, Orthopaedic Svc, BGH, BAMC, FSHT, 24 Oct 1970.

Galloway KF: Nursing Care of the Burned Patient. Nursing In-Svc Program, Kelly AFB Dispensary, Kelly AFB,Texas, 28 Oct 1970.

Pruitt BA Jr: Use of Homograft and Heterograft in the Care of Burn Patients; and, Early Hemodynamic Changes in Burn Patients. Surg Staff, Ft Jackson Army Hosp, Ft Jackson, SC, 4 Nov 1970.

Pruitt BA Jr: Panel Member, Panel on Problems in Burn Management. Presentations: Use of Homograft an Heterograft in the Treatment of Burn Patients; and, Sulfamylon Topical Therapy of Burns. McGuire Lecture Series, Med Coll of Virginia, Richmond, Va, 5,6 Nov 1970.

Galloway KF: Nursing Care of the Burned Patient. Missouri State Nurses Assoc, St Louis, Mo., 5 Nov 1970.

Pruitt BA Jr: The Application of Clinical Investigation to the Care of the Extensively Burned Patient. Staff, Dept of Surgery, Med Univ of South Carolina, Charleston, S C, 7 Nov 1970.

Pruitt BA Jr: Overview and Current Concepts in Burn Management. Panel Discussion on, Current Concepts in Acid and Thermal Burn Care. Symp on Burns, Bannock Mem Hosp, Pocatello, Idaho, 10 Nov 1970.

Pruitt BA Jr: Recent Advances in the Treatment of Burns. Staff, Dept of Surgery, Univ of Utah Coll of Med, Salt Lake City, Utah, 11 Nov 1970.

Curreri PW: Energy Requirements Following Thermal Injury. Univ of Miami Sch of Med, Grand Rounds, Miami, Florida, 12 Nov 1970.

Pruitt BA Jr: Current Therapy of Thermal Injury. Southern Med Assoc Sect on Industrial Medicine &Surgery, and Physical Medicine and Rehabilitation, Dallas, Texas, 16-19 Nov 1970.

Pruitt BA Jr: Western Surg Assoc Mtg, Colorado Springs, Col., 19,20 Nov 1970.

Silverstein P: Treatment of Burns. AMEDD Off Basic Course, MFSS, BAMC, FSHT, 25 Nov 1970.

The following presentations were made at the Meeting of the US Army Surgeon General's Advisory Committee on the Metabolism of Trauma, BAMC, FSHT, 30 Nov-1 Dec 1970:

Pruitt BA Jr: Chairman, Section on, "Define Clinical Value and Limits of Hyperalimentation".
Curreri PW: Longterm Supranormal Caloric Dietary Programs in Extensively Burned Patients.
Moylan JA Jr: Objective Measurement of the Circulation of the Extremities with Circumferential Burns.
Curreri PW: Intracellular Cation Alterations after Major Trauma.
Silverstein P: Clinical and Laboratory Evaluation of Porcine Xenograft as a Physiologic Dressing.
Asch MJ: Assessment of Regional Blood Flow in Early Postburn Patients Using Radioactive Microspheres.

Galloway KF: Nursing Care of the Burned Patient. Clin. Spec. Course, BGH, BAMC, FSHT, 2 Dec 1970.

Galloway,KF: Intensive Nursing Care. Health Care Admin Course, MFSS, BAMC, FSHT, 8 Dec 1970.

Galloway KF, Asch MJ: Management of the Burned Patient. Intensive Care Nursing Course, BGH, BAMC, FSHT, 9 Dec 1970.

Pruitt BA Jr: Viral and Fungal Complications of Burns; and, Sulfamylon Topical Therapy. Amer Academy of Dermatology, Symp on Burns, Chicago, Ill, 9 Dec 1970.

Silverstein P: Treatment of Burns. AMEDD Off Adv Course, MFSS, BAMC, FSHT, 14 Dec 1970.

Galloway KF: Principles of Intensive Care Nursing. Intensive Care Nursing Course, BGH, BAMC, FSHT, 17 Dec 1970.

Exhibits

"Pulmonary Complications in Burns", displayed at AMA Mtg, Chicago, Ill, 21-26 Jun 1970.

The following exhibits were displayed at the ACS Anl Mtg, Chicago, Ill, 12-16 Oct 1970:

"Burns in Children".
"Emergence of Opportunistic Infection in the Burn Wound".

Motion Pictures

"Burns and Fractures", shown at AMA Anl Mtg, Chicago, Ill, 24 Jun 1970.

The following motion pictures were shown at the Third Internatl Congr for Res in Burns, Prague, Czech, 20-25 Sep 1970:

"Management of Severe Thermal Injury Involving the Calvarium".
"Electrical Trauma".
"Burns and Fractures".
"Control of Pseudomonas Burn Wound Sepsis".

The following motion pictures were shown at the ACS Anl Mtg, Chicago, Ill, 12-16 Oct 1970:

"Suppurative Thrombophlebitis: A Complication of Intravenous Therapy".
"The Management of Burns in Children".
"Use of Mesh Autograft in Treatment of Burns".

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0A 6983	71 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
70 07 01	D. CHANGE	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A061102B71R	01	168
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Procede with Security Classification Code)

(U) Clinical Operation, Trauma Study Branc. for Treatment of Injured Soldiers (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 Clinical Medicine

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

17. CONTRACT/GRANT: Not Applicable

a. DATES/EFFECTIVE — EXPIRATION

b. NUMBER

c. TYPE — d. AMOUNT

e. KIND OF AWARD — f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR — PRECEDING 71	1.6	20.2
FISCAL YEAR — CURRENT 72	1.6	21.2

19. RESPONSIBLE DOD ORGANIZATION

NAME: US Army Institute of Surgical Research

ADDRESS: Ft Sam Houston, Texas 78234

RESPONSIBLE INDIVIDUAL

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

TELEPHONE: 512-221-2720

20. PERFORMING ORGANIZATION

NAME: US Army Institute of Surgical Research
Trauma Study Branch

ADDRESS: Ft Sam Houston, Texas 78234

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

NAME: Andrew M. Munster, LTC, MC

TELEPHONE: 512-221-5712

SOCIAL SECURITY ACCOUNT NUMBER: [redacted]

ASSOCIATE INVESTIGATORS

NAME: Lois A. Johns, LTC, ANC

NAME: Alan H. Morris, MAJ,MC

NAME: Kenneth W. Spitzer,CPT,MSC DA

21. GENERAL USE

FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Procede EACH with Security Classification Code)

(U) Trauma; (U) Immunity; (U) Pulmonary Function; (U) Joints; (U) Hemodialysis

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

23. (U) Clinical and laboratory investigations pertaining to severe physical trauma.

24. (U) Planned clinical and laboratory studies relating to acute and chronic injury. Studies conducted by the Branch have included both purely clinical studies, involving patients on the ward, laboratory studies involving animal models, and a combination of the two.

25. (U) 70 07 - 71 06 Studies on the pulmonary function of burned patients have continued. Extensive measurements with regard to ventilation, lung volumes, diffusion, compliance, and shunting have been carried out. During the year 1970, responsibility for supervision of these studies was transferred to the Pulmonary Section, and will be reported on in detail by that section. The Branch has continued extensive laboratory investigations on the effects of thermal and mechanical injuries, and will present a detailed description of the depression of the immune response following thermal and mechanical injury. Studies have commenced extending some of these principles to investigation of patients. A prospective study into calcification about the elbow joint following burns has now been completed, having surveyed a total of 100 patients. Members of the Branch have continued active participation in patient care and air evacuation,both from Japan and CONUS. In addition, continued surgical support is being rendered to the Renal Branch in the form of insertion of arteriovenous shunts and fistulae for the maintenance of chronic hemodialysis.

*Available to contractors upon originator's approval.

ANNUAL PROGRESS REPORT

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

REPORT TITLE: CLINICAL OPERATION, TRAUMA STUDY BRANCH

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

1 January - 31 December 1970

Investigators:

Andrew M. Munster, M.D., LTC, MC
Lois A. Johns, LTC, ANC
Alan H. Morris, M.D., MAJ, MC
Kenneth W. Spitzer, CPT, MSC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

REPORT TITLE: CLINICAL OPERATION, TRAUMA STUDY BRANCH

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

Period covered in this report: 1 January - 31 December 1970

Investigators: Andrew M. Munster, M.D., LTC, MC
Lois A. Johns, LTC, ANC
Alan H. Morris, M.D., MAJ, MC
Kenneth W. Spitzer, CPT, MSC

Reports Control Symbol MEDDH-288(R1)

The Trauma Study Branch has continued to render clinical care to burn patients admitted to this unit and its members take part in air evacuation, both from Japan and CONUS to the Institute of Surgical Research. In addition, clinical and laboratory investigation on problems connected with burns and trauma and teaching activities have been carried out. Surgical support is rendered by the Branch to the Renal Unit for the maintenance of patients on chronic hemodialysis and other related surgical problems. Investigations have centered on pulmonary function following burns, immunological changes following thermal and mechanical injury, heterotopic calcification in the area of the elbow joint, the evaluation of immunological changes following the application of split-thickness porcine xenograft to burned patients,and studies of graft versus host reaction in animal models. In addition, retrospective clinical reviews on cardiac infections in burns and acute acalculous cholecystitis in burned patients have also been carried out. Members of the unit continue to engage in teaching activities connected with Brooke General Hospital, the Medical Field Service School, in-service training of nursing staff, as well as local, national and international presentations of work carried out at this unit.

Trauma
Immunity
Pulmonary function
Joints
Hemodialysis

CLINICAL OPERATION, TRAUMA STUDY BRANCH

Members of the Trauma Study Branch continue to participate in aerial evacuation of burned patients from CONUS and Japan and to take part in their subsequent clinical care. Surgical support to the Renal Unit has been rendered in the form of insertion and maintenance of Scribner-type arteriovenous shunts for the maintenance of chronic hemodialysis, and, on one occasion, the creation of a subcutaneous arteriovenous fistula for the same purpose. During this year, one patient under the care of the Renal Unit underwent his 500th hemodialysis, using Scribner shunts maintained by this branch. This patient was subsequently transferred out of the unit for renal transplantation. Supervision and treatment of surgical complications of shunts for hemodialysis have included control of infection, bleeding problems, and other technical problems which sometimes accompany hemodialysis.

The pulmonary function study, which has made extensive progress during the past year, with measurements of postburn lung volumes, arteriovenous shunting, lung compliance and diffusion, has contributed some important findings. Personnel involved with this study, initiated in this branch, are now responsible to a separate section, the Pulmonary Section, created during the year 1970, and report of this study will be given separately. A prospective study of heterotopic calcification about the elbow joint has now been completed in 100 patients, including 180 limbs. It was found, as suspected, that the extent of deep burn of the arm and the extent of subsequent immobilization of adjoining joints, such as the wrist and shoulder, contributed in a statistically significant manner in the development of heterotopic calcification around the elbow. It was found, contrary to what had been expected, that the extent of total body burn, the positioning of the patient in a circolectric bed, supine or prone, and the patient's motivation, as evaluated by psychological testing, did not make any material difference to whether calcification developed or not. A number of these patients underwent surgery for the correction of heterotopic calcification about the elbow joint, and the results of this study will be given elsewhere.

The Trauma Study Branch participated with the Clinical Division in the evaluation of split-thickness porcine xenograft for use as a biological dressing in burned patients. The role of the branch has been principally in the prosecution of an immunological evaluation. It was found that split-thickness

porcine xenograft did not induce sensitivity in either human or animal recipients, and therefore appears to be safe for clinical use. In an extensive series of laboratory investigations, to be detailed later, it was found that lymphocyte function, a measure of central immunity, is severely depressed following thermal injury for a few days but recovers in the absence of complications, such as sepsis, within 10 days of injury. This holds true whether injury is thermal or mechanical, although the depression engendered by thermal injury is somewhat more extensive than that caused by mechanical injury. The principles underlying this investigation have now been extended to humans who have commenced to undergo a series of skin tests for the measure of delayed hypersensitivity and a test of their peripheral lymphocytes for the presence of depressed immunological capability.

Publications

Munster AM, Hoagland HC, Pruitt BA Jr: The effect of thermal injury on serum immunoglobulins. Ann Surg 172: 965, 1970.

Munster AM: Alterations of the host defense mechanism in burns. Surg Clin N Amer 50: 1217, 1970.

Munster AM, DiVincenti FC, Pruitt BA Jr: Cardiac infections in burns. Amer J Surg (In press)

Johns LA: Combat nursing: Acute renal failure. ANA Clin Conf (Appleton,Century Crofts)(In press).

Presentations

Munster AM: Electrical burns. Sydney Hospital, Sydney, Australia, 27 January 1970

Munster AM: Postgraduate surgical training in the USA. Sydney Hospital, Sydney, Australia, 31 January 1970.

Munster AM: Treatment of burns. Univ of Sydney students, Royal Prince Alfred Hospital, Sydney, Australia, 9 February 1970.

Munster AM: Treatment of burns. Royal Northshore Hospital, Sydney,Australia, 9 February 1970.

Munster AM: Treatment of burns. Sydney Hospital, Sydney, Australia, 10 February 1970.

Munster AM: Recent advances in research in burns. Dept Surgery,

Univ of Sydney, Sydney, Australia, 16 February 1970.

Munster AM: Treatment of burns. Concord Repatriation Hospital, Sydney, Australia, 17 February 1970.

Munster AM: Cardiac infection in burns. Amer Burn Assn, Boston, Mass. 10 April 1970.

Johns LA: Study of a nursing procedure for urinary catheter care. Amer Burn Assn, Boston, Mass. 10 April 1970.

Munster AM: The effect of thermal injury on cellular immunity. Amer Burn Assn, Boston, Mass. 11 April 1970.

Munster AM: The burn patient. Army Chaplain Trng. Program. BGH, BAMC, FSHTex, 21 April 1970.

Johns LA: Nursing service and the chaplain. Army Chaplain Trng. Program, BGH, BAMC, FSHTex, 21 April 1970.

Johns LA: Combat nursing: Acute renal failure. Amer Nurses Assn. Miami Beach, Florida, 6 May 1970.

Johns LA: Ethics in nursing research. Incarnate Word Coll. School of Nursing, San Antonio, Texas, 11 and 13 May 1970.

Munster AM: Recent advances in surgical immunology. Dept. Surg. Univ of Wisconsin Med School, Madison, Wisconsin, 26 June 1970.

Johns LA: Nursing care of the burned patient. Flight Nurses, School of Aerospace Med, Brooks AFB, Texas,30 June 1970.

Munster AM: Emergency treatment of burned patients. Emergency Care Trng School, Texas Dept of Health and Bexar County Med Soc, San Antonio, Texas,15 July 1970.

Munster AM: Burns. VA Hospital, Charleston, South Carolina, 17 July 1970.

Munster AM: Surgical immunology. Med Univ of South Carolina, Surgical staff, Charleston, South Carolina, 18 July 1970.]

Munster AM: Burns. AMEDD ANC Career Course, MFSS, BAMC, FSHTex, 21 August 1970.

Johns LA: Management and nursing care of individuals with burns.

Mississippi Nurses Assn, Jackson, Miss., 6 October 1970.

Munster AM: Procedures used in the treatment of burn patients. Clinical Pastoral Education Program for Chaplains, BGH, BAMC, FSHTex, 19 October 1970.

Johns LA: Nursing and the burn patient. Clinical Pastoral Education Program for Chaplains, BGH, BAMC, FSHTex, 19 October 1970.

Munster AM: Immunology of burns. Louisiana State Univ staff, New Orleans, La, 22 October 1970.

Johns LA: Nursing care of the burned patient. Flight Nurses and Medical Technicians, School of Aerospace Med, Brooks AFB, Tex, 6 November 1970.

Johns LA: Nursing care of the burned patient. Nursing In-service Program, Santa Rosa Hospital, San Antonio, Texas, 19 November 1970.

Munster AM: Host defense mechanisms following thermal injury.US Army Surgeon General's Advisory Committee on Metabolism of Trauma, BAMC, FSHTex, 1 December 1970.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6395	71 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
70 07 01	D.CHANGE	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A061102B71R	01	242
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U)Bacterial Flora on Military Burn Patients at Time of Admission to Institute of Surgical Research (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS:
003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
66 07	Cont	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 71	0.55	14.6
FISCAL YEAR CURRENT 72	0.6	17.5

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

RESPONSIBLE INDIVIDUAL
NAME: Basil A Pruitt, Jr, LTC, 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: Ft Sam Houston, Texas 78234

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic (not civilian))
NAME: Robert B Lindberg, Ph.D.
TELEPHONE: 512-221-2018
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: Virginia C English, M.A.
NAME: Ruth L Latta, B.S. DA

22. KEYWORDS (Precede EACH with Security Classification Code): (U) Burns; (U) Microbiology of burns; (U) Pseudomonas; (U) Providence

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

23. (U) Determine qualitative and quantitative burn flora on admission of injured soldiers. Thermal injury results in maximum threat of infection, and miliary personnel, in war and training, incur burn risk which calls for detailed knowledge of infecting agents to aid in therapy.

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

25. (U) 70 07 - 71 06-Bacterial flora of 174 admissions in 1970 confirmed ubiquitous colonization, negating significance of reverse isolation to maintain sterile wound. P.aeruginosa prominent at 3-5 days; a marked rise in Providencia stuartii incidence showed source of this new and problematic enteric form to be exogenous. Staph aureus, rising in incidence, shifted to type 47,54,75,84,85, then shifted to a monotype epidemic pattern of types 84 and 84,85. High rate of methicillin resistance shows the re-emergence of St. aureus as a clinical problem.

*Available to contractors upon originator's approval

ANNUAL PROGRESS REPORT

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

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

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

1 July 1970 - 30 June 1971

Investigators:

Robert B. Lindberg, Ph.D.
Virginia C. English, M.A.
Ruth L. Latta, B.S.
Arthur D. Mason, Jr., M.D.
Basil A. Pruitt, Jr., M.D., LTC, MC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

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

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

Period covered in this report: 1 July 1970 - 30 June 1971

Investigators: Robert B. Lindberg, Ph.D.
Virginia C. English, M.A.
Ruth L. Latta, B.S.
Arthur D. Mason, Jr., M.D.
Basil A. Pruitt, Jr., M.D., LTC, MC

Reports Control Symbol MEDDH-288(R1)

Contact cultures of burned patients at the time of arrival on the Institute of Surgical Research wards showed that colonization is so prompt that sterile burn surfaces are non-existent. Pseudomonas aeruginosa was present on 37% of burns within five days of wounding, and this rate was seen also with Klebsiella-Enterobacter sp. Staph. aureus rose to its peak concentrations after six days, whereas the other pathogenic opportunist of major import, Providencia stuartii, did not approach its highest incidence (over 40%) until after 11 days postburn. The two species that conspicuously increased in sepsis in 1969 and 1970, Prov. stuartii and Staph aureus, increased in incidence on patients at time of admission; this epidemiologic fact has bearing on the pathogenesis of burn wound infections in this Institute. The altered bacteriologic picture in the past two years is exogenous, not endogenous.

Burns
Microbiology of burns
Pseudomonas
Providencia

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

The Institute of Surgical Research admits burned patients from all parts of the United States and from overseas areas; in 1970, this included primarily patients from Vietnam. The flora of the burn at the time of arrival presents information on the input of new species and strains into the burn ward population, and also offers a picture of the temporal sequence with which burns are seeded in the population at large, without influence by the specific milieu of the Institute burn ward. This report summarizes the microbiologic picture as seen on arrival cultures on 174 patients examined within two hours of admission.

Methods

Contact plates have been described previously. As in earlier studies, each patient was cultured on an available burn site immediately after being cleansed in the Hubbard tank. The more severely burned areas were selected; healing second degree burns were avoided. The intervals in which the bacterial population were recorded were described in a previous progress report. Zero to two days constitutes a period when the burn may be almost sterile, although heavy contamination may occur if circumstances permitted early heavy seeding, and even burn wound sepsis has, in infants, been seen at 36 hours postburn. Three to five days is a period when rapid colonization and build-up of populations occur, although tissue invasion is rare. If patients are treated correctly with sulfamylon burn cream, this period may still be characterized by low colony counts. Days 6 to 10 often see subeschar colonization and an opportunity for more diverse flora to enter the burn. Between day 11 and day 20, eschar separation usually begins, while after day 20, the diverse courses of healing, dressing and manipulation of the wound result in a heterogeneous environment that presents varying flora.

Determinative procedures were carried out in greater detail than has previously been done. Thus, the coagulase-negative Staphylococci were differentiated into *S. epidermidis*, and *Micrococcus sp.* *Candida albicans* was differentiated from other Candida species. *Herellea vaginicola* and *Mima polymorpha* were distinguished, as were *Enterobacter cloacae*, *E. aerogenes*, and *Klebsiella pneumoniae*.

Results

The incidence of all strains and species recovered from 174 patients on admission is shown in Figure 1. S. aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae, E. coli, Pr.mirabilis and Prov. stuartii were the principal species recovered. With the exception of E. coli and Pr. mirabilis, these species encompass those involved in the sepsis problems prominent in this burn population.

The chronologic sequence of colonization of these burns, prior to their accession to the burn ward, is graphically shown in Figure 2. Staph aureus was present on 21% of the patients in the 0-2 day group, and its incidence increased promptly until over half the patients harbored this organism. Staph epidermidis was extremely common in the earliest stage of the burn, but fell thereafter to a low level of 8% in the 11 to 20 day period. Thereafter it rose in frequency in the post-21-day group.

The most important wound contaminant is P. aeruginosa. It was present in only 7% of the patients in the 0-2 day period, but rose rapidly in frequency to 55% of incoming patients by day 3-5. There was an inexplicable drop in recovery of this organism in the 6-10 day period, but its incidence returned to 55% in the next interval and to 70% at 21+ days. This extremely high colonization rate was not typical of previous experience with this organism.

In earlier observations the Klebsiella-Enterobacter group was not further defined. The differentiation of Enterobacter sp from Klebsiella was carried out in this series. It was apparent that Enterobacter cloacae was the most common form; it was present in 25% of the 0-2 day admissions, and rose rapidly to over half the patients in the 3-5 day group. After this point, its frequency fell steadily, to only 7% in the 21+ day group. E. aerogenes was never common. Klebsiella sp (almost entirely K. pneumoniae) paralleled the incidence of E. cloacae in its prompt rise at 3-5 days. However, this species remained present in almost half of the patients up to the 21+ day group.

Escherichia coli, a common fecal form, was present in 17% of the admissions at 0-2 days, and increased to about one-third of the admissions thenceforth.

Proteus mirabilis, the indol-negative species, was the only member of this genus recovered in burns. It was absent until the 6-10 day period; from this time onward, it was present in approxi-

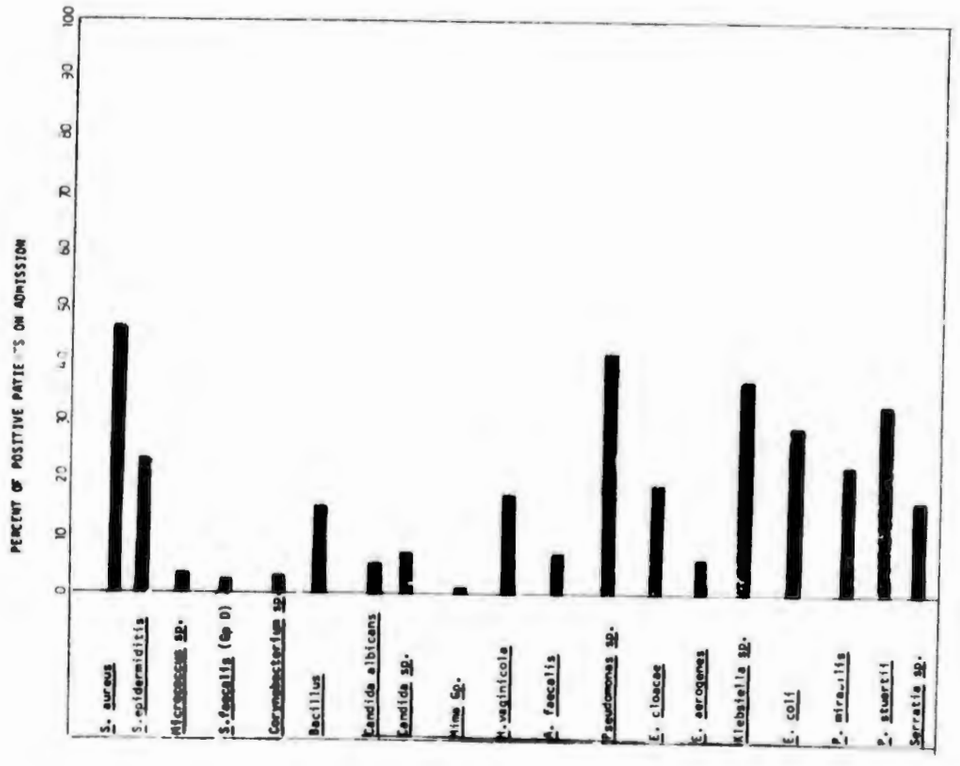

PERCENT OF POSITIVE PATIENTS ON ADMISSION
0
10
20
30
40
50
60
70
80
90
100
S. aureus
S. epidermidis
Micrococcus sp.
S. faecalis (Gp D)
Corynebacterium sp.
Bacillus
Candida albicans
Candida sp.
Mima Sp.
M. vaginicola
M. faecalis
Pseudomonas sp.
E. cloacae
E. aerogenes
Klebsiella sp.
E. coli
P. mirabilis
P. stuartii
Serratia sp.

Figure 1

PERCENT OF POSITIVE PATIENTS ON ADMISSION
AT POSTBURN TIME INTERVALS

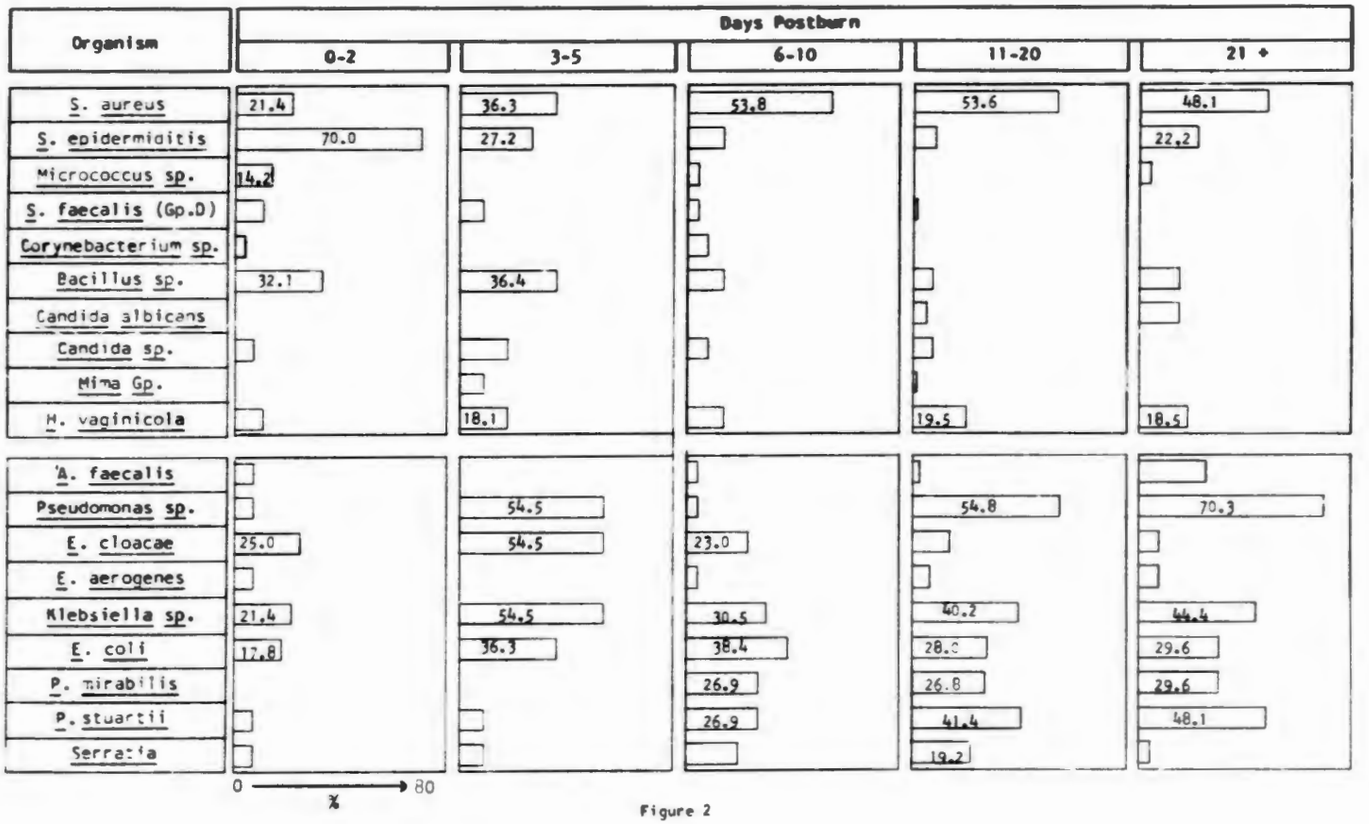


Figure 2

mately one-fourth of the admissions. This organism was less frequently encountered than in previous years. Simultaneously, its role in sepsis in burned patients diminished sharply.

Providencia stuartii, which has become a major problem in sepsis in burns in the past two years, was present in 7% of the 0-2 day admissions, and in 9% of the 3-5 day patients. Its numbers rose rapidly to 26.9% in the 6-10 day group, and almost 50% of admissions at 21 days or more were positive. The epidemiologic implications of this finding are significant. Its appearance on patients arriving here indicates that Prov. stuartii is not unique to this Institute. It must be common in other areas as well, as evinced by its rate of colonization of burn wounds at the time of admission.

A final species, Serratia marcessens, is noteworthy as one which has often been scrutinized as a potential burn pathogen of consequence. It is relatively uncommon up to five days; older burns exhibit it in as much as 20% of patients, but beyond 21 days it once more falls to a low number. This opportunistic invader has caused burn wound sepsis in former years, but its role in this series was that of a fortuitous contaminant.

Changes in Incidence of Burn Flora on Admission. The burn wound flora at admission is of primary interest for its implications in controlling seeding of the burn and the subsequent opportunity for invasive infection. There is a persistent impression in the field of burn treatment that if one could only avoid contamining the burned patient, he could be kept pathogen-free during his course. The presence of a wide spectrum of bacteria on patients at admission negates this concept. Since the cultures are taken on a single contact plate, the recovery is undoubtedly less than a full representation of the quantity of bacteria present on even the early burn.

A comparison of the incidence of Pseudomonas aeruginosa, Klebsiella pneumoniae, and the Enterobacter group over the past four years is presented in Table 1. The pattern is one of relatively few patients being seeded within the first two days, but this changes abruptly, most often on day three. On arrival, an average of 12% of patients are seeded with Pseudomonas on the first two days. This value rises to 50% on the third to fifth postburn day, and after three weeks, is often at the 70% level. We have no explanation for the anomalous value of 3.8% recorded in 1971 for days 6 to 10.

Table 1. Pseudomonas, Klebsiella and Enterobacter on Burn Wounds at Time of Admission to the Institute of Surgical Research

Postburn Interval (Days)	No. Patients for Years				Organism and % of Patients Positive, 1967-1970											
					Pseudomonas				Klebsiella sp				Enterobacter*			
	1967	1968	1969	1970	1967	1968	1969	1970	1967	1968	1969	1970	1967	1968	1969	1970
0 - 2	60	47	59	28	16	21	6	7	13	29	25	21	40	40	30	32
3 - 5	42	56	19	11	50	46	52	54	45	55	47	54	39	48	26	54
6 -10	75	97	54	26	64	50	44	4	40	67	53	30	40	37	44	27
11 -20	41	78	66	82	54	52	51	55	40	51	56	40	25	26	27	21
21+	10	11	13	27	70	54	76	70	30	45	61	44	20	18	23	15

* Includes E. cloacae + E. aerogenes.

Klebsiella sp are, in all probablity, Klebsiella pneumoniae. The average incidence of seeding during the first two days is 22%. This figure rises by days 3-5 to 45% to 55%, and remains at that level until the fourth week, when it may fall slightly. Enteric flora is ubiquitous, and source of seeding need not be sought beyond the patient himself.

Enterobacter sp consist primarily of E. cloacae (see Fig. 1). The initial seeding rate is very consistent at an average level of 35% of admissions. This figure rises by day 3-5, but the pattern fluctuates from year to year; in general, the incidence up to day 10 is little changed from day 0-2; then, there is a distinct drop. From day 11-20 about 25% of admissions were positive, and an irregular fall-off follows in the post-21-day period.

Proteus mirabilis is virtually the only species of this genus in burns in the Institute of Surgical Research as well as on incoming burn patients at the time of admission as shown in Table 2. The unique predominance of the indol-negative mirabilis, as opposed to the other three indol-positive species, has as yet no explanation; certainly there is ample opportunity for seeding with the indol-positive strains. Proteus mirabilis has diminished in frequency in the past two years; it was always uncommon in the fresh burn, and reached a maximum incidence at two to three weeks on 50% of the incoming patients. In 1971 it never increased beyond a seeding rate of 29%. The ebb and flow of bacterial populations has as yet no clearcut explanation, but its occurrence emphasizes the existence of a large number of imponderables as far as opportunistic colonization of burns is concerned.

Providencia stuartii has achieved the status of a major pathogen in sepsis associated with severe burns. Among patients with positive blood cultures in 1971, 38% had Providencia stuartii at some time. It was considered possible that this unusual pathogen was unique to this institute,but the culturing of the incoming patients negates this speculation. As Table 2 shows, the organism was rare in 1967 and,in the early stage of burn, in 1968; but of older burns (11-20 days), one-third harbored it on entry. It is still relatively uncommon up to five days postburn, but it does occur on up to 9% of admissions. In 1969, it was present in 40% of burns at 6-10 days, and its numbers have remained elevated since that time. If any control procedures can be devised, they will have to cope with an admission incidence of 30% to 50% in 70% of our total admissions.

Table 2. *Proteus mirabilis*, *Providencia stuartii* and *Serratia marcessens* on Burns at Time of Admission to the Institute of Surgical Research

Postburn Interval (Days)	No. Patients For Years				Organism and % of Patients Positive, 1967-1970											
					Pr. mirabilis				Prov.stuartii				Serratia marcessens			
	1967	1968	1969	1970	1967	1968	1969	1970	1967	1968	1969	1970	1967	1968	1969	1970
0 - 2	60	47	59	28	15	10	10	0	6	6	3	7	6	14	1	7
3 - 5	46	52	19	ii	24	23	31	0	< 1	7	5	9	11	27	31	9
6 -10	75	97	54	26	32	43	38	27	4	17	40	27	16	22	9	19
11 -20	41	78	66	82	35	38	57	27	8	32	31	41	8	14	13	19
21+	10	11	13	27	50	36	46	29	0	27	23	48	0	9	0	3

Table 3. Staph aureus and Staph epidermidis on Burns at Time of Admission to the Institute of Surgical Research

Postburn Interval (Days)	Organism and % of Patients Positive, 1967-1970							
	Staph aureus				Staph epidermidis			
	1967	1968	1969	1970	1967	1968	1969	1970
0 - 2	16	14	20	21.4	45	48	52	70
3 - 5	6	13	26	36.3	26	30	26	27.2
6 -10	16	24	64	53.8	20	17	11	15.3
11- 20	13	28	40	53.6	25	30	21	8.5
21+	20	18	30	48.1	20	27	23	22.2

Serratia marcessens, some of which have shown ability to invade burn wound, has shown no tendency to increase in incidence. It remains an interesting curiosity for the greater part, and is, if anything, decreasing in incidence in arriving patients. As an infecting organism, it remains, as does any other enteric form, a formidable pathogenic agent when it actually becomes invasive. This is, fortunately, an infrequent occurrence.

Table 3 presents the pattern of colonization with Staph.aureus in the Institute over the past four years. There has been, in 1969 and 1970, a marked rise in the frequency with which arriving patients are colonized with Staph. aureus. A high percentage of these have been type 84, or 84,85 - the phage type which has dominated staphylococcal populations in the Institute in 1970. One out of every five newly arrived patients harbored Staphylococci at 0-2 days in 1970. The rates rose rapidly to one-half of the admissions. Here, as in the preceding species, seeding with Staph. aureus has increased markedly in 1969 and 1970 over the two preceding years. This is not the case with Staph. epidermidis, which is shown on the same table, illustrating a seeding incidence which has fluctuated from year to year; the population is probably not a homogeneous one.

Discussion

A rational basis for detecting typical seeding patterns in burns of various ages is the sampling of incoming patients with burns of varying duration. Chronologic changes in this pattern have been scrutinized in terms of changes that may correlate with an increase in mortality rate in the Institute of Surgical Research population in 1969 and 1970. The admission flora does not reflect changes in this unit; it does reflect changes in the flora on a diverse and scattered population, prior to its arrival here. The opportunistic flora and trends in its occurrence are as follows:

Ps. aeruginosa. There has been little change in the past four years. The incidence on wounds remains high.

Klebsiella pneumoniae. There has been little change, if any has occurred; the organism is today less common after 10 days postburn than it was three years ago.

Enterobacter cloacae. No consistent change in incidence has occurred.

Proteus mirabilis. A decrease in incidence in 1970 was very evident.

Providencia stuartii. An increased incidence in the last two years, and a marked rise in 1970 have occurred.

Serratia marcessens. There has been no change in incidence of this organism.

Staph. aureus. A rise in incidence has occurred since 1969; it was more marked in 1970.

Staph. epidermidis. No consistent change in incidence has occurred.

The implication that altered host susceptibility to Providencia stuartii and Staph aureus has occurred in the burn population outside this Institute is recognized. It is entirely possible that the altered pathogenesis of burns is associated with this change. The observation should be continued.

References

1. Lindberg RB, English VC, Latta, RL, Pruitt BA Jr, Mason AD Jr: Bacterial flora on burn patients at time of admission to the Institute of Surgical Research. Ann Prog Rpt FY 1970, US Army Institute of Surg Res, BAMC, Fort Sam Houston, Tex, Sect. 15.

2. Lindberg RB, Moncrief JA, Contreras AA: Providencia sp in burns: A potential invasive pathogen not previously described in burns. Bact Proc. 19: 1963.

Presentation

Lindberg RB: Changing patterns in the role of enteric bacteria in burn wound infection. Presented at Amer Burn Association, Boston, Mass. 11 Apr 1970.

Publications

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0A 6397	71 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
70 07 01	D. CHANGE	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A061102B71R	01	132
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Procede 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 71	0.18	4.8
	CURRENT 72	0.18	5.3

19. RESPONSIBLE DOD ORGANIZATION

NAME: US Army Institute of Surgical Research

ADDRESS: Ft Sam Houston, Texas 78234

RESPONSIBLE INDIVIDUAL

NAME: Basil A Pruitt, Jr, LTC, 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, Ph.D.

TELEPHONE: 512-221-2018

SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS

NAME: A A Conteras, M.S.

NAME: C E Townsend, SP 6 DA

21. GENERAL USE

FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Burn Wound Flora; (U) Antibiotic Sensitivity; (U) Pseudomonas; (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) Continued assessment of new antibiotics is necessary in laboratory support of the study of trauma, since in the three major areas of burn therapy, trauma study, and renal study, systemic or local infections with resistant microorganisms pose a constant threat.

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

25. (U) Antibiotic therapy is the main thrust of sepsis in tramatically injured combat troops and the senstivity of bacteria from burn patients is to be determined. Antibiotic sensitivity of bacterial isolates from burn patients is to be assessed and used as a major tool for assessing current status of a major group of antibiotic agents used in treatment of pneumonia and sepsis in military burn patients. Broad spectrum reistance in a group of 1447 strains from 457 patients was found. Gentamicin was effective with Pseudomonas, Klebsiella-Enterobacter, Proteus, Providencia and staphylococci; Colymicin was a drug of choice for Pseudomonas and Klebsiella-Enterobacter. Providencia was cross-resistant to a high degree to the entire spectrum of antibiotics. Staph aureus, in a monotype distribution, was methicillin resistant in 65% of strains; lincocin, gentamicin and unipen were the most effective but still left over 60% of strains resistant. A sharp rise in resistance to tetracycline has rendered this agent virtually useless in this population. Proteus mirabilis and E. coli, formerly increasing in sepsis cases, have receded to an inconspicuous status, and although refractory to most antibiotics, are not today of major concern. Multiple resistance has now becoma a major problem in control of sepsis in trauma; no answer is yet available.

*Available to contractors upon originator's approval

ANNUAL PROGRESS REPORT

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

REPORT TITLE: SENSITIVITY OF BURN WOUND FLORA TO ANTIBIOTICS, 1969-1970

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

1 July 1970 - 30 June 1971

Investigators:

Robert B. Lindberg, Ph.D.
Anthony A. Contreras, M.S.
C. E. Townsend, SP-6
Basil A. Pruitt, Jr., M.D., LTC, MC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

REPORT TITLE: SENSITIVITY OF BURN WOUND FLORA TO ANTIBIOTICS, 1969-1970

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

Period covered in this report: 1 July 1970 - 30 June 1971

Investigators: Robert B. Lindberg, Ph.D.
Anthony A. Contreras, M.S.
C. E. Townsend, SP-6
Basil A. Pruitt, Jr., M.D., LTC, MC

Reports Control Symbol MEDDH-288(R1)

Antibiotics play a major role in the control of wound infection today, and the sensitivity of pathogens or potential pathogens encountered in sepsis associated with burns is of immediate value to physicians treating traumatic injuries, especially in combat casualties. Detailed assays can also forecast areas where bacterial resistance may be occurring, warn of potential future treatment failures and guide physicians in emergency therapy. A total of 1447 strains of Pseudomonas, Klebsiella-Enterobacter, Proteus spp, Providence spp, Escherichia coli, Staphylococcus aureus and Staphylococcus epidermidis, from 457 burn patients during 1969 and 1970 were tested by tube dilution technic. A picture of relatively high antibiotic resistance, and an increasing incidence of cross-resistance in this flora emerged. Sixty-three per cent of Pseudomonas aeruginosa strains were sensitive to Colymycin, and 70% to gentamycin. Seventy per cent of Klebsiella-Enterobacter group isolates were sensitive to gentamycin, and 38.5% to Colymycin. Providencia stuartii, now a major pathogen in burn patients, was essentially resistant to antibiotics. Staph. aureus showed a high level of methicillin resistance. Lincocin, gentamycin and Unipen were effective in 36 to 38% of Staph.aureus strains. The level of resistance has moved markedly higher during the past year.

Burn wound flora Antibiotic sensitivity
Pseudomonas Providencia

SENSITIVITY OF BURN WOUND FLORA TO ANTIBIOTICS, 1969-1970

The major cause of morbidity and death in patients with severe burns has always been bacterial infection, and although striking progress has been made in controlling invasive burn wound infection, septic complications frequently with systemic involvement remains the most common cause of death in burn patients.

Topical therapy has controlled Pseudomonas burn wound sepsis (1), but sepsis from pulmonary and other infections which may evolve despite topical chemotherapy, demand effective antibiotics as the principal therapeutic approach. Gram-negative bacilli have played an increasingly important role in nosocomial, or hospital-engendered infections, not only in this Institute but in hospitals throughout the world (2).

Genera of the Enterobacteriaceae have been a conspicuous problem in opportunistic burn infections, since they display a high degree of antibiotic resistance. The resistance transfer factor (RTF) as reviewed by Anderson (3) offers an explanation for the ease with which these organisms acquire resistance to antibiotics. There is no present prospect of resolving this growing impasse, and its existence underscores the need for detailed information on antibiotic sensitivity.

Topical therapy with sulfamylon burn cream resulted in a marked reduction in Pseudomonas burn wound sepsis (4), but since the occurrence of bacterial sepsis due to Pseudomonas, Staphylococci and Enterobacteriaceae from sources other than the burn wound has persisted, sepsis, whether originating in the wound or elsewhere, calls for prompt and energetic systemic antibiotic therapy; and it is often essential that therapy be started prior to determining the specific antibiotic sensitivity of the offending organism(s). Compilation of sensitivity of the bacterial species from this population offers a guide for the selection of antibitiotics. This report summarizes the sensitivity of major bacterial genera and species observed in burn patients in 1969 and 1970.

Materials and Methods

Strains tested were primarily from blood cultures and other clinical specimens in which sensitivity test was requested by the attending physician. The antibiotic battery used included those antibiotics which would offer the greatest likelihood of

including an effective agent. This list is reviewed at least twice yearly, and new antibiotics may be added when desired. The antibiotic screen for gram-negative organisms was as follows:

Antibiotics Used on Gram-Negative Organisms

Tetracycline	
Chloramphenicol	(Chloromycetin)
Kanamycin	(Kantrex)
Keflin	(Cephalothin)
Colymycin	(Colistimethate sulfate)
Gentamycin	(Garamycin)

Polymyxin B, which had been routinely used for several years of testing, was dropped early in 1969, and gentamycin was substituted. Carbenicillin has been used since 1969, but only for Pseudomonas aeruginosa. In contrast to some reports (5), we have not found isolates of Proteus spp or other Enterobacteriaceae susceptible to carbenicillin. Similarly, Proteus mirabilis has been described as susceptible to Penicillin G, but we have found this organism to be only rarely penicillin-sensitive.

Gram-positive organisms have been tested with the following antibiotics:

Antibiotics Used on Gram-Positive Organisms

Kanamycin	(Kantrex)
Lincocin	(Lincomycin)
Prostaphlin	(Oxacillin)
Staphcillin	(Methicillin)
Tetracycline	
Gentamycin	(Garamycin)
Unipen	(Nafcillin)

Gentamycin was included in this battery during 1969.

The assay technic used in this laboratory is a standard MIC (minimum inhibiting concentration) procedure (1). Test solutions and dilutions of antibiotic are prepared from assay quality antibiotic obtained from the manufacturer. Stock solutions in sterile distilled water were made in decrements from 50 units or µg/ml down to 1.5 µg/ml. Five-tenths ml portions of each dilution were placed in sterile, capped, 10 mm tubes and stored at -20° C. These solutions were replaced at frequent intervals. Inoculum was grown from an isolated colony in Trypticase Soy Broth for 4 - 6 hours at

37° C (water bath). The concentration has been found to approximate 10^6 to 10^8 organisms per ml. The culture is diluted 1:100 in double-strength Trypticase Soy Broth. Five-tenths ml of this dilution is added to each 0.5 ml of dilution of thawed antibiotic. Final concentrations of antibiotic thus range from 25 µg/ml to 0.78 µg/ml. Tubes are incubated at 37° C for 20 hours, then read for MIC. The last tube showing visible growth when viewed beside a control blank by oblique light, constitutes the endpoint of growth; the next highest concentration is the MIC.

The numbers of strains tested and the sources are summarized in Table 1. It must be emphasized that the tests were run on strains as requested by physicians; this is not a random sample of isolates. Thus, the strains recovered at autopsy are not represented. All blood stream isolates were routinely tested, but urine and sputum samples were tested only on request. As noted, a drop in Klebsiella-Enterobacter septicemia occurred in 1970, as did a similar fall in Proteus septicemia and in Proteus spp from sputum. The remaining species showed no marked change in incidence for those two years. Obviously, blood and sputum (including Lukens tube aspirates) constituted the major part of sources of culture; these patients were in greatest need of antibiotic therapy.

Results

The percentage of strains sensitive to ascending levels of antibiotic are shown as a cumulative figure. Each level inhibited all strains inhibited at lower levels. For gram-negative bacilli, an inhibiting level of 12.5 µg/ml was regarded as the upper limit which merited the appellation "sensitive". The sensitivity of 129 strains of P. aeruginosa to the "gram-negative battery" of antibiotics is shown in Table 2. It was evident that only Colymycin and genatmycin offered any reasonable rate of effectiveness against this most important burn pathogen. There were no significant changes in the percentage of strains sensitive in 1969 from that seen in 1970. Indeed, the proportion of strains inhibited by Colymycin and by gentamycin were remarkably consistent during this entire period. There has been a consistent tendency for gentamycin to inhibit a higher proportion of isolates at extremely low levels, but in the range of 3.1 units to 6.25 units/ml, the MIC median is comparable for these two antibiotics.

The Klebsiella-Enterobacter group were pooled for this comparison. The sensitivities are summarized in Table 3. The distribution of sensitivity was notably uniform with all antibiotics except gentamycin. Tetracycline showed a wider range than did

Table 1. Organisms from Burn Patients Tested for Antibiotic Sensitivity, 1969 & 1970, Institute of Surgical Research

Species	No. of Patients	No. Strains	Source			
			Blood	Sputum	Urine	Other
P.aeruginosa	73	123	52	49	8	24
Klebsiella-Enterobacter*	76	116	35	59	9	13
Proteus sp** (mirabilis)	40	58	17	22	12	7
Providencia (stuartii)	82	144	64	51	16	13
E. coli	40	45	16	19	4	6
Staph.aureus	113	187	133	32	3	20
Staph.epidermidis	33	45	30	8	2	5
Totals	457	718	347	240	54	88

* A marked drop in blood stream isolates submitted for test occurred between 1969 and 1970.
1969: 31 Klebsiella strains from blood cultures
1970: 4 Klebsiella strains from blood cultures

** A marked fall in Proteus isolates occurred in 1970. 1969, 49 isolates; 1970, 9 isolates tested. Positive blood cultures: 15 in 1969, 2 in 1970. Positive sputum cultures, 20 in 1969, 2 in 1970.

Table 2. Pseudomonas aeruginosa: Cumulative Inhibitory Levels for Strains Isolated in the Institute of Surgical Research, 1969-1970

MIC µg/ml	Antibiotic and Per Cent of Strains Inhibited					
	T	C	K	Kf	Co	G
> 25	100	100	100	100	100	100
25	33	11.7	16.2	5.0	71.8	84.5
12.5	14.9	3.1	6.9	2.5	62.4	74.5
6.25	7.8	1.5	0	0	49.2	55.4
3.1	5.5	1.5	0	0	26.5	48.1
1.5	0.7	0.7	0	0	7.0	22.7
0.78	0	0	0	0	1.5	4.5
< 0.78	0	0	0	0	0	1.8
No. tested	127	128	129	120	128	110

T - Tetracycline
C - Chloramphenical
K - Kanamycin
Kf- Keflin
Co- Colymycin
G - Gentamycin

Table 3. Klebsiella-Enterobacter Group: Cumulative Inhibitory Levels for Strains Isolated in the Institute of Surgical Research, 1969-1970

MIC µg/ml	Antibiotic and Per Cent of Strains Inhibited					
	T	C	K	Kf	Co	G
> 25	100	100	100	100	100	100
25	38.8	30.6	39.8	45.0	50.4	86.8
12.5	30.9	22.7	27.4	28.4	38.5	69.6
6.25	18.5	14.8	10.6	14.7	21.1	49.4
3.1	12.3	6.9	4.4	6.8	5.5	22.7
1.5	6.1	0.9	0	0	1.8	10.1
0.78	2.6	0	0	0	0.9	1.0
< 0.78	1.7	0	0	0	0	0
No.tested	113	101	113	102	109	99

T - Tetracycline
C - Chloramphenicol
K - Kanamycin
Kf - Keflin
Co - Colymycin
G - Gentamycin

chloramphenicol, Kantrex or Keflin. The latter three antibiotics were almost identical in their range of effectiveness; a maximum level of 25% of isolates were within the range that could be designated as effective. Colymycin affected an encouraging 38% of the strains tested, but the most effective in vitro performance was displayed by gentamycin, with almost 70% of the strains inhibited by 12.5 µg/ml or less. When the years 1969 and 1970 were compared, there was little significant difference; if anything, the population was slightly more sensitive in 1970 than in 1969.

Proteus strains were primarily Proteus mirabilis during the two-years of observation. A peculiar scarcity of indol-positive Proteus spp characterizes this patient population; this phenomenon has as yet no explanation. The sensitivity of Proteus mirabilis isolates is shown in Table 4. It will be recalled that 1970 saw a marked drop in the number of Proteus mirabilis strains collected from burn wounds. Forty-nine strains were tested in 1969, and only nine in 1970. Obviously there were far fewer life-threatening episodes due to Proteus mirabilis in 1970 than in 1969. Only Keflin and gentamycin inhibited a significant proportion of strains. Colymycin was completely ineffective against Proteus mirabilis strains.

There was a slight increase in the number of Providencia stuartii strains tested in 1970 over 1969. The increase was primarily in the number of sputum and Lukens tube isolates. Sensitivity results for this increasingly important group of organisms are summarized in Table 5.

In view of its increasing incidence in pneumonia, burn wounds and septicemia, it is dismaying to see reaffirmed the extremely refractory state of this large collection of Providencia strains with reference to this broad spectrum of antibiotics. There was no significant change in sensitivity in the collection of 1969 and that of 1970; the values were, at times, identical. Multiple resistance is obviously so fixed in this species that it is the rule rather than the exception. Gentamycin, with a cumulative effective level of 23.4% of strains at an MIC of 12.5 µg/ml was the most effective antibiotic; tetracycline was so close that this antibiotic would also merit consideration. But, the best results were still far from encouraging. Combinations of antibiotics were tried repeatedly with strains from severely ill patients, but none of these has shown an augmenting effect. At present there is no antibiotic regimen that holds promise in the case of Providencia sepsis.

Table 4. Proteus mirabilis: Cumulative Inhibitory Levels for Strains Isolated in the Institute of Surgical Research, 1969-1970

MIC µg/ml	Antibiotic and Per Cent of Strains Inhibited					
	T	C	K	Kf	Co	G
> 25	100	100	100	100	100	100
25	10.1	22.0	15.7	67.4	0	75
12.5	10.1	10.9	7.0	39.5	0	50
6.25	5.0	0	3.5	20.9	0	14.5
3.1	1.6	1.8	0	0	0	0
1.5	0	0	0	0	0	4.1
0.78	0	0	0	0	0	0
< 0 78	0	0	0	0	0	0
No. tested	59	55	57	43	56	48

T - Tetracycline
C - Chloramphenicol
K - Kanamycin
Kf - Keflin
Co - Colymycin
G - Gentamycin

Table 5. *Providencia stuartii*: Cumulative Inhibitory Levels for Strains Isolated in the Institute of Surgical Research, 1969-1970

MIC µg/ml	Antibiotic and Per Cent of Strains Inhibited					
	T	C	K	Kf	Co	G
> 25	100	100	100	100	100	100
25	21.1	17.2	16.4	6.7	17.2	49.2
12.5	17.4	8.2	7.8	3.7	15.7	23.4
6.25	11.9	4.5	4.2	1.4	10.5	12.1
3.12	7.7	2.2	2.1	0.7	4.5	5.3
1.5	6.3	0	1.4	0	0	2.2
0.78	1.4	0	0.7	0	0	1.5
< 0.78	0.7	0	0	0	0	0
No. tested	142	133	140	133	137	132

T - Tetracycline
C - Chloramphenicol
K - Kanamycin
Kf - Keflin
Co - Colymycin
G - Gentamycin

Escherichia coli has remained an organism of minor but consistent interest in infections in the burned patients. For 1969 and 1970, the number of specimens was consistent: 22 per year. Most of these were from blood cultures and sputum samples. The sensitivity pattern is shown in Table 6. Three of the six antibiotics used were effective against at least half the strains: Tetracycline, Colymycin and gentamycin. Colymycin was the most active, and as little as 3.1 µg/ml inhibited 23.2% of strains. Interestingly, the marked rise of cross-resistance which has in the past three or four years placed the Klebsiella-Enterobacter group and the Providencia stuartii on a level unreachable by most antibiotics has not been seen with E. coli, although resistance transfer factor is readily demonstrated in this species.

No other gram-negative aerobic bacilli were tested (i.e., were involved in infection to an extent that required testing) in numbers significant for assessing sensitivity to antibiotics. Thus, members of the Mima-Herellea group, Serratia sp, Alcoligenes fecalis and other gram-negative forms are encountered in burns, but are of minor import in actual infections.

Staphylococcus aureus is a conspicuous part of the burn flora, and in the last two years has been present in monotype epidemic status on the burn ward. A corresponding sharp rise in infections which call for sensitivity testing has occurred. Here the problem of methicillin-resistant strains is of great importance. There has been a steady rise in incidence of such strains during 1969 and 1970. The cumulative sensitivity of Staph. aureus strains in 1969-1970 is shown in Table 7. The battery of antibiotics included kanamycin, Lincocin, Prostaphlin, Staphcillin, tetracycline, gentamycin and Unipen (nafcillin). The semisynthetic penicillin represent the family of penicillinase-resistant antibiotics that have, hopefully, restored the patency of the penicillins which are attacked by this enzyme. The source of strains implies their virulent role: 70% of those tested were recovered from blood cultures.

The situation with reference to upper limit of sensitivity range differs from that with gram-negative bacteria; for staphylococci, the cutoff level is 6.25 µg/ml. None of the antibiotic battery can be described as markedly effective. Tetracycline, the least effective, inhibited only 7.7% of the strains. Of the 1970 strains, only 3.8% were inhibited. This antibiotic will no longer be tested routinely; it has virtually lost its usefulness. Kanamycin has also dropped markedly during the past two years in its level of inhibiting potential, from 38% in 1969 to 2.8% in 1970. The average level of 18.2% is weighted by earlier collections. Lincocin

Table 6. Escherichia coli: Cumulative Inhibitory Levels for Strains Isolated in the Institute of Surgical Research, 1969-1970

MIC µg/ml	Antibiotic and Per Cent of Strains Inhibited					
	T	C	K	Kf	Co	G
> 25	100	100	100	100	100	100
25	50	54.7	33.3	12.8	76.7	5.0
12.5	50	38.0	21.4	7.6	72.0	65.0
6.25	42.8	14.2	7.1	2.5	48.8	32.5
3.12	21.4	2.3	0	2.5	23.2	5.0
1.5	11.9	0	0	2.5	11.6	2.5
0.78	2.3	0	0	0	4.6	0
< 0.78	2.3	0	0	0	0	0
No. tested	42	42	42	39	43	40

T - Tetracycline

C - Chloramphenicol

K - Kanamycin

Kf - Keflin

Co - Colymycin

G - Gentamycin

Table 7. Staphylococcus aureus: Cumulative Inhibitory Levels for Strains Isolated in the Institute of Surgical Research, 1969-1970

MIC µg/ml	Antibiotic and Per Cent of Strains Inhibited						
	K	L	Ps	Sc	T	G	U
> 25	100	100	100	100	100	100	100
25	38.7	58.6	51.0	47.1	22.7	72.6	63.0
6.25	18.2	37.9	26.9	21.5	7.7	36.9	36.4
3.12	11.2	24.1	17.0	7.9	5.5	15.4	25.3
1.5	6.9	14.9	8.7	1.1	2.7	7.1	16.2
0.78	3.8	7.4	3.2	1.1	2.2	7.1	7.7
< 0.78	2.6	3.4	1.0	0	1.6	2.9	4.1
No. tested	186	174	182	176	180	168	166

K - Kanamycin
L - Lincocin
Ps - Prostaphlin
Sc - Staphcillin
T - Tetracycline
G - Gentamycin
U - Unipen

gentamycin and Unipen were the most effective agents, but even here at best 36 to 37% of the strains were inhibited by 6.25 µg/ml. These levels have been more stable, although the gentamycin-sensitive level fell from 52% in 1969 to 32% in 1970. Prostaphlin and Staphcillin were disturbingly often ineffective: the mean rate of methicillin-resistance was 78.5% of all strains tested. No other report of such a high incidence of methicillin-resistance has come to our attention. There is little gain in speculating on ultimate causes; the fact that the current Staph. aureus population is virtually entirely one type, 84, makes it probable that if this strain is resistant, then the average resistance of all isolates will be very high.

Staphylococcus epidermidis, and coagulase-negative cocci, potentially related to Staph.aureus, were summarized in one group. It was notably more heterogeneous than the Staph. aureus population. Thirty of the 45 isolates came from blood cultures; however, well-defined septicemia due to coagulase-negative cocci was seldom documented. The sensitivity of these isolates is shown in Table 8. There was little change in the general pattern of sensitivity, which suggests the presence of a mixed bacterial population more than it does a homogeneous one. Only three antibiotics, Prostaphlin, gentamycin and Unipen inhibited over 30% of the strains above the 6.25% concentration. Gentamycin was the most effective antibiotic tested, and it inhibited 47.6% of tested strains.

Discussion

The bacterial flora of greatest consequence in sepsis in burn patients was, during 1969 and 1970, the Enterobacteriaceae (primarily Klebsiella-Enterobacter, and Providencia stuartii), P. aeruginosa and Staph. aureus. In terms of frequency with which these occurred in infections serious enough to demand detailed sensitivity data, blood stream and pulmonary infection were the principal sources. Pseudomonas was inhibited best by Colymycin and by gentamycin. The effective range covering 60% to 75% of the Pseudomonas strains by these two antibiotics was, in point of fact, the most favorable result obtained for any major pathogen. Klebsiella-Enterobacter strains were far more likely to display resistance; again, Colymycin and gentamycin were the two most effective antibiotics, with gentamycin the most effective at a level of 69.6% of all strains tested. Tetracycline, kanamycin and Keflin were in the effective range for approximately 30% of the strains.

Proteus mirabilis, has, for reasons not known, dropped to a very minor role as to incidence in serious infections. The numbers

Table 8. Staphylococcus epidermidis and Coagulase-negative Staphylococci: Cumulative Inhibitory Levels for Strains from the Institute of Surgical Research, 1969-1970

MIC µg/ml	Antibiotic and Per Cent of Strains Inhibited						
	K	L	Ps	Sc	T	G	U
> 25	100	100	100	100	100	100	100
25	29.5	23.8	47.3	35.5	43.3	69.0	55.9
12.5	25.0	21.4	39.4	31.1	33.3	66.6	48.7
6.25	18.1	11.8	34.2	20.0	24.4	47.6	32.5
3.1	11.3	9.0	18.4	8.8	20.0	30.9	30.2
1.5	6.8	7.1	13.1	2.2	13.1	26.1	20.9
0.78	2.2	4.7	7.8	0	4.3	21.4	13.9
< 0.78	0	0	0	0	2.2	2.3	2.3
No. tested	44	42	38	45	45	42	43

K - Kanamycin
L - Lincocin
Ps - Prostaphlin
Sc - Staphcillin
T - Tetracycline
G - Gentamycin
U - Unipen

tested were mainly accumulated in 1969; during 1970, few were regarded as of consequence. The species is notoriously refractory to antibiotics; in this series, gentamycin was most effective and Keflin, with 40% of strains inhibited was close behind. Penicillin G has been reported as effective against Proteus mirabilis, but in tests on 45 strains in this collection, only one was inhibited by 12.5 µg/ml; all others were at the 25 µg/ml level or higher.

The striking rise in Providencia stuartii sepsis has been a major disturbing factor in this period. Here the antibiotic sensitivity tests have been entirely discouraging. The most effective antibiotic, gentamycin, inhibited at best, 23.4% of strains; with tetracycline and Colymycin the figures were 17.4% and 15.7% respectively. Results were the same in 1969 and in 1970. At present, there is no antibiotic available that offers any encouragement for this organism.

E. coli responded with encouraging frequency to tetracycline, Colymycin and gentamycin. There was no indication of any rise in resistance in this species. Resistance transfer factors have undoubtedly been present but have not affected the relative level of susceptibility.

Staphylococci have resumed a more significant role in sepsis, if the frequency of septicemia is a valid indicator. A relative increase in resistance to antibiotics, especially to the semi-synthetic penicillins, was evident. The overall average in Table 7 obscures this change, which may be shown thus:

	Antibiotic and % of Staphylococci Inhibited at 12.5 µg/ml						
	K	L	Ps	Sc	T	G	U
1969	38	48	33	26	13	52	41
1970	2.8	29.8	22.4	18	3.8	32	33.9
Change	-35.2	- 18.2	- 10.6	- 8	- 8.2	-20	- 6.1

Lincocin, gentamycin and Unipen remain the most promising antibiotics. Tetracycline and kanamycin were both so inert that there seems little reason to continue using them in the screening battery. Recent observations with Keflin suggest that it be used in place of one of these.

The behavior of the coagulase-negative cocci, including Staph. epidermidis, suggested that this group was heterogeneous. There

were no highly effective antibiotics; the most promising would be Prostaphlin, gentamycin, and Unipen.

At this point there are no encouraging prospects in the overall problem of antibiotic resistant flora in burns. Pseudomonas aeruginosa, still a major pathogen, has the most promising spectrum; all other flora have a higher proportion of strains relatively resistant to all antibiotics. These strains are not unique to the Institute of Surgical Research; many of them have been brought in on patients from Vietnam and CONUS. A continued search for more effective agents is being carried on.

References

1. Lindberg RB, Moncrief JA, Switzer WE, Mason AD Jr.:Control of bacterial infection in severe burns with a topical sulfonamide burn cream. Antimicrobial Agents & Chemother. 1964, pp. 708-716.

2. Kennedy RP, Plande JJ, Petersdorf RG: Studies on the epidemiology of Escherichia coli infections: Evidence for a nosocomial flora. J Clin Invest 44: 193-201, 1965.

3. Anderson ES: The ecology of transferable drug resistance in the Enterobacteriaceae. Ann Rev Microbiol 22:131-180, 1968.

4. Teplitz C, Davis DD, Mason AD Jr, Moncrief JA: Pseudomonas burn wound sepsis I. Pathogenesis. J Surg Res 4: 200-216, 1964.

5. Ross S, Krayhill EN, Khan W: Treatment of Proteus meningitis with carbenicillin. J Infec Dis 22: 62-70, 1970.

6. Kolmer: Laboratory Technic, 1958.

Presentation

Lindberg RB: Mechanisms of development of antibiotic resistance of microorganisms, presented at Seminar on Modern Concepts of Infection Disease Control, Letterman Gen. Hosp. San Francisco, Calif. 15 May 1970.

Publication

Lindberg RB: Mechanisms of antibiotic resistance in bacteria. Acta Microbiologica (In press).

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0B 6397	71 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
70 07 01	D. CHANGE	U	U	DA	NL	[X] YES [ ] NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A061102B71R	01	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 71	0.35	9.3
	CURRENT 72	0.35	10.2

19. RESPONSIBLE DOD ORGANIZATION

NAME: US Army Institute of Surgical Research

ADDRESS: Ft Sam Houston, Texas 78234

RESPONSIBLE INDIVIDUAL

NAME: Basil A. Pruitt, Jr., LTC

TELEPHONE: 512-221-2720

20. PERFORMING ORGANIZATION

NAME: US Army Institute of Surgical Research
Microbiology Branch

ADDRESS: Ft Sam Houston, Texas 78234

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

NAME: Robert B. Lindberg, Ph. D.

TELEPHONE: 512-221-2018

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: R. L. Latta, B. S.

NAME:

21. GENERAL USE

FOREIGN INTELLIGENCE NOT CONSIDERED

DA

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Pseudomonas; (U) Phage typing; (U) Burn Wounds; (U) Topical Chemotherapy

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

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

24. (U) Phagetyping system, developed in this institute is used to type P.aeruginosa isolates.

25. (U) 70 07 - 71 06 - Four major types recognized in burn ward population. Origin of several severe problem types shown to be in Vietnam casualties. Cross infection via nebulizing equipment could be traced via phagetype, and precise recognition of virulent strains in cross infections was achieved. Heterogeneous character of pseudomonas problem substantiated; control measures can be guided by this concept.

* Available to contractors upon originator's approval.

ANNUAL PROGRESS REPORT

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

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

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

1 July 1970 - 30 June 1971

Investigators:

Ruth L. Latta, B.S.
Robert B. Lindberg, Ph.D.
Russell E. Brame, M.S.
Arthur D. Mason, Jr., M.D.
Basil A. Pruitt, Jr., M.D., LTC, MC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

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

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

Period covered in this report: 1 July 1970 - 30 June 1971

Investigators: Ruth L. Latta, B.S.
Robert B. Lindberg, Ph.D.
Russell E. Brame, M.S.
Arthur D. Mason, Jr., M.D.
Basil A. Pruitt, Jr., M.D.,LTC, MC

Reports Control Symbol MEDDH-288(R1)

The predominant bacteriophage types of Pseudomonas aeruginosa included four which encompassed 37.8% of the 18 significant types recorded. Of the strains tested, 19.4% did not react with the typing set, but this rate was acceptable since it consisted primarily of a small proportion of each patient's Pseudomonas population. Six types new to the significant population were recognized, while five types, previously conspicuous, fell to insignificant numbers. Most types found on incoming Vietnam patients, also were recognized on CONUS arrivals; three types were exclusive, two to the Vietnam population, one to CONUS patients. In septicemia and pneumonia, predominant types were shown to match the overall flora in incidence, rather than to suggest a virulent or invasive predilection by certain types. The heterogeneous pattern of Pseudomonas infection was confirmed; the typing system permitted precise delineation of contamination and seeding patterns in the burn ward.

Pseudomonas
Burns
Phagetyping
Topical chemotherapy

BLANK PAGE

BACTERIOPHAGE TYPES OF PSEUDOMONAS AERUGINOSA FOUND IN BURNED PATIENTS

The control of burn wound sepsis by topical therapy was achieved by suppression of invasive infection due to Pseudomonas aeruginosa. Although this objective has been achieved, as shown by the very low incidence of deaths due to Pseudomonas burn wound sepsis, P. aeruginosa has remained a formidable threat to the survival of burned patients. Pseudomonas septicemia still occurs, although the burn wound may be under control. Pulmonary involvement with P. aeruginosa is not uncommon, and colonization of the burn with the threat of ultimate sepsis occurs commonly. The transmission of this burn pathogen is essentially a passive carrier problem, and its eradication from the environment has not been achieved in any encouraging degree. Comprehension of the epidemiologic pattern of Pseudomonas infection requires precise recognition of strains, and the phage typing system which was evolved in this laboratory (1) has proven to be admirably suited to accomplishing this.

The incidence of P. aeruginosa in patients in the Institute of Surgical Research has continued to be high despite successful suppression of burn wound sepsis. The type differentiation of this species forms the basis of this report.

Methods

The technics of developing a phagetyping set have been described previously (2). Technics of its application have continued to follow the procedures set down in that report. Cultures were collected by contact plate on arrival in the ward, by swab or contact plate of wound surface, biopsy, autopsy tissue sample, from urine and sputum, and from blood culture. Sampling of incoming patients have proven to be of especial importance in recognizing seeding patterns.

Results

Predominant Phage Types

The extent of colonization by Pseudomonas is a function of the time elapsed since injury. For the first two days seeding is rare, but by days 3 to 5, it has reached a rate of 40% or more. Vietnam patients almost always arrive at a later postburn

date than do CONUS patients. The carrier rate is consequently higher in Vietnam patients. There were 325 patients admitted in 1970. Out of 174 Vietnam patients, 131, or 75% were cultured on arrival, as shown in Table 1. Seventy of these (53.4%) were positive for P. aeruginosa. Out of the 70 positive cases, 44 remained positive after admission, 22 were negative, and 4 had no further cultures. Sixty-one (46.6%) of the patients were negative for P. aeruginosa, 27 or 44%, acquired P. aeruginosa at some time during their stay; 32 remained negative, and two were not cultured.

The positive rate for CONUS patients on admission was lower than for the Vietnam group; 44 were cultured on admission, and of these, seven (16%) were positive for Pseudomonas and 37 were negative. Out of the seven positives, three remained positive for P. aeruginosa; two showed no further positive cultures. There were 37 CONUS patients negative on arrival; 17 of these, or 46%, acquired P. aeruginosa. The remaining 20 were cultured with negative results. It was surprising to note that the admission rate for Pseudomonas, at 53.4%, was little changed from the rate in 1968 and 1969. CONUS arrivals exhibited 16% positives on admission; in 1969, the figure was 14%. The total positive rate for Pseudomonas, either on admission or post-admission, was 59%. It had reached 75% in 1969.

In all, there were 740 Pseudomonas strains collected from 162 burned patients in 1970. This was approximately half of the patients admitted during the year; 102 were Vietnam returnees, 60 were from CONUS.

The predominant phage types are listed in Table 2. Fourteen categories are listed; since 19.4% of all isolates failed to react with this typing approach, the nontypables (NT) were numerically the largest single category. The 13 types listed took the smallest group to a total of five strains. As has been done previously, the types are assigned letter and numerical codes for convenience in referring to them. Out of these 13 types, seven had been noted in the predominant category in 1969. Six types: M4,F12, C10, H1,D41 and B9a, had not been numerous enough to be listed in 1969.

Although the total of nontypable strains was relatively large, 59 or 40% of these strains were recovered from only seven patients; the remaining 86 strains appeared on 51 patients. Thus, the incidence on most individuals was less than two isolates per person. The nontypable rate has fluctuated; in 1969 it was only 14%.

Table 1. Incidence of Pseudomonas on Admission and/or Post-Admission Cultures of 325 Burned Patients, 1970

Patient Origin	No. of Patients	Admission Contact Culture	Post-Admission Cultures		
			Positive	Negative	No Culture Obtained
Viet Nam 174 patients	70	Positive	44	22	4
	61	Negative	27	32	2
	43	No culture obtained	7	13	23
Other than Viet Nam 151 patients	7	Positive	3	2	2
	37	Negative	17	20	0
	107	No culture obtained	40	51	16

Table 2. Predominant Pseudomonas Phage Types in ISR Burn Ward Patients, January - December, 1970

Phage Type Code	Phage Type	Patient Origin		Total	
		Other than Viet Nam	Viet Nam	No. of Patients	No. of Strains
		No. of Patients - Strains			
	NT	24-65	34-80	58	145
H 3	1214,68,119X	11-39	29-78	40	117
M 2	119X	5-9	17-24	22	33
H15	1214,68,F8	10-57	7-20	17	77
M 4	119X,F7	7-37	3-16	10	53
F12	31	3-3	7-16		19
I 1	68	4-4	4-5	8	9
B13	7,31	5-41	2-7	7	48
B9a	7,68,119X	0	7-12		12
H14	1214,68	1-1	6-11		
C10	16,21,44,1214,68,F8	2-3	4-7	6	10
H 1	1214	2-2	4-7		9
D41	21,68	5-23	0	5	23
D29	21,119X	1-3	4-8		11
B22	7	2-2	3-3		5

The most prominent phage type was 1214,68,119X, designated H3. Forty patients harbored 117 strains. They were first seen in January 1969 and were the predominant type in 1969; again in 1970, it was the predominant type. H3's were unquestionably brought in on returning Vietnam veterans. In 1970, 14 Vietnam patients had this type on admission, from January through April. From that time on, however, the strain disappeared from the returning Vietnam group on their admission cultures. It did not disappear entirely, but only six patients harbored it in the last six months of 1970.

Phage type 119x is designated as M-2. It was the fifth in prevalence in 1969, and increased to second most common type in 1970. It was almost entirely in Vietnam patients in 1969; in 1970, it was still primarily found in Vietnam returnees.

Type 1214,68,F8 is code H-15. Seventeen patients yielded 77 strains all together. It was first seen in January of 1969 on Vietnam returnees, and it was the sixth most prevalent type in 1969. It became more common in 1970, but not in Vietnam returnees. It was not found on admission cultures. It was not seen after October, and it is possible that it has disappeared from this population.

Type 119x,F7 is code M-4. Ten patients furnished 53 strains for typing. It was rare (seen twice) in 1969, and in 1970 did not appear until August. One strain came in December on a Vietnam patient, so that it may indeed be transmitted from the Far East.

Phage type 31 is designated F-12. It has a long history of occurring in the Institute of Surgical Research; in 1967 it was fourth most common; in 1968 it was the second most common type, while in 1969 it was relatively rare. Two Vietnam admissions had it on their wounds on admission; since it was found previously in CONUS, we may conclude that it is a proven world-wide type.

Phage type 68 (I-1) is also an old type, first found in the Institute of Surgical Research in 1965. It was fourth most common type in 1969,and in 1970, was the sixth most commonly encountered. I-1 has always been generally distributed; it was spread over all patients in 1970, and two Vietnam returnees harbored it at time of admission.

Phage type 7,31 (B-13). Seven patients yielded 48 strains of this type. B-13 was extremely prevalent in 1969 (second most common); however, in 1970 it fell to seventh place. It was found in January and February; then it disappeared until December. One Vietnam patient and one CONUS patient harbored it on admission.

Type 7,68,119X (B-9a) also occurred on seven patients, but there were only 12 isolates. It was a new type not previously seen. It occurred only in Vietnam patients, of whom four carried it on admission. These strains were encountered only during March through June 1970.

Type 1214,68 (H-14). This type also occurred 12 times on seven patients. It was ninth most prevalent in 1969, and in 1970 was in the same range. It was most common in Vietnam patients, although it only once was found on admission.

Type 16,21,44,1214,68,F8 is designated as C-10. Six patients were positive, with 10 strains recovered. In 1969 it was seen on only one patient. It was found, in 1970, on two Vietnam patients at admission, but reached CONUS patients on the ward.

Type 1214 (H-1). Six patients yielded nine strains. In 1969, four patients had harbored it; in 1970, one Vietnam admission was positive.

Type 21,68 (D-14). This pattern reacts with two old, well-defined phages; it would seem logical that it would become common. However, this was not the case. It occurred on five patients each in 1969 and in 1970, when it was found only on CONUS patients, of whom one was positive on admission. It would appear to be persistent since 23 strains were recovered.

Type 7 (B-22) was the last one to involve five patients, with one strain each. It was twelfth most prevalent in 1969. One Vietnam patient harbored it on admission in 1970, but it also occurred on CONUS patients.

The most common type within these major types was H-3. The continued influx of strains from Vietnam contributed to this. However, this prevalence diminished markedly after June 1970. If re-seeding does not occur, this type may well disappear. As has been pointed out previously, the population of P. aeruginosa is a continously changing one. The cause of these changes is not yet clear.

Pseudomonas Septicemia: Phage Types

The close association of blood cultures positive for Pseudomonas and the demise of the patient requires a constant monitoring of these strains for a common type which may be associated with bacteremia.

During 1970, 26 strains of Pseudomonas were collected from the blood of 14 patients. The phage types of these particular strains are given in Table 3. Listed are the phage type and phage type code for each individual patient as each strain was recovered. On the right are the number of strains from each patient. The patient number underlined indicates that the patient expired. Only one patient, Patient Nr. 265, in October, survived.

Phage types 1214,68,F8 and 1214,68,119X were each found in blood cultures of four patients. Type 21,119X was observed in the blood of two patients. Four patients had other types, none of which were the same; one patient, Nr. 288, had two types recovered in separate blood cultures.

From this array of phage types, totalling 26 strains of 8 types, it was evident that blood stream invasion was not restricted to any particular type. The two phage types found most frequently were also among the most prevalent types from all sources. Type 21,119X, however, had a higher incidence in blood cultures than it did from other sources, but the numbers were too small to be meaningful. No nontypable strains were encountered in strains recovered from the blood.

Phage Types of Pseudomonas from Post Mortem Lung Tissues

Due to the increasing importance of pneumonia as a major cause of death in the burn patient, a survey of the phage types of Pseudomonas in postmortem lung tissues was made to determine whether any particular type can be associated with this condition.

Listed in Table 4 are the phage types of Pseudomonas from postmortem lung tissues. This collection consisted of 32 strains from 19 patients.

The most prevalent type was 1214,68,119X (H-3) in tissues from seven patients. 1214,68,F8 (H-15) was found in tissues of five patients. Phage type codes M-4 (119X,F7) and H-1 (1214) were observed in three and two patients, respectively. The remaining five types and a nontypable were seen each in a single patient.

It is apparent that no common type can be found among these strains. The 32 strains were of nine phage types with one nontypable. The incidence of types in this collection closely followed the incidence of types from all sources as shown in Table 2.

Monthly Distribution of Predominant Pseudomonas Phage Types

Table 3. Phage Types of Pseudomonas from Blood Cultures, 1970

Patient No.	Month	Phage Type Code	Phage Type	No. of Strains
20	Jan	B13	7,31	1
	Feb Mar			
107	Apr	M 2	119X	1
81		H 3	1214,68,119X	1
96 VN	May	H15	1214,68,F8	1
153 VN	Jun	D29	21,119X	2
122		H 3	1214,68,119X	1
160	Jul	H 3	1214,68,119X	1
161		D29	21,119X	2
217 VN	Aug	H 3	1214,68,119X	2
214	Sep	H15	1214,68,F8	2
243		H15	1214,68,F8	2
264	Oct	H15	1214,68,F8	7
265		F33	31,F10	1
288 VN	Nov	A45 A185	2,7 2,7,119X,F7	1 1
	Dec			

Table 4. Phage Types of *Pseudomonas* from Post-Mortem Lung Tissues, 1970

Patient No.	Month	Phage Type Code	Phage Type	No. of Strains
20	Feb	B13	7,31	1
297	Mar	A27	2,7,31,73,119X,M4	1
59		H 3	1214,68,119X	2
		H15	1214,68,F8	1
80		D30	21,68,M6	1
96 VN	May	H 3	1214,68,119X	1
153 VN	Jun	H 3	1214,68,119X	1
122		H 3	1214,68,119X	1
138		H15	1214,68,F8	1
137			NT	1
161	Jul	H 3	1214,68,119X	2
211	Aug	H 3	1214,68,119X	1
		H13	1214,109,F8	2
		M 4	119X,F7	1
217 VN	Sep	H 1	1214	1
		H 3	1214,68,119X	2
214		H 1	1214	1
243	Oct	H15	1214,68,F8	1
264		H15	1214,68,F8	1
277		H15	1214,68,F8	3
295	Nov	F35	31,F8	1
307	Dec	M 4	119X,F7	2
282		M 4	119X,F7	3

The sequence of phage types as they occurred over the year 1970 demonstrated the scope of the problem as far as applying any chemotherapeutic method to rid the population of certain strains. The multiplicity of types and the predominance and disappearance of types over a period of time complicate the choice of method of treatment.

Table 5 illustrates the sequence and concentration of the predominant types found in the burn ward flora during each month of 1970. Opposite each phage type is the monthly patient occurrence and total strains of that type. The blocks indicate the most prevalent types during each monthly period: the solid line - most prevalent; double line - second most prevalent, and single line - third most prevalent.

Nontypable strains were found in every month of the year. They were the most numerous or second most prevalent in 10 months of the year. Strains of type code H-3 were the most prevalent from January through March and occupied second or third place from April through August. Only two patients had strains of this type during the last four months. Type code M-2, first appearing in March as the third most prevalent type, was the predominant type in April and in May, along with nontypables. Again in October it was the most prevalent type along with type code H-15. Type code H-15 occurred regularly but with low incidence from January through September, but in October was the most prevalent type along with type code M-2. No strains of this type were observed in November and December. Type code M-4 strains were first observed in August in which they were the second most prevalent type. In December strains of this type were the most prevalent along with nontypable strains. Type code F-12 strains first appeared in March when they were the second most prevalent type along with nontypables. They occurred in low incidence in a following five-month period. Strains of type code I-1 were scattered over a five-month period but concentrated mainly in July as the second most prevalent type. Type code B-13 strains appeared in only three months of the year, being the third most prevalent in January and second along with type code C-10 in December. Type code B-9a strains, observed in only a consecutive four-month period, were second in prevalence in April and May. Type code H-14 was found over a seven-month period, but always with a low incidence. The remainder of the types listed occurred in low frequency and during the time intervals indicated. Type code D-41 strains were found during eight months of the year but never in a high incidence.

Discussion

Table 5. Monthly Distribution of Predominant *Pseudomonas* Phage Types

Phage Type Code	Phage Type	Month											
		Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
	NT	7-23	4-10	5-8	3-8	4-8	7-13	11-17	10-29	8-13	2-2	1-1	5-13
H 3	1214,68,119X	8-20	10-19	12-26	3-6	2-7	6-18	2-8	2-8	1-4			1-1
H 2	119X			4-5	8-11	4-5	1-3	1-1	1-1		4-5		1-2
H15	1214,68,F8	1-1	3-17	3-4	1-2	1-1	3-4	1-9	1-2	2-15	4-22		
H 4	119X,F7								5-14	2-7		1-7	5-25
F12	31			5-10	1-1	1-1		2-2	2-4	1-1			
I 1	68	1-1					1-1	5-5	1-1				1-1
B13	7,31	6-28	2-16										2-4
B9a	7,68,119X			1-1	4-8	2-2	1-1						
H14	1214,68	1-1	2-4	2-3				1-1	1-1	1-1	1-1		
C10	16,21,44,1214,68,F8		1-1				2-4		1-1				2-4
H 1	1214		1-2	2-2				1-1	1-1	2-3			
D41	21,68	1-2	1-1	2-4	1-1	1-6	1-6	1-2	1-1				
D29	21,119X				1-2		1-2	1-3	2-4				
B22	7		1-1	1-1	2-2			1-1					

Most prevalent phage type, Patients-Strains

2d most prevalent phage type, Patients-Strains

3d most prevalent phage type, Patients-Strains

The occurrence of P. aeruginosa on burn wounds and in systemic involvement was a major feature of burn pathogenesis in 1970. Vietnam patients, arriving later and with greater opportunity to acquire P. aeruginosa enroute, had a higher rate of positive culture on admission, and during their hospital stay. However, predominant types, many of which first entered the ward on burn patients from Vietnam, permeated the population readily; only with three types was there a marked discrepancy between incidence on Vietnam patients and CONUS patients. B-9a and H-14 were solely, or almost entirely, found on Vietnam patients, D-41 only on CONUS patients. But when numbers were larger, interseeding was readily shown.

The input of previously unknown types has continued but at a slower pace. The implications for immunotherapy in this diversity of identities is obvious; while phage type does not correspond to envelope or cell wall antigen identity, there is great likelihood that infectivity and immunity are extremely heterogeneous with this population.

It must be borne in mind that there is a population of pseudomonads that react with the phage typing set but which occur in small numbers, involving less than five patients. These are not listed in the tabulation, but among them are types that may, in the future, assume predominance. The control of burn wound sepsis demands knowledge of the identity of individual infecting strains; phagetyping is still the most sensitive tool for making such distinctions.

References

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

2. Lindberg RB, Latta RL, Brame RE, Moncrief JA: Bacteriophage typing of Pseudomonas aeruginosa. Epidemiologic observations based on type distribution. Bact Proc. 1963, pp 72.

Presentations

Lindberg RB. The differentiation of Pseudomonas aeruginosa by bacteriophage typing. Presented at 10th International Congress of Microbiology, Mexico City, D.F., 12 Aug 1970.

Lindberg RB. Pseudomonas aeruginosa as a factor in nosocomial infections. Presented at International Conf. on Nosocomial Infections. NCDC, Atlanta, Ga., 3 Aug 1970.

Publications

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6970	71 07 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DOD'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
70 07 01	D. CHANGE	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO. CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A061102B71R	01	267
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Provide with Security Classification Code) (44)

(U) Sensitivity of Pseudomonas Aeruginosa Recovered from Burned Soldiers to Sulfamylon

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	71	0.35	9.3
FISCAL YEAR — CURRENT	72	0.55	16.1

19. RESPONSIBLE DOD ORGANIZATION

NAME: US Army Institute of Surgical Research

ADDRESS: Ft Sam Houston, Texas 78234

RESPONSIBLE INDIVIDUAL

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

TELEPHONE: 512-221-2720

20. PERFORMING ORGANIZATION

NAME: US Army Institute of Surgical Research
Microbiology Branch

ADDRESS: Ft Sam Houston, Texas 78234

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

NAME: Robert B. Lindberg, Ph. D.

TELEPHONE: 512-221-2018

SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS

NAME: Virginia C. English, M.A.

NAME: DA

21. GENERAL USE

FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Provide EACH with Security Classification Code)

(U) Pseudomonas; (U) Burns; (U) Sulfamylon; (U) Topical Therapy

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

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

24. (U) Sensitivity to sulfamylon determined by a drug in agar technic with controlled inoculum.

25. (U) 70 07 - 71 06 - 296 strains tested showed that the 1970 population of P. aeruginosa, the major wound invader, had increased with input of a new population of organisms. The range of sensitivity, had narrowed; continued monitoring is essential to insure continued efficacy of the drug in burn therapy.

*Available to contractors upon originator's approval

DD FORM 1 MAR 66 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65 AND 1498-1 1 MAR 66 (FOR ARMY USE) ARE OBSOLETE

ANNUAL PROGRESS REPORT

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

REPORT TITLE: SENSITIVITY OF PSEUDOMONAS AERUGINOSA TO SULFAMYLON

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

1 July 1970 - 30 June 1971

Investigators:

Robert B. Lindberg, Ph.D.
Virginia C. English, M.A.
Ruth L. Latta, B.S.
Basil A. Pruitt, Jr., M.D., LTC, MC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

REPORT TITLE: SENSITIVITY OF PSEUDOMONAS AERUGINOSA TO SULFAMYLON

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

Period covered in this report: 1 July 1970 - 30 June 1971

Investigators: Robert B. Lindberg, Ph.D.
Virginia C. English, M.A.
Ruth L. Latta, B.S.
Basil A. Pruitt, Jr., M.D., LTC, MC

Reports Control Symbol MEDDH-288(R1)

Monitoring of 296 isolates of Pseudomonas aeruginosa from burn patients showed that this species, a major hazard in burn treatment, was more sensitive to sulfamylon than it had been in 1968 and 1969. The constant introduction of new strains probably accounts for this freedom from the appearance of sulfamylon-resistant strains. The maximum inhibiting level was 0.312%, in contrast to the 0.625% to 1.25% level previously seen. Sensitivity was compared to phage type and showed no specific resistant types, although individual outbreaks of more tolerant strains did occur. Tolerant strains were not found to be directly related to treatment failures. Comparison with sulfadiazine showed that inhibition occurred with 0.0097% sulfadiazine in 46% of isolates; but 54% of 95 isolates were sulfadiazine resistant. Reliance on sulfadiazine as a therapeutic agent requires assurance that the strains involved are sensitive.

Burns
Sulfamylon
Topical therapy
Pseudomonas

SENSITIVITY OF PSEUDOMONAS AERUGINOSA TO SULFAMYLON

Sulfamylon burn cream was first used in full-scale treatment of a burn ward population in 1964. It has now been the mainstay of chemoprophylactic treatment in the Institute of Surgical Research for seven years. As with other forms of chemotherapy, it was assumed that strains resistant to this agent would emerge under conditions of continued use, and in consequence, a close and continued monitoring of the sensitivity of burn wound flora to this drug has been made. From the outset, it was intended that sulfamylon would control Pseudomonas burn wound invasion; thus, this organism has remained the primary target for assessments of the status of sulfamylon as an antibacterial agent. The dilution of sulfamylon active against Pseudomonas aeruginosa is not high and, indeed, the 10% concentration which is used in treatment implies a relatively weak agent. It is of great importance that the incursion of relatively tolerant strains into the burn ward be detected, since a strain requiring 1.25% for inhibition could be regarded as approaching a resistant level.

Methods

The testing procedure has been previously described (1). Dilutions of sulfamylon from 0.019% to 1.25% in nutrient agar were prepared and poured in grid-marked plates. When hardened, each 1 cm square was seeded with a drop of 4-hour broth culture diluted to contain 200 ± 50 colonies per 0.025 ml drop. This required a dilution of 10^{-3} in most instances. Plates were inverted and read for visible growth at 20 hours. The endpoint of inhibition was read at the level which showed a barely visible granularity representing minimal growth, instead of total inhibition. The latter procedure proved too often to create a broad end-zone instead of an endpoint.

Results

Two hundred and ninety-six strains of clinical material from a large succession of patients were tested. The inhibitory levels are presented in comparison with series tested from 1967 to 1970. Since the significance of any given level of sensitivity has greater meaning when it is viewed in light of preceding increments of microorganisms, the 1970 experience is set down in comparison with results obtained from 1967 through 1969 in Table 1. The upper limit of tolerance for sulfamylon, which was 1.25% in 1967, has fallen steadily since then. In 1968 and 1969 a small

Table 1. Inhibiting Concentrations of Sulfamylon for Pseudomonas aeruginosa, 1967-1970

Year	Concentration of Sulfamylon in % and Number Inhibited							
	2.5	1.25	0.625	0.312	0.156	0.078	0.039	0.019
1967	0	15	43	28	96	70	145	74
% of Total (471)	0	3.1	9.1	5.9	20.3	14.8	30.7	15.2
1968	0	0	12	103	43	94	37	5
% of Total (294)	0	0	4.0	35.0	14.6	31.7	12.4	1.7
1969	0	0	13	179	89	74	28	2
% of Total (385)	0	0	3.4	46.5	23.1	19.2	7.3	0.5
1970	0	0	0	65	83	83	59	6
% of Total (296)	0	0	0	21.9	28.0	28.0	19.9	2.03

percentage of strains required 0.625% of sulfamylon to be inhibited; in 1970, no strains tolerated any amount above 0.312%.In the two preceding years,between 40 and 50% of isolates required 0.312% or higher for inhibition;in 1970, only 21.9% required this amount for inhibition. Median inhibiting concentration,at which point one-half of all strains required more,and one-half required less sulfamylon for inhibition,was 0.063% in 1967;0.124% in 1968; 0.234% in 1969, and the median value dropped to 0.117% in 1970.There were fewer phage patterns represented among the strains typed in 1970 than had been the case in earlier years,but there was still a broad spectrum of phage types included in every level of sensitivity. For 0.312%,there were 20 phage types included;for 0.156% MIC,22 phage types.For 0.078%, 27 types; for 0.039%, 28 types. With only six strains at the 0.019% level,there were only two phage types represented.

Cumulative sensitivity to sulfamylon shows the effective level of the agent in relation to all strains tested.This relationship is presented in Table 2.The population of P.aeruginosa collected from clinical specimens was more sensitive than that for any year since 1967.The absence of any strains that tolerated more than 0.312% of sulfamylon had not previously been encountered.The 0.156% level also inhibited a higher proportion of the total strains than had been seen since 1967.The results can only mean that with the constant input of new strains which occurs with receipt of new patients,the trend has been toward an increasing number of sensitive strains,following a two-year period in which a pattern of increasing resistance had been observed.It may be concluded that the efficacy of sulfamylon in controlling Pseudomonas burn wound sepsis has not diminished as far as in vitro susceptibility to the drug is concerned.

The sensitivity of the entire population of P.aeruginosa was greater than in preceding years,but there remains the question of the existence of groups of strains that may constitute exceptions. Individual phage types,predominant for a time in the patient population, offered an opportunity to assess this possibility. Table 3 summarizes the sensitivity to sulfamylon of strains of two well-defined phage types,conspicuous in our ward populations during 1970.Type H-3,with 53 isolates from 33 patients,had sensitivities distributed essentially between 0.156% and 0.078%.This is close to the median level of all strains tested.There was no reason to consider that this type was atypical in its sensitivity.

Type H-15,another well-defined type,was recovered from 15 patients,with 23 strains tested. In this instance,the sensitivity

Table 2. Cumulative Sensitivity to Sulfamylon of Pseudomonas aeruginosa, 1970

Year	No.of Strains	Concentration and % of Total Strains Inhibited						
		1.25	0.625	0.312	0.156	0.078	0.039	0.019
1967	471	100	96.5	87.4	81.5	61.2	46.4	15.7
1968	294	100	100	95.4	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

Table 3.
Sulfamylon Sensitivity Reactions of Two
Predominant Phage Types

	PATIENT	ISOLATES WITH INHIBITING CONCENTRATIONS* AT 0.625	0.312	0.156	0.078	0.039	0.019
Type H-3	34				1		
	125				1		
	217				4		
	115				3		
	2					1	
	32				1		
	37				1		
	7		1				
	96		1				
	56			3			
	5				2		
	11			1	2		
	13			1			
33 Patients	76		1				
	59			3			
	33			5			
	31				1		
	55			2			
	12			1			
	153			1			
	157			1			
	211					1	
53 Isolates	63			1			
	64				1		
	77			1			
	57				3		
	54				1		
	10			1			
	144			1			
	160			2			
	46			1			
Total - each inhibiting strain	30				1		
	67			1			
			3	26	22		2
Type H-15	115				1		
	96		1				
	11			1			
	59			1			
15 Patients	243		3				
	33		1				
	277		3				
	150			1			
	138		1			1	
23 Strains	54				1		
	43					1	
	272					1	
	214			2			
	264		2				
Total-each inhibiting strain	76			1	1		
			11	6	3		3

* All concentrations in Gms.%

level is definitely skewed from the typical distribution which was seen with type H-3. Eleven strains from six patients were all inhibited at the upper limit of the sensitivity level detected in the total population. The conclusion appears justified that this strain did appear at intervals, with a sensitivity level not typical of the whole population. One of the major reasons for monitoring sensitivity of the population of P. aeruginosa in a burn ward is the possibility that a resistant clone will make its entrance and proceed to colonize the ward and create a local problem in drug resistance.

In view of the interest in alternative substances for use in topical therapy, sulfadiazine has been tested as a topical agent. The in vitro sensitivity of P. aeruginosa to sulfadiazine was compared with sensitivity to sulfamylon for 95 strains. This selection from 17 phage types and a group of nontypable strains is shown in Table 4. The types most extensively represented included those currently conspicuous in the burn population: F12(5); H-3(17);H-15 (5);M2(5);M4(19); and nontypable strains included isolates from two patients who were treated with topical 2% sulfadiazine cream. Comparative sensitivities are summarized in Table 5. The limited solubility of sulfadiazine made 0.312% the highest test concentration possible. All strains which were sensitive were inhibited at 0.0195% or below; thus, the strains tolerating 0.312% were resistant strains. Of strains sensitive, 39 or 31%, were inhibited by 0.0048% or less. However, the relatively large number of sulfadiazine-resistant strains included 30 strains from four patients treated with topical 2% sulfadiazine. It appeared that emergence of resistant forms in patients treated with sulfadiazine is a real possibility. The recovery of 25 strains which were sensitive to 0.312% of sulfamylon and resistant to sulfadiazine suggested that there may be a cross-relationship in sensitivity to these two entities; however, sulfamylon sensitivity at 0.312% also was seen in strains sensitive to 0.0048% and to 0.0024% sulfadiazine.

Discussion

The sensitivity level of P. aeruginosa to sulfamylon showed a shift in the direction of greater sensitivity than was observed during 1968 and 1969. Despite broad use of this drug, resistant strains have not been encountered nor have they been recovered when sought in patients treated over long periods with recurrent positive Pseudomonas cultures. The maximum level of sulfamylon tolerated was 0.312% in contrast to previous years when tolerance of 0.625% was seen and even 1.25% failed to inhibit a small proportion of the strains. There were no specific phage types that

Table 4

A COMPARATIVE SENSITIVITY STUDY OF PSEUDOMONAS AGAINST SULFAMYLON AND SULFADIAZINE

PHAGE TYPE	NUMBER OF ISOLATES	END POINT* SULFAMYLON	SULFADIAZINE
	1	0.039	0.0195
A	1	0.039	0.0097
	1	0.312	0.0048
A180	1	0.039	0.0097
A184	1	0.078	0.0048
B9a	1	0.312	0.312 or >
	1	0.312	0.0024
B13	1	0.312	0.312 or >
B62	2	0.312	0.0048
C10	1	0.039	0.0024
	1	0.039	0.0048
D40	1	0.039	0.0048
D41	1	0.039	0.0048
D29	1	0.078	0.0024
F12	2	0.078	0.0048
	1	0.078	0.312 or >
	1	0.312	0.312 or >
	1	0.156	0.0024
H	2	0.039	0.0048
H3	1	0.078	0.0048
	3	0.312	0.312 or >
	1	0.312	0.0048
	10	0.156	0.0048
	1	0.078	0.0024
	1	0.156	0.312 or >
H15	1	0.156	0.0012
	1	0.156	0.0024
	3	0.312	0.0048
M2	2	0.156	0.312 or >
	1	0.156	0.0048
	1	0.078	0.312 or >
	1	0.312	0.312 or >
M4	11	0.312	0.312 or >
	2	0.156	0.312 or >
	6	0.078	0.312 or >
N1	1	0.312	0.312 or >
NT	1	0.078	0.0097
	2	0.078	0.0048
	6	0.312	0.312 or >
	2	0.156	0.312 or >
	2	0.039	0.0048
	2	0.312	0.0024
	11	0.078	0.312 or >

* % of drug in test media.

Table 5. Sensitivity to Sulfamylon and Sulfadiazine of 95 Strains of Pseudomonas aeruginosa from Clinical Isolates

No. of Strains	% Sensitive to Sulfamylon	% Sensitive to Sulfadiazine
25	0.312	> 0.312
8	0.156	> 0.312
19	0.078	> 0.312
1	0.039	0.0195
1	0.078	0.0097
2	0.039	0.0097
7	0.312	0.0048
10	0.156	0.0048
6	0.078	0.0048
7	0.039	0.0048
3	0.312	0.0024
2	0.156	0.0024
2	0.078	0.0024
1	0.039	0.0024
1	0.156	0.0012
95		

were resistant as types; however, type H-15, which was relatively common on the burn ward for a time, did indeed exhibit a disproportionate number of strains requiring 0.312% for inhibition. The presence of patients with a large number of isolates weighted this finding.

Sulfadiazine sensitivity,tested in a series of 95 strains, showed sulfadiazine resistance in 54% of these strains. The figure was weighted by a large number of isolates from patients being treated with 2% sulfadiazine burn cream, but the result emphasized the hazard in the ready emergence of resistant forms with this agent.

Reference

1. Lindberg RB, Calvert J, Brame RE, Dent RE: The sensitivity of burn wound flora to sulfamylon. USA Surgical Research Unit Ann. Prog. Rpt. FY 1965, BAMC, Ft Sam Houston, Texas. Sect. 15.

Presentation

Lindberg RB. Comparison of topical sulfamylon, silver-sulfadiazine, sulfadiazine and gentamycin in control of experimental Pseudomonas burn wound sepsis. Presented at annual meeting American Burn Assn., San Antonio, Tx. April 11, 1971.

Publication

Lindberg RB, Mason AD Jr, Brame RE, Pruitt BA Jr: Relative effectiveness of topical sulfonamides and antibiotics in control of experimental Pseudomonas burn wound sepsis. Bact Proc 1971: p. 69.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0B 6982	71 07 01	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
71 07 01	D.CHANGE	U	U	NA	NL	[X] YES [ ] NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A061102B71R	01	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 71	0.40	10.6
	CURRENT 72	0.4	11.7

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

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

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

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

ASSOCIATE INVESTIGATORS
NAME: R. E. Brame, M. S.
NAME: DA

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Burn wound; (U) Sulfamylon-Sulfadiazene; (U) Pseudomonas

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

23. (U) Assessment of topical antimicrobial agents on the prevention of burn wound sepsis using a laboratory animal model.

24. (U) A systematic comparison of sulfamylon, sulfadiazene, silver-sulfadiazene and gentamicin, each in topical aqueous gel vehicle, was carried out on burned rats challenged with 5 selected strains of p. aeruginosa of increasing virulence. The tests were run simultaneously on groups of 5 to 8 rats treated with the test agents.

25. (U) 70 07 - 71 07 - Cumulative survival rates over a total of 50 to 60 animals per challenge strain and per therapeutic agent showed 2% sulfadiazene to be slightly more effective than sulfamylon, while silver sulfadiazene was markedly less effective on 3 of 5 challenge strains and only moderately effective on one of the two least virulent strains. Only with the least virulent challenge strain was silver sulfadiazene effective in a range approximating that shown by sulfamylon and sulfadiazene-therapeutic trial of 2% sulfadiazene cream on burned patients in progress.

*Available to contractors upon originator's approval

ANNUAL PROGRESS REPORT

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

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

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

1 July 1970 - 30 June 1971

Investigators:

Robert B. Lindberg, Ph.D.
Russell E. Brame, M.S.
Arthur D. Mason, Jr., M.D.
Basil A. Pruitt, Jr., M.D., LTC, MC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

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

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

Period covered in this report: 1 July 1970 - 30 June 1971

Investigators: Robert B. Lindberg, Ph.D.
Russell E. Brame, M.S.
Arthur D. Mason, Jr., M.D.
Basil A. Pruitt, Jr., M.D.,LTC, MC

Reports Control Symbol MEDDH-288(R1)

Combat and military training situations engender large numbers of burn injuries. Modern burn therapy depends upon use of bacteriostatic surface treatment to control burn wound sepsis, caused by Pseudomonas aeruginosa. The continued occurrence of septic complications in burns prompted a comparison of the principal burn creams now in use, together with assessment of sulfadiazine in topical burn treatment. Sulfamylon, sulfadiazine, silver-sulfadiazine, and gentamycin, each in an aqueous cream, were used to treat burned rats seeded with five strains of P. aeruginosa of relatively high virulence. The least virulent strain permitted survival of animals treated with sulfamylon and with 2% sulfadiazine in the 90% range; silver-sulfadiazine effected an 83% survival, while gentamycin saved 68% of animals. With the more virulent strains, survival rates were highest with 2% sulfadiazine and with sulfamylon; silver-sulfadiazine was moderately effective with the less virulent strains but ineffective with the most virulent forms. Using a range of virulent challenge strains increased the plausibility of the animal assay as a means of selecting effective topical agents.

Burns wound
Sulfamylon-sulfadiazine
Pseudomonas

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

The control of burn wound sepsis by use of 10% topical sulfamylon is now an established clinical method, and the procedure is widely used (1). The syndrome of burn wound sepsis can be reproduced in burned animals by seeding a burn wound with Pseudomonas aeruginosa, but this is the only bacterial species with which burn wound sepsis can be consistently created, and indeed, unless a Pseudomonas species is found in the human burn, the clinical diagnosis of burn wound sepsis is open to question. The relative efficacy of different forms of topical therapy for burns is still a question of major interest, and since direct comparisons of different formulations have not been adequately carried out, three currently used agents plus an older sulfonamide in a new formulation were studied. When the original sulfamylon formulation was being developed, various criteria of effectiveness, such as bacterial content of wounds, histologic evidence of suppression of invasive sepsis, prolongation of survival, and destruction of P. aeruginosa were used. In the present study, however, the basis of comparison was confined to death or survival of the animal.

Materials and Methods

Burn Model. The infection model has been described previously (2). Briefly, male, Sprague-Dawley rats of the Holtzman strain, weighing from 195 to 210 grams, were used. They were anesthetized with nembutal, clipped, and scalded for 10 seconds over 20% of the body surface. The burned animals were seeded with one ml of an 18-hour broth culture of the test strain, diluted to contain 100 million organisms. Seeding was performed within 90 minutes after burning, to assure maximum virulence of the test organism, since delay in seeding may result in decreased lethality.

Test Strains. Five strains of P. aeruginosa were selected from a collection of over 100 strains which were tested for virulence in this animal model. These organisms came from burn surfaces, blood, urine, sputum, and tissues collected at biopsy and autopsy. It had earlier been found that there is no correlation between the source of a strain and its virulence for the burned rat (3). The strains used in this study included the following:

12-4-4 collected in 1959 from a blood culture in a burn

patient with fatal septicemia.

8-28-3 collected in 1963 from the burn wound in a patient with a fatal burn injury.

3-24-5 collected in 1964 from a focus of subeschar invasion in a patient with a fatal burn.

4-18-9 collected in 1970 from the sputum of a burn patient.

VA-134 collected from the urine of a patient with fatal burn wound sepsis.

Each strain was documentedly virulent for the burned rat. Since variation in virulence has in some instances occurred when strains were maintained in serial subculture, the stability of challenge was achieved by preparing large stocks of aliquots of seed bacterial suspensions from each fresh isolate. The agar slant cultures were suspended in sterile skim milk, sealed in glass and stored at -70° C. Each challenge culture was made from one of these aliquots and each inoculum was viewed as identical with its predecessors.

Therapeutic Agents Compared.

a. 10% sulfamylon hydrochloride, as originally compounded in a water dispersible gel (4). The therapeutic form of the drug, sulfamylon acetate, was compared with the hydrochloride in an earlier report (5).

b. 2% sulfadiazine, suspended in the same base as that used for sulfamylon HCl.

c. 1% silver-sulfadiazine, provided as an experimental drug by Marion Laboratories (6). This drug is in a pharmaceutical base comparable to that used for "a" and "b".

d. 0.1% gentamycin cream (7), supplied by Schering Corporation. The base for this preparation is water dispersible, and gentamycin can be shown to diffuse from it.

Therapeutic Procedure. Treatment was started 24 hours after the animal was seeded. It consisted of applying 3.5 gm of medication to the back of the burned rat, once daily, for a total of 10 treatments if the animal survived that long. The animals were observed for 21 days postburn at which point survival was registered

as complete. It was originally pointed out (1) that earlier and more frequent treatment will, in the case of sulfamylon, enhance survival. However, in this comparative series, once daily treatment gave a more meaningful spread in survival rates.

Results

All strains that have been tested against topical chemotherapeutic agents have shown some degree of variation in their response to therapy. Thus, fluctuations in death rate have occurred, and the assessment of a given agent's effect on the lethality of a specific strain required that such variation be recognized. Each comparison was therefore made with a single set of animals and a fresh suspension of each of the challenge strains.

The selection of the optimal strength for sulfadiazine was made after parallel tests with 1% and 2% suspensions. The table summarizes this comparison.

There was a marked increase in survival with 2% sulfadiazine over the 1% suspension. Sulfadiazine is relatively insoluble in water, and the 2% suspension appears to represent an upper limit of drug solubility. Thus, 3% and 4% suspensions did not further increase the survival rate beyond that achieved with 2%. Since the minimum effective level of sulfadiazine was sought, the test strength was set at 2%.

Not only can there be wide variation between individual experiments in numbers of survivors, but the logistics of testing limited each test to 5 to 6 animals per drug. Survival rates were found to most effectively portray the effect of the drug when they were presented as cumulative results of successive groups of animals challenged with an individual strain of *P. aeruginosa*.

Figure 1 shows the survival rates with strain 12-4-4. The untreated controls showed a consistent survival rate of 16%. Sulfamylon and 2% sulfadiazine both permitted survival of animals in the 90% range; silver sulfadiazine was effective in a comparable degree, but its consistent level of 83% survival was lower than the first two agents. The gentamycin ointment was effective at a significantly lower range of only 68%.

Figure 2 summarizes the survival rates in animals challenged with strain 8-28-3. It was far more lethal than strain 12-4-4,

EFFECT OF SULFADIAZENE CONCENTRATION ON SURVIVAL
OF PSEUDOMONAS-SEEDED BURN RATS

	CHALLENGE STRAIN AND % SURVIVING				
	12-4-4	8-28-3	3-24-5	4-18-9	VA-134
1% SULFADIAZENE	69.6	42.9	31.3	30.2	0
2% SULFADIAZENE	87.0	72.8	62.5	45.9	16.0

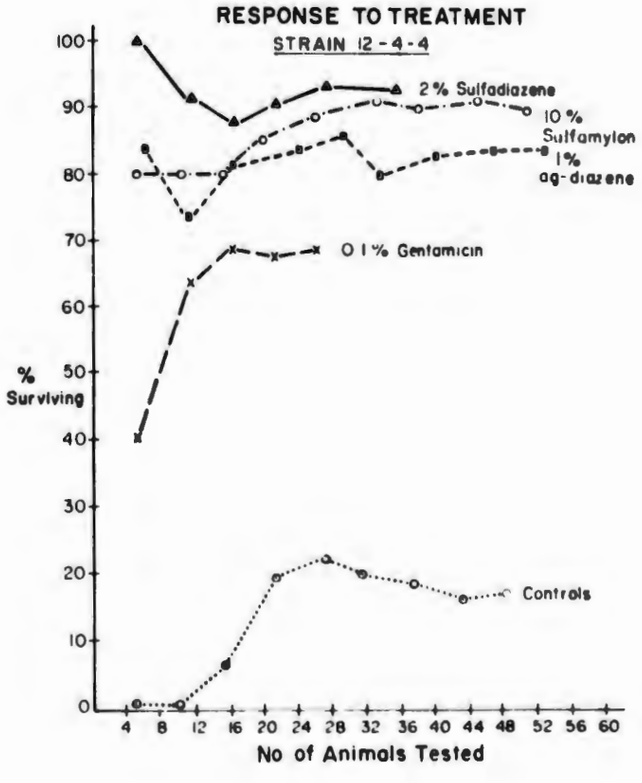

RESPONSE TO TREATMENT
STRAIN 12-4-4
2% Sulfadiazene
10% Sulfamylon
1% ag-diazene
0.1% Gentamicin
Controls
% Surviving
100
90
80
70
60
50
40
30
20
10
0
4 8 12 16 20 24 28 32 36 40 44 48 52 56 60
No of Animals Tested

Figure 1

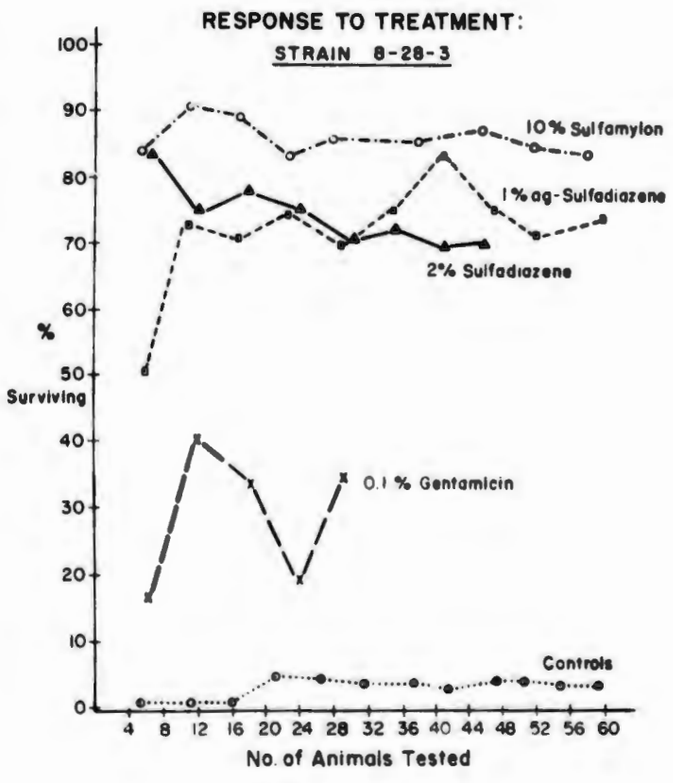

RESPONSE TO TREATMENT:
STRAIN 8-28-3
100
90
80
70
60
50
40
30
20
10
0
%
Surviving
10% Sulfamylon
1% ag-Sulfadiazene
2% Sulfadiazene
0.1 % Gentamicin
Controls
4 8 12 16 20 24 28 32 36 40 44 48 52 56 60
No. of Animals Tested

Figure 2

as is seen in the negligible survival rate among untreated controls. Sulfamylon gave a survival rate of 85% of the animals; sulfadiazine and silver-sulfadiazine were virtually identical in therapeutic effect, but at a lower range of 70% to 72%. With this more virulent challenge, gentamycin was effective only at a far lower level; survival occurred in the 20% to 35% range.

Figure 3 summarizes the survival rates with strain 3-24-5. This was a strain considerably more lethal and more rapidly invasive than strain 8-28-3. With this strain, survival rates for all drugs were significantly lower than with the two preceding strains. The most effective agent was sulfadiazine; with this, 65% of the animals lived. Sulfamylon was consistently effective at the 44% survival level, while silver-sulfadiazine was far less effective, and permitted survival of only 24% of the animals. Gentamycin exerted a slight effect; 7% of the treated animals survived.

Figure 4 shows survival rates with strain 4-18-9, which was an extremely virulent invasive strain isolated from sputum. Survival of untreated controls was negligible. With this strain 2% sulfadiazine was the most effective agent, but the best survival rate was only 40%. Sulfamylon was effective, too, but in only 33% of the animals. Parallel treatment with silver-sulfadiazine permitted survival of only 13% of the animals, while gentamycin was virtually ineffective.

The most virulent and intractable strain in the challenge battery, VA-134, is summarized in Figure 5. It was isolated from urine in a fatal case of burn wound sepsis in 1963. Only rarely did an untreated control survive this strain. The effective therapy with this strain was minimal, although even in this instance, the agents in descending order of effectiveness were sulfamylon, sulfadiazine, silver-sulfadiazine and gentamycin. The highest survival rate, with a cumulative level of 24%, occurred with sulfamylon, while the least effective agent, gentamycin, exerted a minimal but real survival effect on 9% of the animals.

The gradation of survival effect is in inverse proportion to the virulence of the organism. The relative effectiveness of the compounds on a series of challenge strains is evident, but the behavior of each compound is more clearly shown by comparison in terms of the drug itself. Figure 6 summarizes the cumulative survival rates of the challenge strains with 10% sulfamylon. The uniform and consistent fall in therapeutic effectiveness with increasing virulence is apparent. The initial fluctuations in

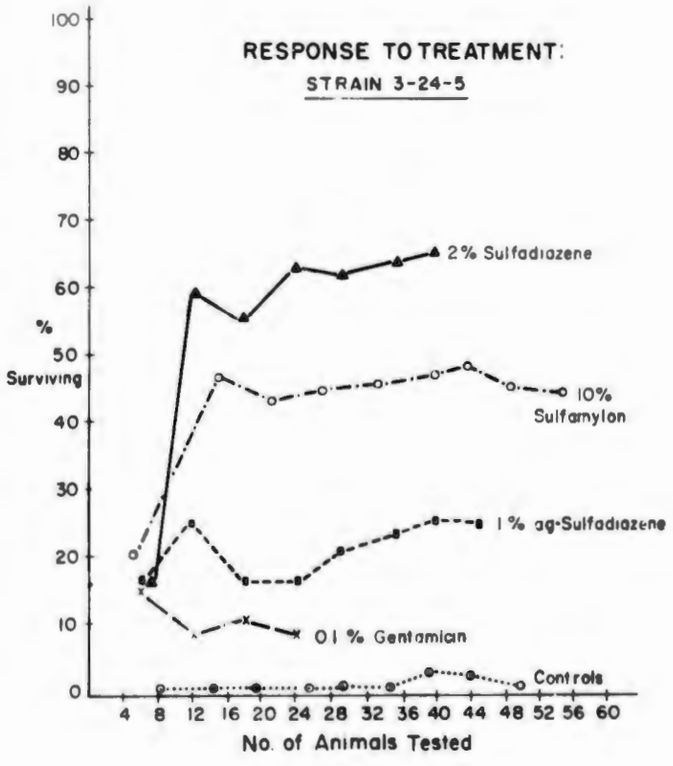

RESPONSE TO TREATMENT:
STRAIN 3-24-5
100
90
80
70
60
%
50
Surviving
40
30
20
10
0
2% Sulfadiazene
10%
Sulfamylon
1% ag-Sulfadiazene
0.1 % Gentamicin
Controls
4 8 12 16 20 24 28 32 36 40 44 48 52 56 60
No. of Animals Tested

Figure 3

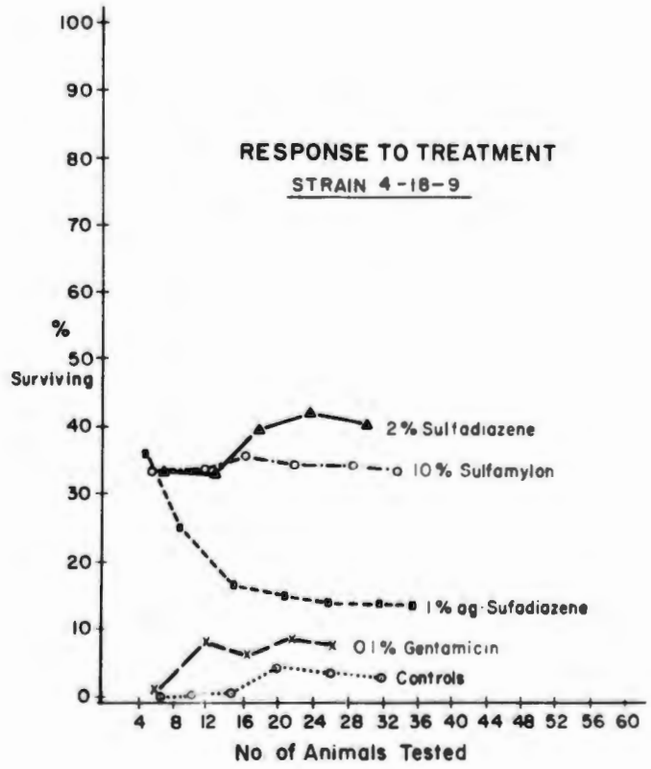

RESPONSE TO TREATMENT
STRAIN 4-18-9
% Surviving
100
90
80
70
60
50
40
30
20
10
0
2% Sulfadiazene
10% Sulfamylon
1% ag·Sufadiazene
0.1% Gentamicin
Controls
4 8 12 16 20 24 28 32 36 40 44 48 52 56 60
No of Animals Tested

Figure 4

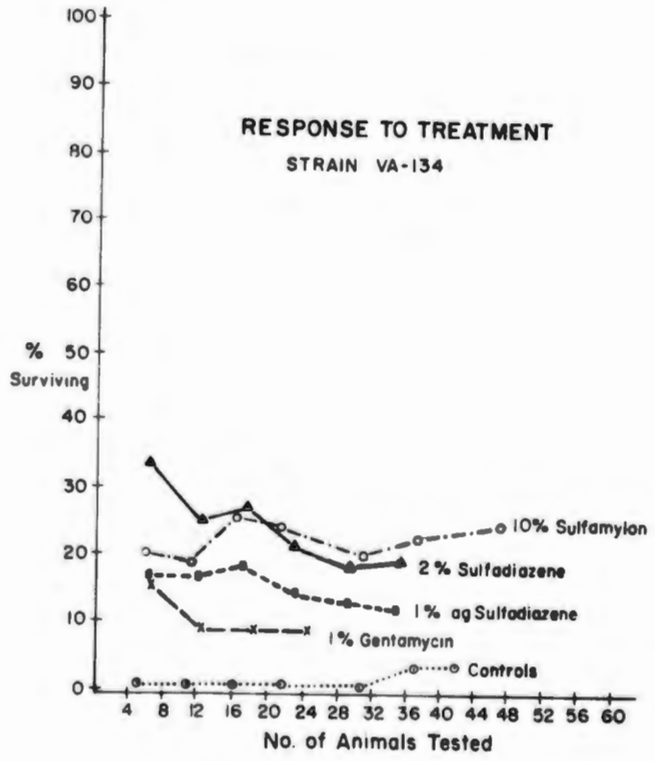

RESPONSE TO TREATMENT
STRAIN VA-134
% Surviving
100
90
80
70
60
50
40
30
20
10
0
4 8 12 16 20 24 28 32 36 40 44 48 52 56 60
No. of Animals Tested
10% Sulfamylon
2% Sulfadiazene
1% ag Sulfadiazene
1% Gentamycin
Controls

Figure 5

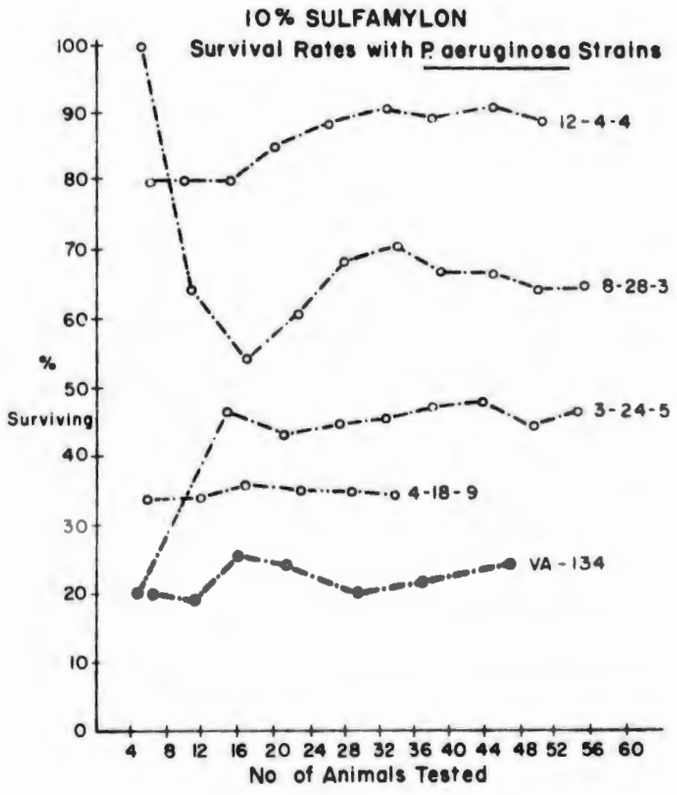

10% SULFAMYLON
Survival Rates with P. aeruginosa Strains
100
90
80
70
60
%
50
Surviving
40
30
20
10
0
12-4-4
8-28-3
3-24-5
4-18-9
VA-134
4 8 12 16 20 24 28 32 36 40 44 48 52 56 60
No of Animals Tested

Figure 6

successive tests with strains 8-28-3 and 3-24-5 as noted above are accounted for by plotting results.

Figure 7 illustrates the behavior of 2% sulfadiazine with the same challenge series. Again a consistent drop in survival with increasing virulence was evident. The behavior of sulfadiazine against strain 3-24-5 resulted in a cumulative survival rate approaching that observed with strain 8-28-3. In this respect, sulfadiazine was more effective than sulfamylon in control of infection in the seeded rat.

Figure 8 summarizes the performance of 1% silver sulfadiazine. Two strains, 12-4-4 and 8-28-3 were "sensitive" to this drug, but the remaining three challenge strains were associated with negligible survival rates. With these highly virulent challenge strains survival ranged from 25% to 12% of the test animals.

In Fig. 9 the performance of topical gentamycin is summarized. Only one strain, 12-4-4, was affected to a significant degree.The remaining five strains were susceptible to treatment only to a slight degree. Survival of animals seeded with those strains exceeded that of the control animals, but was by far the least of any of the agents tested. Strain 8-28-3 fluctuated more widely between successive tests than did other agents tested against gentamycin. There is no explanation available for this phenomenon.

Blood levels of sulfadiazine are an obvious factor to be considered in use of this drug. With the 2% sulfadiazine cream, blood levels in the treated animals reached a peak of 4 to 5 mg% on the fourth postburn day. After this time there was a steady fall in sulfadiazine level. By the tenth postburn day, the mean level was 2 to 3 mg%. Sulfadiazine also appeared in the blood of silver-sulfadiazine treated rats. Its source was obviously the sulfadiazine component of the complex. The highest level was reached on the fourth postburn day, with mean high levels of from 3 to 4 mg%. As with sulfadiazine, this level dropped after one week, with amounts of from 1 to 3 mg% detected.

Discussion

The system of testing described represents a comprehensive battery of challenge strains of high virulence, each possessing a varying degree of response to topical therapy. The relative effectiveness of the four agents used were consistent in successive tests, and the multiple trials cancelled out the effect of fluctuations which occur in this as in all biological test systems.

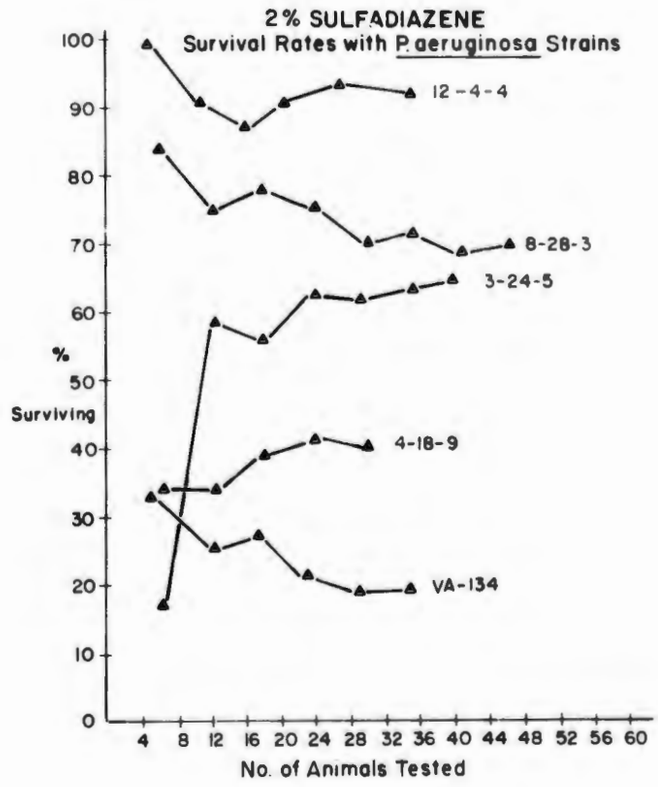

2% SULFADIAZENE
Survival Rates with P. aeruginosa Strains
12-4-4
8-28-3
3-24-5
4-18-9
VA-134
% Surviving
100
90
80
70
60
50
40
30
20
10
0
4 8 12 16 20 24 28 32 36 40 44 48 52 56 60
No. of Animals Tested

Figure 7

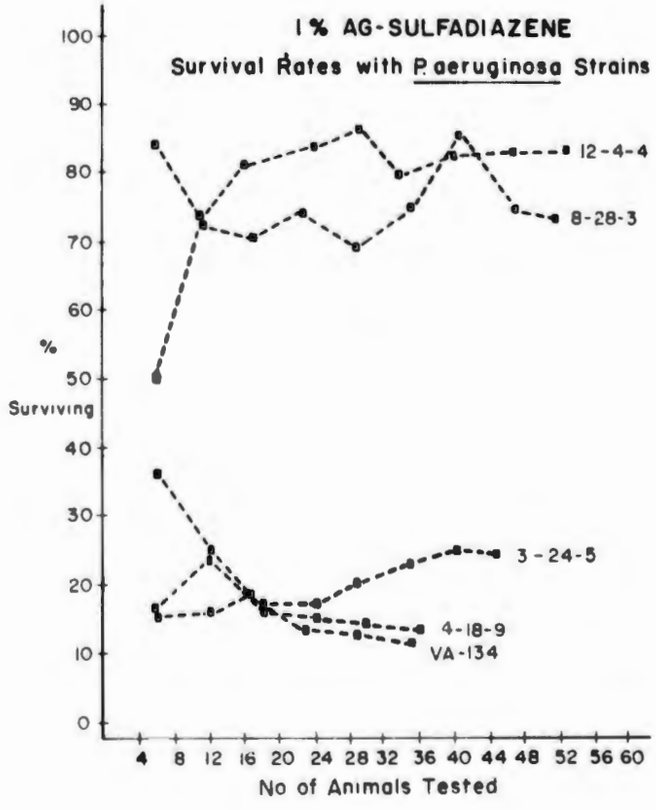

1% AG-SULFADIAZENE
Survival Rates with P. aeruginosa Strains
100
90
80
70
60
%
50
Surviving
40
30
20
10
0
4 8 12 16 20 24 28 32 36 40 44 48 52 56 60
No of Animals Tested
12-4-4
8-28-3
3-24-5
4-18-9
VA-134

Figure 8

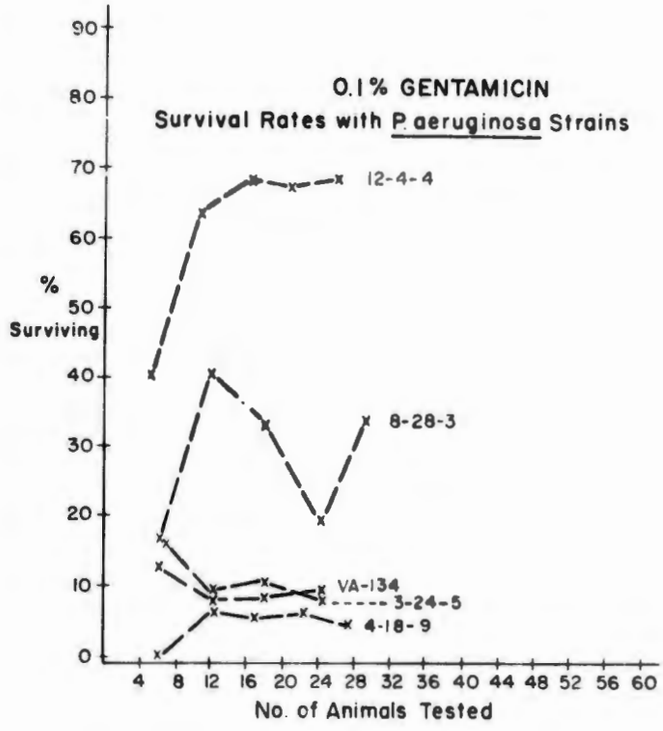

0.1% GENTAMICIN
Survival Rates with P. aeruginosa Strains
% Surviving
90
80
70
60
50
40
30
20
10
0
12-4-4
8-28-3
VA-134
3-24-5
4-18-9
4 8 12 16 20 24 28 32 36 40 44 48 52 56 60
No. of Animals Tested

Figure 9

The high survival rate with sulfadiazine in animals seeded with strain 3-24-5 was an exception to the essentially orderly sequence of therapeutic effect. Fluctuations in survival rate were more evident when successive trials of silver-sulfadiazine was used than was the case with sulfamylon or sulfadiazine. Gentamycin results were minimal and in this infection model it could not be termed to have promising potential.

Sulfadiazine was the most effective drug in tests against 3 out of the 5 test strains; sulfamylon was slightly more effective than sulfadiazine in tests against two strains. Silver-sulfadiazine was ineffective, to a marked degree, with 3 of the 5 strains used.

It should be noted that P. aeruginosa may exhibit resistance to sulfadiazine in vitro, and it is entirely possible that if this drug were used in burn treatment, such resistance might be an adverse factor. None of the challenge strains shown were resistant to any of the test drugs, but the possibility must be considered. Sulfadiazine is not an inhibitor of carbonic anhydrase, in contrast to sulfamylon, nor is a 2% suspension of sulfadiazine in an aqueous gel painful on second-degree burns. These factors could prompt its trial in topical therapy of burns. Should this be considered, it would be advisable to monitor the wound flora and to test P. aeruginosa strains for resistance. If such resistance appeared, it could be a factor in the selection of a topical agent. On the basis of its behavior in the experimental model shown, it merits consideration as an additional agent for clinical trial in control of Pseudomonas burn wound sepsis.

References

1. Lindberg RB, Moncrief JA, Switzer WE, Order SE, Mills W Jr.: The successful control of burn wound sepsis. J Trauma 5: 601-616, 1965.

2. Walker HL,Mason AD Jr, Raulston GL: Surface infection with Pseudomonas aeruginosa. Ann Surg 160: 297-305, 1964.

3. Lindberg RB, Moncrief JA, Brame RE, Mason AD Jr: Variation in virulence of Pseudomonas aeruginosa from severe burns. Fed Proc 24: 571, 1965.

4. Lindberg RB, Moncrief JA, Switzer WE, Mason AD Jr: Control of bacterial infection in severe burns with a topical sulfonamide burn cream. Antimicrobial Agents & Chemother. 1964. pp. 708-716.

5. Lindberg RB, Moncrief JA, Brame RE, Mason AD Jr: A comparison of sulfamylon hydrochloride and sulfamylon acetate in control of experimental burn wound infections. USA Surg Res Inst Ann Progress Rpt FY 1966, Sect 40.

6. Fox CL: Silver sulfadiazine: A new topical therapy for Pseudomonas in burns. Arch Surg 96: 184-188, 1968.

7. Stone HH: Review of Pseudomonas sepsis in thermal burns: Verdoglobin determination and genatmycin therapy. Ann Surg 163: 297-305, 1966.

Publication

Lindberg RB , Mason AD Jr, Pruitt BA Jr: Relative effectiveness of topical sulfonamides and antibiotics in control of experimental Pseudomonas burn wound sepsis. Bact Proc 1971, p. 85.

Presentation

Lindberg RB. A comparison of topical sulfamylon, silver sulfadiazine, sulfadiazine and gentamycin in control of experimental burn wound sepsis. Amer Burn Assn 3rd Annual Meeting, San Antonio,Tx, 17 April 1971.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0B 6950	71 07 01	DD DR&E(AR)636

3. DATE PREV SUM	4. KIND OF SUMMARY	5. SUMMARY SCT	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
71 07 01	D.CHANGE	U	U	DA	NL	[X] YES [ ] NO	A. WORK UNIT

10. NO. CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A061102B71R	01	191
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code) (U) Pathogensis of Burn Wound Infection: Bacterial Flora of Burn Wounds of Military Personnel Receiving Sulfamylon 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 71	.0.38	10.1
FISCAL YEAR — CURRENT 72	0.58	16.9

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

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

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

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

ASSOCIATE INVESTIGATORS
NAME: A. A. Contreras, M. S.
NAME: R. L. Latta, B. S. DA

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

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

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

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

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

25. (U) 70 07 - 71 06 - Staph aureus and Providencia stuartii have in the past 2 years appeared as major burn pathogens - the former after a long quiescent period, the latter as a new pathogen. New monotype staph epidemics have replaced the former heterogeneous phagetype population, and methicillin resistance is at a new high. P. stuartii, presumably an inocuous enteric form, now colonizes wounds intensively, is the major cause of septicemia, and is broadly resistant to antibiotics.

* Available to contractors upon originator's approval

ANNUAL PROGRESS REPORT

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

REPORT TITLE: PATHOGENESIS OF BURN WOUND INFECTION: BACTERIAL FLORA OF BURN WOUNDS RECEIVING SULFAMYLON TREATMENT. ROLE OF STAPHYLOCOCCI AND PROVIDENCIA

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

1 July 1970 - 30 June 1971

Investigators:

Robert B. Lindberg, Ph.D.
Ruth L. Latta, B.S.
Anthony A. Contreras, M.S.
Evan Thomas, LTC, MSC*
Basil A. Pruitt, Jr., M.D., LTC, MC
Arthur D. Mason, Jr., M.D.

* Present address: 5th Army Area Lab, Fort Sam Houston, Texas 78234

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

REPORT TITLE: PATHOGENESIS OF BURN WOUND INFECTION: BACTERIAL FLORA OF BURN WOUNDS RECEIVING SULFAMYLON TREATMENT. ROLE OF STAPHYLOCOCCI AND PROVIDENCIA

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

Period covered in this report: 1 July 1970 - 30 June 1971

Investigators: Robert B. Lindberg, Ph.D.
Ruth L. Latta, B.S.
Anthony A. Contreras, M.S.
Evan Thomas, LTC, MSC*
Basil A. Pruitt, Jr., M.D., LTC, MC
Arthur D. Mason, Jr., M.D.

Reports Control Symbol MEDDH-288(R1)

The microbial flora in sulfamylon-treated burns is viewed with emphasis on two organisms: Staphylococcus aureus and Providencia stuartii. The former, after almost a decade of behavior as a controllable pathogen, has shown a marked rise in incidence as an organism associated with septicemia and other infections. Phage typing showed this resurgence corresponded to replacement of a heterogeneous population with two successive epidemic types, one with 47,54,75,84,85 and later with type 84,85. Methicillin resistance in these strains was very high and no consistently effective therapeutic agent has been identified. Providencia stuartii has become the most important cause of gram-negative sepsis; although burn wound sepsis has not been shown, a high level of colonization in the burn appears, with frequent septicemia and with a high rate of Providencia pneumonia. The strains are broadly resistant to antibiotics. The problem is defined, but solutions to these refractory infections await further research.

Burns
Staph aureus
Providencia stuartii
Sepsis

* Present address: 5th Army Area Lab, Fort Sam Houston, Texas 78234

PATHOGENESIS OF BURN WOUND INFECTION: BACTERIAL FLORA OF BURN WOUNDS RECEIVING SULFAMYLON TREATMENT.ROLE OF STAPHYLOCOCCI AND PROVIDENCIA

Sepsis in burned patients has continued to constitute a major cause of death, although the control of burn wound sepsis remains an accomplished fact (1).At the same time,an apparent increase in mortality rate, and a decrease in the LA_{50} (lethal burn area at which 50% of patients succumb) have occurred. Thus, an ongoing scrutiny of the bacterial flora of the burn wound is called for, since regardless of its source, lethal infection remains a major concern in burn therapy. Burn mortality fell consistently between 1964 (when sulfamylon was first used clinically) and 1968; the rise in mortality since then has not been due to reappearance of uncontrolled burn wound sepsis, but much of the increase may be due to a greater incidence of pneumonia. Altered bacterial flora may not be due to specific resistance to antibacterial therapy; it may well be associated with altered pathogen population, reflecting those labile biological processes which control the microbial flora of species. Certainly an increase in positive blood cultures reflects an increase in sepsis, and when its cause is a previously obscure enteric organism of no pathogenic reputation, the actual causal mechanisms may not be recognizable. Even exhaustive scrutiny of microbial parameters in the infected patient does not of necessity clarify the pathogenesis of lethal sepsis, but this avenue must be constantly explored in the hope that an etiological relationship may be recognized.

This report is directed at three species which are part of the total flora but whose recent behavior prompts scrutiny. The remaining flora may well play a role not presently recognized, but viewing a whole population at once presents a picture of such complexity that it is unintelligible.

Bacterial Flora of Burn Patients

Clinical culture results are summarized in Table 1. The predominant organisms were Staphylococcus aureus, Pseudomonas aeruginosa, and Providencia stuartii. The Klebsiella-Enterobacter group comprise at least three species; individual species rates were obviously lower. An interesting change from previous years was the marked drop in incidence of Proteus mirabilis. It is now an inconsequential part of the flora.

Bacterial Flora of Postmortem Tissues

Table 1. Bacteriology of Antemortem Burn Patients

Organism	Source and Number						Total Isolates
	Wound Surface	Blood	Sputum	Urine	Cath. Tip	Biopsy	
Staphylococci							
Coag. Positive	120	47	119	42	39	48	415
Coag. Negative	47	14	110	69	32	27	299
Streptococci							
Alpha Hemo	6	1	110	0	1	2	120
Beta Hemo *	3	0	18	0	1	0	22
Non Hemo	6	2	23	2	1	3	37
Bacillus sp.	18	7	7	79	32	14	157
Pseudomonas sp.	81	16	240	45	29	39	450
Klebsiella-Enterobacter gp	70	6	185	91	27	28	407
Serratia sp.	3	1	35	4	5	3	51
E. coli	23	6	93	44	24	21	211
Proteus sp.							
mirabilis	41	2	66	38	8	23	178
rettgeri	3	0	6	1	0	1	11
morganii	0	0	0	0	0	0	0
vulgaris	2	0	0	0	1	1	4
Providencia sp. **	96	31	295	117	89	65	693
Mima-Herella gp.	0	1	2	0	1	0	4
Neisseria sp.	1	0	40	0	0	0	41
Candida sp.	8	7	17	117	39	5	193
Number of specimens	338	1674	536	819	401	165	
Patients cultured	135	221	109	216	147	72	
Total patient specimens received:		3933					
Total isolates:		3293					

* With two exceptions, these strains were not group A by Bacitracin sensitivity. Most of them, on the basis of parallel cultures, were group D, primarily Strep. fecalis.

** These are almost entirely P. stuartii on the basis of parallel culture data.

An obvious major source of information about burn pathogenesis is the bacteriology of wounds and of viscera at autopsy. Detailed autopsy bacteriology including extensive quantitative study of the wound is carried out (2). Results are summarized in Table 2. Again, predominant flora included Staph. aureus and Providencia stuartii. Unlike previous years, the total of strains of Pseudomonas isolated diminished in relation to the other two organisms; it is implied that invasive infection diminished, as far as Pseudomonas was concerned.

Staphylococcus aureus: Its Role in Burn Wound Pathogenesis

The role of Staph.aureus in the current status of burn patient infection has changed in the past three years. For a time, after the introduction of topical sulfamylon therapy, Staph. aureus appeared to have assumed a minor role in burns. It was ubiquitous, but the type distribution was heterogeneous and evidence of a major pathogenic role was not seen. In the past two years (1969-1970) a higher incidence of Staph. aureus and a major change in severity of involvement have occurred. They have coincided with a change in phage type of strains from a heterogeneous to a homogeneous population.

Phage Types of Staph. aureus from Burned Patients, ISR, 1970

Monitoring of Staph. aureus strains from burned patients has been carried on at this Institute for a number of years to detect the emergence, if it occurs, of any lethal, antibiotic-resistant, epidemic strain.

A resume of the more prevalent phage types, as well as monthly patient occurrence, during the years 1967 through 1969, is given in Table 3.

Proceeding from the left, the "Phage Type Code" refers to a specific phage type and has been employed as a convenient method of sorting strains and for easier reference, especially to lengthy phage types. Listed beside each Type Code is the specific phage type to which it refers. Each month of the year is listed; the figures underneath represent the number of patients having a particular phage type during that month. A heavy black block indicates the most prevalent phage type during the month; a double line block, the secondary type; and a single line block, the third most common phage type. Listed in the next column are the number of patients having a particular phage type and the number of strains. This column is filled in only when two or more

Table 2. Bacteriology of Postmortem Burn Patients, 1970

Organism	RUL (34)	RLL (34)	LUL (28)	LLL (32)	Liver (41)	Spleen (45)	Kidney (10)	Tissues (423)	Total Isolates
Staphylococci									
Coag. Positive	10	11	11	6	9	8	3	140	198
Coag. Negative	6	8	8	10	9	11	3	136	191
Bacillus sp.	2	0	1	2	2	3	0	81	91
Pseudomonas sp.	6	9	7	9	6	7	0	55	99
Klebsiella-Enterobacter gp	11	12	12	12	13	16	4	98	178
Serratia gp	1	0	1	0	3	1	1	17	24
E. coli	7	6	4	5	6	5	0	46	83
Providencia stuartii	21	22	18	22	18	20	5	233	359
Proteus									
mirabilis	3	2	2	2	6	4	2	34	55
rettgeri	0	0	0	0	0	0	0	2	2
morganii	0	0	0	0	0	0	0	0	0
vulgaris	0	0	0	0	0	0	0	0	0
Candida sp.	3	2	2	3	1	2	1	28	42
Streptococci									
Alpha hemo.	0	1	0	0	0	0	0	0	1
Beta hemo.	0	0	0	0	0	0	0	0	0
Non hemo.	0	0	0	0	0	0	0	1	1
Neisseria sp.	0	0	0	0	0	0	0	0	0
Hafnia gp.	1	0	1	1	0	0	0	1	4
Mima gp.	0	0	0	0	0	1	0	0	1
Corynebacterium sp.	0	1	0	0	0	0	0	2	3
Total isolates									1330

Patients cultured: 62
Patients positive: 61
Patients viscera cultured: 53
Patients tissues cultured: 62

Table 3. Predominant Staph. aureus Phage Types
1967 - 1968 - 1969

	Phage Type Code	Phage Type	Month J	F	M	A	M	J	J	A	S	O	N	D	Patients-Strains Each Type	Total
1967	NT	Non-typable	2	5	4			1		1		1	3	2		18-31
	J 2	42E,53	8	3	2							1				13-19
	B 2	52,52A,80,81										1		1	2-4	
	B 4	52,52A,80			2	1	1	1					1		5-7	10-15
	R 2	80,81		1								1		1	3-4	
	H 5	6,42E,47,54		4												4-10
	J 1	42E	1	1				1								3-3
	J 4	42E,54		2	1											3-3
	J 8	42E,53,54,77		1	1											2-4
	L 1	53				1	1									2-2
	L 3	53,54					1	1								2-2
	L 8	53,54,84			1							1				2-2
	T 1	84				1							1			2-2
	V 1	187				1								1		2-2

Total Patients-Strains: 68-124 -95

1968	T 1	84	2	5	6	4							2		17-22	22-32
	T 2	84,85		1	2	1	2						2		7-10	
	NT	Non-typable	3	2	2	6		3					4			20-29
	B 4	52,52A,80			3	3	2								6-11	
	R 1	80		1	1										2-2	12-19
	R 2	80,81						2					3		5-6	
	S 1	81				1						1	2			3-4
	E 1	3A	2													2-2
	L 1	53	1	1												2-2

Total Patients-Strains: 63-108 -88

1969	T 1	84		1	4	7	8	11	4	7		7	8		53-96	78-168
	T 2	84,85			2	3	7	8	8	8		5	7		46-72	
	NT	Non-typable	2	4	5	6	5	9	5	6		3	9			50-70
	L 1	53		1			4	2	1			1	1			10-10
	R 1	80								1					1-1	8-8
	R 2	80,81	1	4	1		1								7-7	
	P 2	77,84	1	1	1	2	1								6-11	7-13
	P 3	77,84,85					2								2-2	
	S 1	81	3			1	1					1				6-7
	P 1	77			1	3				2						5-9
	L 2	53,77		2	1	1	1									5-6

Total Patients-Strains: 141-340 -291

different phage types considered to be the same strain are encountered. For example, Phage Type Codes B-2, B-4, and R-2 in 1967 are considered one strain and in 1968, Phage Type Codes T-1 and T-2 have been deemed to be the same although varying in reaction with phage 85. At the far right is the total patients and strains of a particular phage type or of combined types.

1967 - During this year, 124 strains of Staph. aureus were collected from 68 patients during a ten-month period. 76.6% of the total strains are represented in this listing.

The most commonly occurring strains were nontypable: 18 patients with 31 strains, the latter figure representing 25% of total strains. Second in prevalence were strains of phage type 42E,53: 13 patients - 19 strains. This represented an additional 15.3% of total strains. Twelve per cent of strains were of the next three listed phage types, assumed to be the same strain. Phage Type Code H-5 accounted for 8% of total strains. 60% of the total strains were in one of these four categories. The remaining types occurred in a much lower incidence as shown.

1968 - During the year, 108 strains were recovered from 63 patients. No collection was made during a four-month period, July through October, and very little in November. 81.5% of the total strains are represented in this tabulation. The most prevalent strain encountered was of either phage type 84 or 84,85: 22 patients - 32 strains. 29.6% of all strains fell into this category. Next in frequency were the nontypable strains: 20 patients - 29 strains, the latter figure representing 26.8% of total strains. 17.6% of strains were of Type Codes B-4, R-1, or R-2 regarded to be the same. 74% of total strains fell into one of these three categories. Nontypable and 52,52A,80; 80; and 80,81 types remained prominent as in 1967; however, phage type 42E,53 disappeared and was replaced by strains of type 84 or 84,85 which became predominant.

1969 - 340 strains of Staph. aureus were derived from 141 burned patients during the year. No collection was made during the months of September and October. Phage types of 85.6% of total strains are summarized. Strains of phage types 84 and 84,85 remained the most common: 78 patients - 168 strains. In fact, 49.4% of all strains were of one of these phage types. Nontypable strains also remained prevalent accounting for 20.6% of total strains. 70% of the total strains fell into either of these categories. None of the remaining types accounted for more than 4% of total strains.

In summary, during this three-year period, nontypable strains occurred in a high incidence in each year. During 1968 a type 84 and 84,85, which had been seen in only two patients in 1967, became established and became the most prevalent type during 1968 and 1969. Strains of types 52,52A,80,81; 52,52A,80; 80; and 80,81 were usually the third most prevalent strains recovered. Type 84 and 84,85 were,of course, the new type originally described as UC-18, which became world-wide in distribution in this period.

1970 - During the year,624 strains of Staph. aureus were recovered from 169 patients. A summary of the predominant types in sequence as well as monthly patient occurrence is given in Table 4.

The designations have been mentioned previously; however, it should be stated that the number of patients listed in the last column is not obtained by merely adding the number of patients from the previous column as in the case of number of strains. A single patient may have several of the various types and while being counted each time as far as types are concerned, is counted only once in the last column.

The predominant strain encountered consisted of a series of 12 different phage types denoted by Phage Type Code K in the left hand column. These strains had in common a reaction with phage 47, but varied in reaction with phages 54,75,84 and 85. The overall pattern was 47,54,75,84,85. With closer examination of multiple strains from individual patients, and observation of variability, it could be ascertained that these strains were in all probability indeed the same type. This phenomenon is comparable to that of strains of types 52,52A,80,81; 52,52A,80; 80; and 80,81, which are essentially 80,81 in category. The question as to whether they are really the same will continue to arise.

The phage type most often seen in 1970 was 47,75,84,85 denoted by Phage Type Code K-5. It appeared in 56 patients - 131 strains. 44% of the Phage Type Code "K" strains were of this type. Strains of phage type 47,84,85 (Phage Type Code K-3) were next most frequent: 33 patients - 70 strains, and 23.6% of Phage Type Code "K" strains of this type. The next three Phage Type Codes, K-2, K-23, and K-9 accounted for another 20% of the strains; thus, 87.8% of the 296 strains were of the first five listed phage types. The remaining seven types, of course, each occurred much less frequently. A total of 94 patients had one or more of these types with a total of 296 strains. 47% of all strains were of one of these phage types.

Table 4. Predominant Staph. aureus Phage Types, 1970

Phage Type Code	Phage Type	Month												Patients-Strains	
		J	F	M	A	M	J	J	A	S	O	N	D	Each Type	Total
K 5	47, 75,84,85		7			2	15	13	12	11				56-131	94-296
K 3	47, 84,85		6	15	1	7	7	1						33-70	
K 2	47, 85		2	4	6	3	1							13-19	
K23	47, 75,84							4	4	4	1			13-19	
K 9	47,54,75,84,85		1			1	5	2		3				12-21	
K 7	47,54, 84,85		3	7										9-12	
K11	47, 75						2		1	4	2			8-11	
K 4	47, 75, 85		1				1	1	1					4-5	
K 1	47		1		3									4-5	
K 6	47,54, 85		1											1-1	
K 8	47,54,75,84		1											1-1	
K12	47,54,75		1											1-1	
T 1	84	4	9	6	1	3	7	4	2	4	13	15	12	70-155	88-220
T 2	84,85	9			5		8	3	2	5				31-65	
NT	Non-typable	9	2	3	3		3	3	2		6	6	4		40-63
U 1	85				3		6	1		1					11-14

Listed next in order of incidence are strains of Phage Type Codes T-1, phage type 84, and T-2, phage type 84,85. Again, the question arises as to whether these two types were indeed the same strain. Sufficient duplication of variability within isolates from a single patient was seen so that it can be justifiably assumed that these were the same strain. Phage type 84 occurred more often than 84,85. Eighty-eight patients had 220 strains of either or both of these types, and 35% of total strains were of these phage types.

Nontypable strains,which are part of every phage typed population, were next in occurrence: 40 patients - 63 strains. This group accounted for 10% of total strains.

Less often encountered were strains of Phage Type Code U-1 or phage type 85. Whether this strain has any relationship to types 84 and 84,85 is doubtful on the basis of its occurrence in patients. It was not observed in combination with either of these types often enough to warrant inclusion. Only 2% of the total strains were of this type.

95% of all strains fell into one of the four listed categories: Phage Type Code K types, T-1 or T-2, nontypable, or U-1. It is readily apparent that a survey of this years' strains represented an outbreak of epidemic proportions, as revealed by the restriction of 95% of strains to only four categories.

The monthly occurrence of phage types based on total number of patients with each type allows us to observe the change in predominance of certain types, appearance or complete disappearance of others.

In January, strains of types 84, and 84,85 were the most prevalent. In February, Type Code K strains first appeared.Strains of type 84 were predominant followed closely by Phage Type Codes K-5 and K-3. From March through September, the Type Code K strains were predominant with 84 and 84,85 types second in incidence. In October, only two patients had strains of the Type Code K, and no strains of Type Code K were found in November and December. Instead, type 84 became the most common type during the last three months, with nontypable strains second in occurrence. The sudden appearance, predominance, and then the sudden disappearance of strains such as those of Type Code K is difficult to explain. However, the persistence of types 84 and 84,85, which obviously were decidedly pathogenic in terms of their effect on patients, merited categorizing as of major importance.

Some insight into the matter of the origin of particular phage types, and possible causes of the upsurge and persistence or disappearance of types, can be gained by observing the phage types of strains recovered from admission contact cultures on patients at time of arrival at this Institute. From June through December, a collection was made of some of the admission contact strains. These findings are presented in Table 5.

Thirty-three strains from 27 patients having Staph. aureus on arrival comprised this collection. Twenty-four patients were Vietnam evacuees. In June, seven Vietnam patients had Staph.aureus on arrival. All had Type Code K-5, and in addition, one each of Type Codes T-1, T-2 and nontypable. In July, three Vietnam evacuees had two Type Code K types, and O-1 type and nontypable strains. On October 19 and November 16, all 10 Vietnam patients admitted had type 84 and no other type. Two patients from Alaska on November 29 had a type 84 and a nontypable strain on admission. In December, three of four Vietnam patients had phage type 84, the other a nontypable strain. One non-Vietnam patient had Type Code B-14. From this information, it is apparent that the Phage Type Code K types were introduced in June and July by Vietnam evacuees. Suddenly, in October, there was a change-over in these patients to phage type 84. This change was reflected in our own burn ward flora. It is probable that many times the changes seen in the Institute of Surgical Research wards are reflections of drastic changes in the flora brought in by Vietnam admissions, or potentially by CONUS admissions.

Strains of Staph. aureus from blood cultures are of special interest. In previous years, the problem of staphylococcal infection was regarded as under control, but in the past year and a half, these organisms have taken on a new and disturbing prominence. During the year 1970, 100 strains were collected from 46 patients. Twenty-one patients were from Vietnam; the remaining 25 were of other origins. Twenty patients expired - five of the Vietnam admissions and 15 from other sources. Tabulation of these phage types is presented in Table 6.

On the left is listed the patient number. The underline signifies that the patient expired. Next is listed the month, phage type code of the strain and number of strains of each patient, and finally, the total strains from each patient.

The predominance of Phage Type Codes "K" and "T" was readily apparent. Thirty-two patients had 57 strains of Type Code K and

Table 5. Phage Types of Staph. aureus from Admission Contact Cultures, 1970

Origin	Date	Number of Patients-Strains								Total
		Phage Type Code								
		B14	K 4	K 5	K23	O 1	T 1	T 2	NT	Patients-Strains
VN	6-8			1-1						1-1
	6-29			6-6			1-1	1-1	1-1	6-9
	7-27		1-1		1-1	1-1			1-1	3-4
	10-19						4-4			4-4
	11-16						6-6			6-6
Alaska	11-29						1-1		1-1	2-2
VN	12-14						3-5		1-1	4-6
non-Viet Nam	12-15	1-1								1-1

27-33

Table 6. Phage Types of Staph. aureus from Blood Cultures, 1970

Patient No.	Month	Phage Type Code-Strains			Total Strains
20	Jan		T 1-1		1
13			T 2-1		1
42	Feb	K 3-1			1
40		K 4-1 K 5-1 K 8-1	T 1-2		5
29		K 9-1			1
30 VN			T 1-1		1
32 VN		K 2-1			1
70	Mar	K 3-1			1
67 VN		K 7-1			1
81	Apr	K 1-1			1
106			T 1-1 T 2-1		2
129 VN	May	K 3-1			1
125	Jun	K 9-1			1
138		K 5-2 K 9-1			3
158		K 5-1	T 2-2		3
153 VN		K 5-2	T 2-1	NT-1	4
157		O 1-1	T 2-1		2
136			T 1-1		1
151 VN		K 3-2 K 5-2 K 9-2	U 1-1		7
197 VN	Jul	K 5-1			1
173 VN		K 5-1			1
192			T 1-1		1
115		K 5-2	T 1-1		3

Patient No.	Month	Phage Type Code-Strains			Total Strains
189 VN	Jul	K 5-2 K23-1			3
196 VN	Aug	K 5-1			1
211		K 5-1	T 1-2		3
221 VN		K23-1			1
217 VN		K 5-1 K23-2			3
231 VN		K 1-1			1
216		K 4-1			1
214		K 5-2			2
191 VN		K23-1			1
232 VN		K 5-1	T 2-1		2
218 VN	Sep		T 1-1		1
247 VN		K 5-5 K 9-1 K11-2			8
240		K 5-1			1
246 VN		K23-1			1
248 VN		K11-1			1
243		K11-2 K23-1	T 1-1 T 2-2		6
262	Oct	K11-1	T 1-1		2
283	Nov		T 1-1		1
281			T 1-4		4
289 VN			T 1-1		1
288 VN			T 1-10		10
282	Dec		T 1-1		1
311			T 1-1		1
					100

22 patients had 40 strains of either type 84 or 84,85, or both. This parallels the incidence of these types from all sources. Only three strains were of types other than Type Codes K or T. Type 84 was the predominant type observed: 17 patients - 31 strains; phage type 84,85 was found in seven patients - nine strains. Phage Type Code K-5 was the predominant Type Code K found: 16 patients - 26 strains; and K-23 was second: 6 patients - 7 strains.

Of the 20 patients who expired, nine had Type Code K types, six had "T" types and five had both. On the basis of this information, the ability of either of these types to produce a fatal outcome must be regarded as closely comparable.

It was evident that during the period of 1969 and 1970 a marked change in phage type of Staph. aureus occurred in this Institute. The local ward has an epidemic situation, which may well have been triggered by introduction of these strains from Vietnam returnees. Methicillin-resistance has been emphasized in this population, in another section of this report. Corrective measures, if they exist, have not as yet been discovered; the rapid spread of these strains does not augur well for removing them by a specific program.

Providencia stuartii: Role in Burn Wounds

The delineation of type identity, which made it possible to recognize specific changes in wound flora with successive years in the case of staphylococci and pseudomonads, is not yet readily achieved with Providencia stuartii, the other new problem organism in burn patients at this Institute. The species is clearly delineated and its identification is unequivocal (3), but it has not yet become widely known. This may be due to lack of interest in characterizing this erstwhile "paracolon" bacillus, although it cannot justifiably be so called any longer. A typing system was described by Ewing, Tanner and Dennard (4), but it had little occasion to be used, and the sera for the system will require renewal.

It is of note that the species recovered in burn patients is still primarily P. stuartii. P. alcalifaciens can be detected in stools of normal individuals but has not been recovered from burn wounds. Ewing (3) described the distribution of species as 85% alcalifaciens and 15% stuartii.

The frequency with which P. stuartii was encountered in

clinical and autopsy samples is shown in Table 7.

The incidence on surface cultures was over one-third of samples, and almost half the patients harbored the organism on the wound during their illness.

Biopsy samples, which more clearly detect tissue involvement, yielded an even higher proportion of samples positive than did surface cultures, and 45.6% of patients biopsied harbored the organism in the tissue sample.

Blood cultures were positive at some point in 14% of patients cultured. The large number of cultures minimized the meaning of the total positive samples.

In view of the problem of pneumonia in burn patients, the incidence of P. stuartii in sputum and Luken's tube samples is of great interest. Fifty-five per cent of all sputa were positive, and 67.7% of the patients cultured harbored the organism in their sputum at some point during their illness. This was an extremely high incidence of a coliform bacillus which has not been described as associated with pulmonary sepsis in any significant degree.

Urinary tract seeding was lower than might be expected for a gut organism. Twenty-eight per cent of the patients cultured had at least one positive urine culture.

A significant source of sepsis in burn patients can arise from infected indwelling I.V. catheters. Culturing these catheter tips on removal is not an entirely reliable source of information about the population of the tip; it is obvious that surface contamination can reach the tip at its moment of withdrawal. The incidence of positive I.V. cannula tip cultures was, however, disturbingly high; 27% of the patients cultured harbored P. stuartii on the tip at the time of its removal.

Autopsy tissues were a remarkable source of positive P. stuartii. Eighty-five per cent of the tissue blocks cultured harbored the organism, and 75% of the patients with tissues cultured harbored the organism. When the lung was scrutinized, the positive rate of samples was 20% lower than that of other tissues. But, 70% of the patients had P. stuartii in the lung at autopsy.

Discussion

There has been a marked rise in Staph. aureus infections in

Table 7. Providencia stuartii: Isolates from Clinical and Autopsy Specimens, 1970

Source	No. Isolates/ Total Specimens	Per Cent Positive	No. Patients Positive/Total Patients Cultured	Per Cent of Cultured Patients Positive
Burn wound,swab, clincal	96/338	28.4	59/135	43.7
Biopsy, wound	65/165	39.3	33/72	45.6
Blood culture	64/1674	3.8	31/221	14.0
Sputum (and Lukens)	295/536	55.0	74/109	67.7
Urine (and Foley)	152/983	15.4	62/216	28.7
I.V. catheter tip	30/237	12.6	30/110	27.2
Autopsy:burn	359/423	84.8	47/62	75.8
Autopsy: lung	83/128	64.8	38/53	69.8

terms of positive cultures in the burn patient population since 1968. This has been accompanied, and in fact preceded by, a shift of Staph. aureus flora from a heterogeneous flora with relatively wide spectrum of phage types, to a monotype flora, in which an initial type 47,54,75,84,85 pattern populated the burn ward, to be supplanted by a classic type 84 and 84,85 population. Admission contact culture data suggests that this change may have initially been introduced by Vietnam returnees, but the same types have been recovered on admission from CONUS patients.

The Providencia stuartii picture is one of marked rise in incidence of this supposedly inocuous enteric organism to a point where it is prominent on many wound surfaces and especially in sputa. At autopsy it is found to have colonized (? and invaded) burn wounds to a large extent, and it is especially conspicuous in autopsy lung samples.

The microbiologic aspect of burn wounds has changed significantly in the past two years. Although the control of Pseudomonas burn wound sepsis is still effected with topical therapy, the change is strongly implicated in the current rise in burn mortality. Further intensive study of these two organisms is projected.

References

1. Lindberg RB, Moncrief JA, Switzer WE, Order SE, Mills, W Jr: The successful control of burn wound sepsis. J Trauma 5:601-616, 1965.

2. Teplitz C, Lindberg RB, Switzer WE, Mason AD Jr: Necropsy documentation of burn wound sepsis. USA Surg Res Unit Ann Prog Rpt FY 1963, BAMC, Ft Sam Houston, Texas. Sect. 41.

3. Edwards PR, Ewing WH: Identification of Enterobacteriaceae. Burgess Publishing Co., 1962.

4. Ewing WH, Tanner KE, Dennard DA: The antigenic structure of Providencia sp. J Infect Dis 94: 134, 1954.

Publication

Lindberg RB,Mason AD Jr, Pruitt BA Jr, Brame RE: Potentiating effect of Providencia organisms in experimental burn infections. Fed Proc 29:638, 1970.

Presentations

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OD 6954	71 07 01	DD-DR&E(AR)636

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

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A061102B71R	01	084
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Procode with Security Classification Code): (U) Organ and Subcellular Metabolism of Sulfamylon as Used in the Treatment 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
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 71	.40	10.6
FISCAL YEAR CURRENT 72	.40	11.7

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

RESPONSIBLE INDIVIDUAL
NAME: PRUITT, B. A., JR, LTC, MC
TELEPHONE: 512-221-2720

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

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME: George M Helmkamp, Jr, CPT, MSC
TELEPHONE: 512-221-4106
SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS
NAME:
NAME: DA

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Procode EACH with Security Classification Code)
(U) Sulfamylon; (U) Metabolism; (U) Monamine Oxidase

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

23. (U) Study the oxidation of the aminomethyl group of sulfamylon (p-aminomethylbenzene sulfonamide) to a carboxylic acid function (p-carboxybenzenesulfonamide), to locate and identify the specific enzymes involved (presumably amine oxidase and an aldehyde oxidase or dehydrogenase), and to provide a fundamental basis for subsequent studies in the human body.

24. (U) Cell-free homogenates of various rat organs will be assayed for amine and aldehyde oxidizing activities initially using simple benzene derivatives, but evantually employing the sulfonamide series. It will be necessary to establish reliable and reproducible methods for these determinations. Spectrophotometric labelled substrates could serve as useful alternatives. Along these lines the synthesis and characterization of the putative intermediate, p-sulfamylbenzaldehyde, will be accomplished. Experiments will also be designed to detect possible changes in the levels of enzyme activity following controlled burn and sulfamylon treatment.

25. (U) 70 07 - 71 06 Four enzyme systems have been studied to date: rat plasma, rat liver, human plasma, and human liver. In none of these was the oxidation of sulfamylon (p-aminomethylbenzenesulfonamide) to p-sulfamoylbenzaldehyde observed to occur at a measurable rate. With the liver preparations, the mitochondrial fraction was isolated and solubilized to release a benzylamine-oxidizing activity. Human plasma monoamine oxidase was partially purified, but still exhibited no activity toward sulfamylon. Rat plasma appeared to contain no monoamine oxidase.

Available to contractors upon originator's approval.

ANNUAL PROGRESS REPORT

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

REPORT TITLE: ORGAN AND SUBCELLULAR METABOLISM OF SULFAMYLON AS USED IN THE TREATMENT OF BURN PATIENTS

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

1 July 1970 - 30 June 1971

Investigator:

George M. Helmkamp, Jr., CPT, MSC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

REPORT TITLE: ORGAN AND SUBCELLULAR METABOLISM OF SULFAMYLON AS USED IN THE TREATMENT OF BURN PATIENTS

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

Period covered in this report: 1 July 1970 - 30 June 1971

Investigator: George M. Helmkamp, Jr., CPT, MSC

Reports Control Symbol MEDDH-288(R1)

The metabolism of Sulfamylon or p-aminomethylbenzene-sulfonamide, a topical antibacterial agent employed in the routine treatment of burns, has been undertaken. In particular, the oxidation of Sulfamylon to p-sulfamoylbenzaldehyde, a reaction presumably catalyzed by monoamine oxidase, was examined in several tissues of the rat and human. The aldehyde product, p-sulfamoylbenzaldehyde, was synthesized and characterized in order to establish reliable assay techniques. Although the monoamine oxidases from rat and human liver mitochondria and human plasma exhibited normal activity toward benzylamine and other selected substrates, Sulfamylon was not oxidized. Rat plasma appeared to lack altogether a capacity to oxidize monoamines.

Sulfamylon
Metabolism
Monoamine oxidase

BLANK PAGE

ORGAN AND SUBCELLULAR METABOLISM OF SULFAMYLON AS USED IN THE TREATMENT OF BURN PATIENTS

The biological oxidation of Sulfamylon (p-aminomethylbenzenesulfonamide) to p-carboxybenzenesulfonamide was first reported in the 1940's through the work of Blaschko and Duthie[1] and Hartles and Williams.[2] In rabbits over 80% of ingested Sulfamylon was recovered in the urine as the free acid.[2] Furthermore, it was exceedingly difficult to maintain high concentrations of Sulfamylon in the blood of dogs and humans owing to its rapidoxidative deamination to p-carboxybenzenesulfonamide.[3] More recently in this institute similar experiments with dogs have confirmed these observations.[4] On this basis the participation of two enzymes, monoamine oxidase and aldehyde oxidase or dehydrogenase, was proposed to account for the metabolic fate of Sulfamylon.[2] The aldehyde, p-sulfamoylbenzaldehyde, was a presumed intermediate in the over-all reaction.

It was therefore decided to undertake initially a study of the action of monoamine oxidase on p-aminomethylbenzenesulfonamide. A review of the recent literature reveals a widespread distribution of monoamine oxidase in mammals (Table 1). While this list is by no means exhaustive, it does point to the primary role of the liver, or more specifically, the mitochondrial fraction of this organ, in the catabolism of amines. Plasma also appears to be a ready source of monoamine oxidase. In general, the enzymes are metalloproteins and tolerate a wide variety of aliphatic and aromatic substrates.

Table 1 also notes the instances in which Sulfamylon oxidation was considered. Two of these, rabbit liver[1] and guinea pig liver,[5] followed the complete oxidation to p-carboxybenzenesulfonamide, while in the other the individual reactions were examined.[6] Indeed, partially purified monoamine oxidase from beef plasma catalyzed the oxidation of p-aminomethylbenzenesulfonamide to p-sulfamoylbenzaldehyde with a concurrent release of ammonia and hydrogen peroxide. Corresponding activities in various organs of the rat and human are the object of the present investigations.

Materials and Methods

1. Synthesis of p-sulfamoylbenzaldehyde

The synthesis of p-sulfamoylbenzaldehyde was accomplished

Table 1. Distribution of Monoamine Oxidase Activity Among Mammals

Animal	Organ				
	Plasma	Liver	Kidney	Intestine	Brain
Man	+	+	+		+
Rat		+	+	+	+
Rabbit	+	0			
Guinea pig		0			
Monkey		+	+	+	
Cow	0	+			
Ox		+	+		
Pig	+	+	+		+

Note: + designates activity in general; 0 designates activity toward Sulfamylon

by controlled oxidation of p-toluenesulfonyl chloride with chromium trioxide in a mixture of acetic anhydride and sulfuric acid, followed by formation of the amide and hydrolysis of the benzal diacetate.[7,8] Crude product was further purified by formation, crystallization, and subsequent hydrolysis of its aniline Schiff base, p-sulfamoylbenzylidene aniline.[9] The desired compound was then crystallized from hot water to give essentially colorless needles with a melting point (uncorrected) of 121-123°; reported m.p. 122°,[7] 123-124°,[8] 122-124°.[9] The thiosemicarbazone derivative melted at 229-230°; reported m.p. 230-231°,[7] 227°.[8] Ultraviolet absorption of an aqueous solution showed λ_{max} 249 nm, $\epsilon = 1.64 \times 10^4$; a broad, low-intensity shoulder appeared in the 280-300 nm region.

Examination of the aldehyde by thin layer chromatography in vapor-saturated tanks showed the material to be free of contaminants. In addition, its migration was compared with the corresponding amine and acid; the solvent systems employed and results are summarized in Table 2. As the silica gel plates contained fluorescent indicator, visualization of the compounds was achieved with a mercury lamp (254 nm). Alternatively the plates were sprayed first with t-butylhypochlorite and then with starch-iodide reagent to yield blue spots on a white background.

II. Fluorescent properties of p-sulfamoylbenzaldehyde

The fluorescent characteristics of p-sulfamoylbenzaldehyde were studied with an Aminco-Bowman spectrophotofluorometer equipped with a Varian X-Y recorder. In 90% sulfuric acid the excitation and fluorescence spectra had maxima at 335 nm and 450 nm respectively. Furthermore, in the presence of equal quantities of p-aminomethylbenzenesulfonamide or p-carboxybenzenesulfonamide, the fluorescence was virtually unquenched. Finally, if excitation is performed at 290 nm, the fluorescence of both the acid and the aldehyde may be observed, the former at 360 nm. In this case a reasonably linear concentration dependence of the aldehyde fluorescence results.

III. Extractability of p-sulfamoylbenzaldehyde

From buffered or unbuffered aqueous media, p-sulfamoylbenzaldehyde could be extracted into two or three volumes of water-saturated diethyl ether or n-butanol. Less than 10% of the aldehyde remained in the aqueous phase, while, more importantly, greater than 90% of p-aminomethylbenzenesulfonamide was retained by the water layer under identical conditions. These two compounds

Table 2. Thin Layer Chromatographic R_F Values of Various Benzenesulfonamides

Compound	System I n-butanol:acetic acid:water (7:1:2)	System II n-butanol:pyridine:water (1:1:1)
p-sulfamoylbenzaldehyde (p-formylbenzenesulfonamide)	0.90	0.79
p-carboxybenzenesulfonamide	0.79	0.62
Sulfamylon (p-aminomethylbenzenesulfonamide)	0.48	0.55

can easily be distinguished by their aqueous ultraviolet spectra: amine λ_{max} 220 nm, $\epsilon = 1.24 \times 10^4$; aldehyde λ_{max} 249 nm, $\epsilon = 1.64 \times 10^4$.

IV. Monoamine oxidase assay procedures

Several spectrophotometric methods were used to assay crude and partially purified enzyme preparations. With benzylamine as substrate, product released could be monitored directly at 249 nm or quantitatively extracted into cyclohexane and measured at 242 nm.[10] Other oxidizable amines included kynuramine (2-(2-aminobenzoyl)ethylamine), followed at 360 nm,[11] and p-dimethylaminobenzylamine, followed at 355 nm.[12] The last two substrates offer the advantages of larger extinction coefficients and absorption maxima in a region distinct from typical protein absorptions.

When p-aminomethylbenzenesulfonamide was used as substrate, the assays were usually performed in potassium phosphate buffers of pH 7.2 or 7.6 at 30 or 37°. The product aldehyde was measured directly at 249 nm or, if first extracted into n-butanol, at 251 nm. In some cases aliquots of the deproteinized reaction mixture were analyzed in the spectrophotofluorometer.

Results

I. Rat plasma

Plasma was prepared from heparinized whole blood obtained by heart puncture. Using benzylamine or p-dimethylaminobenzylamine as substrates and up to 2 ml of plasma in a total volume of 3 ml, no monoamine oxidase activity could be demonstrated. Neither whole blood nor washed red cells were active.

II. Rat liver

Fresh rat livers were homogenized in potassium phosphate-buffered sucrose according to Dr. F.J. Ruzicka. After an initial centrifugation to remove cell debris and fat droplets, a mitochondrial pellet was obtained. The latter was washed several times in sucrose, resuspended in buffer, and frozen at -70°. The mitochondrial content of the preparation was verified by the difference spectrum of oxidized and reduced samples, in which the characteristic cytochrome absorptions were apparent.

Separation and purification of the metochondria resulted in over an 8-fold increase in benzylamine oxidizing activity

compared with the crude homogenate. In most spectrophotometric assays it was necessary to add a small amount of detergent in order to solubilize and clarify the mitochondrial suspension. Up to a weight ratio of about 1, enzyme activity remained relatively constant; however at higher ratios of sodium deoxycholate to mitochondria the activity decreased significantly.

In Table 3 are depicted the results of a variety of substrates for rat liver mitochondrial monoamine oxidase. It is immediately obvious that the unsubstituted benzylamine is the most active substrate; introduction of other functional groups on the aromatic ring greatly reduces the oxidation of the aminomethyl moiety and in the case of p-aminomethylbenzenesulfonamide, there is no activity whatsoever.

III. Human plasma

Samples of plasma obtained from the clinical chemistry section were shown to have considerable monoamine oxidase activity with benzylamine as substrate. It was therefore desirable to prepare a partially purified enzyme of higher specific activity. This was in fact accomplished by the method of McEwen.[13] After ethanol fractionation, stepwise ammonium sulfate cuts were isolated and dialyzed. Only the 45-60% saturated fraction contained monoamine oxidase. At this stage the specific activity was 0.34 nmole/min/mg protein, comparing favorably with the value of 0.68 reported previously.[13] Over-all purification was about 20-fold. Against p-aminomethylbenzenesulfonamide and p-dimethylaminobenzylamine the partially purified plasma enzyme showed no activity. Kynuramine has been shown to be an active substrate,[13] but it was not examined in these experiments.

IV. Human liver

A 27-gram specimen of liver was procured at autopsy from a burned patient approximately six hours post mortem. Mitochondria were prepared according to McEwen et al.[14] The twice-washed material was resuspended in buffer and stored at -70°. Half of this suspension was thawed and treated with Triton X-100 at a final concentration of 1.5 g/100 ml. Nearly all the activity remained in the 27,000xg supernatant. Ammonium sulfate was then added to 45% saturation. Upon centrifugation at 20,000xg, a bright yellow gelatinous material was observed floating on the liquid surface; there was no precipitate. The gel was redissolved in phosphate buffer and found to contain greater than

Table 3. Activity of Deoxycholate-solubilized Metochondria from Rat Liver Toward Monoamine Oxidase Substrates

Substrate	Concentration	Temperature	Specific Activity
			nmole/min/mg protein
Benzylamine	3×10^{-3} M	30°	5.3
		37°	6.3
Kynuramine	1×10^{-4} M	30°	4.8
p-Dimethylaminobenzylamine	3×10^{-3} M	30°	1.5
p-Aminomethylbenzene-sulfonamide	3.3×10^{-3} M	37°	not detectable

99% of the original benzylamine oxidase activity. However, overnight dialysis led to a 55% loss of this activity. The half-life at -20° was about two months.

The remainder of the mitochondrial suspension was ultrasonically disrupted (140 watts, 20 MHz) in the presence of benzylamine. Despite cooling in ice the temperature of the solution rose to 29° and sonication was discontinued after 30 minutes. The solution was next brought to 1% (w/v) in sodium cholate and centrifuged at 37,000xg. At this point most of the activity had been lost, and what little remained (7%) was associated with the supernatant fraction.

Attempts to demonstrate oxidase activity toward p-aminomethylbenzenesulfonamide were fruitless and fraught with experimental difficulties. Following incubation with the solubilized enzyme, extraction of the putative aldehyde was complicated by high concentrations of the detergent Triton X-100, whose absorption was too intense for quantitative spectroscopic analysis of the product. Nevertheless, no aldehyde appeared to be present. In an assay utilizing ^{14}C-Sulfamylon less than 1% of the radioactivity was extractable into ether, and when this material was chromatographed on silica gel thin layers, there was no significant radioactivity coincident with authentic p-sulfamoylbenzaldehyde. In this case, too, Triton X-100 interfered by causing some streaking on the thin layer plates.

Discussion

Despite detailed analysis of four potentially rich enzyme sources, namely, rat plasma, rat liver, human plasma, and human liver, catalysis of the oxidation of p-aminomethylbenzenesulfonamide to the corresponding aldehyde could not be detected. Indeed, of the above systems, only one, rat plasma, was completely devoid of monoamine oxidase activity as measured by the benzylamine-benzaldehyde conversion. The others exhibited activity in varying degrees toward benzylamine, p-dimethylaminobenzylamine, and kynuramine. Monoamine oxidases from human plasma and human liver mitochondria were partially purified in order to concentrate their activities. However, Sulfamylon remained unoxidized. With the liver enzyme there were particular problems arising from the use of Triton X-100 as a solubilizing agent. The detergent complicated the chromatographic and spectrophotometric analysis of p-sulfamoylbenzaldehyde. Thus, it is still quite possible that a Sulfamylon oxidizing activity is present, but so far undetected in human liver. In any event, one must eventually account for

the rapid appearance of the Sulfamylon metabolite, p-carboxybenzenesulfonamide, in the blood and urine of rats and humans treated with the drug. The participation of other organs, such as the kidney and the intestine, cannot be disregarded.

References

1. Blaschko H,Duthie R ,Biochem J 39, 347, 1945.

2. Hartles RL, Williams RT, Biochem J 41, 206, 1947

3. McChesney EW, Auerbach ME, McAuliff JP, Eckert HW, J Pharmacol Exptl Therap 96, 356, 1949.

4. Seraile LG, unpublished observation.

5. Beyer KH, Govier WM, Science 101, 150, 1945.

6. Tabor CW, Tabor H, Rosenthal SM, J Biol Chem 208, 645, 1954.

7. Sekikawa I, Kakimoto S, J Chem Soc (Japan) 73, 587, 1952. Chem.Abst 48, 1985g (1954).

8. Sycheva TP, Shchukina MN, Sbornik Statei Obshchei Khim, Akad Nauk SSSR 1, 527, 1953; Chem Abst 49, 932c, 1955.

9. Dakin HD, Biochem J 11, 79, 1917.

10. McEwen CM Jr, Cohen JD, J Lab Clin Med 62, 766, 1963.

11. Weissbach H, Smith TE, Daly JW, Witkop B, Udenfriend S, J Biol Chem 235, 1160, 1960.

12. Deitrich RA, Erwin VG, Anal Biochem 30, 395, 1969.

13. McEwen CM Jr, J Biol Chem 240, 2003, 1965.

14. McEwen CM Jr, Sasaki G, and Lenz WR Jr, J Biol Chem 243, 5217, 1968.

Presentations and/or Publications

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0D 6382	71 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
70 07 01	H.TERMINATION	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A061102B71R	01	310
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)

(U) Prevention of Chondritis of the Ears 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 08	70 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 71	0.31	8.2
FISCAL YEAR — CURRENT 72	0	0

19. RESPONSIBLE DOD ORGANIZATION

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

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

20. PERFORMING ORGANIZATION

NAME: US Army Institute of Surgical Research
Burn Study Branch
ADDRESS: Ft Sam Houston, Texas 78234

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME: Andrew M. Munster, LTC, MC
TELEPHONE: 512-221-5712
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: Morris J Asch, LTC, MC
NAME: Paul Silverstein, MAJ, MC DA

21. GENERAL USE

FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Ears; (U) Chondritis

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

23. (U) To compare the effects of prophylactic treatment of burned ears of Carbenicillin cream and aqueous solution with Sulfamylon cream and aqueous solution in the prevention of chondritis.

24.(U) This double-blind study was designed so that successive patients with burns of the ears received topical treatment with one of the four modalities mentioned above or no treatment (controls). The end point of the study was the development of chondritis, or complete healing of the ear.

25. (U) 70 07 - 71 07- This study could not be completed because of insufficient numbers of patients qualified to enter the study and proceed to the designated end-point. The study has been terminated and no conclusions drawn.

* Available to contractors upon originator's approval

FINAL REPORT

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

REPORT TITLE: PREVENTION OF CHONDRITIS OF THE EARS

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

1 July 1970 - 30 June 1971

Investigators:

Andrew M. Munster, M.D., LTC, MC
Morris J. Asch, M.D., LTC, MC
Paul Silverstein, M.D., MAJ, MC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

REPORT TITLE: PREVENTION OF CHONDRITIS OF THE EARS

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

Period covered in this report: 1 July 1970 - 30 June 1971

Investigators: Andrew M. Munster, M.D., LTC, MC
Morris J. Asch, M.D., LTC, MC
Paul Silverstein, M.D., MAJ, MC

Reports Control Symbol MEDDH-288(R1)

In this study, difficulties were encountered in generating enough patients suitable for inclusion to render this study statistically significant. As the study was designed to focus on patients admitted early postburn with burns of the ears, and who were followed for long enough to allow either complete healing of the ear or the development of chondritis, and as the experimental design included four groups of patients to be treated with Sulfamylon cream or Sulfamylon soaks, carbenicillin cream or carbenicillin soaks, it was found that to generate a statistically sufficient number of patients to justify continuation of this study, at least several years would be involved. The selection of patients, the division of the patients into four groups, the procurement and labelling of the compounds to be used, and the regular evaluation of the results involved logistical work of considerable proportions. It was considered by the investigators that the number of patients who could enter the study was insufficient to warrant continuation of this particular study. This study has therefore been terminated and no conclusions made.

Ears
Chondritis

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6981	71 07 01	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8A. DOD'N INSTR'N	8B. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
70 07 01	D. CHANGE	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A061102B71R	01	303
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)
(U) Urinary Tract Infections in Burned Military Patients (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS
003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
69 05	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 71	0.47	12.5
FISCAL YEAR CURRENT 72	0.47	13.7

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

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

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

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic institution)
NAME: Jon M Reckler, MAJ, MC
TELEPHONE: 512-221-4906
SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS
NAME: Andrew M. Munster, LTC, MC
NAME: Robert B. Lindberg, Ph.D. DA

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Bacteriuria; (U) Urinary tract infection

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

23. (U) To study the effect of the presence and duration of an indwelling urethral catheter on the incidence of urinary tract infections. To assess the relation, if any, between organisms in the urinary tract and in the blood stream.

24. (U) Blood and urine cultures will be obtained at time of catheterization, 48 hours, one, two, four and eight weeks postburn.

25. (U) 70 07 - 71 07 Thirty-four patients have been entered in this study since May 1969. When complete information has been obtained on patients still hospitalized, data will be tabulated and analyzed.

*Available to contractors upon originator's approval

ANNUAL PROGRESS REPORT

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

REPORT TITLE: URINARY TRACT INFECTIONS IN BURNED PATIENTS

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

1 July 1970 - 30 June 1971

Investigators:

Jon M. Reckler, M.D., MAJ, MC
Andrew M. Munster, M.D., LTC, MC
Robert B. Lindberg, Ph.D.

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A061102B71R-01, RESEARCH IN BI MEDICAL SCIENCES

REPORT TITLE: URINARY TRACT INFECTIONS 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 1970 - 30 June 1971

Investigators: Jon M. Reckler, M.D., MAJ, MC
Andrew M. Munster, M.D., LTC, MC
Robert B. Lindberg, Ph.D.

Reports Control Symbol MEDDH-288(R1)

This study is designed to permit a prospective examination of the incidence of urinary tract infections in burned patients. A urinary tract infection will be defined as the presence of a number of organisms equal to or greater than 10^5 organisms per cc of urine. Of particular interest is the effect of the presence and duration of an indwelling urethral catheter on the incidence of urinary tract infections, the possibility of a correlation between organisms found in the urinary tract and on the surface of the burn or in the blood stream, and the incidence of persisting urinary tract infections at the time of discharge of patients from the hospital.

All male patients between the ages of 15 and 45 admitted to the Institute of Surgical Research within 48 hours of burn with or requiring an indwelling urethral catheter, have been and will be entered in this series. All patients accepted into this study will have urine and blood cultures on admission. Subsequent urine and blood cultures will be obtained at 48 hours one week, two weeks, four weeks and at eight weeks following the burn. All positive urine cultures will be repeated to verify their accuracy.

At the time of this report, 34 patients have been entered in this series. Complete information has not yet been obtained on

all patients, as studies are still in progress on the individuals most recently entered in the study. Because of the small number of patients on whom complete information is available, firm conclusions based upon tabulation of available data at this time are unwarranted. It would appear, however, that the majority of patients who have developed significant urinary tract infections have not done so until some time during the second week postburn, and then only with the continued presence of an indwelling urethral catheter. The possibility of a correlation between organisms in the urinary tract and in the blood stream appears likely, though based on available information it is not possible to determine the sequence in which these materials have become infected and if there is cross-infection between the two sites. To date, only three patients have had persistent bacteriuria at time of discharge from the hospital, although all had colony counts of less than 10^5 organisms per cubic centimeter.

Bacteriuria
Urinary Tract Infection

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0C 6396	71 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. DOD'N INSTR'N	8A. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
70 07 01	D.CHANGE	U	U	NA	NL	[X] YES [ ] NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A061102B71R	01	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 71	0.55	14.6
FISCAL YEAR CURRENT 72	0.3	8.8

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

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

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

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

ASSOCIATE INVESTIGATORS
NAME: Virginia C 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

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

23. (U) Serratia marcessens is one of the Enterobacteriaceae with documented capability for wound invasion;thermal injury and its complications, a continued threat to military personnel, require detailed delineation of opportunistic invaders in the program controlling nosocomial infection.

24. (U) A phage typing set,devised for this purpose, is propagated to give high potency typing fluids for delineating wound, biopsy, lung and autopsy strains.

25. (U) 70 07 - 71 06 - Three predominant phage types plus a heterogeneous population were shown in 117 strains; 94% were typable. A direct correspondence between type in sputum, lung, and nebulizer showed one path for airborne cross-infection; the tissue invasiveness of two types was suggested by autopsy results.Typing will be continued.

*Available to contractors upon originator's approval.

ANNUAL PROGRESS REPORT

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

REPORT TITLE: BACTERIOPHAGE TYPES OF SERRATIA MARCESSENS FROM BURN WOUNDS

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

Investigators:

Virginia C. English, M.A.
Robert B. Lindberg, Ph.D.
Ruth L. Latta, B.S.
Basil A. Pruitt, Jr., M.D., LTC, MC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

REPORT TITLE: BACTERIOPHAGE TYPES OF SERRATIA MARCESSENS FROM BURN WOUNDS

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

Period covered in this report: 1 July 1970 - 30 June 1971

Investigators: Virginia C. English, M.A.
Robert B. Lindberg, Ph.D.
Ruth L. Latta, B.S.
Basil A. Pruitt, Jr., M.D., LTC, MC

Reports Control Symbol MEDDH-288(R1)

From 49 patients 117 strains of Serratia marcessens were recovered and phagetyped from burns in 1970. Incidence was episodic, as was seeding of the burn ward. Both CONUS and Vietnam patients were shown to be capable of seeding the burn ward. Predominant types exist; in this period, a long established type, 5,7,9,11,15, 18 continued to predominate. Type 15 was the next most common. A third type, distinct in identity, was 5,7,9,15,18. This latter type was less persistent than the other two. All types were capable of seeding nebulizer reservoirs, and in view of current heightened concern over pneumonia in burn patients, this vector path merited close attention. The same type was found in Lukens culture and in reservoirs; contamination could proceed in both directions. The persistence of S.marcessens in lung tissue when it had been cleared from sputum was demonstrated.

Burns
Serratia marcessens
Phagetyping

BLANK PAGE

BACTERIOPHAGE TYPES OF SERRATIA MARCESSENS FROM BURN WOUNDS

The Enterobacteriaceae family includes the major part of the burn wound flora,(1),and in recent years control of burn wound sepsis with topical chemotherapy has, if anything, increased the incidence of coliform bacteria on burns by reducing the incidence of the more susceptible pseudomonads (2). Serratia marcessens has been observed in burn wounds and has, indeed, appeared to cause invasive sepsis in burns on rare occasions. Its record as an opportunistic invader has prompted more detailed scrutiny of its behavior as part of the burn wound flora. Since it is an organism essentially refractory to antibiotics, it can present a major problem when it produces sepsis.

Methods

S. marcessens is the only member of the genus commonly encountered (3). Although it classically produces a brick-red pigment, at least half of the isolates are non-pigmented, and non-pigmented variants are common. Its differentiation from other Enterobacteriaceae involves gelatin liquefaction, lysine and ormithine decarboxylase, arginine dihydrolase, and arabinose and adamitol fermentation at the least. Rhaminose and raffinose fermentation may be needed when gelatin liquefying Enterobacter cloacae must be differentiated.

The bacteriophage system used for differentiating types was developed in this laboratory, using classic isolation procedures from sewage effluent. The procedures follow those described by Adams (4). The system involved selection of seven phages, with which virtually all strains can be differentiated. As a check on specificity, 30 strains of Klebsiella, Enterobacter and Providencia were tested against the typing set. No lysis occurred with any strain of these coliforms.

Results

From 49 patients, 117 strains of S. marcessens were recovered during 1970. The incidence was sporadic. On several occasions, flurries of S. marcessens appeared to drift through the burn ward following arrival of patients from Vietnam who were harboring the organism. Twenty-five different phage patterns were recognized, plus eight isolates which could not be typed. This represents a 6.7% nontypable rate, which reflects a high level of sensitivity for the typing system. In 1969, the nontypable rate was 6.0%.

The phage patterns distinguished are set down in Table 1. The type designations are set down in ascending numerical order; this arbitrary sequence permits recognition and organization of the phage types. There were nine types that occurred only once; six more were seen twice. This diversity of type pattern is characteristic of sensitive phage typing systems.

Predominant types included three with a probable fourth closely related type. 5,7,9,11,15,18 was the most common type; it is probable that 5,7,9,15,18 is a variant of this type. 5,7,9,11, 15,18 was the numerically predominant type in 1969 as well. Type 15 was the second most common type in 1970.

A comparison of predominant types is presented in Table 2. The predominant type 5,7,9,11,15,18 has been a major feature of the species every year since 1968. It has been recovered from wound surfaces, on admission and later, from amputation stumps, from sputum and Lukens tube aspirates and from ultrasonic nebulizer devices. It has also been recovered from autopsy tissues, including liver and lung.

The distribution during 1970 of three predominant types: 5, 7,9,11,15,18; 5,7,9,15,18, and 15 is presented in Table 3. The most common type, 5,7,9,11,15,18 occurred on wounds in seven out of 10 patients; in sputum (Lukens tube) in one; in urine in one; in autopsy tissues in two; and in water reservoirs of nebulizers in two patients. One of these harbored the organism in sputum as well. Type 5,7,9,15,18 was never as persistent as was the previous type. It appeared in eight patients over an eight-month period; in one of these, it was recovered from two samples of tissue obtained at autopsy. The remaining seven patients had one Lukens tube and six burn surfaces positive.

Phagetype 15 was also recovered from eight patients. In one, Nr. 63, it was involved in thrombophlebitis, since it was recovered from the i.v. tip and from two vein excisions. One Lukens tube sample was positive and the remaining six patients had the organism on the burn wound. The distribution of the latter two types was scattered and episodic; seldom did it appear on successive cultures. Type 5,7,9,11,15,18 is far more prone to persist and be seen repeatedly on a given patient.

Persistence of a single type or seeding with a succession of strains has direct bearing on the clinical significance of Serratia. If it is a fortuitous contaminant, then it would be expected that different types might appear on a patient as he remained in the

Table 1.
Phage Types of *Serratia marcesscens*
Recovered from 49 Burn Patients

Phage Type	Number of Patients	Number of Isolates
3	1	2
3,5,7,18	2	2
3,5,7,15,18	1	1
3,5,7,9,15,18	1	2
3,7	1	1
3,7,11,15	1	1
5,7	1	1
5,7,11	1	1
5,7,18	2	2
5,7,9,11	1	1
5,7,9,15,18	8	9
5,7,9,11,15,18	10	29
5,7,11,15,18	2	2
5,9,15,18	1	1
5,9,11,15,18	1	1
7,15	3	3
7,18	1	6
7,9,15	1	5
9,15,18	2	2
11	2	5
11,15	1	6
11,18	1	1
15	8	13
15,18	3	5
18	5	7
Nontypable	7	8

Table 2.

Comparsion of Yearly Predominant Phage Types

	Type	No.Patients	No.Isolates
1971	5,7,9,11,15,18*	10	29
	5,7,9,15,18	8	9
	15	8	13
1970	5,7,9,11,15,18*	10	19
	5,7,9,15,18	4	4
	7,9,15	7	5
	15	5	5
1969	5,7,9,11,15,18*	7	18
	5,7,15	7	7
	11,15	7	16
	15	8	16
1968	3,5,7,9,11,15,18	6	8
	3,5,7,11,15	5	21
	3,5,7,11,15,18	5	7
	5,7,9,11,15,18*	12	21
	11	5	5

* Note that this type was among predominant each year.

Table 3.

Distribution of Three Predominant *Serratia marcessens* Types in the ISR Burn Ward

Phage Type 5,7,9,11,15,18			
Patient Number	Admission Date	Isolate	Source
8	1-5-70	1-5-20(2)	Thigh
		1-5-20(5)	Thigh
		1-5-23(2)	Thigh
		1-13-24(4)	R.thigh
		1-13-25(4)	L.thigh
		3-17-10	L.stump
49	2-23-70	2-23-20(3)	Thigh
		2-25-21(1)	Liver
		2-25-23(1)	RUL
131	7-13-70	7-17-9	Lukens
		7-28-8	Urine
		7-22-5	Lukens
		7-25-4	Lukens
		7-27-5	Lukens
		7-23-3	Lukens
		8-7-10	Lukens
		P # 2	H_2O
		P # 3	H_2O
200	7-27-70	7-27-15(2)	Arm
		7-27-15(5)	Arm
		7-31-57(3)	L.leg
		8-5-30(2)	?
201	7-27-70	7-27-22(3)	Arm
		7-27-22(4)	Arm
		7-31-48(3)	L.arm
219	8-11-70	8-13-13(3)	Back
264	10-9-70	10-15-108	H_2O
266	10-12-70	10-22-17(1)	LUL
272	10-13-70	10-13-16(4)	Arm
297	11-23-70	11-23-3(3)	L.Shoulder

Phage Type 5,7,9,15,18			
Patient Number	Admission Date	Isolate	Source
96	4-20-70	4-25-4 B	Lukens
141	6-8-70	6-14-6(2)	L.U.thigh
190	7-13-70	8-25-26(1)	PM Tissue # 1
		8-25-27(1)	PM Tissue # 2
277	8-11-70	8-12-30(2)	L.U.arm
256	9-26-70	9-28-18(2)	R.forearm
274	10-13-70	10-13-14(1)	L.arm
284	11-16-70	11-17-6(1)	Chest
314	12-14-70	12-14-24(2)	Thigh

Phage Type 15			
Patient Number	Admission Date	Isolate	Source
63	3-9-70	3-11-13	Groin vein
		3-11-14	R.ankle vein
		3-11-15	I.V. tip
67	3-9-70	3-9-15(1)	L.arm
79	3-23-70	3-24-1(4)	Shin
141	6-8-70	6-8-42(2)	Arm
		6-8-42(5)	Arm
		6-14-6(3)	L.U.thigh
150	6-8-70	6-8-43(1)	Arm
245	9-7-70	9-7-13(6)	Abdomen
264	10-9-70	10-15-2	Lukens
288	11-16-70	11-17-7(3)	Shin

hospital, presuming that concurrent sources of contamination existed.

Table 4 presents the detailed sequence of infection in 13 patients who had more than one type recovered. These occurred from February through December. Patient 30 had one type on admission; a month later he exhibited another type, 7,9,15 at autopsy. Between these times Serratia was not seen in numerous cultures, including those of wounds, sputum and blood. The predominant organism in wound tissues was Providencia stuartii. Patient 49 had type 5,7,9,11,15,18 on the burn on admission; he died two days later with a pulmonary embolism. The admission type was found in liver and lung; but another type, 5,7,9,11 was found in the burn wound.

Patient 96 harbored three types: an 11,18 on admission and later in urine, Lukens tube and i.v. tip; a type 18 on a later urine culture; and a 5,7,9,15,18 in sputum later.

In the case of three patients, their nebulizer water reservoirs were positive. In Nr. 189, the same type was found in sputum, prior to the positive nebulizer culture. In the second, the Lukens tube culture was positive before the nebulizer culture. In a third, three types were found at once; a 15 in the Lukens tube culture and the nebulizer culture; a 3,5,7,18 in the Lukens culture; and a third type in the reservoir culture only. In Nr. 322, two different types were recovered from separate lung autopsy samples.

The diverse identity of Serratia types implies more an intermittent seeding with an opportunistic colonizing organism, rather than a specific pathogen, able to predominate in a susceptible host. It is pertinent to note that in none of these patients with multiple types was a blood stream isolate recorded. This does not, of course, negate that a role in sepsis is played by Serratia. The postmortem visceral samples indicate that bacteremia may very well occur with Serratia, but it was not detected in clinical cultures.

There were 17 out of the 49 patients positive for Serratia who came from Vietnam. The diminished tempo of the war was reflected in this minority of all positives; there were 32 patients from CONUS who were positive. In the previous two years, the major part of Serratia carriers were from Vietnam. Table 5 summarizes this Vietnam group. Eighteen of the soldiers had positive cultures on admission but never exhibited the species again. Nine were negative on admission but later yielded a positive culture.

Table 4. *Serratia* Phage Types in Patients with Multiple Types

	Patient No.	Adm. Date	Phage Type	Date	Source
FEB	30	2-2	5,9,15,18	2-2	R.Arm
			7,9,15	3-3	PM Tissue 9 PM Tissue 10 PM Tissue 11 PM Spleen PM Liver
	49	2-23	5,7,9,11,15,18	2-23	Thigh
				2-25	PM Liver PM RUL
			5,7,9,11		PM Tissue 9
	57	2-23	7,15	2-23	Thigh
			5,7	2-24	Head
APR	96	4-20	5,7,9,15,18	4-25	Lukens
			11,18	4-20	R.Calf R.Thigh
				4-24	Urine Lukens
				4-25	I.V.tip Lukens
			18	4-30	Foley tip
JUN	141	6-8	18	6-8	Arm Arm
			15		Arm Arm
				6-14	L.Up.Thigh
			5,7,9,15,18		L.Up.Thigh
JUL	189	7-13	11	7-14	Lukens
				8-3	Nebulizer water Nebulizer water
				8-4	Nebulizer water
			NT	8-3	Nebulizer water
				8-4	Nebulizer water
	191	7-13	5,7,9,11,15,18	7-17	Lukens
				7-21	Urine
				7-22	Lukens
				7-25	Lukens
				7-27	Lukens
				8-7	Lukens
				-	Nebulizer water Nebulizer water
			15	8-4	Nebulizer water
	200	7-27	5,7,9,11,15,18	7-27	Arm Arm
				7-31	L.Leg
				8-5	-
			5,7,18	7-31	L.Leg
OCT	264	10-9	15	10-15	Nebulizer water Lukens
			3,5,7,18		Lukens
			5,7,9,11,15,18		Nebulizer water
			3,7	10-17	Legs
	267	10-13	3,7,11,15	10-15	Lukens
			3,5,7,18		Lukens
			7,15	12-2	Tracheal ring
NOV	288	11-16	5,9,11,15,18	11-17	Shin
			15		
DEC	314	12-14	5,7,9,15,18	12-14	Thigh
			9,15,18		Arm
	322	12-17	5,7,18	12-21	PM RLL
			7,18		PM LLL PM LUL PM RUL PM Tissue 3 PM Tissue 4
			NT		PM RLL

Table 5
SURVEY OF SERRATIA PHAGE TYPES FROM VIET NAM ADMISSIONS

Admission Date	Patient No.	Admission Cultures		Subsequent Cultures	
		Phage Type	Source	Phage Type	Source
1-5-70	8	5,7,9,11,15,18	Thigh	5,7,9,11,15,18	L.thigh R.thigh L.stump
2-2-70	30			7,9,15	PM Tissue 9, 10,11; Spleen, Liver
2-23-70	49			5,7,9,11 5,7,9,11,15,18	PM Tissue 9 Liver; RUL
	57			5,7	Head
3-9-70	63			15	R.groin vein R.ankle vein I.V. tip
	67	15	L.arm		
3-23-70	79	15	Shin		
4-6-70	87	18	Lukens		
4-20-70	103	18	Leg		
	96	11,15	R.calf, thigh	11,15 5,7,9,15,18 18	Lukens; Urine I.V.tip Lukens Foley tip
6-8-70	141	18 15	Arm Arm	18 5,7,9,15,18	L.Up.thigh L.Up.thigh
	150	15	Arm		
7-13-70	187	5,7,11	Thigh		
	190			5,7,9,15,18	PM Tissue 1,2
7-17-70	193			7,15	L.leg
7-27-70	196	15,18	Thigh		
	200	5,7,9,11,15,18	Arm	5,7,9,11,15,18 5,7,18	L.leg L.leg
	201	5,7,9,11,15,18	Arm	5,7,9,11,15	L.arm
	202	3,5,7,15,18	Thigh		
8-11-70	217			5,7,9,15,18	L.Up.arm
	218	15,18	L.calf		
	219			5,7,9,15,18	Back
9-7-70	245	15	Abdomen		
9-28-70	256	5,7,9,15,18	R.forearm		
10-19-70	272	5,7,9,11,15,18	Arm		
	274	5,7,9,15,18	L.arm		
11-16-70	284	5,7,9,15,18	Chest		
	288	5,9,11,15,18	Shin		
12-14-70	314	5,7,9,15,18 9,15,18	Thigh Arm		
	315			NT	L.leg
	317	11,18	Thigh		

In five cases they were positive on admission and later. These latter exhibited the same type on admission and later, although in three instances there were later strains of different type as well. Incoming positives included 12 different types, although the predominant 5,7,9,11,15,18 was present in four. The later positives included 11 types. None of the three patients who had positive postmortem cultures had been positive on admission. The types present later in the patients illness were in large part related to the 5,7,9,11,15,18 pattern.

Discussion

Serratia marcessens is shown in this series to occur in burn patients originating in CONUS as well as those received from Vietnam. The persistence of a few dominant types, with a periphery of types which are seen only once or twice in a year is indicated. The typing system adequately differentiated types; whether the more common types can be differentiated into invading and colonizing types remains to be revealed with further study. The tissue predelection of this species has been emphasized by this study. Its movement through the ward is consistent with its being transported by the personnel attending the burn patients. The potential for epidemic spread of this species exists; continued observations are in order.

References

1. Lindberg RB, Contreras AA, Pruitt BA Jr, Mason AD Jr, Townsend CH: Pathogenesis of burn wound infection. Bacterial flora of burn wounds receiving sulfamylon.Ann Prog Rpt FY 1970. USA Institute Surg Res, BAMC, Ft Sam Houston,Texas.Sect. 16.

2. Lindberg RB, Moncrief JA, Switzer WE, Order SE, Mills W Jr: The successful control of burn wound sepsis. J Trauma 5: 601-616, 1965.

3. English VC, Latta RL, Brame RE, Lindberg RB: Development of a bacteriophage typing system for organism of the genus Serratia. Ann Prog Rpt FY 1968. USA Surg Res Unit, BAMC, Ft Sam Houston, Texas. Sect. 32.

4. Adams MH: Bacteriophages. New York, Interscience Pub. 1959.

Publications and/or Presentations

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0D 6386	71 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
70 07 01	D. CHANGE	U	U	NA	NL	[X] YES [ ] NO	A. WORK UNIT

10. NO. CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A061102B71R	01	314
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Procede with Security Classification Code): (U) Studies on Occurrence and Significance of Fungi in Burn Wounds of Injured Military Personnel - Development of Laboratory Model (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 71	.68	18.1
FISCAL YEAR CURRENT 72	.68	19.9

19. RESPONSIBLE DOD ORGANIZATION
NAME: US Army Institute of Surgical Research
ADDRESS: Ft Sam Houston, Texas 78234
RESPONSIBLE INDIVIDUAL
NAME: PRUITT, B.A., JR, LTC, MC
TELEPHONE: 512-221-2720

20. PERFORMING ORGANIZATION
NAME: US Army Institute of Surgical Research Clinical Division
ADDRESS: Ft Sam Houston, Texas 78234
PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME: Harold M Bruck, MAJ, MC
TELEPHONE: 512-221-3302
SOCIAL SECURITY ACCOUNT NUMBER:
ASSOCIATE INVESTIGATORS
NAME: F. D. Foley, MD
NAME: R. B. Lindberg, PhD DA

21. GENERAL USE: FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Procede EACH with Security Classification Code): (U) Burns; (U) Fungi; (U) Mycotic; (U) Colonization; (U) Invasion; (U) Alloxan-Diabetes

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

23. (U) Development of an experimental model of fungal burn wound infection is required to define some of the factors which predispose burned patients to this recently recognized complication.

24. (U) Rats burned and seeded with a suspension of spore and hyphal fragments of Rhizopus sp develop superficial and poorly reproducible examples of phycomycotic burn wound infection without systemic dissemination. Since invasive phycomycosis is a recognized although infrequent complication of diabetes mellitus, rats with alloxan-induced hyperglycemia were burned and seeded with Rhizopus sp.

25. (U) 70 07 - 71 06 Rats pretreated with alloxan consistently developed deep burn wound infection with Rhizopus sp. One-third of animals so treated also had disseminated hematogenous lesions in spleen, kidneys, liver and lung. Further studies will define the occurrence, sequence and type of inflammatory response, effects of prior sensitization with Rhizopus spores and the effects of topical therapy with mystatin, on the development or containment of phycomycotic burn wound infection in the model.

*Available to contractors upon originator's approval

ANNUAL PROGRESS REPORT

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

REPORT TITLE: STUDIES ON OCCURRENCE AND SIGNIFICANCE OF FUNGI IN BURN WOUNDS--DEVELOPMENT OF LABORATORY MODEL

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

1 July 1970 - 30 June 1971

Investigators:

Harold M. Bruck, LTC, MC
F. Daniel Foley, M.D.
Robert B. Lindberg, Ph.D.

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

REPORT TITLE: STUDIES ON OCCURRENCE AND SIGNIFICANCE OF FUNGI IN BURN WOUNDS--DEVELOPMENT OF LABORATORY MODEL

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

Period covered in this report: 1 July 1970 - 30 June 1971

Investigators: Harold M. Bruck, LTC, MC
F. Daniel Foley, M.D.
Robert B. Lindberg, Ph.D.

Reports Control Symbol MEDDH-288(R1)

Fungal burn wound infection has continued to occur in 3% of our burn population. Invasive infection of the burn wound with Phycomycetes and Aspergillus sp is attended by a 50% mortality. Twenty-seven per cent of patients with fungal burn wound invasion demonstrate visceral dissemination. Forty rats burned and seeded with a spore suspension of Rhizopus sp developed inconstant and only superficial wound invasion without visceral dissemination. In contrast, 103 burned rats pretreated with alloxan developed consistent deep invasive infection of burn wounds when seeded with Rhizopus spores with frequent direct extension of infection through the entire rat back into abdominal viscera. Deep infection in these animals was accompanied by extensive tissue necrosis. Hematogenous spread to one or more visceral organs was noted in 36% of the alloxan diabetic animals. The pathologic features noted in the diabetic rats closely parallel those seen in the human host.

Infection in these animals was monitored by histologic sections of the burn wound, liver, spleen and kidney. The infecting organisms was recovered on culture in each experiment and a fresh growth of the recovered organism was used to infect succeeding animals, thereby fulfilling Koch's postulates.

Although this Rhizopus sp was shown to be sensitive

to nystatin *in vitro*, topical treatment of the burn wound with nystatin either alone or in combination with mafenide, failed to eradicate wound infection and to prevent vascular invasion and systemic dissemination, whether treatment was begun 24 hours after, 6 hours after, 1.5 hours after or 1.5 hours before seeding of the burn wound. Therefore, while this model is satisfactory for reproducing the pathologic features of fungal burn wound invasion, it does not appear to be satisfactory for the assessment of topical antifungal therapy.

Additional studies are needed and are underway to elucidate the mechanisms of increased host susceptibility to fungal infection in the alloxan diabetic rat and to find another model more suitable for the assessment of effectiveness of topical antifungal therapy.

Burns
Fungi
Mycotic
Colonization
Invasion
Alloxan-Diabetes

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OD 6966	71 07 01	DD-DR&E(AR)636

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

10. NO / CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A061102B71R	01	162
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code) (U) Bacteriologic Survey of Inhalation Therapy Equipment in Burn Unit - Potential Source of Airborne Pneumonia in Burned Troops (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS
003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
70 07	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 71	.43	11.4
FISCAL YEAR — CURRENT 72	.43	12.6

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

RESPONSIBLE INDIVIDUAL
NAME: PRUITT, B.A., JR, LTC, MC
TELEPHONE: 512-221-2720

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

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME: Alan H Morris, MAJ, MC
TELEPHONE: 512-221-4307
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) Nosocomial pneumonia; (U) Nebulized Decontamination; (U) Inhalation Therapy; (U) Nebulizers; (U) Aerosols; (U) Bacterial Contamination

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

23. (U) To define the extent of contamination of inhalation therapy equipment in our burn unit, and to develop effective decontamination programs in order to prevent the generation of bacteria-containing aerosols when nebulizers are used in the course of inhalation therapy treatments.

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

25. (U) 70 07 - 71 06 Forty-six percent of the initial 389 specimens were contaminated with bacteria. Seventeen percent were contaminated with gram negative rods alone, seven percent were contamined with gram negative and gram positive organisms, and twenty-two percent were contaminated with gram positive organisms alone. After the institution of a decontamination program, involing the use of Cidex (gluteraldehyde), acetic adic (nebulized), and ethylene oxide sterilization, the degree of contamination of nebulizer chambers was significantly reduced. Of the last 125 specimens obtained, only 0.8 percent were contaminated with bacteria (only one positive specimen).

*Available to contractors upon originator's approval

ANNUAL PROGRESS REPORT

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

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

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

1 July 1970 - 30 June 1971

Investigator:

Alan H. Morris, M.D., MAJ, MC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

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

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

Period covered in this report: 1 July 1970 - 30 June 1971

Investigator: Alan H. Morris, M.D., MAJ, MC

Reports Control Symbol MEDDH-288(R1)

The purpose of this study was to define the extent of contamination of inhalation therapy nebulizers in use in the burn unit of the US Army Institute of Surgical Research and to develop a decontamination program which would successfully eliminate the generation of bacterial aerosols from such equipment.

Puritan all-purpose nebulizers and Devilbiss ultrasonic nebulizers in use in the US Army Institute of Surgical Research were studied both before and after the application of a decontamination program. The fluid in the nebulizer chamber, or reservoir jars of these instruments, was sampled aseptically at the time the instrument was set up for patient use, and at 2, 4, 6 and 24 hours after use began. The collection of these specimens in no way interfered with the use of these nebulizers in the routine administration of inhalation therapy as commonly practiced at the US Army Institute of Surgical Research. Before decontamination was attempted, there was a 46% incidence of contamination of the reservoir chambers or reservoir jars of these nebulizers. Of 389 specimens collected, 17% were contaminated with gram negative organisms alone, 7% were contaminated with gram negative and gram positive organisms, and 22% were contaminated with gram positive organisms alone. After the institution of a decontamination program suggested by the American Thoracic Society, involving the use of Cidex (glutaraldehyde), nebulized acetic acid, and ethylene oxide sterilization (Am Rev Resp Dis 98:3, 1968), the incidence of

contamination of the reservoir or nebulizer chambers of these instruments fell to 0.8% (of 125 specimens collected most recently, only one was contaminated).

These preliminary results indicate that, as has been found in other institutions, nebulizers used in the administration of inhalation therapy can easily and frequently become contaminated with bacteria. Other workers have demonstrated that such contamination results in the generation of bacteria-containing aerosols and is associated with a striking increase in the incidence of gram negative necrotizing pneumonia occurring in seriously ill hospitalized patients. The institution of an effective decontamination program can effectively eliminate this program.

Inhalation Therapy
Nebulizers
Aerosols
Bacterial Decontamination
Nosocomial Pneumonia
Nebulizer Decontamination

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA 0D 6975	71 07 01	DD-DR&E(AR)636

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

10. NO./CODES.*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A061102B71P	08	065
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)* (U) Study of Tracheal Damage Due to Tracheostomy Tube Cuffs - Development of Improved Device for Use in Burned Soldiers (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*
003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
70 08	71 04	DA	C. In-House

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

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 71	.80	21.2
	CURRENT 72	0	0

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

RESPONSIBLE INDIVIDUAL
NAME: PRUITT, B.A., Jr, LTC, MC
TELEPHONE: 512-221-2720

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

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME:* James Bowen, CPT, VC
TELEPHONE: 512-221-4951
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: Gerald Nash, MAJ, MC
NAME: DA

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Tracheostomy; (U) Tube Cuffs

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 type of tracheostomy cuff that causes the least tracheal injury.

24. (U) The standard latex tracheostomy cuff, a low pressure Sanders cuff, and a newly designed non-inflatable cuff made from fine cell medical grade silicone rubber sponge were surgically placed in the trachea of a series of goats in a manner that would prevent leakage and could be maintained in place for up to 14 days.

25. (U) 70 08 - 71 04 Fifteen goats following tracheostomy were maintained with cuffed tubes in place for 4-14 days. Latex cuffs were inflated and the sponge cuffs used were of sufficient size to prevent leakage at 30 cm water respirator pressure. Both gross and microscopic evaluation after sacrifice revealed tracheal changes which were minimal and much less severe in the goats with the sponge cuffs than the damage caused by the inflatable cuffs tested.

*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. 3A061102B71R-01, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: TRACHEOSTOMY TUBE CUFFS: A COMPARISON OF THREE TYPES IN GOATS

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

1 July 1970 - 30 June 1971

Investigators:

James A. Bowen, CPT, VC
Gerald Nash, M.D., MAJ, MC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

REPORT TITLE: TRACHEOSTOMY TUBE CUFFS: A COMPARISON OF THREE TYPES IN GOATS

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

Period covered in this report: 1 July 1970 - 30 June 1971

Investigators: James A. Bowen, CPT, VC
Gerald Nash, M.D., MAJ, MC

Reports Control Symbol MEDDH-288(R1)

The purpose of this study was to evaluate the type of tracheostomy cuff that causes the least tracheal injury. The standard latex tracheostomy cuff, a low pressure Sanders cuff, and a newly designed noninflatable cuff made from fine cell medical grade silicone rubber sponge were surgically placed in the trachea of a series of goats in a manner that would prevent leakage and could be maintained in place for up to 14 days.

Fifteen goats following tracheostomy were maintained with cuffed tubes in place for 4-14 days. Latex cuffs were inflated and the sponge cuffs used were of sufficient size to prevent leakage at 30 cm water respirator pressure. Both gross and microscopic evaluation after sacrifice revealed tracheal changes which were minimal and much less severe in the goats with the sponge cuffs than the damage caused by the inflatable cuffs tested.

Tracheostomy
Tube Cuffs

TRACHEOSTOMY TUBE CUFFS: A COMPARISON OF THREE TYPES IN GOATS

The production of tracheal injury by tracheostomy tubes and their cuffs is recognized as a serious problem.[1] Erosion and perforation of the tracheal wall by tips of rigid tracheostomy tubes have been common. Silicone rubber tubes, which are soft, flexible, and biologically inert, show great promise in reducing this problem. Injury by cuffs occurs consistently in every human trachea through which ventilatory assistance has been given using a standard cuffed tracheostomy tube for a period of 48 hours or more.[2] A spectrum of lesions occurs at the site of the cuff which commences with superficial tracheitis, progresses to necrosis and loss of cartilage, and in an occasional case, to perforation with tracheo-esophageal fistula. Tracheal stenosis is a common sequela. Cuff size and shape, duration of inflation, and inflation pressure play important roles in the development of this complication. The purpose of this study was to evaluate a new design non-inflatable cuff and to compare its performance with inflatable cuffs.

Materials and Methods

The new design tracheostomy tube cuff, composed of medical grade fine cell silicone rubber sponge, is constructed by wrapping a strip of sponge around a silicone rubber tube and bonding it to the tube with Type A Silastic adhesive. A keel of sponge is bonded to the cuff on the greater curvature for the purpose of filling the muscular groove located on the posterior aspect of the trachea. The tubing under the cuff is thinner than that of the remainder in order to allow the cuff to conform more easily to the elliptical shape of the trachea. A dacron-reinforced strip of silicone rubber sheeting is bonded to the upper end for attachment of ties.

The study was designed to compare the amount of tracheal injury produced by silicone rubber sponge cuffs with that produced by standard design latex cuffs, and low pressure Sanders cuffs.[3]

Five groups of three Angora goats had tracheostomies constructed.[4] Each goat in every group of three received a different type cuff. The two types of latex cuffs were inflated only to the point of sealing,using a Bird Respirator set at

an inspiratory pressure of 30 centimeters of water. The sponge cuff was selected according to size to prevent leakage at the same pressure. All tubes were removed and washed once daily, and inflatable cuffs were reinflated twice daily. In order to maximize damage by all cuffs, hourly deflations (or removal in the case of the non-inflatable cuff) were not done. Mucous secretions were removed twice daily with cotton tipped applicators. Group 1 was sacrificed at 4 days, group 2 at 6 days, group 3 at 8 days, and groups 4 and 5 at 14 days. At sacrifice tracheas were removed, photographed, and submitted for pathological evaluation. By use of a code, the pathologist performed each gross and microscopic evaluation without knowing which cuff was used. The gross evaluation was done by comparing each trachea with the other two in the same group. They were judged best, middle and worst according to the degree of erythema, exudation, and ulceration. Microscopically each trachea was graded individually according to depth of inflammation and necrosis.

Results

The results of the gross and microscopic examinations are given in Tables 1 and 2 respectively. Note that when the silicone rubber cuff was compared to the others with respect to the gross extent of injury it was uniformly associated with the least amount of damage in each of the five experimental groups. On microscopic examination the silicone rubber never caused necrosis extending deeper than the lining epithelium of the trachea. In one animal that was sacrificed after 14 days, there was not even evidence of epithelial necrosis. In contrast both the standard latex and Sanders cuffs usually caused necrosis that extended well into the lamina propria or even down to a cartilage plate.

Conclusion

The performance of the silicone rubber sponge cuff in an animal model shows promise and merits testing in human beings.

References

1. Pruitt BA Jr, Flemma RJ, DiVincenti FC, et al: Pulmonary complications in burned patients: A comparative study of 697 patients. J Thorac Cardiovasc Surg 59:7-20, 1970.

2. Cooper JD, Grillo HC: The evolution of tracheal injury due to ventilatory assistance through cuffed tubes: A pathologic study. Ann Surg 167: 334, 1969.

TABLE 1

Gross evaluation: Comparison of extent of tracheal damage caused by each cuff.

1=best(least damage) 2=middle 3=worst

Group	Standard Latex	Sanders	Silicone Rubber
I	2	3	1
II	3	2	1
III	3	2	1
IV	1	3	1
V	2	3	1
TOTAL	11	13	5

TABLE 2

Microscopic evaluation: Depth of injury in each trachea.

0=no necrosis

1=necrosis of epithelium only

2=necrosis into lamina propria

3=necrosis down to cartilage plate

Group	Standard Latex	Sanders	Silicone Rubber
I	2	2	1
II	3	2	1
III	2	2	1
IV	1	2	0
V	3	3	1
TOTAL	11	11	4

3. Carroll R, Hedden M, Safar P: Intratracheal cuffs; Performance characteristics. Anesthesiology, Vol 31, No 3, Sep 1969.

4. Bowen JA, Nash G, Feldmann RJ: The goat as an artificial ventilation model. Laboratory Animal Science, Vol 21, No. 2, April 1970.

Presentation

Bowen, J.A.: A New Design Silicone Rubber Tracheostomy Tube Cuff. Amer Burn Assoc Mtg, San Antonio, Texas, 17 Apr 1971.

Publications

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0D 6978	71 07 01	DD-DR&E(AR)636

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

10. NO. CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A061102B71R	01	194
b. CONTRIBUTING				
c. CONTRIBUTING				

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

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
70 07	Cont	DA	C. In-House

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

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
FISCAL YEAR PREVIOUS: 71	.1	2.0
FISCAL YEAR CURRENT: 72	.2	4.0

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

20. PERFORMING ORGANIZATION
NAME: US Army Institute of Surgical Research
ADDRESS: Ft Sam Houston, Texas 78234
PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic institution)
NAME: Basil A Pruitt, Jr, LTC, MC
TELEPHONE: 512-221-2720
SOCIAL SECURITY ACCOUNT NUMBER:
ASSOCIATE INVESTIGATORS
NAME: J A Moylan, MAJ, MC
NAME: R B Lindberg, PhD DA

21. GENERAL USE: FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Procede EACH with Security Classification Code): (U) Operating room based infections; (U) Surgical drapes; (U) Surgical gowns

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

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

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

25. (U) 70 07-71 06 "Tightly bonded" material found to resist passage of bacterial broth throughout a 24-hour incubation period. Sheeting had poorer draping characteristics than cotton, and less fluid absorbency with more run-off than cotton drapes. Bacterial barrier maintained throughout surgical procedures and no significant temperature rise noted. The material was generally acceptable to the surgeons. Surgical gowns of synthetic sheeting were also acceptable. More "loosely bonded" material supplied by the manufacturer showed no transmission of bacterial broth during one-hour incubation periods but in occasional test runs passage of Proteus, Klebsiella and Serratia organisms were noted subsequent to one-hour incubation periods. Further clinical evaluation and laboratory testing 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. 3A061102B71R-01, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: EVALUATION OF SYNTHETIC SHEETING AS OPERATING ROOM DRAPE MATERIAL

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

1 July 1970 - 30 June 1971

Investigators:

Basil A. Pruitt, Jr., M.D., LTC, MC
Joseph A. Moylan, Jr., M.D., MAJ, MC
Robert B. Lindberg, Ph.D.

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

REPORT TITLE: EVALUATION OF SYNTHETIC SHEETING AS OPERATING ROOM DRAPE MATERIAL

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

Period covered in this report: 1 July 1970 - 30 June 1971

Investigators: Basil A. Pruitt, Jr., M.D., LTC, MC
Joseph A. Moylan, Jr., M.D., MAJ, MC
Robert B. Lindberg, Ph.D.

Reports Control Symbol MEDDH-288(R1)

Conventional cotton surgical drapes quickly become saturated by water and/or body fluids, thereby losing their bacterial barrier function and serving as potential vehicles of microorganisms capable of causing wound infection. Conventional cotton surgical gowns are also wettable and may permit the passage of bacteria from an operating team member onto the previously sterile operating field. Synthetic material evaluated by others has either lacked satisfactory draping characteristics or has been impermeable to even water vapor, leading to undesirable heat retention. A disposable Spunbonded Olefin-cellulosic laminate material has been shown to have acceptable absorbency and to be permeable to water vapor. Most importantly, the material has been bacteriologically evaluated and found to be virtually impermeable to bacteria even when wet. The originally evaluated drape material was somewhat stiff, and a less tightly matted form of the sheeting is being evaluated. The surgical gown which is of a lighter "weave" is scheduled for bacteriologic study.

Surgical Drapes
Operating Room-Based Infections

EVALUATION OF SYNTHETIC SHEETING AS OPERATING ROOM DRAPE MATERIAL

Surgical drapes may be defined as aseptic materials placed so as to prevent the spread of microorganisms from the environment into an aseptic area, usually the surgical wound. The microorganisms concerned are usually bacterial but fungi may be of importance in the severely injured or debilitated patient. Water is the most common bacterial vehicle in the form of blood, serous exudates, saline, and breath droplets. Easily wettable, cotton drapes readily permit bacterial passage from nonsterile areas into the operating field if, as commonly occurs during surgery, they become wet. Postoperative wound infections represent a significant loss of manpower and money which may well be minimized by use of draping and gowning materials, providing a more satisfactory bacterial barrier. The necessary properties for adequate isolation of a surgical incision by drapes are shown in Table 1, and the desirable, although not absolutely necessary properties, are shown in Table 2.

Materials and Methods

A synthetic sheeting material supplied by the Textile Division of the Dupont Corporation consists of a network of 1/5000th inch diameter polyethylene fibers laminated to a cellulosic material. This Spunbonded Olefin drape weighs 2.1 ounces per square yard, is 8 mills in thickness, has a strip tensile strength of 10.8 pounds per inch and a grab tensile strength of 22 pounds per inch. This material was evaluated in the operating room, both in the form of surgical drapes and surgical gowns. Assessment was made of physician acceptability, draping qualities, body temperature changes during surgery, absorbency, maintenance of integrity, and pre- and postoperative bacterial cultures of the operating field. Further bacteriologic study was carried out in the laboratory. Discs of the synthetic material were placed on agar plates, seeded with bacterial broth cultures and incubated for 24 hours. The test material was then removed and the agar plates incubated for a second 24 hours to detect any transmission of bacteria through the sheeting material. Initial test organisms consisted of Pseudomonas, Enterobacter, Providencia stuartii, Serratia marcescens and Staphylococcus aureus, with Klebsiella pneumoniae, Proteus mirabilis, and E. coli added in the later tests.

Table 1. Necessary Properties of Drapes

1. Impermeability to bacteria and bacterial vehicles
2. Permeability to water vapor
3. Adequate heat transmission
4. Strength to maintain integrity throughout use
5. Pliability with capacity to conform to body and/or wound contours
6. Absence of static electricity
7. No antigenicity or tissue toxicity

Table 2. Desirable Properties of Drapes

1. Easy detectability of loss of protectivity
2. Easy to apply and remove
3. No special storage requirements
4. Indefinite shelf life
5. Flame resistance
6. Esthetic acceptability
7. Heat sterilizable
8. Reasonable cost

Results

The drape and gown material, which are treated with an antistatic agent and have high abrasion resistance, were found to be potentially reusable and both were found to resist passage of water and other fluids well. There was no observed significant body temperature rise suggesting that the material does indeed transmit water vapor and gases. The materials were generally well accepted by the operating surgeons in the test with the qualification that improved draping characteristics of the drapes would be desirable. The surgeons also felt that a somewhat thicker cellulosic material providing greater absorbency and minimizing fluid runoff would be desirable. In six operative cases, four showed negative cultures both preoperatively and postoperatively and one showed a negative preoperative culture with a positive postoperative culture from an area removed from the operative field. The sixth case was positive both on pre- and postoperative culture from an area of open burn wound.

The laboratory, bacteriological testing revealed no passage of Pseudomonas, Enterobacter, Providencia, Serratia or the staphylococcal organisms upon 24-hour incubation on the draping material placed on agar plates. Subsequent modification of the drape to enhance its draping characteristics has necessitated a change in the "tightness" of the weave, and this material has been retested against Serratia marcescens, Pseudomonas aeruginosa, Klebsiella pneumoniae, Enterobacter cloacae, Proteus mirabilis, E.coli, Providencia stuartii,and Staph, aureus.These latter tests revealed occasional penetration of Proteus, Klebsiella and Serratia with no penetration of the other organisms following 4-hour incubation. With none of these test organisms was penetration apparent sooner than one hour following application of the broth culture.

Discussion

The advantages of the Spunbonded Olefin-cellulosic laminate drape material as compared to conventional cotton drapes consist of (1) good resistance to bacterial transmission which, as is apparent from testing of the most recent

material, depends upon the tightness of the weave; (2) permeability to water vapor with no significant impairment of normal body heat loss; (3) strength, with maintenance of integrity during use; (4) flame resistance, and (5) no apparent antigenicity or toxicity. It appears as if excellent draping characteristics and bacterial impermeability may be antithetical, and that some compromise will need to be made in terms of draping characteristics in order to maintain the bacterial impermeability throughout a time period sufficient to encompass virtually all operative procedures.

The disadvantages of the Spunbonded Olefin-cellulosic laminate consist of the draping limitations noted above, the fact that it is difficult to detect loss of barrier function, and greater fluid runoff than with conventional drapes. A thicker cellulosic laminate may correct this relatively minor runoff problem. Further studies are underway to improve the draping characteristics of the synthetic sheeting while simultaneously maintaining its promising bacterial barrier properties.

Publication

Pruitt BA Jr: Surgical dressings and drapes, in, Lecture Outlines, Postgraduate Course on Pre- and Postoperative Care. Engineering in Surgery. 56th Amer Clin Congr, ACS Chicago, 12-16 Oct 70, pp. 7-10.

Presentation

Pruitt BA Jr: Surgical Dressings and Drapes. Postgraduate Course, Pre- and Postoperative Care, Amer Coll of Surgeons Mtg, Chicago, Ill,12-16 Oct 70.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0C 6959	71 07 01	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DOD'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
70 07 01	K.COMPLETION	U	U	NA	NL	[X] YES [ ] NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A061102B71R	01	256
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) Plasma Immunoglobulin Determination in Burned Patients-Changes Affecting Survival 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 01	71 06	DA	C. In-House

17. CONTRACT/GRANT
a. DATE/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 71	0.17	4.5
	CURRENT 72	0	0

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

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

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

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic institution)
NAME: Andrew M Munster, LTC,MC
TELEPHONE: 512-221-5712
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) Immunoglobulin; (U) Immunodiffusion; (U) Immune Response in Burns; (U) Injection in 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 investigate the immunoglobulin levels in the plasma of burned patients and determine whether any patterns exist which may be of diagnostic, prognostic or therapeutic value.

24. (U) Measurements were made on the plasma of burned patients using the method of radial immunodiffusion at under 48 hours postburn, 3 days, 7 days, 14 days and one month postburn. Control values were obtained from normal patients and non-burned surgical patients.

25. (U) 70 07 - 71 06 - Fifty patients were investigated. Serum immunoglobulin G levels were found to be profoundly depressed immediately following injury, and recover towards normal by one month. Depression was more profound in fatal cases and was uninfluenced by burn size. Immunoglobulin M and A levels remained normal. Patients with invasive phycomycosis showed excessive elevation of IgM levels, which returned to normal following eradication of their disease. The study has now been terminated and the information gained is being used in the clinical care of burn patients.

*Available to contractors upon originator's approval

FINAL REPORT

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

REPORT TITLE: PLASMA IMMUNOGLOBULIN DETERMINATION IN BURN PATIENTS

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

1 July 1970 - 30 June 1971

Investigators:

Andrew M. Munster, M.D., LTC, MC
H. Clark Hoagland, M.D.*
Basil A. Pruitt, Jr., M.D., LTC,MC

* Present Address:Mayo Clinic, Minneapolis, Minn. 55901

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

REPORT TITLE: PLASMA IMMUNOGLOBULIN DETERMINATION IN BURN PATIENTS

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

Period covered in this report: 1 July 1970 - 30 June 1971

Investigators: Andrew M. Munster, M.D., LTC, MC
H. Clark Hoagland, M.D.*
Basil A. Pruitt, Jr., M.D., LTC, MC

Reports Control Symbol MEDDH-288(R1)

The determination of serum immunoglobulin levels in burn patients follows recent interest in the surgical world in serum immunoglobulin patterns in other surgical disorders. As Immunoglobulin G carries most antibodies to gram-positive and gram-negative infections, Immunoglobulin M to tuberculosis and some gram-negative infections as well as fungi, and Immunoglobulin A the antibody to most viruses, it was thought possible that there might be a correlation between postburn levels of these immunoglobulins and the presence or absence of various clinical complications due to infection. Accordingly, serial analysis by radial immunodiffusion on 50 patients admitted to the Institute was made, and the levels of these immunoglobulins serially measured in the serum at various intervals up to two months postburn. A very clearcut pattern did indeed emerge whereby Immunoglobulin G levels dropped very markedly immediately postburn and rose slowly to normal after about one month, and Immunoglobulin A and M levels were essentially unaffected. Immunoglobulin G levels did not show a rise when the patient was infected with an appropriate organism whereas Immunoglobulin M did show a sharp response in the presence of a fungal infection. Because of the statistical comparison of immunoglobulin levels and burn size, as well as the changes of levels as related to immunoglobulin molecules molecular weight, it was concluded that the changes observed were not caused by mechanical leakage of proteins from the burn wound but by some as yet unknown mechanism.

After investigating these 50 patients, the following con-

* Present address: Mayo Clinic, Minneapolis, Minn. 55901

clusions were drawn: Immunoglobulin G levels are markedly depressed postburn. A very severe prolonged depression implies a poor prognosis. Antibody levels in the Immunoglobulin G class do not respond well to invasion by microorganisms. Immunoglobulin M remains normal postburn and responds rapidly when invasion occurs by fungi, particularly Phycomycetes. It can be used to gauge the adequacy of surgical treatment as levels drop rapidly back to normal when the invaded area is surgically excised. Immunoglobulin A shows no relevant changes and remains essentially normal. There is no difference in the basic pattern of immunoglobulin concentrations between large burns and small burns. Also, it is of interest that Immunoglobulin G and A behave so differently seeing that they are approximately the same molecular size. Because of these reasons, it was concluded that mechanical leakage of protein was not the sole cause of the observed changes and that other mechanisms are at work. These results have been applied as a clinical aid in the diagnosis and management of burn patients with invading infection.

Immunoglobulin
Immunodiffusion
Immune response in burns
Infection in burns

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0D 6960	71 07 01	DD-DR&E(AR)636

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

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A061102B71R	01	130
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) A Study of Altered Cellular Immunity in Military Personnel Following Thermal and Mechanical Injury (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
71 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 71	.21	5.6
FISCAL YEAR — CURRENT 72	.21	6.1

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

RESPONSIBLE INDIVIDUAL
NAME: PRUITT, B. A., JR, LTC, MC
TELEPHONE: 512-221-2720

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

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME: A. M. Munster, LTC, MC
TELEPHONE: 512-221-5712
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: P Silverstein, MAJ, MC
NAME: R M Katz, MAJ, MC DA

21. GENERAL USE: FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code): (U) Skin Tests; (U) Serum Factors; (U) Immunity; (U) Trauma; (U) Thermal Injury; (U) Graft-Versus-Host Reaction; (U) Hypersensitivity

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 by means of studying lymphocyte function the state of central immunity, following thermal and mechanical injury, in animal models and in patients.
24. (U) The animal model depends on the ability of spleen cells from allogeneic rats to induce a graft-versus-host reaction in the popliteal node of an incompatible rat following footpad injection of the cells. This model can be adapted to investigation of human peripheral lymphocytes postburn. Serum factors - serum from burned patients will be investigated for the presence of soluble factors which are capable of suppressing normal macrophage inhibition, the mixed lymphocyte reaction in vitro, and blast transformation following stimulation by poke-weed antigen. Skin tests - a series of skin tests of patients with thermal injury to Monilia, Trichophyton, and 3-5 Dinitrofluobenzene will be carried out. Patients will be tested immediately postburn and then about two months later.
25. (U) 71 04 - 71 06 In the animal model, it has been shown that the induction of graft-versus-host reaction in the rat popliteal node is a sensitive and reproducible assay system to measure lymphocyte function in the donor. Thermal injury of 30% of the total body surface and mechanical injury induced by dermatome removal of skin from the back of rats has been demonstrated to depress immunological capability of their spleen cells considerably. Recovery occurs, in the absence of complications, within 10 days of injury. Human peripheral lymphocytes induce the same reaction, except that this is a bidirectional reaction. Results on the human studies are not yet available at this time.

*Available to contractors upon originator's approval.

DD, FORM 1 MAR 66 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65 AND 1498-1, 1 MAR 66 (FOR ARMY USE) ARE OBSOLETE.

ANNUAL PROGRESS REPORT

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

REPORT TITLE: A STUDY OF ALTERED CELLULAR IMMUNITY IN MILITARY PERSONNEL FOLLOWING THERMAL AND MECHANICAL INJURY

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

1 April - 30 June 1971

Investigators:

Andrew M. Munster, M.D., LTC, MC
Paul Silverstein, M.D., MAJ, MC
Roger M. Katz, M.D., MAJ, MC*
Luis Canales, M.D., LTC, MC**
Richard F. Mortensen, M.S., Sp5

* Present Address: Allergy Clinic, Brooke General Hospital, BAMC, Fort Sam Houston, Texas 78234

** Present Address: Pediatric Service, Brooke General Hospital, BAMC, Fort Sam Houston, Texas 78234

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

REPORT TITLE: A STUDY OF ALTERED CELLULAR IMMUNITY IN MILITARY PERSONNEL FOLLOWING THERMAL AND MECHANICAL INJURY

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

Period covered in this report: 1 April - 30 June 1971

Investigators: Andrew M. Munster, M.D., LTC, MC
Paul Silverstein, M.D., MAJ, MC
Roger M. Katz, M.D., MAJ, MC *
Luis Canales, M.D., LTC, MC **
Richard F. Mortensen, M.S. Sp5

6. Reports Control Symbol MEDDH-288(R1)

The introduction of a sensitive model which measures the graft-versus-host reaction in popliteal nodes of recipient rats following an injection of donor spleen cells into the hind foot-pad has been instrumental in enabling us to measure changes in lymphocyte function in the donor rat following thermal and mechanical injury. Both these injuries have been found to cause a profound depression in lymphocyte function, which lasts about 10 days, following which recovery occurs. Recovery is slower following thermal injury than that following mechanical injury, but the differences are not statistically significant. The testing of human peripheral lymphocytes in this system is being commenced, and is done parallel with a search for serum factors capable of inhibiting normal molecular phase migration inhibition, a mixed lymphocyte reaction in vitro, and blast

---

* Present Address: Allergy Clinic, Brooke Gen. Hosp., BAMC, Fort Sam Houston, Texas 78234

** Present Address: Pediatric Service, Brooke Gen Hosp., BAMC, Fort Sam Houston, Texas 78234

transformation by poke weed antigen. At the same time, a series of skin tests, involving testing to Monilia, Tricophyton, and 3-5 dinitrofluobenzene is being carried out to test skin delayed hypersensitivity in burn patients.

Trauma
Thermal Injury
Graft-versus-Host Reaction
Delayed Hypersensitivity
Skin Tests
Serum Factors

A STUDY OF ALTERED CELLULAR IMMUNITY IN MILITARY PERSONNEL FOLLOWING THERMAL AND MECHANICAL INJURY

This work involves an extensive investigation of the state of central immunity following thermal and mechanical injury. The investigation was commenced with the establishment of a model in the rat. Parental F344 spleen cells, dose 40×10^6, in TC199 medium and 20% calf serum were injected into the right hind footpad of filial (F344 x Lewis)F1 rats, with only medium injected into the left footpad as a control. Present methods of measuring the GvH reaction in the rat are limited by poor reproducibility and sensitivity. We described here a method which we believe overcomes these difficulties.

Method

Parental (F344) spleen cells, 40×10^6 in TC 199 and 20% calf serum were injected into the right hind footpad of filial (F344 x Lewis)F1 rats, with only medium injected into the left footpad as control. One week later, the draining popliteal node of each leg was excised, weighed, the cells counted and their tritiated thymidine uptake measured. Of the weights, counts, and uptake, the last was the most consistently sensitive measure of increased activity. Five groups of animals were studied as follows:

Group I: P → F1; Group II: F1 → P; Group III: P → F1 but P sensitized by a prior F1 skin graft; Group IV; P → F1, with P radiated with 450 R; Group V: P → F1 with F1 radiated with 450 R.

Results

The mean total node uptakes, with 95% confidence limits, were:
Group I (n = 10) 10,130 (3,600-23,120), control 1,076 (324-3,367, $p < 0.001$);
Group II (n = 10) 35,400 (20,890-58,880), control 1,349 (550-3,311, $p < 0.001$);
Group III (n= 10) 54,080 (30,200-95,500), control 955 (482-2,143, $p < 0.001$);
Group IV (n = 5) 1,750 (955-3,090, control 912 (532-1585, $p < 0.05$);
Group V (n = 5) 1,202 (518-1,941), control 560 (280-1,050, $p > 0.5$).

Conclusion

The activity of the popliteal nodes was heightened by prior sensitization of the parental donors and severely depressed by radiating either the parental donors or the filial recipients, thus conforming to classic theory on the behavior of the GvH reaction.

This model was next employed to study the effect of thermal and mechanical injury on the graft-versus-host reaction. Donor rats were injured either with the standard 30% total body surface scald burn or with the dermatome removal of a corresponding area of skin under anesthesia. Results were as shown in the table.

It can be seen that in this sensitive graft-versus-host assay, a severe depression of rat lymphocyte function occurs following trauma, slightly more marked after thermal than after mechanical injury, although the differences are not statistically significant between these two modalities of injury. Recovery appears to occur within 10 days and is more rapid following mechanical injury than thermal injury.

Next, investigation was undertaken to see if human peripheral lymphocytes could provoke a reaction in the rat popliteal node. Human lymphocytes were drawn in synchronized equipment, separated, counted, and a dose of 15×10^6 cells injected into Lewis F344, F_1 hybrid rats hind footpads, again using the left side as control with just medium injected. It was found that blast transformation in this model occurred with greater rapidity than in the rat model, and that maximum uptake by the popliteal nodes occurred after three days rather than seven days as with the rat cells. The height of the counts, namely, about 20,000-25,000, was of the same order of the counts in the allogeneic rats. This might appear surprising, since heterogeneic reaction is stronger than the allogeneic, unless one remembers that according to clonal selection theory, one population of lymphocytes only is responsible for each reaction and, therefore, maximal stimulation of a single population of lymphocytes would possibly produce the same quantitative amount of blast transformation. We are at present injecting rats with burned patients' peripheral lymphocytes to determine whether a depression in this reaction will occur. One will then need to determine whether the depression is due to loss of immunological capability of the human lymphocyte to react against

Lymphocyte Function Following Thermal and Mechanical Injury

Group	Experiment	n	CPM Experimental Node	CPM Control Node	P	Analysis of Variance
1	1 day postburn	3	1,832	301	<0.01	Gps 1 and 2 vs 7 $f = 17.4$, $p<0.01$
2	4 days postburn	3	3,952	842	<0.01	
3	10 days postburn	3	7,007	1,475	<0.01	Gp 3 vs 7 not significant
4	1 day post-trauma	3	3,636	1,864	<0.01	Gps 4 and 5 vs 7 $f = 6.9$, $p<0.05$
5	4 days post-trauma	3	5,092	3,313	<0.05	
6	10 days post-trauma	3	11,632	1,328	<0.01	Gp 6 vs 7 not significant
7	Uninjured rats	10	10,130	1,076	<0.01	

the rat, or a loss of immunological identity of the human lymphocyte to react against the rat, or a loss of immunological identity of the human lymphocyte, preventing the rat from reacting against it. If such a depression indeed does occur we then plan to treat the human lymphocytes with Mitomycin-C to arrest blast transformation in human lymphocytes without effecting their own immunological identity. Any further depression of the reaction could then be related to a loss of the immunological capability of the human lymphocyte.

Parallel with these tests, human serum is frozen and preserved to assay any serum factor present in thermal injury which may be responsible for immunologic inhibition. Among those functions planned for study are macrophage migration inhibition, mixed lymphocyte reaction, and blast transformation in response to poke weed. A factor which is capable of depressing all these parameters has been found in the serum of patients suffering from chronic mucocutaneous Candidiasis, and it is possible that this factor might be found in the serum of burn patients. Patients will also be subjected to a series of skin tests, one series after admission and one prior to discharge, to Monilia, Tricophyton, and 3-5 dinitrofluobenzene. Monilia and Tricophyton antigen induce a positive skin reaction to 70% of the military population, and the study will aim at determining whether a comparable series of burn patients can react in the immediate postburn stage or, if not, whether they will react upon recovery from the thermal injury. Dinitrofluobenzene, on the other hand, causes no reaction the first time, but induces delayed hypersensitivity to itself, so that all individuals are positive to a second challenge. The purpose of this particular study will be to determine if dinitrofluobenzene can induce a state of delayed hypersensitivity in the postburn phase.

Publications and/or Presentations

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OD 6959	71 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. DIST'N INSTR'N	8A. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
	H. TERMINATION	U	U	NA	NL	[X] YES [ ] NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A061102B71R	01	129
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Procede with Security Classification Code): (U) Inhibition of Lymphocyte Migration in Burns - Model to Assess Immunocompetence Alterations 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
70 08	71 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 71	.17	4.5
	CURRENT 72	0	0

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

RESPONSIBLE INDIVIDUAL
NAME: PRUITT, B.A., JR, LTC, MC
TELEPHONE: 512-221-2720

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

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME: Andrew M Munster, LTC, MC
TELEPHONE: 512-221-5712
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: R F Mortensen, SP5, MS
NAME: DA

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Procede EACH with Security Classification Code)
(U) Immunology; (U) Lymphocytes; (U) Lymphocyte Migration; (U) Burns

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

23. (U) Inhibition of lymphocyte migration has been reported to be an accurate method of assessing cellular immunocompetence in various conditions. The state of cellular hypersensitivity in burn patients and burned animals has been questioned, and the delay in graft rejection and depression of lymphocyte volumes has been reported in the literature. The detection of lymphocyte volumes has been reported in the literature. The detections of migration inhibition among the peripheral lymphocytes of animals and patients, when stimulated with appropriate antigen, would prove that these lymphocytes in the burn patients are immunologically competent; the absence of such migration inhibition would attest immunological incompetence.

24. (U) The following experimental groups were planned: Homogenized subcellular antigenic material was extracted from organs and skin of donor animals. These were injected into genetically disparate-matched recipient animals. Following this procedure, the recipient animals were burned and their lymphocytes incubated with the same antigenic material that had been used to immunize the host rat. The degree of migration of lymphocytes from the test animals were then compared in the presence and absence of antigen. The precise technique of measuring lymphocte migration was that described in 1967 by Soborg and Bendixen, in which lymphocytes were separated from plasma grown in capillary tubes and allowed to migrate in small culture chambers, the area of migration measured by paper planemetry and compared with controls.

25. (U) 70 08 - 71 01 It was discovered that the rat, which is the animal that we have used as the standard burn model at this institution for some years, is singularly unsuitable for the study of migration inhibition. Because of the impracticality of establishing a large scale investigation of burn animals in large numbers other than rats, this study was discontinued.

*Available to contractors upon originator's approval

FINAL REPORT

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

REPORT TITLE: INHIBITION OF LYMPHOCYTE MIGRATION IN BURNS

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

1 July 1970 - 30 June 1971

Investigators:

Andrew M. Munster, M.D., LTC, MC
Richard F. Mortensen, M.S., SP5

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

REPORT TITLE: INHIBITION OF LYMPHOCYTE MIGRATION IN BURNS

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

Period covered in this report: 1 July 1970 - 30 June 1971

Investigators: Andrew M. Munster, M.D., LTC, MC
Richard F. Mortensen, M.S., SP5

Reports Control Symbol MEDDH-288(R1)

When antigenic stimulation of the central immune response occurs, the small lymphocyte undergoes blast transformation and produces a number of soluble factors, one of which is the migration inhibition factor. This factor is so described because it is capable of inhibiting the migration out of the end of a small capillary tube of peritoneal macrophages of various animal species. The inhibition of macrophage migration is, therefore, an index of central immunological competence of tested animals. It was proposed to investigate this model in the burned rat. First, a reliable model of macrophage migration from a capillary tube had to be established in an animal suitable for burning, such as the rat. When rat lymphocytes or peritoneal macrophages were processed in the appropriate manner and placed in capillary tubes, spontaneous migration either did not occur at all, or occurred in such variable and unpredictable manner that the development of an adequate assay was impossible. It was then determined that the guinea pig macrophage was the cell used for such testing by other workers.

Unfortunately, guinea pigs tolerate thermal injury poorly; with a high mortality following anything but a minimal scald burn. Because of these difficulties another assay, reported elsewhere, which more easily quantifies the central immune response in rats has been developed and this study of macrophage migration has been terminated.

Immunology Lymphocyte Migration
Lymphocytes Burns

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6982	71 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
70 07 01	D. CHANGE	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO. CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UN.T NUMBER
a. PRIMARY	61102A	3A061102B71R	01	304
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code) (U) Cell Mediated Immunity in the Experimental Burn - A Laboratory Analogue of Changes Occurring in Burned Soldiers (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS
003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
69 08	70 12	DA	C. In-House

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

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR — PRECEDING 71	0.23	6.1
FISCAL YEAR — CURRENT 72	0.23	6.7

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

20. PERFORMING ORGANIZATION
NAME: US Army Institute of Surgical Research Renal Section
ADDRESS: Ft Sam Houston, Texas 78234
PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME: Karl Eurenius, MAJ, MC
TELEPHONE: 512-221-4264
SOCIAL SECURITY ACCOUNT NUMBER:
ASSOCIATE INVESTIGATORS
NAME: Richard Mortensen, Sp5, M.S.
NAME: DA

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Delayed Hypersensitivity in Burns; (U) Lymphocyte Transformation; (U) Immunity

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

23. (U) To examine quantitatively the cellular immune response of rat lymphocytes after thermal injury, using lymphocyte transformation as an indicator, and phytohemagglutinin (PHA) and tuberculin and the mixed lymphocyte reaction as non-specific and specific immunologic stimuli.

24. (U) In vitro lymphocyte cultures of rat lymph node lymphocytes in 20% fetal calf serum and tritiated thymidine. Effect of burn sera and steroid levels.

25. (U) 70 07 - 71 06 - Enchancement of PHA transformation in lymphocytes from 4 and 10 day old burns, not explained by differences in cell viability, rate of culture cell death, hypercorticosteroidism or burn sera factors. Specific transformation studies have been unsuccessful but studies using other investigative technics are planned.

*Available to contractors upon originator's approval

DD FORM 1 MAR 66 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65 AND 1498-1, 1 MAR 66 (FOR ARMY USE) ARE OBSOLETE

ANNUAL PROGRESS REPORT

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

REPORT TITLE: CELL MEDIATED IMMUNITY IN THE EXPERIMENTAL BURN

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

1 July 1970 - 30 June 1971

Investigators:

Karl Eurenius, M.D., MAJ, MC
Richard F. Mortensen, M.S., SP5

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

REPORT TITLE: CELL MEDIATED IMMUNITY IN THE EXPERIMENTAL BURN

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

Period covered in this report: 1 July 1970 - 30 June 1971

Investigators: Karl Eurenius, M.D., MAJ, MC
Richard F. Mortensen, M.S., SP5

Reports Control Symbol MEDDH-288(R1)

The status of cellular immunity following thermal injury has not been well defined. This mechanism is certainly crucial since it is either a closely related or the causal effector in host defense against many pathogenic organisms, and graft rejection.

The purpose of this study is to quantitate the lymphocyte response to specific and nonspecific stimuli. The response used is lymphocyte growth or transformation as measured by triatiated thymidine incorporation (DNA synthesis), in rat lymph node lymphocyte cultures from animals sacrificed 1, 24, 96, and 240 hours after a 30% scald burn. Cultures were grown in 20% fetal calf serum with Difco medium TC199.

The stimuli used were phytohemagglutinin (PHA), a non-specific mitogen, and tuberculin protein, a specific antigen to which animals had been previously sensitized.

In a series of eight matched experiments, it was demonstrated that lymphocytes from burned animals had an enhanced response to PHA. This enhanced response was most prominent 96 hours postinjury when thymidine incorporation was roughly five times control PHA levels, and was still present twice control levels at 240 hours. Lymphocytes from burned animals were

similar to control cells with regard to cell number in culture and cell viability. Rat serum from both control and burn animals had equal inhibitory effects on thymidine incorporation. Serum corticosterone levels were markedly elevated one hour and again 96 hours postburn and were regarded as results of "release" and "synthesis" activity. Elevated corticosterone levels induced in normal rats with ACTH administration did not result in subsequent augmentation of the PHA response.

Pending information regarding specific antigenic response the following conclusions can be made.

Lymphocyte transformation to PHA is enhanced in the burned rat. This enhancement is maximum 96 hours postinjury and is still present at 10 days. Rat PHA lymphocyte transformation is depressed equally with normal and burned rat serum. Elevated corticosterone levels observed in burned rats both immediately and 4 days postburn are not responsible for this enhanced response, which might reflect either increased cellular immunity or "sterile activation" following thermal injury.

Specific *in vivo* immune reactions have not been successful in the rat model. Preliminary *in vivo* studies, using the one way GVH reaction, indicate suppression of this response after thermal injury.

Delayed Hypersensitivity in Burns
Lymphocyte Transformation
Immunity

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
	DA OD 6381	71 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
70 07 01	H. TERMINATION	U	U	NA	NL	[X] YES [ ] NO	A. WORK UNIT

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A061102B71R	01	309
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code) (U) Homograft Immunity in Burns-Models of Changes in Immunity Affecting Use of Physiologic Dressings 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 09	71 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 71	0.17	4.5
	CURRENT 72	0	0

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

20. PERFORMING ORGANIZATION
NAME: US Army Institute of Surgical Research
Burn Study Branch
ADDRESS: Ft Sam Houston, Texas 78234
PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME: Andrew M Munster, LTC, MC
TELEPHONE: 512-221-5712
SOCIAL SECURITY ACCOUNT NUMBER:
ASSOCIATE INVESTIGATORS
NAME:
NAME: DA

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Immunity; (U) Allograft; (U) Homograft; (U) Rejection; (U) Transplantation; (U) Defense Mechanisms

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

23. (U) Skin homograft depression following thermal injury is well documented in the literature.Whether this is an expression of the depression of central immune response or alternately merely a defect in the afferent arc from skin to the seat of the central immune response is unknown.A series of internal organ grafts was carried out to see if this defect could be localized.A series of xenografts was also carried out to see if really strong sensitization could overcome whatever defect there may be.Finally,systemic sensitization by a subcellular antigenic extract was carried out to see if a second-set rejection could be precipitated.

24. (U) Three models have been developed for the study of this problem:(a)a rat model, employing spleen slices on the renal cortex;(b) a model employing pig heterograft on the back of Sprague-Dawley rats; and (c) the same model with sensitization of the recipient rat with subcellular extracts obtained from the pig skin.

25. (U) 70 07 - 71 07 This study is concluded.It has been found that a 30% total body surface thermal burn in a recipient rat does not influence its ability to reject spleen slice allograft on its own renal cortex,as judged by histological criteria,after 10 days. Similarly,canine and porcine skin xenografts on the back of burned rats were rejected in the normal fashion,both first and second sets.This held true whether the grafts were placed on the burn area or not.Finally,sensitization by the systemic injection of subcellular antigenic extracts did produce a second-set rejection in these skin grafts,again regardless of whether the burn was present or not.It was concluded that thermal injury does not influence the rate of rejection of internal organ grafts or of skin grafts strong enough to provide major antigenic challenge,and that the central immune response,as measured in this model was capable of mounting a second-set rejection.

*Available to contractors upon originator's approval

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

FINAL REPORT

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

REPORT TITLE: HOMOGRAFT IMMUNITY IN BURNS--MODELS OF CHANGES IN IMMUNITY AFFECTING USE OF PHYSIOLOGIC DRESSINGS

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

1 July 1970 - 30 June 1971

Investigator:

Andrew M. Munster, M.D., LTC, MC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

REPORT TITLE: HOMOGRAFT IMMUNITY IN BURNS--MODELS OF CHANGES IN IMMUNITY AFFECTING USE OF PHYSIOLOGIC DRESSINGS

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

Period covered in this report: 1 July 1970 - 30 June 1971

Investigator: Andrew M. Munster, M.D., LTC, MC

Reports Control Symbol MEDDH-288(R1)

A 30% total body surface thermal burn in a recipient rat does not influence its ability to reject a spleen slice allografted onto its own renal cortex, as judged by histological criteria after 10 days. Similarly, canine and porcine skin xenografts on the back of rats were rejected in the normal fashion, both first set and second set, in a manner totally uninfluenced by the presence or absence of a burn. This held true whether the grafts were placed on the burn area or not. Finally, sensitization by the systemic injection of subcellular antigenic extracts did indeed produce a second-set rejection in these skin grafts, again regardless of whether the burn was present or not. It was concluded that thermal injury does not influence the rate of rejection of internal organ grafts or of skin grafts strong enough to provide major antigenic challenge, and that the central immune response, as measured in this model, was capable of mounting a second-set rejection.

Immunity	Rejection
Allograft	Transplantation
Homograft	Defense Mechanisms

HOMOGRAFT IMMUNITY IN BURNS--MODELS OF CHANGES IN IMMUNITY AFFECTING USE OF PHYSIOLOGIC DRESSINGS

Three sets of experiments were carried out to test the ability of burned rats to reject allografts and xenografts. Firstly, a thin slice of donor spleen was transplanted onto the cortex of the kidney of recipient rats, using Lewis and BN rats, which are different at the strong AGB locus. The operation was done as a laparotomy under general anesthesia using pentobarbital, following which the kidney bearing the graft was carefully replaced in the abdomen and the abdomen closed. Evaluation, using a scoring system, was done histologically following sacrifice of the animals 10 days after surgery. A score of 0 was given for total rejection and 6 for perfect survival. Recipient animals were then given a 30% total body surface burn one day, four days, or 10 days prior to grafting and the results evaluated. Controls included isografts from Lewis to Lewis and from F_1 to F_1. The experimental animals consisted of Lewis X BN)F_1 donors to Lewis recipients; this was done in order to eliminate histological confusion by the donor spleen causing a rejection reaction in the recipient kidney. In this way, a unit rejection reaction by the host against the donated kidney or spleen was the only reaction observed. In the second group of experiments, canine skin xenografts onto rats with 20% and 30% burns were carried out, and porcine skin xenografts onto rats with 30% burns. In another group of experiments, rat skin allografts were carried out on rats with 30% burn but this allograft was not placed on the burn area, rather it was placed away from it. Next, sensitization was carried out by injecting the recipient rat intraperitoneally with subcellular antigenic abstract manufactured from either pigskin if the graft was pigskin, or dog spleen if the graft was dog skin. Results are shown in the table.

Conclusion

Thermal injury in these models did not adversely affect the normal development of the rejection process. In the internal graft model, spleen slices were rejected in the normal fashion, as assessed 10 days postburn. Xenografts were also rejected in the normal fashion, and second-set rejection occurred in an accelerated fashion whether the mode of presensitization was a previous skin graft or the injection of subcellular antigenic extract. It is therefore concluded that (1) the central immune

GRAFT REJECTION IN EXPERIMENTAL THERMAL INJURY

	Score
1. Spleen slice grafts on renal cortex	
(Lewis x BN) F_1 donors to Lewis recipients	1.6
30% burn 10 days prior to graft	2.2
30% burn 4 days prior to graft	2.0
30% burn 1 day prior to graft	1.6
Control groups (isografts, with and without burns)	4.2 - 5.4 **
	Rejection Day
2. Canine skin xenografts on rats, 20% burn	8.66 ± 0.46
Control	8.00 ± 0.75
3. Canine skin xenografts on rats, 30% burn	6.4 ± 0.8
Control	8.8 ± 1.1*
4. Porcine skin xenografts on rats, 30% burn	6.6 ± 1.2
Control	11.1 ± 2.0 **
5. Rat skin allografts, 30% burn	6.83 ± 0.48
Control	10.0 ± 0.81 ***
6. Second set rejection; canine xenograft on rats, 30% burn	4.66 ± 0.46
Control	4.88 ± 0.44
7. Rejection following sensitization	
Porcine skin xenograft, single immunization	6.1 ± 2.5
Control	6.0 ± 2.5
Porcine skin xenograft, serial immunization	4.2 ± 2.2
Control	4.6 ± 4.3
Canine skin xenograft, single immunization	2.8 ± 1.3
Control	10.2 ± 2.7 **

response is either not depressed or depressed only for a very short period following thermal injury and it recovers enough to mount an effective rejection reaction of internal spleen slices by 10 days; (2) the central immune mechanism is capable of mounting a second-set response.

Publications and/or Presentations

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0A 6382	71 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
70 07 01	D. CHANGE	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A061102B71R	01	117
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) Hemodynamic Changes in the Early Postburn Period - Observations 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
65 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 71	0.17	4.5
	CURRENT 72	0.47	13.7

19. RESPONSIBLE DOD ORGANIZATION

NAME: US Army Institute of Surgical Research

ADDRESS: Ft Sam Houston, Texas 78234

RESPONSIBLE INDIVIDUAL

NAME: Basil A Pruitt, Jr, LTC, MC

TELEPHONE: 512-221-2720

20. PERFORMING ORGANIZATION

NAME: US Army Institute of Surgical Research Clinical Division

ADDRESS: Ft Sam Houston, Texas 78234

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

NAME: Thomas W. Newsome, Maj, MC

TELEPHONE: 512-221-2943

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Basil A Pruitt, Jr, LTC, MC

NAME: P William Curreri, LTC, MC DA

21. GENERAL USE: FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code): (U) Burn; (U) Blood volume; (U) Cardiovascular system; (U) Resuscitation fluids

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 changes in blood volume and other cardiovascular indices in extensively burned patients during the early postburn period and to determine the adequacy and effectiveness of resuscitation fluids.

24. (U) Selected patients with extensive burns greater than 50% total body surface admitted within the first 24 hours postburn were followed by means of serial measurement of blood volume, vital signs, and serum and urine chemistries.

25. (U) 70 07 - 71 06 - Five patients with extensive burns over greater than 50% total body surface and 100% mortality rate were studied. Resuscitation fluids in three patients were administered in excess of that predicted for 50% burn, more closely approximating that predicted for the exact burn size. In all five patients resuscitation was adequate as measured by blood volume, pulse rate and urine volume. The blood volumes were initially depressed and began to return toward normal in the second 24 hours postburn as reported in the previous annual report. In only one patient was a post resuscitation diuresis documented. This was associated with continued administration of high volumes of intravenous fluids.In three patients a marked natriuresis accompanying resuscitation disappeared 12 hours after the administration of salt containing solutions was discontinued.

*Available to contractors upon originator's approval

ANNUAL PROGRESS REPORT

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

REPORT TITLE: HEMODYNAMICS OF THE EARLY POSTBURN PATIENT

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

1 July 1970 - 30 June 1971

Investigators:

Thomas W. Newsome, M.D., MAJ, MC
P. William Curreri, M.D., LTC, MC
Martin G. White, M.D., MAJ, MC
Basil A. Pruitt, Jr., M.D., LTC, MC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

REPORT TITLE: HEMODYNAMICS OF THE EARLY POSTBURN PATIENT

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

Period covered in this report: 1 July 1970 to 30 June 1971

Investigators: Thomas W. Newsome, M.D., MAJ, MC
P. William Curreri, M.D., LTC, MC
Martin G. White, M.D., MAJ, MC
Basil A. Pruitt, Jr., M.D., LTC, MC

Reports Control Symbol MEDDH-288(R1)

The adequacy of the Brooke Burn Formula for fluid resuscitation was evaluated by serial measurements of blood volume, vital signs, and serum and urine chemistries in five patients with burns greater than 50% of the total body surface. Each arrived at the US Army Institute of Surgical Research within the first 24 hours postinjury. In three patients, fluids were administered qualitatively in accordance with the Brooke Burn Formula but quantitatively in approximation to their true burn size rather than the 50% maximum suggested by the formula. In one the burn size was 50% and fluids were given accordingly. In the fifth, a 68% burn, the fluid volume was calculated based on a 50% burn but the proportion of colloid to electrolyte was increased. No resuscitative deaths occurred. Blood volumes were initially depressed, beginning to return to normal in the second 24 hours postburn as reported in Annual Report 1970. Serum electrolytes and blood gases were unremarkable. The two patients with burns greater than 75% of total body surface had initial and persistent elevations of serum creatinine and BUN levels and depressed creatinine clearances. In only one patient was a postresuscitative diuresis documented. This was associated with the continued administration of high volumes of intravenous fluids. In three patients a marked natriuresis accompanied resuscitation, disappearing twelve hours after the administration of salt-containing solutions was discontinued.

Burn
Blood Volume
Cardiovascular System
Resuscitation Fluids

HEMODYNAMICS OF THE EARLY POSTBURN PATIENT

As a part of the continuing evaluation of the adequacy of resuscitation with the Brooke Burn Formula for fluid administration during the first 48 hours postburn, parameters of cardiovascular and renal function were monitored in patients admitted to the US Army Institute of Surgical Research less than 24 hours following thermal injury of at least 50% of their total body surface. Previous cardiovascular studies had documented an initial obligatory plasma volume loss with spontaneous repletion in the second 24-hour postburn period.[1]

Method

Five previously healthy young adult males with burn size ranging from 50-87% of the total body surface (average 68%) were studied. Three patients were administered fluids qualitatively according to the Brooke Burn Formula but quantitatively received a volume calculated on true burn size. The fourth patient was given fluids appropriate for his 50% burn. In the fifth patient, with a 68% burn, fluid volume was calculated for a 50% burn but with a disproportionately high colloid to electrolyte ratio. After 48 hours, salt free fluids were administered except for treatment of hypotension or low urine volumes suggesting hypovolemia. Indwelling arterial catheters were inserted in two with measurement of arterial pressure and cardiac output. Because of technical difficulties these measurements were discontinued in the next three patients. Measurements of weight, pulse, and respiration; $R^{131}ISA$ blood volumes counted in the Volemetron; hematocrit; serum Na, K, Cl, CO_2, creatinine, osmolality and UUN were monitored in each patient at 6-8 hour intervals for the first five days postburn. Excretion rates of Na, K, Cl and creatinine in the urine were calculated.

Results

No deaths occurred in the resuscitation stage of postburn care. All patients in the study group eventually succumbed to their injuries, the postburn day of death ranging from 6 to 9. All deaths were attributable to pulmonary complications.

Body weight in four patients revealed an increase from 2-10 kg occurring over the first three days postburn with subsequent weight loss.

In three of four patients blood volume in the first 24 hours was less than the predicted normal. In each of these patients and in the fifth, whose initial determination was in the second 24 hours, blood volume rose progressively during the second 24 hours.

Hematocrits fell progressively in all patients. Serum sodium and chloride concentrations were normal in four patients and high in one during the first 72 hours postinjury. The last patient had a deep third degree burn over 87% of his total body surface with a high predicted evaporative water loss. Serum electrolyte values after 72 hours followed no consistent pattern. Serum potassium concentrations were within normal limits except for high values noted 30 hours postburn in one patient with decreased renal function on admission. Serum bicarbonate concentration fell below 20 mEq/L within 72 hours in two patients, within 96 hours in two patients, and remained normal in one. Significant correlations with type of topical therapy, i.e., carbonic anhydrase inhibiting or noncarbonic anhydrase inhibiting sulfa creams, could not be made.

Creatinine concentrations and BUN remained normal during the resuscitation of three patients. In two, admission levels were significantly elevated. The latter were the largest burns in the group, i.e., 75% and 87%.

Arterial pH was increased for at least 48 hours in all patients following institution of fluid resuscitation. These values were associated with low pCO_2's as previously reported. In all five patients subsequent respiratory failure was associated with diminished pH levels and a rising pCO_2. Arterial pO_2 was normal during the first 72 hours postburn. Subsequent hypoxia occurred with pulmonary insufficiency.

Urine volumes during the first 48 hours ranged from 25-170 cc/hour. The former levels were obtained in the patient resuscitated with fluids restricted to a 50% burn size despite a true 68% burn. A postresuscitation diuresis disproportionate to postresuscitative intravenous fluid administration occurred in no patient. In the one patient whose urine volume began to rise 48-72 hours postburn, the associated rate of intravenous fluid administration remained at intraresuscitative levels.

Creatinine clearances were low in the two largest burns. Sodium excretion in three patients were greater than 60 microequivalents per minute during the 48-hour resuscitative period,

dropping to less than 5 microequivalents per minute in two of the three within twelve hours after discontinuing administration of salt containing solutions. The third of these patients had persistent hypernatremia and with continued administration of a large volume of fluids postresuscitation had a persistent natriuresis with an even more pronounced diuresis. Chloride excretion paralleled or remained below sodium excretion. Potassium excretion ranged from 60-130 microequivalents per minute throughout the resuscitative phase and into the post-resuscitative phase in each patient. Urinary urea nitrogen and osmolality levels were unremarkable.

Discussion

Survival into the sixth postburn day demonstrated the adequacy of initial fluid resuscitation in these five patients with extensive burns. Parameters of cardiovascular and renal function reflecting adequate resuscitation were rising blood volume during the second 24 hours and sustained urine output. The weight gain and hematocrit fall have been previously documented. Serum chemistries were normal except for depressed renal function studies in two patients. With resuscitation no further increase in the BUN or creatinine occurred in either. Arterial gases and pH were unremarkable.

Urine volumes were adequate in each patient. In three patients they ranged from 30-190 cc per hour. Each was administered fluids according to true burn size without the 50% maximum suggested by the Brooke Burn Formula. In the two patients given fluids for a 50% burn, the urine volumes were lower, 20-120 ml/hr, but resuscitation was no less satisfactory as evidenced by blood volume and vital signs. All five patients had pulmonary deaths, making the postresuscitative significance of the different resuscitative fluid loads difficult to assess.

The appearance of a postresuscitative increase in urine volume in only the single patient with a continued high intravenous input attests to the propriety of decreasing the rate of fluid administration on the second postburn day. With a return to normal capillary permeability, the mobilization of extravascular resuscitation fluids is expected. Although theoretically this could reach high levels, in no patient in this study was an excessive curtailment of intravenous fluid administration necessary to maintain a urine output less than 100 ml/hr. This may reflect the large burn size with the excessive evaporative water loss seen in this particular patient group.

The natriuresis in three patients probably reflects favorably on the adequacy of electrolyte administration. The equally significant sodium conservation after discontinuing salt administration would suggest that sodium lost extravascularly during early resuscitation does not re-enter the vascular space as rapidly as previously thought. It may also reflect inadequate sodium administration after the classic 48-hour resuscitation period after which the Brooke Burn Formula prescribes only salt free fluids except for specific indications.

Most importantly this study has shown the relevance of systematic observation of parameters such as blood volume and urinary sodium excretion. These are readily obtainable, and added to the urine volumes and measurable vital signs should lead to a more accurate assessment of the physiologic status of the extensively burned patient.

Summary

Serial measurements of blood volume, vital signs, and serum and urine chemistries were made in five patients with lethal burns of greater than 50% of the total body surface. No resuscitation period occurred. Resuscitation as measured by blood volume and urine output was adequate in all patients. In three patients fluids were administered according to true burn size rather than restricted to those for a 50% burn as described by the Brooke Burn Formula. In only one patient was a post-resuscitative diuresis documented. This was associated with a necessary continued administration of high fluid volumes. The natriuresis accompanying resuscitation seemed to cease abruptly when patients were given no electrolyte solutions. Blood volume and urine sodium excretion are easily obtainable data which are useful in monitoring the extensively burned patient.

Reference

1. Pruitt BA Jr, Mason AD Jr, Moncrief JA: Hemodynamic changes in the early postburn patient: The influence of fluid administration and of a vasodilator (Hydralazine). J. Trauma 11:36-46, 1971.

Publication

Pruitt BA Jr, Mason AD Jr: Hemodynamic studies of burn patients during resuscitation. Proc Third Internatl Congress for Research in Burns, P.Matter, T.L. Barclay, Z. Konickova (eds), Hans Huber Publishers, 1971, pp 83-87.

Presentation

Pruitt BA Jr: Hemodynamic studies of burn patients during resuscitation. Presented to Third International Congress for Research in Burns, Prague, Czechoslovakia, 21 Sep 1970.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0D 6970	71 07 01	DD-DR&E(AR)636

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

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A061102B71R	01	189
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Procede with Security Classification Code): (U) Hypertonic Lactated Saline Resuscitation in Thermal Injury - Evaluation of Modified Fluid Therapy for Burned Soldiers (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
70 09	Cont	DA	C. In-House

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

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 71	.62	16.5
FISCAL YEAR CURRENT 72	.4	11.7

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

RESPONSIBLE INDIVIDUAL
NAME: PRUITT, B.A., JR, LTC, MC
TELEPHONE: 512-221-2720

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

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic institution)
NAME: Joseph A Moylan, Jr, MAJ, MC
TELEPHONE: 512-221-2943
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: J M Reckler, MAJ, MC
NAME: A D MASON, JR, MD DA

21. GENERAL USE: FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Procede EACH with Security Classification Code): (U) Hypertonic Lactated Saline; (U) Thermal; (U) Brooke Formula

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

23. (U) Evaluate the naturesis effect of hypertonic lactated saline resuscitation in thermal injuries.

24. (U) Using four groups of dogs, 40% scald burns were administered. Group I were controls. Group II were resuscitated according to the Brooke formula; Group III dogs by one-half the Brooke formula; Group IV by 300 mEq/L lactate saline solution. Parameters studied were hematocrit, serum and urine sodium and potassium, serial cardiac outputs and plasma volumes, serum pH, heart rate and blood pressure, sodium balance and weight changes for a 24 hour period.

25. (U) Group I dogs received no resuscitation. Group II dogs received 10.8 mEq Na/Kg, and Group IV received 12.0 mEq Na/Kg while the one-half Brooke formula animals received 5.4 mEq Na/Kg. In all dogs except controls, resuscitation appeared adequate using the parameters noted above. Cardiac outputs and plasma volumes returned to pre burn levels by 24 hours in Groups II, III and IV. Serum Na levels were significantly higher in Group IV as compared to the other animals. Serum K were similar in all groups. Urine flows were also not significantly different in all groups. The animals receiving HLS solution excreted urine sodium loads approximately ten times larger than the other resuscitated groups. Group II and III retained 94% and 92% respectively of the administered Na load while Group IV retained only 64%. However, Group III had the lowest net Na gain. Group IV showed over all weight loss whereas Groups II and III had weight gains of 3.5% and 2.4% respectively. Studies are in progress to evaluate increasing concentrations of sodium with HLS resuscitation and to measure the fluid shifts using labelled isotopes.

*Available to contractors upon originator's approval

ANNUAL PROGRESS REPORT

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

REPORT TITLE: HYPERTONIC LACTATED SALINE RESUSCITATION IN THERMAL INJURY

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

1 July 1970 - 30 June 1971

Investigators:

Joseph A. Moylan, Jr., M.D., MAJ, MC
Jon M. Reckler, M.D., MAJ, MC
Arthur D. Mason, Jr., M.D.

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

REPORT TITLE: HYPERTONIC LACTATED SALINE RESUSCITATION IN THERMAL INJURY

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

Period covered in this report: 1 July 1970 - 30 June 1971

Investigators: Joseph A. Moylan, Jr., M.D., MAJ, MC
Jon M. Reckler, M.D., MAJ, MC
Arthur D. Mason, Jr., M.D.

Reports Control Symbol MEDDH-288(R1)

Clinical reports have suggested the value of hypertonic lactate saline in the resuscitation of burn patients. A study was undertaken to evaluate this mode of resuscitation against conventional isotonic resuscitation. Four groups of dogs subjected to a 40% total body surface scald burn and resuscitated by one of four methods: control, Brooke formula, one-half Brooke formula and 300 mEq Na/L lactate saline. All dogs receiving fluids were adequately resuscitated using the usual clinical parameters. The hypertonic lactate saline resuscitation effected a natriuresis and weight loss with retention of 64% of the administered dose. However, the one-half Brooke formula dogs had the lowest net Na gain.

Studies are in progress to evaluate increasing concentrations of Na with HLS resuscitation and to measure fluid shifts in HLS using labelled isotopes.

Hypertonic lactated saline
Thermal
Brooke formula

HYPERTONIC LACTATE SALINE RESUSCITATION IN THERMAL INJURY

Continued interest in the optimal mode of fluid therapy for the successful treatment of burn shock has led to the study of many different intravenous fluids. No single fluid or combination of fluids currently in use stands out as being clearly superior in treating previously healthy young adults. These patients have a great physiologic reserve permitting accommodation to extremes of volume and composition of resuscitation fluids. In these patients it is unusual to encounter serious early problems secondary to adequate resuscitation except in very large burns. Children, older individuals and those with limited physiologic reserve, when thermally injured, often experience complications associated with cardiovascular overload and excessive fluid and sodium retention.

The search for a better mode of fluid resuscitation has been directed toward effecting minimal positive fluid and sodium load while simultaneously providing adequate resuscitation. Secondary desirable qualities of this new solution would be to minimize wound and peripheral edema, eliminate the need for colloid, diminish postburn intestinal ileus, and maintain good urine flow. Recent investigations using hypertonic lactate saline solution for resuscitation suggest that this fluid may fulfill these requirements.[1]

The purpose of this study was to evaluate hypertonic lactate saline solution for resuscitation in thermal injury and to compare it with isotonic resuscitation according to the Brooke formula. In addition, the study was designed to evaluate the individual effects of volume and sodium loading by administering similar sodium loads in unequal fluid volumes.

Methods

The experimental design was as follows: 20 healthy, adult, mongrel dogs in which weights ranged from 10 to 22 kg were randomly assigned to four treatment groups, five dogs in each group. Resuscitation for each group was as shown in Table 1. Group I received no resuscitation. Group II was resuscitated according to the Brooke formula, receiving a total of 2 ml/kg/% burn in an electrolyte/colloid ratio of 3:1 in addition to daily maintenance water requirements which were calculated on the basis of 50 ml/kg body weight of the dog. Group III was resuscitated with fluid volume equivalent to one-half of that predicted by the Brooke formula, that is, 1 ml/kg/% burn, with a 3:1 electro-

lyte/colloid ratio plus, daily maintenance water requirement of 50 ml/kg. Group IV received hypertonic saline solution in a volume of 1 ml/kg/% burn. The lactate saline solution was prepared under sterile conditions and was composed of 300 mEq of sodium, 200 mEq lactate and 100 mEq of chloride.

Table 1. Treatment Groups

Group I	- No resuscitation
Group II	- Brooke formula 2 ml/kg/% burn + maintenance water
Group III	- 1/2 Brooke formula 1 ml/kg/% burn + maintenance water
Group IV	- Hypertonic lactate saline (300 mEq/L) 1 ml/kg/% burn

Dogs were conditioned to metabolic cages for three days prior to each study. Twenty-four hours before each study, dogs were subjected to a brief intravenous brevane anesthesia for insertion of two central arterial and venous catheters. The dogs were returned to the metabolic cages and when awake allowed oral alimentation until 12 hours prior to the study at which time only access to water was permitted. Measured parameters included cardiac output, plasma volume, heart rate, blood pressure, hematocrit, arterial pH, serum sodium, potassium and chloride. All were measured before the injury and again at one, 6, 12 and 24 hours postinjury, except for the plasma volumes which were measured prior to the burn and at 8 and 24 hours postburn. Twenty-four hour urine collections were analyzed for volume, sodium, potassium and chloride content. Body weight was recorded at the initiation and completion of each experiment.

Following baseline measurements the dogs were lightly anesthetized with intravenous brevane, subjected to a 40% third-degree scald burn and returned to the metabolic cages and allowed to wake up. All dogs were awake within 20 minutes of the time of the burn. One hour postburn, indices were again measured and then treatment was begun according to the group to which the dogs were assigned. Dogs in Groups II and III received their fluid at rates recommended by the Brooke formula, that is, half during the first 8 hours postinjury and the remaining half during the subsequent 16 hours. The dogs in Group IV received the hyper-

tonic solution at a constant rate between one and 24 hours postburn. No further anesthesia or analgesia was administered.

Results

The volume and sodium loads for each treatment group are presented in Table 2. Group I received no fluids. The sodium load was very nearly equal for Group II, the Brooke formula group, and Group IV, the hypertonic saline group, though their volume loads were quite different. The sodium load for the half-Brooke formula group, Group III, was half that of the Brooke formula group and the volume administered roughly half way between the Brooke formula and hypertonic saline groups.

Table 2. Na+ and Volume Load

Group	I	II	III	IV
Na+ (mEq/kg)	0	10.88	5.44	12.0
Volume (ml/kg)	0	130	90	40

All dogs in each of the four treatment groups survived the 24-hour postburn period without difficulty. All dogs receiving intravenous fluids appeared adequately resuscitated and remained calm and alert. There were no differences among treatment groups with regard to changes in heart rate or blood pressure.

The mean arterial hematocrit for each treatment group at the various points in time are shown in Figure 1. For each group, there was a rise in hematocrit during the first hour postburn. Resuscitation produced a progressive decrease in the hematocrits of Groups II,III, IV, while the unresuscitated control group, I, became more hemoconcentrated.

Inspection of the mean arterial pH, Figure 2, reveals little difference between treatment groups at any point in time; the values for the three resuscitated groups of dogs are consistently above those for the unresuscitated control group. The mild alkalosis in the three resuscitated groups is anticipated and is of no clinical significance.

The outstanding difference in serum sodium concentrations, Figure 3, is between that of Group IV, which received the hyper-

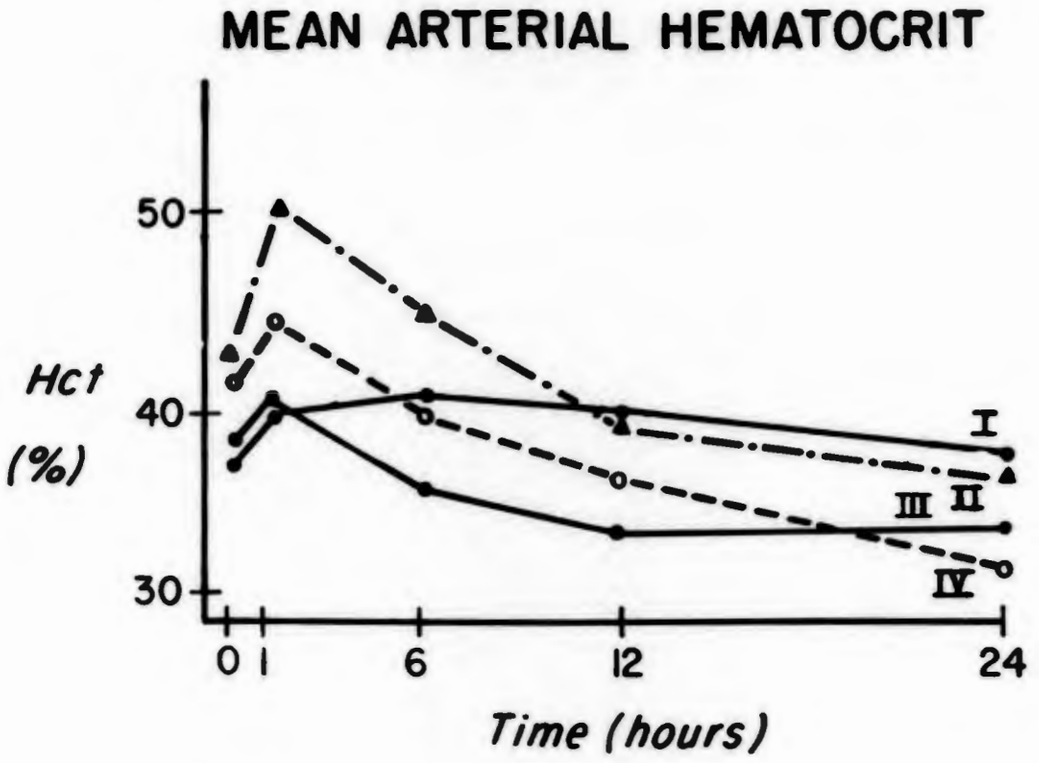

MEAN ARTERIAL HEMATOCRIT
Hct
(%)
50
40
30
0
1
6
12
24
Time (hours)
I
II
III
IV

Figure 1

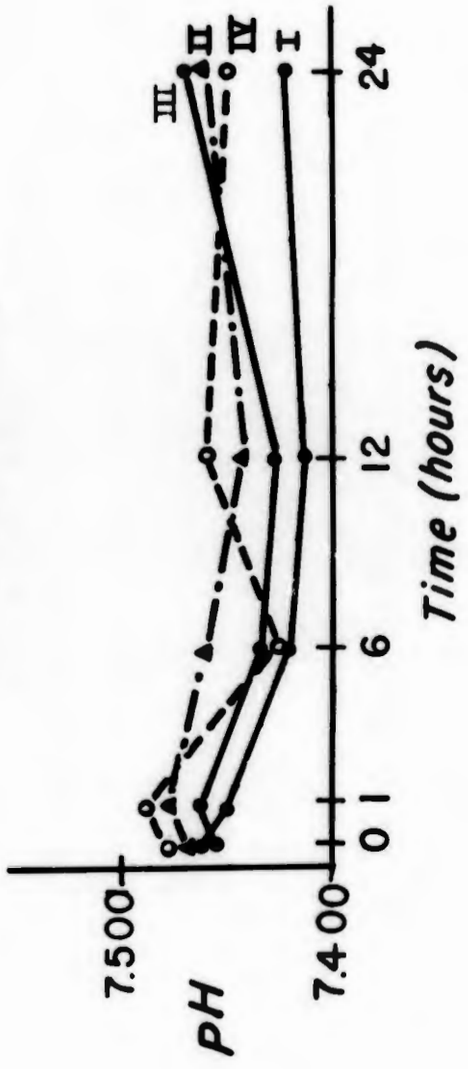


Figure 2

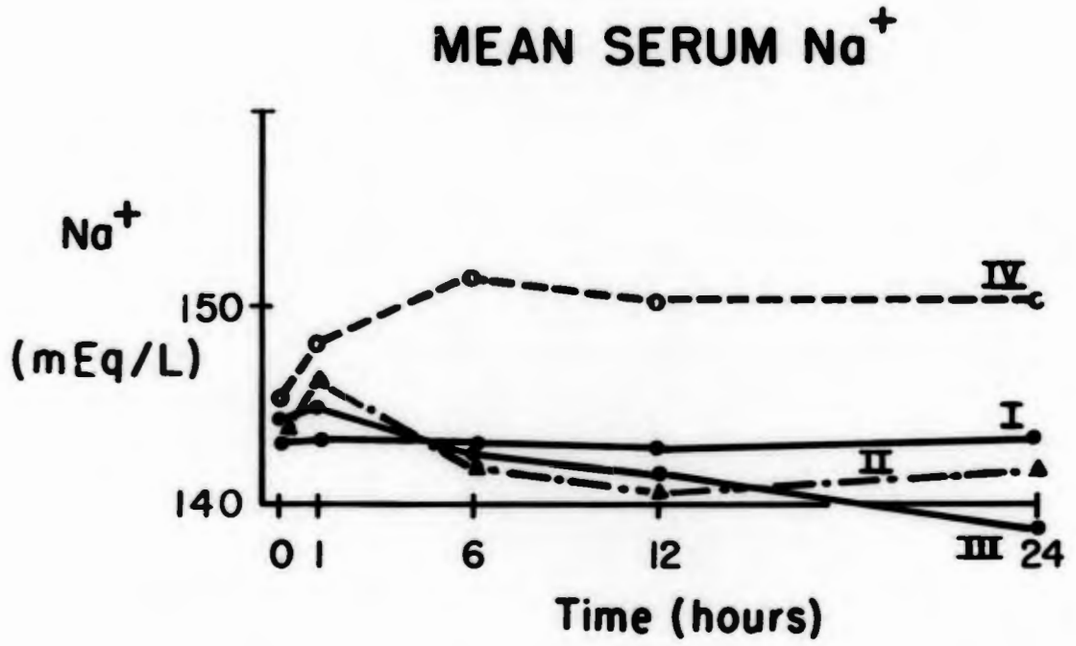


Figure 3

tonic solution, and the other three groups which received either hypertonic fluid or no fluid whatsoever. This elevated serum sodium level of Group IV is consistent with clinical observations in patients treated with hypertonic saline. Statistical analysis by the technic of analysis of variance revealed that there is a significant difference between Group IV and the combination of the other three treatment groups, at the 1% level.

No statistical analysis was carried out on the various serum potassium levels (Figure 4) as there was no difference between the treatment groups of clinical importance.

Mean urine flows in ml/hour and the standard deviation of the means are presented in Table 3. While urine flow was lowest in the unresuscitated control group, I, and highest in the Brooke formula group, II, there was no statistically significant difference between the rates of flow in any of the four groups. These experimental results with the hypertonic saline group do not corroborate the clinical reports of increased urine flows with hypertonic saline in burn patients exceeding those achieved with standard Brooke formula.

Table 3. Urine Flow ml/hr

Group	I	II	III	IV
$\bar{Y}$	9.40	18.0	15.1	11.1
$S\bar{Y}$	3.41	5.94	4.64	2.58

In Table 4 are the mean urine sodium concentration in mEq/L for each of the four treatment groups. The values at time zero represent the mean concentrations for each treatment group prior to injury. All the 24-hour values represent the mean sodium concentrations of the total volume of urine excreted during the 24-hour postburn period. Analysis of variance of the urine sodium concentrations reveals a significant difference between Group IV, the hypertonic saline group, and each of the other three groups. There are no significant differences among the urine sodium concentrations in the other three groups. The excellent natriuresis with hypertonic saline and the lack thereof with the Brooke formula parallels the clinical experiences with these different forms of resuscitation.

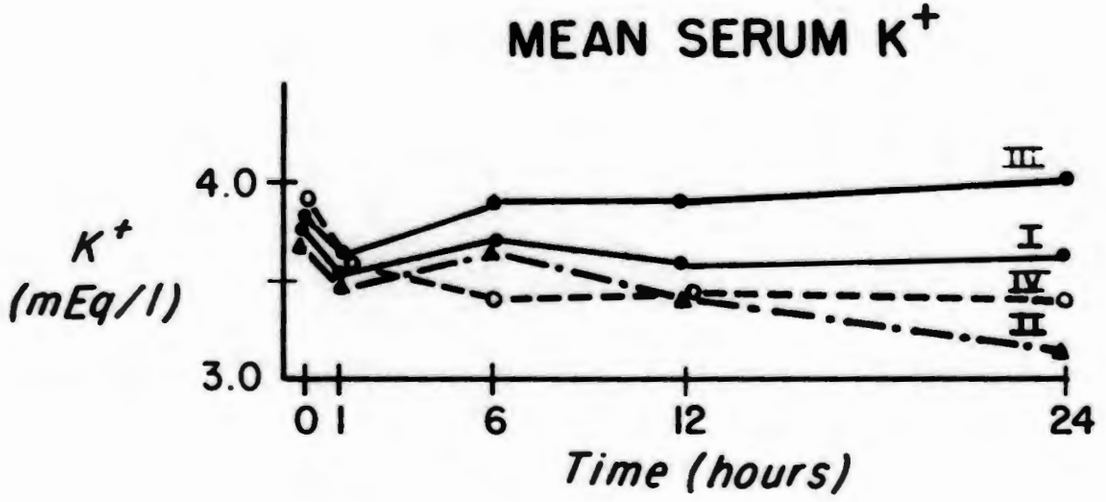


Figure 4

Table 4. Urine Na+ mEq/L

Time Hrs.	Group I	II	III	IV
0	47.7	80.1	49.7	46.3
24	25.2	39.7	16.1	226.2

Urine potassium concentrations were measured on the preburn and total volume of the 24-hour postburn collection (Table 5). It is interesting to note that during the postburn period the urine potassium concentration appeared to be greatest in the hypertonic saline treated group.

Table 5. Urine K+ mEq/L

Time Hrs.	Group I	II	III	IV
0	108.6	81.2	73.8	144.8
24	96.0	75.0	52.3	139.4

The mean cardiac outputs for each treatment group are shown in Figure 5. The preburn output is plotted as 100% and subsequent outputs are plotted as the per cent of normal. All four treatment groups underwent initial decrease in cardiac output during the first hour postburn prior to resuscitation. The unresuscitated group, I, continued to fall until six hours postburn after which there was an increase to 80% of the preburn level stabilizing there. The Brooke formula group, Group II, in contrast to the half-Brooke formula, Group III, and the hypertonic group, IV, had returned to normal level earlier. At 24 hours postburn, there was marked overshoot by the Brooke formula group, a less impressive overshoot of the half-Brooke formula group and stabilization at the preburn level by the hypertonic group.These changes were all analyzed by analysis of variance. There was no

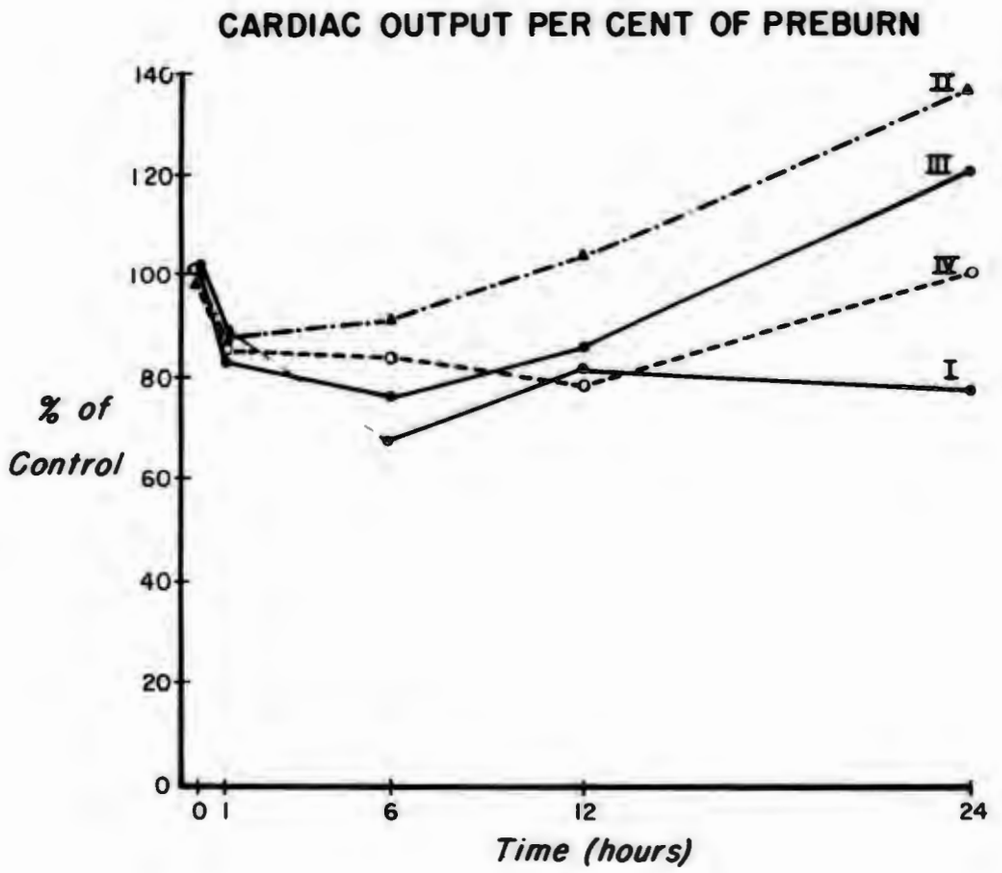

CARDIAC OUTPUT PER CENT OF PREBURN
140
120
100
80
60
40
20
0
% of
Control
0 1
6
12
24
Time (hours)
II
III
IV
I

Figure 5

significant difference in any of the outputs at the 6 and 12 hour points, though at 24 hours, there was a statistical difference between the three resuscitated groups and the unresuscitated group. Within the three resuscitated groups there were no significant differences.

Plasma volumes, Figure 6, are plotted in a fashion similar to that of the cardiac outputs with control representing 100% and subsequent measurements plotted as per cent of normal. Plasma volume fell in the unresuscitated group at all points in time whereas between 8 and 24 hours there was return to normal or supernormal levels in all of the resuscitated groups. The magnitude of the overshoot at 24 hours in each of the resuscitated groups paralleled that seen in the cardiac outputs. The greatest overshoot was observed with Brooke formula, less with half-Brooke formula and the more minimal overshoot with the hypertonic saline group. Statistically there was a significant difference at 24 hours between all resuscitated groups and the unresuscitated group, though there was no significant difference between Groups II, III, IV indicating that the three treatment groups were all equally effective in restoring plasma volume.

The mean sodium balance for each treatment group in mEq/kg is shown in Table 6. The mean per cent retained is shown in the bottom row. These differences are statistically significant ($p < 0.01$). The hypertonic group retained only 64.3% of the administered dose in distinction to the other two resuscitated groups. This, of course, is consistent with the natriuresis seen in the sodium concentration in the experimental hypertonic-saline treated group as well as in clinical reports.

The patterns of weight change (Table 7) for each treatment group are consistent with the total positive fluid and sodium gain. The hypertonic group had a natriuresis with an accompanying fluid volume producing a weight loss while the other two resuscitated groups gained weight.

Discussion

Many burn resuscitation formulae have been devised since it has been realized that burn shock and renal failure can be prevented by intravenous fluid administration. Using the clinical indices of blood pressure, urine output, electrolyte balance and early survival, all appear clinically adequate except in the pediatric and geratic age groups. The ideal mode of fluid resuscitation would provide adequate resuscitation while effecting a minimal positive fluid and sodium loading. All conventional

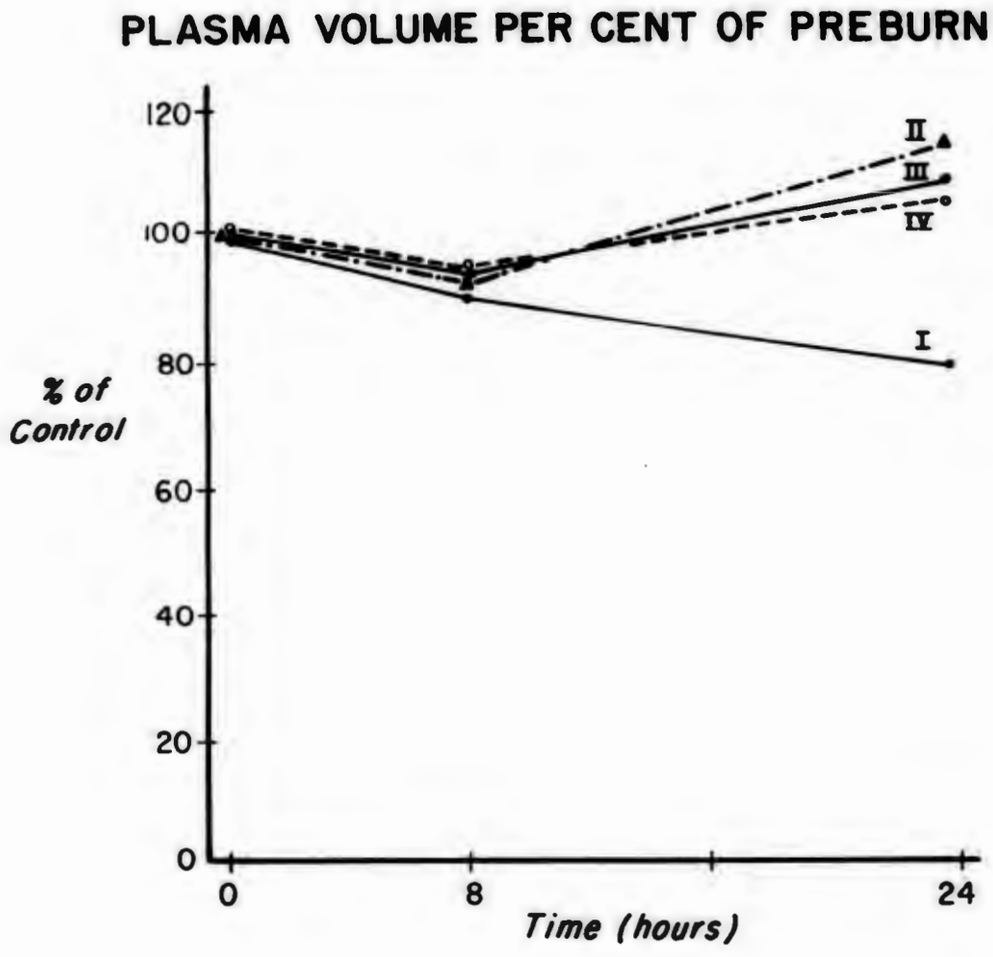

PLASMA VOLUME PER CENT OF PREBURN
120
100
80
60
40
20
0
% of Control
II
III
IV
I
0
8
24
Time (hours)

Figure 6

Table 6. Na+ Balance (24 hrs)

	Group			
	I	II	III	IV
Na+ Given mEq/kg	0	10.88	5.44	12.0
Na+ Excreted mEq/kg	0.67	0.66	0.45	4.28
Na+ Balance mEq/kg	-0.62	+ 10.22	+4.99	+ 7.72
% Retained	0	94.03	91.8	64.33

Table 7. Weight

	Group			
	I	II	III	IV
Δ Weight	-4.2%	+3.5%	+2.4%	-1.73%

isotonic resuscitation results in an appreciable positive fluid and sodium load often more than 10% of the preburn weight, while achieving adequate resuscitation.

In this study all animals receiving intravenous fluids were adequately resuscitated by the usual clinical indices. The hypertonic resuscitation produced a natriuresis, retaining only 64% of the administered dose; which coupled with a smaller administered volume of fluid resulted in no weight gain. The shift of fluids from the intracellular to extracellular space to maintain osmolality and expand the plasma volume to slightly above normal levels appeared well tolerated. There was no evidence of decreased cellular metabolism or metabolic acidosis in any of the Group IV animals. In fact, all resuscitated animals were slightly alkalotic.

It is interesting to note that dogs receiving half Brooke formula resuscitation, while retaining almost 92% of the administered dose, had the smallest net sodium gain. This group did gain weight because of the larger administered fluid load.

There were no statistical differences in urinary output, cardiac output or plasma volume between the modes of therapy. The Brooke formula resuscitation returned indices to normal levels earlier than the other methods and this may be of clinical significance.

We conclude that the efficacy of resuscitation as evaluated by the selected indices was roughly equal with the Brooke formula, half-Brooke formula and hypertonic lactated saline solution. No statistically significant advantages can be ascribed to any of these three therapeutic approaches, though all else being equal, the minimization of positive fluid and sodium loading achieved by the half-Brooke formula and the hypertonic lactated saline is attractive, particularly in patients with limited cardiopulmonary reserves.

Summary

Four groups of animals were resuscitated by various methods following a 40% scald burn. All animals receiving intravenous fluids responded equally well when evaluated by selected indices. Hypertonic saline resuscitation produced a natriuresis and no weight gain. Animals receiving half-Brooke formula resuscitation had the smallest net sodium gain. These two types of resuscitation may be of advantage clinically in patients with limited cardiopulmonary reserve.

Reference

1. Monafo WW: The treatment of burn shock by the intravenous and oral administration of hypertonic lactated saline solution. J Trauma 10: 575-586, 1970.

Presentation

Reckler JM: Hypertonic lactate saline resuscitation following thermal injury. Presented at Amer Burn Assn meeting San Antonio, Texas, 17 April 1971.

Publications

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0D 6964	71 07 01	DD-DR&E(AR)636

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

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A061102B71R	01	149
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Procede with Security Classification Code)

(U) Study of Renal Function of the Thermally Injured Soldier (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
70 09	71 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 71	.15	4.0
	CURRENT 72	0	0

19. RESPONSIBLE DOD ORGANIZATION

NAME: US Army Institute of Surgical Research

ADDRESS: Ft Sam Houston, Tx 78234

RESPONSIBLE INDIVIDUAL

NAME: PRUITT, B. A., JR, LTC, MC

TELEPHONE: 512-221-2720

20. PERFORMING ORGANIZATION

NAME: US Army Institute of Surgical Research
Renal Section

ADDRESS: Ft Sam Houston, Tx 78234

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

NAME: Martin G White, MAJ, MC

TELEPHONE: 512-221-4307

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME:

NAME: DA

21. GENERAL USE

FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Procede EACH with Security Classification Code)

(U) Burn Patients; (U) Renal Insufficiency; (U) Sodium and Chloride Excretion

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

23. (U) To evaluate the cause of azotemia in burn patients in order to improve administation therapy to these traumatized pat'ents.

24. (U) Glomerular filtration rate and electrolyte excretion patterns were measured in thermally injured patients who had azotemia. Glomerular filtration rates were measured before and after volume expansion or before and after septic shock.

25. (U) 70 09 - 71 06 Sixteen patients were studied, and nine of these patients were studied on two occasions. Results: The clearance data and electrolyte excretion patterns suggested that the primary cause of azotemia in these patients was a reduced effective arterial blood volume superimposed upon a mildly diseased kidney.

*Available to contractors upon originator's approval

FINAL REPORT

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

REPORT TITLE: STUDY OF RENAL FUNCTION OF THE THERMALLY INJURED

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

1 July 1970 - 30 June 1971

Investigator:

Martin G. White, M.D., MAJ, MC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

REPORT TITLE: STUDY OF RENAL FUNCTION OF THE THERMALLY INJURED

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

Period covered in this report: 1 July 1970 - 30 June 1971

Investigator: Martin G. White, M.D., MAJ, MC

Reports Control Symbol MEDDH-288(R1)

Renal function was assessed in 16 burned patients; nine studies were done before and after major hemodynamic changes. The primary cause of renal failure appears to be due to a decreased effective arterial blood volume superimposed on mild intrinsic renal disease.

Burn patients
Renal insufficiency
Sodium and chloride excretion

STUDY OF RENAL FUNCTION OF THE THERMALLY INJURED

In the burn patient population, the incidence of acute tubular necrosis leading to uremia and requiring hemodialysis has greatly diminished. The decline in this complication is probably a consequence of a wide-spread application of aggressive regimens of fluid resuscitation such as the "Brooke Formula". The techniques of fluid resuscitation prevent vascular collapse, renal ischemia, and the deposition of nephrotoxic substances in the renal tubules. Nevertheless, azotemia remains a frequent problem, particularly during the latter course of the burn injury. Azotemia may occur for several reasons:

1) The production of urea may be increased as from increased tissue catabolism, gastrointestinal hemorrhage, tissue necrosis, steroids, antibiotics, such as tetracycline, or the ingestion of inordinate amounts of nitrogen.

2) Decreased excretion of urea as in

a) Decreased renal blood flow (states of vascular collapse, shock,or vascular disease in the large and small arteries of the kidney).

b) Increased urea back diffusion as in obstructive uropathies.

c) Intrinsic glomerular and tubular disease as glomerulitis or acute tubular necrosis.

Any of these abnormalities may occur to different degrees so that acute tubular necrosis may only affect a variable per cent of the overall nephron population. If such a situation would occur, the remaining functional nephrons would be overperfused and, although retaining a remarkable functional capability, would not be expected to eliminate sodium from the urine. If all the tubules were damaged, then decreased reabsorption of sodium from the tubular lumen might occur much as is seen in some patients during the polyuric recovery phase of acute tubular necrosis. In neither hypothetical instance would the urine sodium concentration be expected to be very low. On the other hand, if the effective arterial volume is reduced as in hypoalbuminemic states, the nephron will be stimulated to very active sodium reabsorption and little sodium should appear in the urine. This analysis requires that nothing interfere with

sodium reabsorption, particularly in the proximal tubule. Inhibition of carbonic anhydrase is of particular importance in the proximal tubule, a consequence of this inhibition is impaired reabsorption of bicarbonate and, possibly sodium; therefore, despite other stimuli leading to increased proximal reabsorption, some sodium will leave the proximal tubule. This sodium may ultimately be reabsorbed, particularly at the distal site in exchange for potassium, thereby augmenting potassium excretion. Chloride excretion may serve as a better indicator of the state of the effect of arterial volume in patients receiving a carbonic anhydrase inhibitor. A previous study from this unit suggested "non-oliguric renal failure" existed in traumatically injured patients, and characterized these patients as having azotemia, urine outputs between 540 and 1100 ml/day, and very low levels of sodium concentration in the urine (3-31 mEq/L).[1] The study has several objectionable features, including the fact that the observation of urine output, BUN, and urine sodium concentration was made on different days - as far as 12 days apart. Finally, the findings of a low urinary sodium concentration is not compatible with a model of a diffuse renal injury.

Methods

In an attempt to evaluate the cause of azotemia in a series of burn patients, glomerular filtration rates and urine chemical compositions were studied in patients whose BUN was greater than 29 mg%. A series of two to three one-hour clearance periods were performed. The patient had been given a single 20 microcurie subcutaneous dose of iothalamate-125 iodine, administered one hour prior to the beginning of collection periods. A blood specimen was collected midway through the collection period. Both plasma and urine were analyzed for iothalamate-125 iodine, sodium, potassium chloride, urea, osmolality, and creatinine. The plasma total solids were also measured, using an AO refractometer. Sixteen patients were studied on 25 occasions; in five instances, studies were done before and after attempts to expand intravascular volume, and in four patients studies were done before and after the intervention of septicemia and shock. In the remaining cases, volume expansion was not performed because it did not seem reasonable on the basis of clinical grounds.

Results

Detailed results of the studies of three patients who were volume expanded between the first and subsequent studies are shown in Table 1. Table 2 presents the data on all the patients

Table I. Detailed Data in the Burn Patients who Were Volume Expanded

PATIENT	DATE	URINE FLOW (ml/min)	GFR (ml/min)	U_{Na} (mEq/L)	U_{Na} V (μEq/min)	U_K (mEq/L)	U_K V (μEq/min)	U_{Cl} (mEq/L)	U_{Cl} V (μEq/min)	UUN (mg/100 ml)	U_{Osm} (mOsm/kg)	U_{Cr} (mg/100 ml)	U_{p-c} (mg/100 ml)	P_{Na^+}	P_{K^+}	P_{Cl^-}	$P_{HCO_3^-}$	P_{Cr}	P_{BUN}	P_{p-c}	$P_{Total\ Solids}$ (Per Cent)
														mEq/L			mg/100 ml				
1	10 Sep	1.6	38	12	19	71	114	43	69	770	419	46	250	131	5.5	99	28.8	2.5	65	5.8	7.8
	15 Sep	3.6	66	47	169	51	184	63	227	507	347	27	–	136	4.3	102	23.3	1.2	35	—	8.1
	24 Sep	2.6	79	54	140	77	200	63	164	452	382	26	–	133	4.3	100	30.0	.6	20	—	8.0
2	9 Oct	1.4	41	1	1	99	139	4	6	1040	623	84	920	145	3.7	109	18	2.7	101	8.5	8.7
	10 Oct	2.0	95	2	4	108	216	41	82	1160	726	77	730	151	3.9	126	14	1.4	43	6.0	8.0
3	10 Aug	1.1	33	0.5	1	50	55	18	19	1140	558	92	500	131	3.8	107	15.6	1.8	41	6.0	7.2
	12 Aug	1.3	48	5	7	62	81	27	35	740	–	64	320	129	2.9	100	18.2	1.6	33	5.0	7.4

Table 2. Studies of Renal Function in 16 Burn Patients

PATIENT	DAYS*	GFR (ml/min)	U_{Na} (mEq/L)	$U_{Na}V$ (μEq/min)	U_K (mEq/L)	U_KV (μEq/min)	U_{Cl} (mEq/L)	$U_{Cl}V$ (μEq/min)	BUN (mg/100 ml)	Cr	URINE SOLIDS (Per Cent)
1(a)		20	12	19	71	114	43	69	65	2.5	5.8
(b)	14	79	54	140	77	200	63	164	20	.6	8.1
2(a)		41	1	1	99	139	4	6	101	2.7	8.7
(b)	1	95	2	4	108	216	41	82	43	1.4	8.0
3(a)		33	.5	1	50	55	18	19	41	1.8	7.5
(b)	2	48	5	7	62	81	27	35	33	1.6	7.2
4(c)		63	2	6	73	234	52	105	42	1.2	6.0
(d)	1	40	2	3	98	136	11	15	59	1.6	5.8
5		37	3	5	5	7	8	11	79	1.4	7.0
6(a)		33	70	122	49	87	10	17	59	2.3	7.3
(b)	3	94	31	97	72	214	53	165	27	1.3	7.0
7(c)		114	43	109	85	216	55	139	32	1.2	7.6
(d)	21	7	5	6	92	111	2	2	119	3.1	6.0
8		50	0	0	68	85	7	9	41	–	–
9		17	16	10	92	55	3	2	59	1.6	7.4
10		122	.3	.6	80	168	49	103	36	1.2	6.2
11(c)		50	23	9	136	56	36	13	43	1.6	6.6
(d)	8	18	3	3	136	114	10	8	71	2.6	6.9
12		4	84	63	42	32	97	73	75	5.8	5.3
13		71	4	5	139	190	8	11	29	1.1	5.6
14		44	1.4	1.2	62	55	9	8	61	1.2	5.4
15(c)		20	2	1.5	130	99	13	10	45	1.8	5.9
(d)	6	17	5	4	75	62	5	4	40	1.5	5.6
16(a)		46	56	130	82	191	39	91	39	2.4	8.9
(b)	8	42	53	228	73	314	74	318	34	1.8	8.6

* Interval between studies

a = Before volume expansion

b = After volume expansion

c = Before the onset of septicemia

D = After the onset of septicemia

studied.

In five patients, Numbers 1, 2, 3, 6, 16, the attempts at volume expansion increased GFR in four of five studies. In each of these studies, a significant increase in chloride and potassium excretion occurred. If urinary sodium excretion and urinary potassium excretion were summed in each instance, the effect of volume expansion resulted in a marked increase in the sum of urinary sodium plus potassium excretion in each of the five patients.

In four patients, renal function deteriorated from one study to the next (Numbers 4, 7, 11, 15). This deterioration was usually associated with generalized sepsis. In each patient, the glomerular filtration rate decreased. The urinary chloride excretion fell in each instance; and the urinary sodium plus potassium excretion fell in three of four studies. One patient (No. 12) was studied during the evaluation of acute tubular necrosis, ultimately requiring repeated hemodialysis. In this instance, a very low glomerular filtration rate was associated with a modestly elevated sodium and chloride excretion pattern.

The remaining six patients studied had BUN's ranging from 29 to 79 mg%, and glomerular filtration rates ranging from 17 to 122 ml/min, with all but one below 71 ml/min. In each instance, sodium excretion was very low, and in five of the six studies chloride excretion was low as well.

Discussion

Analysis of these data suggests that azotemia in burn patients is frequently due to decreased renal perfusion, presumably due to a decrease in the effective arterial blood volume. The effective arterial blood volume is decreased in some burn patients due to (1) absolute volume contraction due to loss of body fluids in excess of intake, (2) decreased plasma oncotic pressure, and (3) arteriovenous shunting - presumably in the area of the burn injury.

Absolute volume contraction is most likely to occur after the initial phase of resuscitation (when the large volumes of fluid used in resuscitation have been discontinued), when the patients may receive dextrose and water and continue to lose body fluids through (1) draining wounds, (2) at times of submersion in hypotonic solutions ("tanking"), (3) bleeding associated with debridement, and (4) via nasogastric suction.

The plasma oncotic pressure is reduced as a consequence of hypoalbuminemia, hypoalbuminemia being a manifestation of (1) extensive transudation of fluid through the burn wound, particularly when some element of venous obstruction is present, and (2) extensive metabolic demands in a situation of relative starvation. This finding of course varies a great deal with the size of the burn, duration and course of the postburn illness, and the nature and extent of alimentation.

The mean per cent total solids in these 16 subjects was 6.85% + SD 1.08. The normal per cent total solids ranges from 7 - 9%, and suggests that these patients were mildly hypoproteinemic. The observation has been made that after the first 48-72 hour period after injury, when resuscitation has been completed, that the cardiac output and plasma volumes are both above normal.[2] These findings suggest, in conjunction with the normal to low mean arterial pressure, that peripheral resistance is reduced. This setting would best be explained on the bais of increased shunting, presumably peripherally in the area of the burn wound.

These factors in various combinations and of varying degrees of severity are probably playing a role in the patients described here. Only an absolute decrease in the effective arterial volume and a low plasma oncotic pressure are amenable to treatment. In those patients studied before and after attempts at volume expansion, an increase in glomerular filtration rate and chloride excretion strongly suggest that a decrease in the effective arterial volume had been present, and that therapy aimed at correcting these abnormalities had resulted in both (1) an increase in renal blood flow - as indicated by an increased glomerular filtration rate, and (2) an increase in the effective arterial blood volume is indicated by an increased chloride excretion.

Renal carbonic anhydrase appears to be inhibited during the initial days of mafenide acetate topical treatment of burn patients.[3] As a consequence, the rate of bicarbonate reabsorption is decreased in the proximal tubule, and increased delivery of bicarbonate and sodium out of the proximal tubule would be expected. Net bicarbonate excretion is increased. Indirect evidence for that in these patients is that the sum of sodium and potassium is greatly in excess of chloride excretion, and the other anion presumably is bicarbonate. However, the increased sodium delivered out of the proximal tubule may not appear in the urine. Active exchange of sodium for potassium in the distal

tubule, driven by aldosterone, may result in the appearance of large concentrations of potassium in the urine but little sodium. Increased levels of aldosterone production have not been documented but seem very likely on the basis of the excretion of large amounts of potassium in the urine and the hemodynamic setting of a decreased effective arterial blood volume.

A prior study emphasized that patients similar to these described here had "nonoliguric acute renal failure". This implies that the kidneys of these patients were primarily damaged. One of the features emphasized in this study was the low level of sodium excretion in the urine, a feature which may be regarded as indicating the preservation of renal function. Azotemia, which occurs in the patients described in this report, appears to be primarily due to a decrease in renal blood flow, superimposed upon a kidney which no longer has a capacity for normal function. The response to volume expansion in improving renal function or the effect of septicemia and shock in reducing renal function tend to support a decrease in renal blood flow. The generally reduced glomerular filtration rate under any circumstance support the concept of a defective kidney. The persistently increased urine output in these patients represents a solute diuresis from a kidney which no longer is able to maximally concentrate the urine. Therefore, when azotemia appears in burn patients treated with mafenide acetate, the first issue to be examined is whether the effective arterial blood volume is low. Blood pressure, pulse, the response to tilting towards the erect position, and central venous pressure are helpful parameters. The pattern of urine electrolyte excretion should be examined with particular emphasis on chloride excretion. Finally, the response to expanding the intravascular volume with salt and colloid solutions is the most helpful guide to further therapy.

References

1. Vertel RM, Knochel JP: Nonoliguric acute renal failure. JAMA 200: 598-602, 1967.

2. Pruitt BA Jr, Mason AD Jr, Moncrief JA: Hemodynamic changes in the early postburn patient. The influence of fluid administration and of a vasodilator (Hydralazine). J Trauma 2: 36-46, 1971.

3. White MG, Asch MJ: The acid base effects of mafenide acetate in burned patients. NEJM 284: 1281-1286 (June 10), 1971.

Publications and/or Presentations

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OD 6394	71 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
70 07 01	H, TERMINATION	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A061102B71R	01	081
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) Assessment of Regional Organ Blood Flow and Influence of Fluids - Laboratory Study of Changes Seen in Burned Soldiers (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
70 01	71 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 71	.30	8.0
	CURRENT 72	0	0

19. RESPONSIBLE DOD ORGANIZATION
NAME: US Army Institute of Surgical Research
ADDRESS: Ft Sam Houston, Texas 78234
RESPONSIBLE INDIVIDUAL
NAME: PRUITT, B.A., Jr, LTC, MC
TELEPHONE: 512-221-2720

20. PERFORMING ORGANIZATION
NAME: US Army Institute of Surgical Research
Laboratory Division
ADDRESS: Ft Sam Houston, Texas 78234
PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME: M J Asch, MAJ, MC
TELEPHONE: 512-221-4440
SOCIAL SECURITY ACCOUNT NUMBER:
ASSOCIATE INVESTIGATORS
NAME: P M Meserol, SP5, MS
NAME: A D Mason, Jr, MD DA

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Regional Organ Blood Flow; (U) Radioactive Microspheres

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 regional blood flow in burn shock.

24. (U) 50 micron radioactive microsphere injection; large volume Tabor counter.

25. (U) 70 07 - 71 06 Ability of microsphere technique to assess regional flow has been validated by injection of Cr51, SR 85 and serium 141 into the left ventricle of mongrel puppies and assessing the percentage of radioisotope recovered in total body counting and the isotope distribution. Percentage recovery of isotope injected has been 94-98% in six whole body count experiments. Quite routinely, the paired organs, i. e., cerebral hemispheres and the kidneys, receive equal amounts of radioactivity and the percentage of isotope going to the individual organ system is what one would expect with respect to their blood flow. There has been some difficulty in separating the three isotopes when they are injected into the same animal and therefore single isotope injections will have to be performed. A series of burned dog experiments comparing radioactive microsphere distribution when injected into control animals, non-resuscitated burned animals two hours postburn and burned animals four hours after fluid resuscitation has been completed revealing significant early decrease in hepatic and renal blood flow which is largely restored by resuscitation therapy.

*Available to contractors upon originator's approval.

FINAL REPORT

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

REPORT TITLE: REGIONAL BLOOD FLOW IN THE BURNED UNANESTHETIZED DOG

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

1 July 1970 - 30 June 1971

Investigators:

Morris J. Asch, M.D., LTC, MC
Peter M. Meserol, M.S., SP5
Arthur D. Mason, Jr., M.D.
Basil A. Pruitt, Jr., M.D., LTC, MC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

REPORT TITLE: REGIONAL BLOOD FLOW IN THE BURNED UNANESTHETIZED DOG

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

Period covered in this report: 1 July 1970 - 30 June 1971

Investigators: Morris J. Asch, M.D., LTC, MC
Peter M. Meserol, M.S., SP5
Arthur D. Mason, Jr., M.D.
Basil A. Pruitt, Jr., M.D., LTC, MC

Reports Control Symbol MEDDH-288(R1)

Using a recently developed microsphere injection technique, this study delineates changes in organ blood flow following burn injury. Blood flow to individual organs and to carcass were measured in 27 dogs divided equally into three groups: control (Group A); burned, nonresuscitated (Group B); and burned, resuscitated (Group C). All dogs were lightly anesthetized during burning and were fully conscious during measurements. Cardiac output was measured prior to and just after injection of 50 μ ^{51}Cr labelled microspheres. After bead injection, the animals were sacrificed and the individual organs were weighed, frozen and counted. Using cardiac output and fractional isotope distribution, flow in ml/min/gm of tissue was calculated for each organ.

Cardiac index in normal dogs was 5.77, one hour after injury it fell to 3.78 and after four hours of fluid administration, it rose to 5.68. Flow after burn was unchanged to brain, heart, stomach, duodenum, large intestine, spleen, and pancreas despite the significant decrease in cardiac index. Specific organ flows which were significantly altered were as follows: kidneys after burn, 59% of control, restored to 71% with resuscitation; liver after burn, 66% of control, restored to normal with resuscitation; small intestine after burn, 40% of control, restored to 70% with resuscitation; carcass after burn, 57% of control, restored to normal with resuscitation.

Regional organ blood flow Readioactive microspheres

REGIONAL BLOOD FLOW IN THE BURNED UNANESTHETIZED DOG

Burn shock is characterized by decreased cardiac output, associated with diminished plasma volume and hemoconcentration.[1] These hemodynamic alterations are responsive to resuscitation with electrolyte and colloid solutions. Restoration of cardiac output and plasma volume does not necessarily mean that normal volume flow is restored to individual organs. Using a recently developed microsphere injection technic,[2] this study attempts to delineate the changes in blood flow to individual organs after burn injury and following the administration of resuscitative solutions.

Methods

Twenty-seven adult, mongrel dogs were randomly divided into three groups of nine: Group I, control; Group II, burned, non-resuscitated; Group III, burned, resuscitated. On the day prior to each experiment, catheters were placed in the left atrium, pulmonary artery and femoral artery. On the day of each experiment, the animals in Groups II and III were briefly anesthetized and burned. The burn used was a 40%, histologically confirmed, full-thickness scald. All measurements were made in fully conscious animals given analgesia as required.

Cardiac output was measured by an indocyanine green injection technic immediately prior to and just after injection into the left atrium of a measured quantity of 50 micron, ^{51}Cr labelled microspheres. These microspheres were injected one hour after injury in Group II and five hours after injury (following four hours of resuscitation according to the Brooke formula) in Group III. Injections in the control animals were made without any sham procedure. After the second determination of cardiac output, each animal was sacrificed and the individual organs were dissected free, weighed and frozen. The microsphere radioactivity in each organ was determined in a large volume scintillation detector. Using cardiac output and fractional distribution of isotope, flow in ml/min/gm of tissue was calculated for each organ.

Results

Cardiac index in the control dogs (Group I) was 5.77 liters/min/meter^2; one hour after injury (Group II) it had fallen to 3.78 liters/min/meter^2, and after four hours of fluid administra-

tion (Group III) it rose to 5.68 liters/min/meter^2. Statistical analysis revealed no difference between the cardiac outputs in Groups I and III, both differed significantly from Group II.

Individual organ flow after burn was not changed in brain, heart, stomach, duodenum, large intestine, spleen or pancreas, despite the 35% decrease in cardiac output. The activity reaching the lung, which is an index of bronchial arterial flow and systemic arteriovenous shunting of 50 micron particles, was not changed by either injury or resuscitation.

Specific organ flows which were significantly altered by injury were as follows: kidney, after burn, 59% of control, restored to 71% of control following resuscitation; liver, after burn, 66% of control, restored to normal with resuscitation; small intestine, after burn, 40% of control, restored to 70% with resuscitation; carcass, after burn, 57% of control, restored to normal with resuscitation. The estimates of flow to right and left kidneys within individual animals agreed well in all groups, corroborating method consistency.

Summary

Burn injury, as measured by this technic, caused early diminution of cardiac output and of arterial blood flow to the kidneys, liver, small intestine and carcass, while sparing flow in a number of organs, including the heart and brain. Though this study does not differentiate between the effect of resuscitation and the effect of the passage of time, restoration of cardiac output after four hours of resuscitation was accompanied by restoration of flow to the liver and, surprisingly, the carcass. A modest flow deficit continued in the kidneys and small intestine, but this deficit was not sufficiently large to be of major importance. For all practical purposes, burn shock, in this model, was reversed and/or prevented after four hours of resuscitative therapy, without residual major alterations of organ flow.

References

1. Pruitt BA Jr, Mason AD Jr, Moncrief JA: Hemodynamic changes in the early postburn patient: The influence of fluid administration and of a vasodilator (Hydralazine). J Trauma 11: 36-46, 1971.

2. Neutze JM, Wyler F, Rudolph AM: Use of radioactive microspheres to assess distribution of cardiac output in rabbits. Amer J Physiol 215: 486-495, 1968.

Presentations and/or Publications

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OD 6956	71 07 01	DD-DR&E(AR)636

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

10 NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A061102B71R	01	116
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) Vasoactive Peptides in Thermal Injury - Laboratory Model of Changes Occurring in Burned Soldiers (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 Clinical Medicine

13 START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
70 08	71 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 71	.53	14.1
	CURRENT 72	0	0

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

RESPONSIBLE INDIVIDUAL
NAME: PRUITT, B. A., JR, LTC, MC
TELEPHONE: 512-221-2720

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

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME: H M BRUCK, MAJ, MC
TELEPHONE: 512-221-4906
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: A D Mason, Jr, MD DA
NAME:

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Bradykinin, (U) Bradykininogen; (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 levels of vasoactive peptides in serum of a laboratory animal model of burn injury.

24. (U) Plasma of burned dogs is extracted in ethanol and bio-assayed for vasoactive peptide activity by measuring contraction of guinea-pig ileum by method of Greenbeam.

25. (U) In six animals with 40% body surface burns only three showed slight elevation of bradykinin levels. Results of bradykininogen assay were similarly inconstant. Technical difficulties and unrewarding results necessitate termination of study.

*Available to contractors upon originator's approval.

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

FINAL REPORT

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

REPORT TITLE: VASOACTIVE PEPTIDES IN THERMAL INJURY

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

1 July 1970 - 30 June 1971

Investigators:

Harold M. Bruck, M.D., LTC, MC
Arthur D. Mason, Jr., M.D.

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

REPORT TITLE: VASOACTIVE PEPTIDES IN THERMAL INJURY

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

Period covered in this report: 1 July 1970 - 30 June 1971

Investigators: Harold M. Bruck, M.D., LTC, MC
Arthur D. Mason, Jr., M.D.

Reports Control Symbol MEDDH-288(R1)

Bradykinin is a vasopeptide that has been shown to cause vasodilation, pain, increased vascular permeability, smooth muscle contraction, and margination of leukocytes. Using L. Greenbaum's modification of the ethanol extraction technique of Rocha e Silva (Am J Phys 156:261-273, 1949), samples of plasma of dogs before and after burning were assayed in vitro against guinea pig ileum for kinin content. A mild elevation of plasma kinin activity was detected in three of six dogs with 40% total body surface area burns. No significant increase could be detected in the plasma of the other three dogs when compared with preburn controls. Similar inconstant results were obtained when plasma was extracted for bradykininogen content after the method of Diniz and Carvalho (NY Acad Sci 104:77, 1963). When lymph obtained from cannulation of the right thoracic duct of two burned dogs was extracted in ethanol and assayed for kinin activity, none was found.

The results of these experiments demonstrate that no significant elevation of plasma kinin can be detected in burned dogs using these methods. Among several possible explanations for these phenomena, the most plausible include:

1. High levels of kininase activity destroying most or all circulating kinin.

2. Leakage of kinins out of the circulation into the interstitial tissues.

3. High levels of a kinin inhibitor that cause deactivation of the kinin system.

4. Inconsistent nature of assay techniques.

Because of technical difficulties and these unrewarding preliminary results, study is being terminated.

Bradykinin
Bradykininogen
Burns

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OD 6962	71 07 01	DD-DR&E(AR)636

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

10. NO CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A061102B71R	01	134
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) Effect of a Supranormal Dietary Intake on Intracellular Sodium and Potassium in Thermally Injured Soldiers (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
69 07	71 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 71	.29	7.7
	CURRENT 72	0	0

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

RESPONSIBLE INDIVIDUAL
NAME: PRUITT, B.A., Jr, LTC, MC
TELEPHONE: 512-221-2720

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

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME: P. W. Curreri, LTC, MC
TELEPHONE: 512-221-3301
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: F.J. Ruzicka, CPT, MSC
NAME: DA

21. GENERAL USE: FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code): (U) Intracellular Cation; (U) Intracellular Sodium; (U) Intracellular Potassium; (U) Parenteral Hyperalimentation; (U) Supranormal Dietary

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

23. (U) To quantitate the effects of the combined enteral-intravenous supranormal caloric dietary program utilized at this Institute in the treatment of patients with severe thermal injury. To describe the effects of supranormal caloric intake on intracellular erythrocyte cation concentration in the above patients.

24. (U) A study was designed to compare intracellurlar cation concentration in two similar groups of patients with extensive thermal injury. One group received standard intravenous solutions containing 5% dextrose to which were added appropriate quantities of electrolytes and vitamins to satisfy daily maintenance requirements. The second group received a combined enteral-intravenous dietary program consisting of 3,000-6,000 kilo-calories per day.

25. (U) 69 07 - 71 06 The above study has been completed, and the results indicate a significant elevation of intracellular sodium concentration and decrease in intracellular potassium concentration in red blood cells from catabolic thermally injured patients. The erythrocyte sodium concentration returns to normal levels within 3-5 days following administration of a combined enteral-intravenous dietary program of 6,000 Kilocalories per day.

*Available to contractors upon originator's approval.

DD FORM 1 MAR 66 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65 AND 1498-1, 1 MAR 66 (FOR ARMY USE) ARE OBSOLETE.

FINAL REPORT

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

REPORT TITLE: EFFECT OF A SUPRANORMAL DIETARY INTAKE ON INTRACELLULAR SODIUM AND POTASSIUM IN THERMALLY INJURED PATIENTS

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

1 July 1970 - 30 June 1971

Investigators:

P. William Curreri, M.D., LTC, MC
Douglas W. Wilmore, M.D.

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

REPORT TITLE: EFFECT OF A SUPRANORMAL DIETARY INTAKE ON INTRACELLULAR SODIUM AND POTASSIUM IN THERMALLY INJURED PATIENTS

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

Period covered in this report: 1 July 1970 - 30 June 1971

Investigators: P. William Curreri, M.D., LTC, MC
Douglas W. Wilmore, M.D.

Reports Control Symbol MEDDH-288(R1)

Previous studies at this institute have indicated that a combined intravenous-enteral feeding program providing up to 8,000 kilocalories per day to patients with extensive thermal injury achieved positive energy balance and prevented extensive weight loss. The present study was undertaken to describe the effects of such a supranormal caloric intake on intracellular erythrocyte cation concentration in these patients and to attempt to quantitate the effects of this dietary program.

Twenty-seven adult male patients were included in this study and divided into two groups of similar age and extent of burn. The mean burn size was 60%, with a 31.5% third-degree involvement. Heparinized blood was obtained from patients in each group every 3-4 days during their first post-burn month. In addition, blood was obtained from 14 convalescent patients and eight unburned, young adult male volunteers for control studies.

The results revealed a significant elevation of intracellular sodium concentration and decrease in intracellular potassium concentration in red blood cells from catabolic, thermally injured patients. Erythrocyte sodium concentration returned to normal levels in three to five days following administration of a combined enteral-intravenous dietary program of 6,000 kilocalories per day. Red blood cell

volume, hemoglobin, and hemoglobin concentration were unchanged in patients treated by standard and supranormal dietary programs when compared to unburned controls and late convalescent patients. No difference in arterial pH was noted between the two test groups, and intracellular sodium concentration in serial samples from the same patient could not be correlated with pH changes.

Intracellular Cation
Intracellular Sodium
Intracellular Potassium
Parenteral Hyperalimentation
Supranormal Dietary Intake

EFFECT OF A SUPRANORMAL DIETARY INTAKE ON INTRACELLULAR SODIUM AND POTASSIUM IN THERMALLY INJURED PATIENTS

Elevation of intracellular erythrocyte or muscle sodium concentration has been described in patients with congestive heart failure,[1] hyperthyroidism,[2] hemorrhagic shock,[3] and thermal injury.[4,5] Resolution of the underlying illness has been associated with return of intracellular sodium concentration to normal levels. However, in the case of thermal injury, acute illness may be prolonged despite optimal therapy, and erythrocyte cation abnormalities often persist up to eight weeks postburn even in patients with small areas of third-degree thermal injury.[4]

Through the achievement of weight stabilization and positive energy balance would appear to be empirically beneficial, almost no information is presently available to quantitate the effects of either the parenteral hyperalimentation regimen developed by Dudrick et al.[6] or the combined enteral-intravenous supranormal caloric dietary program reported from this Institute.[7] The present study was undertaken to describe the effects of supranormal caloric intake on intracellular erythrocyte cation concentration following extensive thermal injury.

Methods

Twenty-seven adult, male patients were included in this study and were divided into two groups. Twenty (Group I) received standard intravenous solutions containing 5% dextrose to which were added appropriate quantities of sodium, potassium, chloride and vitamins to satisfy daily maintenance requirements. In addition, those patients with a functioning gastrointestinal tract were served a high-protein, high-caloric enteral diet and were encouraged to consume as many calories as possible throughout the day. The average daily caloric intake was 1,609 kilocalories (range 93 to 4,807).

A combined enteral-intravenous dietary program was begun between the fifth and tenth postburn day in the seven remaining patients (Group II). All received 2,000 to 4,000 kilocalories intravenously as a hypertonic parenteral solution containing 20% glucose, 5% protein hydrolysate, minerals and vitamins. In addition, each received up to 4,000 kilocalories per day enterally

either in the form of a high-protein, high-caloric hospital diet or as a solution manufactured from semi-synthetic ingredients which matched the composition of the above hospital diet. Minimal caloric intakes of 3,000 kilocalories per day were maintained, and caloric intake was progressively increased as rapidly as possible without inducing diarrhea or significant glucosuria. A daily intake of 6,000 kilocalories per day was sought, although it generally required several days to attain these intakes.

The average age of patients in Group I was 24.5 as compared to 28.0 in Group II. All patients had extensive thermal injury with greater than 35% total body surface burn. The mean burn size was 61.7% (31.3% third-degree) in Group I and 57.8% (31.6% third-degree) in Group II.

Heparinized blood was obtained from patients in each group for the measurement of intracellular and plasma electrolyte concentrations ever three to four days during the first postburn month or until patient expiration. In addition, blood from 14 convalescent burn patients (greater than 40% total body surface burn) one month after complete skin coverage and from eight unburned, young adult, male volunteers was similarly analyzed for red blood cell, plasma, and whole blood sodium and potassium concentration by the method of Bugyi.[8] Mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and arterial blood pH were measured by standard laboratory techniques.

Results

Mean red blood cell intracellular sodium concentrations in the unburned controls, in the late convalescent burn group, and in the Group II patients were almost identical. Only in the Group I patients was intracellular sodium concentration significantly elevated. No consistent differences in plasma sodium concentration were noted. Both Group I and Group II patients had significantly lower mean intracellular potassium concentrations than the unburned or convalescent subjects. No significant differences in plasma potassium concentration were noted.

The normal intracellular sodium concentration in the Group II patients appeared to be specifically related to the

administration of a supranormal caloric intake. Four patients initially placed in the Group I dietary program and then, after two baseline studies, given the supranormal caloric intake of the Group II patients, all showed prompt return of intracellular sodium concentration to normal levels. In these patients, not a single erythrocyte sodium concentration following initiation of supranormal caloric intake was higher than the baseline intracellular sodium concentration in the same subject.

A comparison of the arterial blood pH levels in the Group I and Group II patients revealed no significant pH difference related to administration of the combined enteral-intravenous dietary regimen. Comparison of the red blood cell indices in the four groups by statistical analysis showed no significant alterations.

Discussion

To our knowledge, no therapeutic modality in the treatment of thermally injured patients has previously been reported which has resulted in the correction of this intracellular sodium abnormality. The difference between red blood cell sodium concentration in conventionally fed patients and in those receiving a supranormal caloric dietary program is highly significant ($P < .005$). These results suggest that monitoring of erythrocyte intracellular sodium concentration may provide a simple and reliable means of evaluating the effectiveness of nutritional support in traumatized patients.

Since no significant difference in arterial pH values was found in the two groups of patients tested in this study, the observed findings cannot be attributed to pH alterations resulting from therapy. Moreover, no consistent correlation between serial pH determinations and serial erythrocyte cation concentrations was observed in any single patient. An increase of mean corpuscular volume alone might be associated with alteration of intracellular sodium concentration, and for this reason, red blood cell indices were initially monitored. The absence of significant differences in the measurements of mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration makes any explanation of our results based on cell volume untenable.

There remain only two possible explanations for the burn patient's elevated intracellular sodium concentration and the subsequent intracellular sodium concentration reduction following institution of supranormal caloric intake. The initial trauma either results in primary or secondary inhibition of enzymes responsible for active transport across the red blood cell membrane or causes physical change in membrane structure which in turn inhibits either passive or active molecular transport.

Summary

1. A significant elevation of intracellular sodium concentration and decrease in intracellular potassium concentration was found in red blood cells from catabolic, thermally injured patients.

2. Erythrocyte sodium concentration returned to normal levels within three to five days following administration of a combined enteral-intravenous dietary program of 6,000 kilocalories per day. It is suggested that erythrocyte intracellular sodium concentration may reflect the effectiveness of nutritional support in traumatized patients.

3. Red blood cell volume, hemoglobin, and hemoglobin concentration were unchanged in patients treated by standard and supranormal dietary programs when compared to unburned controls and late convalescent burn patients. No difference in arterial pH was noted between the two test groups, and intracellular sodium concentration in serial samples from the same patient could not be correlated with pH changes.

4. It is postulated that the observed results can be explained by an inhibition of red blood cell active transport or an induced defect in the erythrocyte cell membrane as a result of thermal trauma which is reversed by maintenance of positive energy balance.

References

1. Flear CTG, Crampton RF, Matthews,DM: Observations on the electrolyte and water composition of skeletal muscle in patients in congestive cardiac failure, using an *in vitro* method for determination of inulin space. Clin Sci 21: 381-392, 1961.

2. Smith EKM, Samuel PD: Abnormalities in the sodium pump of erythrocytes from patients with hyperthyroidism. Clin Sci 38:49-61, 1970.

3. Campion DS, Lynch LJ, Rector FC Jr, et al: Effect of hemorrhagic shock on transmembrane potential. Surgery 66: 1051-1059, 1969.

4. Lantsberg LA: Changes of potassium and sodium content of plasma and red cells in burns. Fed Proc (Transl Suppl) 23: 515-518, 1964.

5. Proctor HJ, Smith EKM, Cole C, et al: Active transport defect in red cells from burned patients. Surg Forum 18:69-71, 1967.

6. Dudrick SJ, Wilmore DW, Vars HM, et al: Long-term total parenteral nutrition with growth, development, and positive nitrogen balance. Surgery 64:134-142, 1968.

7. Wilmore DW, Curreri PW, Spitzer KW, et al: Supranormal dietary intake in thermally injured hypermetabolic patients. Surg Gynec Obstet 132:881-886, 1971.

8. Bugyi,Magnier,E,Joseph W,et al:A method for measurement of sodium and potassium in erythrocytes and whole blood. Clin Chem 15:712-719, 1969.

Publications

Curreri PW, Wilmore DW, Mason AD Jr, Newsome TW, Asch MJ, Pruitt BA Jr: Intracellular cation alterations following major trauma: Effect of supranormal caloric intake. J Trauma 11: 390-396, 1971.

Presentations

Curreri PW: Intracellular Cation Alterations Following Major Trauma: Effect of Supranormal Caloric Intake. Amer Assoc for Surgery of Trauma Mtg, Chicago, Ill, 9 Oct 1970.

Curreri PW: Intracellular Cation Alterations Following Major Trauma: Effect of Supranormal Caloric Intake. San Antonio Res Club, San Antonio, Texas, 21 Oct 1970.

Curreri PW: Intracellular Cation Alterations after Major Trauma. USA Surgeon General's Adv Comm on Metabolism of Trauma, BAMC, FSHT, 30 Nov 1970.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OD 6961	71 07 01	DD-DR&E(AR)636

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

10. NO. CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A061102B71R	01	133
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Procede with Security Classification Code): (U) Synthetic Aminosol with Dextrose 20% Used for Hyperalimentation 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
70 06	71 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 71	.27	7.2
FISCAL YEAR CURRENT 72	0	0

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

RESPONSIBLE INDIVIDUAL
NAME: PRUITT, B.A., Jr, LTC, MC
TELEPHONE: 512-221-2720

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

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME: P W Curreri, LTC, MC
TELEPHONE: 512-221-3301
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: W W Inge, Jr, LTC, MC
NAME: T W Newsome, MAJ, MC DA

21. GENERAL USE: FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Procede EACH with Security Classification Code): (U) Parenteral Hyperalimentation; (U) Amino Acid Intravenous Solution; (U) Neo-Aminosol; (U) Synthetic Aminosol with Dextrose 20%

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

23. (U) To investigate the safety and therapeutic value of the administration of a synthetic amino acid solution containing 29% glucose in patients with extensive thermal injury.

24. (U) Patients with burns of greater than 40% who were unable to be fed orally because of complicating medical conditions such as paralytic ileus were administered the solution over variable time periods and evaluated by clinical examination as well as radiological and chemical studies.

25. (U) 70 06 - 71 01 Eight patients were evaluated prior to and after treatment with Synthetic Aminosol with Dextrose 20%. The experimental study was discontinued in January 1971 when all intravenous solutions manufactured by the supplier were withdrawn from the market of defects in the bottling process which resulted in the potential for bacterial contamination of their i. v. solutions. Results obtained in the eight patients studied indicated an increased incidence of metabolic acidosis as compared to previously used solutions. The preparation appeared to be equally as effective in maintaining positive nitrogen balance as those solutions containing fibrin hydrolysates. No other significant side effects were noted.

*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. 3A061102B71R-01, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: SYNTHETIC AMINOSOL WITH DEXTROSE 20%

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

1 July 1970 - 30 June 1971

Investigators:

P. William Curreri, M.D., LTC, MC
Wellford W. Inge, Jr., M.D., LTC, MC
Thomas W. Newsome, M.D., MAJ, MC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

REPORT TITLE: SYNTHETIC AMINOSOL WITH DEXTROSE 20%

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

Period covered in this report: 1 July 1970 - 30 June 1971

Investigators: P. William Curreri, M.D., LTC, MC
Wellford W. Inge, Jr., M.D., LTC, MC
Thomas W. Newsome, M.D., MAJ, MC

Reports Control Symbol MEDDH-288(R1)

Synthetic Aminosol with Dextrose 20% appeared to offer several advantages over the commercially available fibrin or casein hydrolysates for the parenteral administration of intravenous solutions containing nitrogen and carbohydrate. The solution was manufactured in sterile containers, thus reducing the possibility of contamination during mixture in the pharmacy or on the ward. In addition, all the nitrogen in the solution was present as pure synthetic amino acids, and it seemed likely that more nutritive value would be obtained from each bottle as compared to the hydrolysates in which up to half of the nitrogen is present as peptides.

The solution was administered to eight male patients with an average of 44.4% total body surface burn (range 18-82.5%). Five of the eight patients had significant associated injuries in addition to their burn, and all eight of the patients had severe complications of their burn injury. Three of the patients had extensive pneumonia; two each had invasive burn wound sepsis, renal failure, and cutaneous Herpes. The patients received intravenous hyperalimentation for an average of 19.5 days, although four of the patients had therapy for only 5-6 days prior to expiration. No significant chemical side effects were noted except for transient rises in LDH in five patients and a transient elevation of SGOT in two

patients. Plasma proteins either remained unchanged or increased in all except one patient who had an 82.5% total body surface burn. Positive nitrogen balance was attained in all patients except the latter patient, and weight gain was noted in six of the eight patients. Total body weight increased an average of 3 kg during therapy in the seven patients who attained positive nitrogen balance.

In the few patients in whom a metabolic acidosis was noted during administration of the synthetic Aminosol solution, a rapid correction of pH was attained by appropriate manipulation of the sodium and potassium salts which were utilized for supplementation of the solution.

Synthetic Aminosol with Dextrose 20% was a promising preparation for intravenous administration, although several areas for improvement in the formulation of the solution are evident. The concentration of amino acids in the final solution is only 2%, and thus the nitrogen content is considerably less than a comparable volume of previously used formulations. In addition, the quantity of individual crystalline amino acids is probably not ideal for maximum nitrogen utilization. Finally, the Federal Drug Administration has requested withdrawal of all intravenous solutions produced by the manufacturer as a result of bacterial contamination found in other products. No infectious complications in this study could be related to the intravenous administration of the test solution.

Parenteral Hyperalimentation
Amino Acid Intravenous Solution
Neo-Aminosol
Synthetic Aminosol with Dextrose 20%

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0C 6978	71 07 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8A. DOD'N INSTR'N	8B. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
70 07 01	D. CHANGE	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO. CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A061102B71R	01	300
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (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 71	0.45	12.0
FISCAL YEAR CURRENT 72	0.45	13.1

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

20. PERFORMING ORGANIZATION
NAME: US Army Institute of Surgical Research Clinical Division
ADDRESS: Ft Sam Houston, Texas 78234
PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME: Wellford W Inge, Jr,LTC, MC
TELEPHONE: 512-221-3301
SOCIAL SECURITY ACCOUNT NUMBER:
ASSOCIATE INVESTIGATORS
NAME: Mary E Spitzer, CPT, AMSC
NAME: Frank J Ruzicka, CPT, MSC 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

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

24. (U) Daily caloric intakes have been obtained on four severely burned patients. The patients consumed two to two and one-half times that of their predicted basal metabolic rates. Nude body weights were obtained and it has been found that the subjects lost approximately 15 to 20 per cent of their preburn weight. The protein intakes were more than 2 to 3 grams per kilogram for three of the patients. Blood samples were not evaluated except on a clinical basis. Gastrointestinal function studies were not performed on these patients.

25. (U) 70 07 - 71 06 - To date four patients have been studied and results from these are inconclusive. However, the 15 to 20 per cent weight loss is indicative of possible malabsorption and gastrointestinal function studies should be accomplished.

*Available to contractors upon originator's approval

ANNUAL PROGRESS REPORT

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

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

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

1 July 1970 - 30 June 1971

Investigators:

Mary E. Spitzer, CPT, AMSC
Wellford W. Inge, Jr., M.D., LTC, MC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

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

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

Period covered in this report: 1 July 1970 - 30 June 1971

Investigators: Mary E. Spitzer, CPT, AMSC
Wellford W. Inge, Jr., M.D., LTC,MC

Reports Control Symbol MEDDH-288(R1)

With improvement in techniques of fluid resuscitation, control of infection, and early coverage of the burn wound, re-emphasis of supportive care of convalescent thermally injured patients has become necessary. Adequate nutritional support is essential for successful wound healing and rehabilitation. This is usually achieved by the enteral administration of a high caloric, high protein hospital diet. The efficacy of this diet, however, has never been evaluated in burn patients.

Preliminary studies have involved the monitoring of dietary intake in four severely burned patients. Daily determinations of total caloric intake, protein intake and body weight were made for at least forty days in the early postburn stage. The patients consumed two to two and one-half times the amount predicted on the basis of basal metabolic rates. This was accompanied by a 15-20% loss of preburn body weight. All patients took in at least 2 g of protein per kg of body weight and three of the patients took in 3.5 g of protein per kilogram.

Results from these four patients are inconclusive. However, the 15-20% weight loss is indicative of possible malabsorption, and gastrointestinal function studies are planned in subsequent patients.

High Protein Diet
Trace Elements
Gastrointestinal Absorption

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6398	71 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
70 07 01	K, Completion	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A061102B71R	01	245
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) Studies of Dietary Constituents in Diets Administered to Sick and Injured Soldiers (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
68 07	71 05	DA	C. In-House

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

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
FISCAL YEAR	PRECEDING 71	0.40	10.6
	CURRENT 72	0	0

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

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

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

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic institution)
NAME: Mary E. Spitzer, CPT, AMSC
TELEPHONE: 512-221-4733
SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS
NAME: Frank J. Ruzicka, CPT, MSC
NAME: A. D. Mason, Jr., M. D. DA

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Diet; (U) Human volunteer; (U) Electrolytes

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

23. (U) In order to allow the dietitian to accurately formulate prescribed diets, a study of the dietary constituents was carried out.

24. (U) Electrolytes, including those found in trace quantities, in foods were determined by flame photometry, atomic absorption spectroscopy, and other analytical techniques.

25. (U) 70 07 - 71 05 (a) Major and trace elements in beverages have been determined and analysis completed.

(b) A new method for determining electrolytes in foods has been developed and was compared with a standard method of analysis.

(c) The sodium, potassium, and chloride content of a standard hospital sodium-restricted 500 mg diet has been analyzed and the completed reports show that the actual sodium content is twice the prescription.

(d) A comparison of the sodium and potassium in foods from 10 different authors with a standard table has shown that certain foods differ appreciably in sodium content from the content shown in standard tables and since the standard table does not include values for all sodium-restricted products, e.g., sodium-restricted bread, analysis of the total diet and its individual food items is the only accurate method for a true determination of a sodium-restricted diet.

*Available to contractors upon originator's approval.

FINAL REPORT

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

REPORT TITLE: A RAPID METHOD OF PREPARING FOOD FOR SODIUM AND POTASSIUM DETERMINATIONS

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

1 July 1970 - 30 June 1971

Investigators:

Mary E. Spitzer, CPT, AMSC
Ysidro Villarreal, B.S.
Arthur D. Mason, Jr., M.D.
Frank J. Ruzicka, CPT, MSC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

REPORT TITLE: A RAPID METHOD OF PREPARING FOOD FOR SODIUM AND POTASSIUM DETERMINATIONS

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

Period covered in this report: 1 July 1970 - 30 June 1971

Investigators: Mary E. Spitzer, CPT, AMSC
Ysidro Villarreal, B.S.
Arthur D. Mason, Jr., M.D.
Frank J. Ruzicka, CPT, MSC

Reports Control Symbol MEDDH-288(R1)

In the past, methods used for preparing foods for sodium and potassium determinations have involved ashing the sample or digesting it with various acids. Both methods are time consuming, subject to inaccuracies, and, in the case of acid digestion, potentially dangerous. Thus, a faster mechanical means of achieving solution of the sodium and potassium in foods was developed.

This rapid method of preparing food for electrolyte determinations was compared with the standard wet digest technique and was found to be as accurate and reproducible as the standard method. This expedient method of food analysis may be of value to dietitians who wish to measure the sodium and potassium contents of individual foods, recipes, and standard hospital diets.

Diet
Human volunteer
Electrolytes

BLANK PAGE

A RAPID METHOD OF PREPARING FOOD FOR SODIUM AND POTASSIUM DETERMINATIONS

The analysis of foods and the resulting food composition tables form the basis for assessing dietary composition. While food composition tables enable the dietitian to estimate the sodium and potassium content of diets, food analyses define the actual content.[1,2]

Flame photometric technique, used to measure sodium and potassium, requires that the sample be in solution and free of macro particles.[3] Methods have been developed to meet these criteria for analyses of biological material. Presently, there are two basic methods for preparing food for analysis. One method is to digest the food with acid (wet ash);[4] the other to burn the sample to an ash and make the ash soluble for analysis (dry ash).[3] Both of these methods are standard accepted procedures, but each has the disadvantage of requiring considerable time to complete the preparation.

The dietitian involved in developing, preparing, and serving defined sodium and potassium diets needs an expedient method of analysis to define dietary content. For this purpose, a rapid accurate method for analyzing the sodium and potassium content of foods has been developed.

Equipment

The rapid method is a mechanical means of achieving solution of the sodium and potassium in foods. The equipment we have employed to accomplish analysis is as follow:

1. Macro-homogenizer, Virtis Model 16-200, capable of 45,000 r.p.m. with stainless steel shaft-blade assembly and teflon cap.[5]

2. Fluted homogenizing flasks, Pyrex or equivalent, small (20 to 200 ml) and large (50 to 400 ml).

3. Volumetric flasks (1000 ml), Class A tolerance, Pyrex or equivalent.

4. Centrifuge, capable of 1,100 x g.

5. Filter paper, weight of ash per circle less than 0.07mg.

6. Flame photometer, Instrumentation Laboratory Model 143,[6] without the automatic dilutor for sodium and potassium analysis.

7. Standard solutions:

   a. 160 mEq/L sodium, 8 mEq/L potassium

   b. 100 mEq/L sodium, 100 mEq/L potassium

Method

To insure an identical homogeneous sample, the foods, except liquids and butters, were weighed, dried to a constant weight, and pulverized.

Rapid Method

The rapid method of preparation consisted of weighing 10.0 grams of the pulverized sample into a homogenizing flask. A quantity of deionized water was added to the homogenization flask to allow for a fluidized final homogenized sample. With the homogenizing flask setting in an ice bath, the food was homogenized at 45,000 r.p.m. for 20 minutes. After homogenization, the suspension of food in deionized water was quantitatively transferred to a 250 ml volumetric flask. The content of the volumetric flask was allowed to equilibrate with room temperature and then brought to volume with deionized water. After adequate mixing, an aliquot was poured into a centrifuge tube and centrifuged at 2,000 r.p.m. for 20 minutes. To eliminate fiber particles, the supernatant solution was filtered. Finally, the filtered supernatant fluid was analyzed by flame photometry.

Analysis

1. A series of 100 ml volumetric flasks were set up, one for each unknown and one each for a blank and the two standard solutions.

2. To each flask, 1.0 ml of 1,500 mEq/L lithium was added.

3. To one of the standard flasks, 0.5 ml of the 160 mEq/L sodium, 8 mEq/L potassium standard solution was added.

4. To the other standard flask, 0.5 ml of the 100 mEq sodium, 100 mEq potassium standard solution,was added.

5. To each unknown flask, 5.0 ml of the filtrate was added.

6. Each flask was brought to volume (100 ml) with deionized water.

7. Calculations:

a. Rapid Method

The machine readout in mEq/L was multiplied by 2.50 L/10 grams to arrive at mEq/100 grams of dried food sample.

To calculate the original wet food values/100 grams one solves the following equation:

$$\text{mEq/100 gm of dried food } \left(\frac{\text{dry weight}}{\text{wet weight}}\right) = \text{mEq/100 gm wet weight}$$

b. Precautions for the Rapid Method

1) When cleaning glassware, the homogenizer's shaft-blade assembly and its teflon cap, acetone is used to dissolve fats, followed by careful rinsing with deionized water.

2) To prevent air borne contamination and evaporation, flasks and test tubes are sealed with parafilm or its equivalent between procedures.

3) The use of plastic gloves, when food is handled, will help prevent contamination.

4) For already homogenized products, such as milk, the homogenization step may be omitted and the sample weighed directly into the volumetric flask.

5) For plain gelatin type products, such as prepared clear gelatin or jellies, using a funnel, weigh directly into the volumetric flask, add some deionized water, but do not bring to volume, and heat until the product is dissolved. Allow the heated material to come to room temperature, then bring to volume and continue with the method. This applies to clear gelatin and jellies, but not preserves or jams.

6) When analyzing products with a high fat content, for example peanut butter, omit the ice bath. The heat developed

during homogenization will make fat more evenly dispersed.

7) Since the homogenizer incorporates air into the sample, with some foods a large amount of foam is transferred to the volumetric flask,and interferes with bringing the contents to volume. The addition of a few drops of caprylic alcohol to the volumetric flask will dissipate the foam. Caprylic alcohol does not affect the results of sodium or potassium determinations.

8) If too much deionized water is added to the homogenizing flask, foaming may cause the fluidized sample to seep out between the teflon cap, the shaft-blade assembly, and the flask, resulting in a lost sample. By adding only enough deionized water to cover the blades or most of the food sample, this problem can be eliminated.

9) The quantitative transfer from the homogenizing to volumetric flask requires that all of the homogenate from the flask, the shaft-blade assembly, and the teflon cap be transferred by pouring and the use of deionized water. In this instance, the use of a washing bottle aids in the transfer of the hemogenate.

Comparison

To establish the accuracy and reproducibility of the rapid method, it was compared with:

Standard wet ash method. Fifteen different foods varying in estimated sodium and potassium content, from high to low, and consistency from solid to liquid, were selected for comparison. The wet ash method of preparation consisted of weighing 1.0 gm of the pulverized sample into a 50 ml digestion tube. Then, 5 ml of concentrated sulfuric acid and four pyrex glass boiling beads were added. The contents were heated until a clear solution was obtained for analysis.

Sodium and potassium analyses were performed on the final sample, using an Instrumentation Laboratory's flame photometer Model 143. The results were calculated to represent the values for 100 gm of dried sample.

Results

Table 1 lists the food items selected for analysis, and the results of the duplicate sodium analyses for each method. The

TABLE I

SODIUM (mEq/100 GRAM) - RAPID METHOD VERSUS STANDARD METHOD

ITEM	RAPID METHOD		STANDARD METHOD	
	SET I	SET II	SET I	SET II
HAMBURGER	8.500	8.500	9.500	8.500
CHICKEN	9.875	9.500	9.250	9.750
TUNA	42.500	42.750	42.750	43.000
PEANUT BUTTER	24.500	24.250	23.750	24.500
LIVER	11.500	11.500	11.750	11.500
EGG	18.250	19.625	18.500	18.650
MILK	2.200	2.200	2.225	2.250
CORN	0.625	0.500	0.500	0.500
CORN, FLAKED	46.250	46.000	52.000	42.750
BREAD	40.750	35.500	43.000	41.500
PEACHES	0.875	0.875	0.850	0.900
PEAS	22.250	22.375	22.500	22.500
LETTUCE	3.758	3.579	3.700	3.850
RAISINS	0.230	0.220	0.215	0.220
BUTTER	45.125	44.950	45.000	45.100

difference between duplicates for the rapid method was 0.563 ± 1.340 mEq sodium/100 gm of dried food sample; wet ash, 0.932 ± 2.341 mEq sodium/100 gm of dried food sample.

Table II shows the same results but for potassium. The difference between duplicates for the rapid method was 0.279 ± 0.247 mEq potassium/100 gm of dried food sample; wet ash, 0.677 ± 1.102 mEq potassium/100 gm of dried food sample. Liver was chosen for comparison because it had been found that acidification appeared necessary to release potassium from organ meats.[7] However, identical results were found for both methods.

Tables III and IV list the statistical comparison of the rapid method of food analyses versus the wet ash method by analysis of variance for sodium and potassium, respectively.[8] There was no significant statistical difference between the two methods for sodium or potassium determinations. There was no significant statistical difference between sets for sodium or potassium. Thus, statistically, the two methods are essentially the same.

Theoretical Error

When analyzing foods which contain high fat and/or fibrous materials, a theoretical error can be calculated. For example, if 100 grams of bacon, with 50 of the 100 grams being fat, were homogenized, transferred, brought to volume and shaken well to obtain a "representative sample", a representative sample would not be obtained. The reasoning is that fat, being less dense than water, would rise to the top of the flask and even with mixing would not be evenly distributed throughout the total volume: fibrous material would settle to the bottom of the flask. Thus, an error resulting in a determined value 10% greater than the actual value could be present. With this theoretical error, the sodium and potassium values of such foods analyzed by the rapid method would be greater than those of the standard method. The existence of this theoretical error is supposedly eliminated by the standard method. However, Table I (sodium)and Table II (potassium) show that the theoretical error was not of apparent significance with the rapid method, since the sodium and potassium values calculated by this method were either lower than those calculated by the standard method or not significantly greater.

Discussion

The rapid method has several advantages when compared to

TABLE II

POTASSIUM (mEq/100 GRAM) - RAPID METHOD VERSUS STANDARD METHOD

ITEM	RAPID METHOD		STANDARD METHOD	
	SET I	SET II	SET I	SET II
HAMBURGER	29.000	28.750	28.500	24.500
CHICKEN	26.250	26.750	26.000	26.500
TUNA	24.000	23.750	24.000	24.000
PEANUT BUTTER	19.750	20.000	20.000	20.500
LIVER	26.750	26.500	26.750	26.500
EGG	10.750	11.000	10.500	11.000
MILK	3.600	3.750	3.500	3.750
CORN	17.750	17.000	14.500	17.000
CORN, FLAKED	2.500	2.500	3.000	2.500
BREAD	5.250	4.500	5.500	5.500
PEACHES	16.500	16.500	17.000	16.500
PEAS	19.750	20.000	20.000	20.000
LETTUCE	5.386	5.419	5.350	5.450
RAISINS	22.500	23.000	22.000	22.500
BUTTER	0.670	0.670	0.250	0.300

TABLE III

ANALYSIS OF VARIANCE - A COMPARISON OF THE RAPID METHOD OF FOOD ANALYSIS VERSUS A STANDARD METHOD FOR SODIUM

SOURCE	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARES	F	PROBABILITY
TOTAL	59	17,208.61			
FOOD ITEMS	14	17,129.58			
RAPID METHOD VERSUS STANDARD METHOD	1	2.15	2.15	1.24	NOT SIGNIFICANT
SET I VERSUS SET II	1	3.65	3.65	2.11	NOT SIGNIFICANT
METHODS X SETS	1	0.46	0.46	0.27	NOT SIGNIFICANT
ERROR	42	72.77	1.73		

TABLE IV

ANALYSIS OF VARIANCE - A COMPARISON OF THE RAPID METHOD OF FOOD ANALYSIS VERSUS A STANDARD METHOD FOR POTASSIUM

SOURCE	DEGREES OF FREEDOM	SUM OF SQUARES	MEAN SQUARES	F	PROBABILITY
TOTAL	59	5,281.98			
FOOD ITEMS	14	5,259.57			
RAPID METHOD VERSUS STANDARD METHOD	1	0.85	0.85	1.67	NOT SIGNIFICANT
SET I VERSUS SET II	1	0.01	0.01	0.02	NOT SIGNIFICANT
METHODS X SETS	1	0.00	0.00	0.00	NOT SIGNIFICANT
ERROR	42	21.55	0.51		

the standard dry ash and wet ash methods. First, it is very fast, the analytical results can be obtained in four hours. The dry ash method requires a minimal ashing time of eight hours; and, to obtain a clear solution, the west ash method required two weeks to digest the majority of foods tested in this study.Since the dry ash method requires relatively difficult transfers, it is easy to lose part of the ash. Transfers are simple with the rapid method, with only one quantitative transfer, which is a standard laboratory procedure. The use of concentrated sulfuric acid (wet ash) is hazardous and requires the use of an acid-resistant hood to insure that vapors are properly eliminated. The rapid method is essentially hazard free and is reliable and reproducible. The advantages of the rapid method for food analysis may make it preferable to the standard method for sodium and potassium determinations.

The ease of the rapid method in comparison to other methods will enable the dietitian to analyze individual food items and standard hospital diets, thus acquiring food values applicable to the geographical area, food supply, and the institution's recipes. With greater emphasis upon the role of diets in clinical medicine, food analysis by each institution will be essential for accurate dietary conputation. It follows that with the continual introduction of new varieties of foods, the use of different cultural practices in crop production and the increased usage of convenience foods, food analysis should be an inherent aspect of any clinical, research, or dietary activity.

Conclusion

A rapid method of preparing food for sodium and potassium determinations is described, which is as accurate and reproducible as a standard method. This expedient method of food analysis may be of value to dietitians who wish to measure the sodium and potassium contents of individual foods, recipes, and standard hospital diets.

References

1. Harris RS: Reliability of nutrient analyses and food tables. Amer J Clin Nutr 11: 377, 1962.

2. Asenjo CF: Variation in the nutritive values of foods. Amer J Clin Nutr 11: 368, 1962.

3. Hald PM: The flame photometer for the measurement of

sodium and potassium in biological materials. J Biol Chem 167: 499, 1947.

4. Official Methods of Analysis, 10th ed., Washington, DC: Assn. Off. Agric Chemists, 1965.

5. Instructions for the Vir Tis Hi-Speed "45" Homogenizer Model 16-200. Gardiner, NY: The Vir Tis Co.,Inc.

6. Operator's Manual 143, Flame Photometer. Watertown, Mass: Instrumentation Laboratory, Inc., 1967.

7. Mounib MS, Evans JV: Comparison between three methods for the preparation of tissues for determinations of potassium and sodium. The Analyst 82: 522, 1957.

8. Bruning JL, Kintz BL: Computational Handbook of Statistics. 1st ed, Glenview, Ill: Scott, Foresman and Co, 1968.

Presentations

Spitzer ME: A rapid method of preparing food for sodium and potassium determinations. Georgia Dietetic Assn, Atlanta, Ga., 20 October 1970.

Publications

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OD 6952	71 07 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. GRP'N INDIC'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
	K.COMPLETION	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A061102B71R	01	305
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) Red Blood Cell Thiamine Pyrophosphate Level 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
70 08	71 04	DA	C. In-House

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

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
FISCAL YEAR PRECEDING 71	.9	23.9
FISCAL YEAR CURRENT 72	0	0

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

RESPONSIBLE INDIVIDUAL
NAME: PRUITT, B. A., JR, LTC, MC
TELEPHONE: 512-221-2720

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

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME: Jerl Blackwell, BS
TELEPHONE: 512-221-4106
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: George M Helmkamp, Jr, CPT, MSC
NAME: DA

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Thiamine pyrophosphate (TPP); (U) Transketolase; (U) Sedoheptulose

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 thiamine pyrophosphate deficiency occurs in burn patients in order to allow for early recognition and treatment of this deficiency.

24. (U) Twenty adult patients with burns of 30% or more of body surface will be studied on postburn days 5 and 10. Two adult patients with similar burns will be followed serially from the time of admission until termination of treatment. Normals will be used as controls. The activity of total transketolase in the red blood cells of these patients will be measured by determining the amount of sedoheptulose formed by the hemolysate to which exogenous TPP is added. The TPP-augmentable transketalase activity which will provide a measure of thiamine pyrophosphate deficiency is determined as the difference in the transketolase activity of the red blood cells following the addition of exogenous thiamine pyrophosphate.

25. (U) 70 07 - 71 04 Significant thiamine phyophasphate (TPP) deficiency was not observed in the thermally injured patients, possibly due to the fact that supplemental vitamins were administered intravenously or orally upon admission and throughout the treatment of the burn. A slight decrease in TPP levels occurred in those patients who were febrile.

* Available to contractors upon originator's approval

FINAL REPORT

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

REPORT TITLE: RED BLOOD CELL THIAMINE PYROPHOSPHATE LEVELS FOLLOWING THERMAL INJURY

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

1 July 1970 - 30 June 1971

Investigators:

Jerl P. Blackwell, B.S.
George M. Helmkamp, Jr., CPT, MSC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

REPORT TITLE: RED BLOOD CELL THIAMINE PYROPHOSPHATE LEVELS FOLLOWING THERMAL INJURY

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

Period covered in this report: 1 July 1970 - 30 June 1971

Investigators: Jerl P. Blackwell, B.S.
George M. Helmkamp, Jr., CPT, MSC

Reports Control Symbol MEDDH-288(R1)

The relationships of transketolase-determined TPP deficiency, febricity, and burn size in thermal injuries were investigated. While statistical findings did not substantiate any relationship, those patients who were febrile on the day of analysis had a mean deficiency of 19%, while those who remained afebrile elicited a deficiency of only 11%. Burn size and TPP deficiency could not be correlated. One patient followed for 33 days also illustrated the relationship of TPP deficiency and febricity reported by others. On febrile days the TPP deficiency was greater than 20%, but this value dropped back to within normal limits when the body temperature returned to normal.

Thiamine Pyrophosphate (TPP)
Transketolase
Sedoheptulose

RED BLOOD CELL THIAMINE PYROPHOSPHATE LEVELS FOLLOWING THERMAL INJURY

In 1969 Gilbert, Susser, and Nolte demonstrated a deficiency of thiamine pyrophosphate (TPP) during febrile human infections.[1] Deficiencies occurred in patients who were anorexic and feverish for one week or longer and in those patients debilitated by other infectious diseases. The possibility that TPP deficiency might contribute to the morbidity and mortality of these patients was of utmost concern. The recognition of such a deficiency in patients with underlying disease or injury is also of considerable importance as these individuals are frequently susceptible to secondary infections which respond poorly to therapy.

This investigation was designed to find out if a similar TPP deficiency exists in burn patients and plays a role in the metabolic disorders associated with thermal injury.

Methods

Brin et al. noted the presence of transketolase activity in red blood cells and its applicability in monitoring thiamine levels in the body.[2] This enzyme, which has an absolute requirement for thiamine pyrophosphate, catalyzes the following reaction:

xylulose-5-phosphate + ribose-5-phosphate ⇌ sedoheptulose-7-phosphate + glyceraldehyde-3-phosphate

Xylulose-5-phosphate is generated from rubose-5-phosphate via enzymatic epimerization.

The determination of TPP was described by Gilbert et al. who modified the assay procedure of Dreyfus.[3] Erythrocytes from adult male patients with burns of 30% or greater were collected on the 5th and 10th postburn days. One patient with a 42% burn was followed serially from the day of admission. Normals were obtained from personnel in the laboratory.

Approximately 10 cc of heparinized (1 mg/ml) venous blood were centrifuged for 30 minutes at 4°C, the buffy coat removed, and the red cells washed once in three volumes of 0.9% saline. The packed erythrocytes were then lysed in an equal volume of distilled water and stored at -70°. Before use the hemoglobin concentration was determined spectrophotometrically. Reagents used included

Krebs-ringer buffer, 0.002 M thiamine pyrophosphate (Sigma Chemical Company), 0.036 M ribose-5-phosphate(Sigma), 0.25 mg/ml of sedoheptulose-7-phosphate (Sigman), 15% trichloroacetic acid, 70% sulfuric acid, and 30 mg/ml of cysteine. After incubation at 37°, sedoheptulose-7-phosphate was measured colorimetrically according to Dische.[4] The results are expressed in mg of sedoheptulose produced per hour per gram of hemoglobin. The activity of total transketolase is that quantity of product elaborated by the hemolysate to which exogenous TPP (1 mM) was added; the TPP-augmentable activity is obtained by subtracting the sedoheptulose synthesized by the hemolysate alone from that of the total transketolase. Thus, augmentation of transketolase by supplemental TPP reflects the level of TPP-depleted apoenzyme in the red cell. TPP deficiency is merely the per cent of total activity attributable to augmentation.

Results and Discussion

In Table 1 are presented the results of this investigation, together with brief clinical histories of the patients. Patients 4 and 5 were sampled on the 3rd and 5th days as a result of their impending demise. There appears to be no correlation between burn size and TPP deficiency. Rather the magnitude of thiamine requirements may be determined to some extent by the body temperature. Those patients who were febrile on the day of analysis had a mean deficiency of 19%, while those who remained afebrile elicited a deficiency of only 11%; controls averaged 13% (Table 2). A mean deficiency of 10% has been reported for normal, healthy subjects.[1]

The relationship between TPP deficiency and febricity is more dramatically illustrated in the case of patient 8, who was followed for 33 days with 13 blood samples. While febrile from days 1 through 8, his deficiency was greater than 20%; upon returning to normal body temperature on day 9 and remaining there, the deficiency decreased to within normal limits.

Despite these cursory observations and conclusions, the data do not withstand rigid statistical analysis. Thus, as noted in Table 2, no significance can be attached to the differences in total and TPP-augmentable transketolase activities among the three subject categories. However, this treatment is no doubt limited by the small number of samples.

It must be noted that all patients received upon admission the Brooke formula for fluid resuscitation and throughout their

Table 1. Summary of Patient Histories and Transketolase Activities

Patient	Age	% Body Surface Burn	Postburn Day of analysis	Body Temperature *	Activity of RBC transketolase (mg sedoheptulose/hr/g Hb) Total	TPP-augmentable	TPP Deficiency (%)	Final Disposition** of Patient
1	51	66	5	F	4.75	0.56	11.9	D
			10	F	4.00	0.75	18.8	
2	42	32	5	F	5.38	1.12	20.8	R
			10	N	8.07	0.15	1.9	
3	22	51	5	F	8.67	1.17	13.5	D
			10	N	8.67	2.00	20.1	
4	22	83	3	N	7.75	0.38	4.8	D
			5	N	6.42	0.50	7.8	
5	18	60	3	F	7.00	1.57	22.4	D
			5	N	8.71	1.11	12.7	
6	27	41	5	F	8.13	1.57	19.3	R
			10	F	12.00	2.60	21.7	
7	26	58	5	F	8.00	1.40	17.5	R
			10	F	9.25	1.75	18.5	
8	23	42	5	F	5.70	1.20	21.1	R
			10	N	6.30	0.75	11.9	

* N, normal; F, febrile (> 100°F)

** R, recovered; D, deceased

Table 2. Activities of Total and TPP-augmentable Transketolase in Red Blood Cells from Febrile and Afebrile Burn Patients and Controls

Subject	Number of Samples	Activity of RBC Transketolase (mg sedoheptulose/hr/g Hb) Total	TPP-augmentable	TPP Deficiency (%)
Controls	6			
Mean ± SE		8.87 ± 1.13	1.13 ± 0.21	13
Range		6.00 to 13.80	0.64 to 2.00	10 to 14
95% confidence limits *		5.96 to 11.78	0.59 to 1.67	
Burn patients, afebrile	6			
Mean ± SE		7.65 ± 0.44	0.82 ± 0.27	11
Range		6.30 to 8.71	0.38 to 2.00	2 to 20
95% confidence limits		6.53 to 8.77	0.12 to 1.52	
Burn patients, febrile	10			
Mean ± SE		7.29 ± 0.76	1.37 ± 0.18	19
Range		4.00 to 12.00	0.56 to 2.60	12 to 22
95% confidence limits		5.56 to 9.02	0.96 to 1.78	

* Confidence limits calculated by t-test

hospital stay vitamin supplementation, either intravenously or in tablet form. This fact, coupled with generally good preburn physical status, probably accounts for the relatively low levels of thiamine deficiency, regardless of body temperature, in these burn patients. Values in the range 30-50% are not uncommon among patients with severe infection and/or prolonged fever.[1]

References

1. Gilbert VE, Susser MC, Nolte A: Deficient thiamine pyrophosphate and blood alpha-ketoglutarate-pyruvate relationships during febrile human infections. Metabolism 18:789-799, 1969.

2. Brin M, Tai M, Ostashever AS, Kalinshy H: The effect of thiamine deficiency on the activity of erythrocyte hemolysate transketolase. J. Nutrition 71:273-280, 1960.

3. Dreyfus PM: Clinical application of blood transketolase determinations. NEJM 267:596-598, 1962.

4. Dische Z: Qualitative and quantitative colorimetric determinations of heptoses. J Biol Chem 204:983-993, 1953.

Publications and/or Presentations

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0D 6977	71 07 01	DD-DR&E(AR)636

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

10. NO. CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A061102B71R	01	193
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) Diagnosis and Treatment of Acute Gastrointestinal Ulceration 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 08	71 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 71	.1	2.0
FISCAL YEAR CURRENT 72	0	0

19. RESPONSIBLE DOD ORGANIZATION
NAME: US Army Institute of Surgical Research
ADDRESS: Ft Sam Houston, Texas 78234
RESPONSIBLE INDIVIDUAL
NAME: PRUITT, B.A., Jr, LTC, MC
TELEPHONE: 512-221-2720

20. PERFORMING ORGANIZATION
NAME: US Army Institute of Surgical Research
ADDRESS: Ft Sam Houston, Texas 78234
PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME: Basil A Pruitt, Jr, LTC, MC
TELEPHONE: 512-221-2720
SOCIAL SECURITY ACCOUNT NUMBER:
ASSOCIATE INVESTIGATORS
NAME: F D Foley, MD
NAME: A D Mason, Jr, MD DA

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Burns; (U) Curling's Ulcers; (U) Stress Ulcer; (U) Gastrointestinal Hemorrhage

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

23. (U) The description of the natural history of acute ulceration of the stomach and duodenum in burn patients to delineate factors of clinical significance in the pathogenesis of Curling's ulcer. Review and analysis of results of surgery.

24. (U) Review of hospital charts of burn patients with Curling's ulcer treated since Jan 1954. Recording of clinical characteristics, including number and location of ulcers, symptoms and signs, coexisting sepsis, indications for surgery, type of surgical procedure and operative results. Records of all patients treated in 1968 to be reviewed to relate sepsis to Curling's ulcer.

25. (U) 69 08-71 06 Clinical review completed. Curling's ulcer occurred in 11.7% of 2,951 burn patients since Jan 1954. Curling's ulcer is directly related to burn size and the dose-response manner rising to 40% in patients with burns of 70% or more. Ulcers are commonly small, multiple, and gastric in location. Sixteen per cent of patients had both gastric and duodenal lesions. Hemorrhage is most common presenting sign (63% of patients), and in half it is massive. Perforation present in 12% of patients since 1968. Sepsis is an additive stress predisposing to ulcer development which is highly significant in patients with small burns. Sixteen of 43 patients who required surgery survived and were discharged. Seven others regained gastroduodenal function but died of other complications. These two groups represent a potential salvage rate of 54% compared to only 23% of the entire group as a whole. Operation of choice is vagectomy with antrectomy.

• Available to contractors upon originator's approval

FINAL REPORT

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

REPORT TITLE: DIAGNOSIS AND TREATMENT OF ACUTE GASTROINTESTINAL ULCERATION IN BURN PATIENTS

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

1 July 1970 - 30 June 1971

Investigators:

Basil A. Pruitt, Jr., M.D., LTC, MC
F. Daniel Foley, M.D.
Arthur D. Mason, Jr., M.D.

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

BLANK PAGE

ABSTRACT

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

REPORT TITLE: DIAGNOSIS AND TREATMENT OF ACUTE GASTROINTESTINAL ULCERATION IN BURN PATIENTS

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

Period covered in this report: 1 July 1970 - 30 June 1971

Investigators: Basil A. Pruitt, Jr., M.D., LTC, MC
F. Daniel Foley, M.D.
Arthur D. Mason, Jr., M.D.

Reports Control Symbol MEDDH-288(RI)

Acute stress ulceration of the gastrointestinal tract has been diagnosed in 346, or 11.7%, of 2,951 burn patients treated at the US Army Institute of Surgical Research between January 1954 and June 1970. In approximately two-thirds of patients, hemorrhage was the initial sign and in almost one-half of these it was massive and life-threatening. Sudden, unexplained hypotension and/or ileus occurring late in the postburn period is suggestive of Curling's ulcer. Characteristically, the lesions are small, multiple, and gastric in location, but in approximately one out of six of these patients, both gastric and duodenal ulcers were present. Over the past three and one-half years, perforation of a Curling's ulcer had occurred at the time of diagnosis in 12% of patients. Increased overnight gastric acid secretion was noted in only four patients who subsequently developed Curling's ulcer, and three of these had coexisting hypercarbia secondary to pulmonary complications which is known to increase acid secretion. Sepsis is an additive stress contributing to ulcer development and was present prior to or at the time of diagnosis in 77% of recent cases. Another factor of importance in the pathogenesis of Curling's ulcer is size of burn.

Nonoperative treatment was initially employed in all

patients with bleeding, but even if bleeding ceased over one-third of such patients bled again. Forty-three patients required surgical intervention, 32 because of massive hemorrhage, seven because of perforation and four because of prolonged hemorrhage. The operative procedure of choice is vagotomy with antrectomy, but other operations have been employed in specific cases. Rebleeding followed resection in only four of the 32 patients operated upon for hemorrhage. Seven of the surgical patients recovered from surgery and regained gastrointestinal function, but expired later in their hospital course, and represent potential survivors who, when combined with the 16 patients who survived their operation and were discharged from the hospital, constitute a potential survival group of 54%, in marked contrast to the over-all survival of only 23% in this entire population with Curling's ulcer.

Burns
Gastrointestinal Hemorrhage
Stress Ulcer
Curling's Ulcer

DIAGNOSIS AND TREATMENT OF ACUTE GASTROINTESTINAL ULCERATION IN BURN PATIENTS

Acute ulceration of the stomach or duodenum, first described in a burn patient by Swan in 1823 and reported as occurring in 10 burn patients by Curling in 1842, is the most frequent life-threatening gastrointestinal complication in the burn patient. Although no single pathogenetic mechanism has been found to adequately explain the occurrence of this entity, ulcerogenic factors active in other clinical situations are present in burn patients, the additive effects of which may tip the physiologic scales toward ulceration of the gastroduodenal mucosa. This clinical review records the experience of this institute in the treatment of 346 burn patients with a clinical or autopsy diagnosis of Curling's ulcer, and draws attention to those co-existing stresses which appear significant in the development of this complication.

Materials and Methods

The hospital records of the 346 patients treated at the US Army Institute of Surgical Research from January 1954 to June 1970 were reviewed. The clinical characteristics of each patient, the location and number of ulcerations, and the morphology of the ulcer were recorded. In the case of 43 operative patients, the indication for surgery, the quantity of preoperative blood replacement, the operative procedure employed, postoperative complications, and in those who expired the cause of death were noted.

The records of 394 consecutive burn patients treated at this Institute in 1968 were reviewed to determine what, if any, effect pre-existing sepsis had on the subsequent development of Curling's ulcer.

The criteria used to make the diagnosis of Curling's ulcer in these burn patients were: (1) ulceration of the stomach or duodenum present at surgery or autopsy; (2) gastric or duodenal ulcers in the burn patient noted by X-ray; (3) clinical evidence of upper gastrointestinal bleeding after the third postburn day requiring transfusion in excess of that anticipated in the normal course of the burn patient; and (4) massive upper gastrointestinal bleeding at any time resulting in a fall in the hematocrit of 10 points or shock and requiring immediate transfusion. Iatrogenic

"tube" erosions were excluded as was hemorrhagic gastritis where no discrete ulcers were apparent.

Results

The 346 patients with Curling's ulcer included 273 males and 73 females, and range in age from 35 days to 82 years. The mean burn index of the group was 41.7, with a range of 2 to 95. The mortality rate was 77%. In 121 Curling's ulcer patients treated during the past three and one-half years, the average burn size has been 50% of the total body surface with a 23% third-degree component. In this latter subgroup, the presence of Curling's ulcer was diagnosed clinically in 89 patients (74%) and the mean postburn day of diagnosis was the 15th. The diagnosis rested on clinical grounds alone in only 23 patients, was confirmed by X-ray in 9, and confirmed at either surgery or autopsy in 57.

Curling's ulcers are characteristically round, shallow and sharply demarcated, showing no fibrosis and little inflammatory change. Those ulcers associated with massive hemorrhage have characteristically eroded into a mural vessel following extension through the muscularis mucosa. Since 1968, perforation of these ulcers had occurred in 12% of the patients at the time of diagnosis of their ulcer.

There were 246 of these patients in whom the site of the ulcer was confirmed (Table 1). Gastric ulcers (commonly multiple) were present in 47% of the patients, and duodenal ulcers, more commonly solitary, were present in 37%. In one out of six of these patients, both gastric and duodenal ulcers were present. The distribution of lesions in burned children with Curling's ulcer was the same.

Table 1. Site of Curling's Ulcer in 246 Patients, 1954-June 1970

Location	No. of Patients			% of Ulcers
	Single	Multiple	Total	
Gastric	20	96	116	47
Duodenal	64	27	91	37
Gastric and duodenal			39	16

In 63% of these patients, upper gastrointestinal hemorrhage was the initial sign, with hematemesis three times more frequent than melena. The hemorrhage was massive in 44% of the patients with gastrointestinal hemorrhage requiring prompt resuscitation in all, and surgical intervention in 32. Distention was regarded as a presenting sign in only 9% of these patients and its frequent association with sepsis in burn patients makes it relationship to Curling's ulcer unclear. Pain as an initial symptom has in general been associated with perforation or sudden, massive exsanguination and was felt to be the presenting symptom in only 4% of our patients. In 24% of these patients, the diagnosis was first made at the autopsy table and no symptoms or signs had been recorded clinically.

Burn size is directly related to the occurrence of Curling's ulcer in a dose response manner, with the incidence of ulceration rising to approximately 40% in patients with burns of 70% or more of the total body surface. Although no direct relationship between sepsis and Curling's ulcer has been previously confirmed, a frequent association of infection and Curling's ulcer as well as other stress ulcers has been noted by many other authors. Since 1967, sepsis had been diagnosed during life in 88% of the patients who expired with the diagnosis of Curling's ulcer made at the autopsy table, and in 62% of all the patients in whom the diagnosis was made during life. The most common forms of infection present in these patients were pneumonia, suppurative thrombophlebitis, burn wound sepsis, septicemia, and an assortment of other septic processes. A 5-day interval between the diagnosis of sepsis and the diagnosis of the Curling's ulcer was noted.

To further analyze the relationship between Curling's ulcer and sepsis, the incidence of Curling's ulcer in 394 consecutive burn cases treated in 1968 with and without pre-existing sepsis was determined and compared. A significantly greater incidence of Curling's ulcer was noted in those patients with pre-existing sepsis. In patients with burns of greater than 50% of the body surface, although an additive effect of sepsis was apparent, it was not significant. In those patients with burns of less than 50% of the body surface, the greater incidence of Curling's ulcer in those with pre-existing sepsis was highly significant, confirming the additive effect of the stress of sepsis in these burn patients who subsequently developed Curling's ulcer.

Comments

Initial nonoperative treatment should be attempted in all

patients with Curling's ulcer unless massive, exsanguinating hemorrhage is the presenting sign. Even if the initial hemorrhage ceases with medical therapy, one-third of the patients with hemorrhage due to Curling's ulcer will rebleed. Initial blood requirements may be disproportionate to the observed bleeding since the occult nature of this complication may lead to a dangerously diminished blood volume prior to clinical manifestations of hemorrhage. Forty-three patients required surgical treatment, either because of uncontrollable hemorrhage, 36; or perforation, 7. Our reluctance to operate upon these patients has been reflected in the average of almost 10 units of blood given to the patients prior to surgery, although in recent years surgery has been undertaken with less hesitance as manifested by the decrease to approximately 7 units in the average amount of blood given preoperatively.

Excision of the ulcer is of primary importance with addition of a vagotomy to diminish whatever acid secretion is present and for its beneficial effect upon the gastric mural vasculature. The operations employed in the 43 operative cases and the operative results associated with each operation are shown in Table 2. Our experience with nonresectional therapy is both limited and discouraging. Our operation of choice is vagotomy with antrectomy to preserve as much gastric mass as possible consistent with excision of the ulcer. If a more extensive gastric resection is necessary to remove the offending ulcer, we advocate subtotal gastrectomy and omit the vagotomy.

Table 2. Type of Operation

Operations	Total No.	Survivors	Deaths
Subtotal gastrectomy	20	8	12
Vagotomy and antrectomy	14	5	9
Vagotomy and pyloroplasty	4	1	3
Vagotomy and gastrojejunostomy	2	2	-
Plication of ulcer	3	-	3
	43	16 (37%)	27

Postoperative complications in the surgical group were commonly related to conditions which existed preoperatively. Pneumonia was the most frequent postoperative complication, reflecting the fact that it was the most common pre-existing septic

complication in these Curling's ulcer patients. Rebleeding occurred in only 4 of the 32 patients in whom operation had been undertaken because of hemorrhage and then only in those patients in whom a duodenal ulcer could not be excised (2) or patients who developed an anastomotic leak and/or peritonitis.

Thirty-seven per cent of the operative group (16 patients) survived and were discharged from the hospital. Seven of the surgical patients who expired later in their hospital courses with other complications had recovered from surgery and regained gastrointestinal function and represent potential survivors. When the actual survivors are combined with the potential survivors, an overall potential salvage of 54% was achieved compared to the survival of only 23% of this entire population with Curling's ulcer.

Summary

Of 2,951 burn patients treated at this Institute between January 1954 and June 1970, 11.7% developed Curling's ulcer. Pre-existing sepsis is an additive stress significantly predisposing the patients with smaller burns to the development of Curling's ulcer. Curling's ulcer is directly related to burn size. Approximately one-fourth of burn patients with Curling's ulcer will have no symptoms or signs during life, with the diagnosis first being made at the autopsy table. Gastrointestinal bleeding was the presenting clinical sign in two-thirds of the patients and was massive in approximately one-half of these. Solitary Curling's ulcer of the stomach is the most common lesion, but in 16% of patients both gastric and duodenal ulcers coexist, and at surgery, exploration of both stomach and duodenum is essential. Vagotomy and antrectomy is the operation of choice, but excision of the ulcer, which is the goal of surgical therapy, may require greater gastric mass resection with the surgical procedure tailored to meet the needs of the individual patient. Sixteen of 43 patients undergoing surgery for Curling's ulcer survived to be discharged from the hospital.

References

1. Harkins HN: Acute ulcer of the duodenum (Curling's ulcer) as a complication of burns; Relation to sepsis. Surgery, 3:608-641, 1938.

2. O'Neill,JA, Pruitt BA Jr, Moncrief JA, Switzer WE: Studies related to the pathogenesis of Curling's ulcer. J Trauma 7: 275-284, 1967.

3. O'Neill JA Jr, Pruitt BA Jr, Moncrief JA: Surgical treatment of Curling's ulcer. Surg Gynec Obstet 126:40 -44, 1968.

Publications

Pruitt BA Jr, Foley FD, Moncrief JA: Curling's ulcer: A clinical-pathologic study of 323 cases. Ann Surg 172:523-539, 1970.

Pruitt BA Jr, O'Neill JA Jr: Diagnosis and treatment of Curling's ulcer. A report of 346 cases. Proc. Third Internatl Congress for Res in Burns. P.Matter,T.L.Barclay,Z.Konickova(eds).Hans Huber,p.465

Presentations

Pruitt BA Jr: Curling's ulcer. A clinical-pathologic study of 323 cases. Presented at the annual meeting of the American Surgical Assoc, White Sulphur Springs, W Va, April 1970.

Pruitt BA Jr: Diagnosis and treatment of Curling's ulcer. A report of 346 cases. Presented at Third Internatl Congr for Research in Burns, Prague, Czech, 23 Sep 1970.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA OC 6975	71 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
70 07 01	D. CHANGE	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A061102B71R	01	272
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)* (U) Effect of Adrenalectomy on Curling's Ulcer in the Burned Laboratory Rat Model of a Complication Occurring in Injured Military Personnel (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*
003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
69 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 71	0.68	18.1
	CURRENT 72	0.68	19.9

19. RESPONSIBLE DOD ORGANIZATION

NAME:* US Army Institute of Surgical Research

ADDRESS:* Ft Sam Houston, Texas 78234

RESPONSIBLE INDIVIDUAL

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

TELEPHONE: 512-221-2720

20. PERFORMING ORGANIZATION

NAME:* US Army Institute of Surgical Research
Laboratory Animal Branch

ADDRESS:* Ft Sam Houston, Texas 78234

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

NAME:* Gilbert L. Raulston, COL, VC

TELEPHONE: 512-221-4951

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Harrell L. Walker, M.S.

NAME: DA

21. GENERAL USE

FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Adrenalectomy; (U) Stress Ulcer; (U) Curling's Ulcer

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

23. (U) Determine pathogenesis of gastric ulceration following trauma. Definition of pathogenesis will eventually lead to treatment and/or preventive procedures in burn subjects.

24. (U) Standard scald burn (20%BSA) inflicted on anesthetized rats after surgical adrenalectomy and/or other experimental manipulations (ie. starvation and administration of Lente insulin). Rats are sacrificed 24-72 hours postburn with gross and microscopic examination of the glandular stomach.

25. (U) 70 07 - 71 06 - Lesions have been produced in rats as cited above. The lesions are considered identical with those previously identified as Curling's ulcers or stress ulcers in the rat and those identified in the human. Further studies await receipt of a new strain of rats and establishment of baseline values on that strain. The strain change was necessitated due to recognition of a subtle change in response from that observed previously in rats in this laboratory after burning. The change was in wound healing and mortality postburn which appeared to coincide with an observation that ulcer incidence was considerably different from that formerly observed.

*Available to contractors upon originator's approval.

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

ANNUAL PROGRESS REPORT

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

REPORT TITLE: EFFECT OF ADRENALECTOMY ON CURLING'S ULCER FORMATION IN THE BURNED RAT

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

1 July 1970 - 30 June 1971

Investigators:

Gilbert L. Raulston, COL, VC
Harrel L. Walker, M.S.

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

REPORT TITLE: EFFECT OF ADRENALECTOMY ON CURLING'S ULCER FORMATION IN THE BURNED RAT

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

Period covered in this report: 1 July 1970 - 30 June 1971

Investigators: Gilbert L. Raulston, COL, VC
Harrel L. Walker, M.S.

Reports Control Symbol MEDDH-288(R1)

A previous report outlined methods of producing gastric lesions in the burned rat (Anl Prog Rpt, 30 June 1970, Sect 44). Since that report, changes in the response of Holtzman strain rats to adrenalectomy and thermal stress have been noted, both experimental and control rats showing very small lesions at about the same rate of incidence. These lesions are not the frank ulcerations previously reported.

Efforts to identify the reason for this change in response have been unsuccessful. These efforts are continuing in the belief that elucidation of the cause of change in response will lead toward identification of pathogenic mechanisms of the originally reported lesions.

Adrenalectomy
Stress Ulcer
Curling's Ulcer

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0D 6383	71 07 01	DD DR&E(A) 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
70 07 01	H.TERMINATION	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A061102B71R	01	311
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) Myositis Ossificans in Burns: A Prospective Study of a Disabling Complication of Burn Injury in Soldiers (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
69 07	70 11	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 71	.77	20.5
FISCAL YEAR CURRENT 72	0	0

19. RESPONSIBLE DOD ORGANIZATION
NAME: US Army Institute of Surgical Research
ADDRESS: Ft Sam Houston, Texas 78234
RESPONSIBLE INDIVIDUAL
NAME: PRUITT, B.A., Jr, LTC, MC
TELEPHONE: 512-221-2720

20. PERFORMING ORGANIZATION
NAME: US Army Institute of Surgical Research
Clinical Division
ADDRESS: Ft Sam Houston, Texas 78234
PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME: Andrew M Munster, LTC, MC
TELEPHONE: 512-221-5712
SOCIAL SECURITY ACCOUNT NUMBER:
ASSOCIATE INVESTIGATORS
NAME: H M Bruck, MAJ, MC DA
NAME: L A Johns, LTC, ANC

21. GENERAL USE: FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code): (U) Joints; (U) Elbows; (U) Calcium; (U) Ossification; (U) Myositis

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

23. (U) To discover if there are any common factors in patients suffering from thermal injury which lead to the development of calcification of burned joints in some patients and not in others.

24. (U) Each patient with upper limb burns has been evaluated by a team of physicians, nurses, physical therapists, occupational therapists and a nurse psychologist. Regular weekly physical examination of the joints was made, weekly x-rays were taken, serum chemistries were drawn: alkaline pahosphatase, calcium and phosphorus. Careful examination on a basis of range of motion of the elbow joints was made, and the patient subjected to psychological testing. Initially, it was planned to investigate 50 patients; however, a total of 100 patients were investigated.

25. (U) 70 07 - 70 11 The study has now been concluded, with a total of 180 limbs in 100 patients. Calcification occured in 12 patients out of 100. Significant contributing factors were the presence of third degree burns of the extremity and impairment of motion at the adgoining wrist and shoulder joint. Prolonged bedrest in either the supine or prone position did not contribute significantly to the development of calcification, and blood levels of calcium, phosphorus, and alkaline phosphatase likewise were noncontributory.

*Available to contractors upon originator's approval

FINAL REPORT

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

REPORT TITLE: MYOSITIS OSSIFICANS IN BURNS: A PROSPECTIVE STUDY OF A DISABLING COMPLICATION OF BURN INJURY

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

1 July 1970 - 30 June 1971

Investigators:

Andrew M. Munster, M.D., LTC, MC
Harold M. Bruck, M.D., LTC, MC
Lois A. Johns, LTC, ANC
Kilulu Von Prince, MAJ, AMSC
Elaine M. Kirkman, CPT, AMSC
Robert L. Remi[illegible], CPT, AMSC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

REPORT TITLE: MYOSITIS OSSIFICANS IN BURNS: A PROSPECTIVE STUDY OF A DISABLING COMPLICATION OF BURN INJURY

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

Period covered in this report: 1 July 1970 - 30 June 1971

Investigators: Andrew M. Munster, M.D., LTC, MC
Harold M. Bruck, M.D., LTC, MC
Lois A. Johns, LTC, ANC
Kilulu Von Prince, MAJ, AMSC
Elaine M. Kirkman, CPT, AMSC
Robert L. Remig, CPT, AMSC

Reports Control Symbol MEDDH-288(RI)

In a prospective study of 180 burned upper extremities in 100 patients, the incidence of heterotopic calcification about the elbow joint was found to be 10%. Spontaneous resolution occurred in five patients, and operative correction was required in the remaining seven.

Pain and limitation of motion at the elbow preceded roentgenologic evidence of calcification in 11 out of 12 patients. The occurrence of calcification was significantly related to the presence of third-degree burns of the extremity ($X^2 = 14$, $p < 0.001$). In those limbs where calcification was noted, there was a high incidence of impaired movement at the adjoining wrist and shoulder joints as well as the elbow ($X^2 = 57$, $p < 0.001$). By contrast, prolonged bedrest in either the supine or the prone position did not contributed significantly to the development of calcification ($X^2 = 0.83$ $p > 0.51$).

All patients were subjected to formal psychological testing in order to evaluate their insight and ability to project their future as a means of assessing motivation. The results revealed no difference in the response of patients who developed calcification and those who did not. The significance of a higher mean value of serum calcium in patients who developed elbow calcification remains unclear in view of the localization of this abnormality to one anatomical area. No significantly greater incidence of elbow calcification occurred in patients whose limbs were in occlusive dressings during part of their care nor in patients whose hands and elbows were temporarily splinted.

Heterotopic calcification about the elbow joint appears to be connected with factors related to the burned extremity rather than metabolic changes involving the patient as a whole.

Joints
Elbows
Calcium
Ossification
Myositis

MYOSITIS OSSIFICANS IN BURNS: A PROSPECTIVE STUDY OF A DISABLING COMPLICATION OF BURN INJURY

Over the last 18 months, 100 patients and 180 burned upper extremities were evaluated to elucidate factors of importance in the pathogenesis of myositis ossificans. Of the 180 patients, 12 developed myositis ossificans. Spontaneous resolution occurred in five patients, and operative correction was required in the remaining seven.

Materials and Methods

Patients admitted to the study comprised all burn patients who were 15 years of age and older. Each patient was examined weekly by a team of physicians, nurses, occupational therapists, and physical therapists. Blood was drawn for the determinations of calcium, phosphorus, and alkaline phosphatase levels on a weekly basis. X-rays in two planes of the involved elbow joints were taken each week. The study was conducted in blind fashion so that individual teams responsible for recording the various parameters did not know the findings of the other teams until the conclusion of the study, and all attempts were made to ensure that management of these patients with thermal injury was in no way altered by inclusion in the study per se.

In addition, psychological evaluation was carried out at two interviews: the first shortly after admission, at which time the patients answered a number of questions relating to their ability to project their future. The patients were asked how they envisioned themselves within the next few weeks, within a year, and within five years. The responses were graded and compared with the responses at the time of discharge or return from convalescent leave. Since the response of the patients to such questions is regarded as a sign of psychological maturity and motivation, it was felt that evaluation in this manner would contribute to an understanding of how much psychological factors, motivation, and cooperation have to do with the development of calcification about a joint. This was considered an essential part of the study because it had been felt by some members of the occupational therapy team that patients who were more uncooperative than others showed a higher likelihood of the development of myositis ossificans.

The physical therapy team included in the study made regular evaluation of the range of movement of the elbow joints in question as well as the adjoining shoulder and wrist joints. Measurements were accurately done with a goniometer on a weekly basis and recorded. The modality of treatment given by the physical therapy team, such as passive, active, or assisted active movement at each of these joints was also recorded. In addition, regular records were made of the patients' positions in bed, for instance, prone, supine, predominantly prone, predominantly supine, turned, ambulant, etc., and the type and length of splintage of the elbow, shoulder, and wrist joint of the affected limb. Clinical notations were made about the general condition of the patient, chemistries were drawn for calcium, phosphate, and alkaline phosphatase determinations, and x-rays of the elbow joints were taken. Diagrams of the extent and depth of burn injury of the whole patient and of the affected limbs were made. Regular records were kept of the operative procedures of each patient, such as xenografting, homografting, and autografting.

Results and Discussion

Evaluation of the data at the conclusion of the study showed that pain and limitation of motion at the elbow were the first symptoms of developing calcification and preceded roentgenological evidence of calcification in 11 out of 12 patients. The cause of calcificiation was significantly related to third-degree burns of the extremity ($X^2 = 14$, $p < 0.001$). If calcification were noted, there was a high incidence of impaired movement at the adjoining wrist and shoulder joints as well as the elbow ($X^2 = 57$, $p < 0.01$). By contrast, prolonged bedrest in either the supine or the prone position did not appear to contributed significantly to the development of calcification ($X^2 = 0.83$, $p > 0.5$). Psychological testing showed that the ability of the patients to project was in no way relevant to the development of calcification. Mean serum calcium was higher in those patients but the localization of ectopic calcification to the elbow joint suggests that local factors are of primary importance. The type of splints and the type of dressings used (open versus closed) likewise did not materially influence the development of calcification.

Several conclusions can be drawn from this study. Heterotopic calcification about the elbow joints appears to be related more to local factors in the burned extremity than to metabolic or psychological changes involving the patient as a whole. The first warning sign of the development of calcification is pain and limitation of motion. These patients should be X-rayed immediately and passive movement abandoned should calcification be present. The presence of heterotopic calcification does not necessarily lead to surgery as the condition is capable of spontaneous resolution.

Presentation

Munster AM: Myositis Ossificans in Burns. A Prospective Study. Presented at meeting of American Burn Assoc, San Antonio, Texas, 16,17 Apr 1971.

Publications

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA 0C 6388	71 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
70 07 01	H.TERMINATION	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A061102B71R	01	235
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)* (U) Analysis of Joint Range of Motion Following Treatment and Healing of Burn Wounds in Military Personnel (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*
003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
67 01	70 12	DA	C. In-House

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

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 71	.30	8.0
	CURRENT 72	0	0

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

RESPONSIBLE INDIVIDUAL
NAME: PRUITT, B. A., JR, LTC, MC
TELEPHONE: 512-221-2720

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

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME:* Wilma F Hall, MAJ, AMSC
TELEPHONE 512-221-4646
SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS
NAME: Elaine M Kirkman, CPT, AMSC
NAME: W J O'Brien III, Lt, AMSC DA

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Thermal Injury; (U) Limitation of Motion; (U) Normal Range of Motion; (U) Preservation of Function

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 rehabilitation of the thermally injured in terms of objective joint range of motion measurements. This evaluation to help in the determination of an effective exercise program.

24. (U) All patients are measured for joint range of motion initially at weekly intervals and upon final disposition.

25. (U) The data from burned military personnel treated in 1970 reflects continuing good results in maintaining range of motion and function in the joints of thermally injured patients.

*Available to contractors upon originator's approval

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

FINAL REPORT

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

REPORT TITLE: ANALYSIS OF JOINT RANGE OF MOTION FOLLOWING TREATMENT AND HEALING OF BURN WOUNDS

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

1 July 1970 - 30 June 1971

Investigators:

Wilma F. Hall, MAJ, AMSC
Elaine M. Kirkman, CPT, AMSC
William J. O'Brien, III, 1LT, AMSC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

REPORT TITLE: ANALYSIS OF JOINT RANGE OF MOTION FOLLOWING TREATMENT AND HEALING OF BURN WOUNDS

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

Period covered in this report: 1 July 1970 - 30 June 1971

Investigators: Wilma F. Hall, MAJ, AMSC
Elaine M. Kirkman, CPT, AMSC
William J. O'Brien, III, 1LT, AMSC

Reports Control Symbol MEDDH-288(R1)

This report outlines the result of joint range of motion studies in the thermally injured patient for the past four years. All patients received early physical therapy and range of motion records are kept until time of disposition. These records reflect the successful efforts to minimize loss of joint motion and to maintain function of involved joints.

Thermal injury
Limitation of motion
Normal range of motion
Preservation of function

ANALYSIS OF JOINT RANGE OF MOTION FOLLOWING TREATMENT AND HEALING OF BURN WOUNDS

For the past four years range of motion records have been kept on all admissions to the Institute of Surgical Research. Such has been done in order to evaluate the effectiveness of the methods used in the Physical Therapy Section to preserve motion and maintain function in the thermally injured patient.

From 1 June 1970 to 30 December 1970,there were 325 admissions to this Institute; of these 247 were included in the study. Of the patients followed from admission to discharge, 177 had normal range of motion and 70 had loss of motion in one or more joints. About 70% of the patients were discharged with normal range of motion. Table 1 shows the statistics for the four-year period.

In the year 1970 in the 70 patients who demonstrated loss of motion, 138 joints were involved. Table 2 shows the extent and site of the limitation in these joints.

Our definition of the three categories of join limitation are:

(1) Acceptable - The range is limited only in the final arc of normal motion.

(2) Functional - Approximately 50% of normal joint range - these patients function independently in activities of daily living.

(3) Severe - Range of motion is less than 50% of normal - independent activity is limited.

Summary

It is felt that the program of early active and active assistive exercise of thermally injured patients has been consistently effective in preventing severe loss of motion and loss of function in the majority of our patients.

Publication

Dobbs, E.R.: Analysis of Joint Range of Motion Following Treatment and Healing of Burn Wounds. In press (J. Trauma).

Table 1. Per Cent of Patients with Normal Range of Motion - Four-Year Period

Year	Total Patients	Patients with Normal Range of Motion	Patients with Loss of Motion	Per Cent with Normal Range of Motion
1967	318	235	83	74
1968	363	258	105	71
1969	231	150	81	65
1970	247	177	70	70

Table 2. Extent and Site of Joint Limitation

Joint	Acceptable	Functional	Severe	Total
Hand	19	11	6	36
Elbow	29	13	3	45
Shoulder	12	22	5	39
Cervical	0	0	3	3
Knee	7	2	1	10
Foot	3	1	1	5
Total	70	49	19	138

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0D 6380	71 07 01	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA CONTRACTOR ACCESS	9. LEVEL OF SUM
70 07 01	D.CHANGE	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A061102B71R	01	308
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U)An Evaluation of the Use of Enzymatic Debridement of Burn Wound Eschar to Decrease Morbidity in Burned Troops (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
70 01	Cont	DA	C. In-House

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

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 71	0.54	14.3
FISCAL YEAR CURRENT 72	0.54	15.8

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

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

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

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME: Paul Silverstein, MAJ, MC
TELEPHONE: 512 -221-4440
SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS
NAME: Frank J.Ruzicka, CPT, MSC
NAME: Harrel Walker, M.S. DA

21. GENERAL USE: FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code): (U) Enzymatic Debridement; (U) Eschar; (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) Rapid removal of burn eschar by enzymatic means.

24. (U) Purified proteinases and collagenases will be tested for efficacy in eschar debridement by application to male Sprague-Dawley rats which have been given a 30% total body surface third degree scald burn.

25. (U) 70 07 - 71 07 - Initial evaluation of Bromelain, a proteolytic enzyme derived from the pineapple, has shown it unsuitable for use in its present form because of systemic toxicity and incomplete debridement. Laboratory and clinical trials with Sutilains, the proteolytic enzyme of the bacteria B. subtilis, is continuing. Investigation is also under way in the laboratory exploring the possibilities of combining proteases with lipases and phospholipases in an effort to achieve more complete debridement of full thickness eschar.

*Available to contractors upon originator's approval

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

ANNUAL PROGRESS REPORT

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

REPORT TITLE: AN EVALUATION OF ENZYMATIC DEBRIDEMENT OF BURN WOUND ESCHAR

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

1 July 1970 - 30 June 1971

Investigators:

Paul Silverstein, M.D., MAJ, MC
Frank J. Ruzicka, Captain, MSC
Harrel L. Walker, M.S.

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

REPORT TITLE: AN EVALUATION OF ENZYMATIC DEBRIDEMENT OF BURN WOUND ESCHAR

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

Period covered in this report: 1 July 1970 - 30 June 1971

Investigators: Paul Silverstein, M.D., MAJ, MC
Frank J. Ruzicka, Captain, MSC
Harrel L. Walker, M.S.

Reports Control Symbol MEDDH-288(R1)

A laboratory and clinical evaluation of two proteases was undertaken to test the efficacy of eschar debridement by enzymatic means. After completion of the laboratory part of the evaluation, one enzyme, Sutilains, was considered adequate for limited clinical trial.

Further laboratory investigation was undertaken to compare the in vitro digestive indices of the enzymes in reference to pure collagenase by a spectrophotometric method. A diverse group of protein substrates was studied that was believed to be representative of those found in normal and denatured skin. Combinations of proteases plus lipases and phospholipases resulted in no enhanced effect of proteolysis. Disc gel electrophoresis of the individual enzymes all manifested one or two major protein bands and several minor ones, indicating moderately reliable purity of the products.

Enzymatic Debridement
Eschar
Thermal Injury

BLANK PAGE

AN EVALUATION OF ENZYMATIC DEBRIDEMENT OF BURN WOUND ESCHAR

Since the advent of topical antimicrobial burn wound therapy, there has been a prolongation of the period between date of burn and separation of eschar. This delay in eschar separation has been noted in both human and animal populations and is believed to be due to reduction in subeschar bacterial proliferation and hence in bacterial digestion of the eschar. While this control of subeschar infection has been vital to the improvement in burn mortality statistics, it has also contributed to morbidity and increased length of hospitalization by delaying the period of homografting and total autografting.

It would, therefore, seem reasonable to assume that rapid removal of burn eschar would favorably affect recovery by permitting earlier coverage of the wound and thereby expedite the anabolic phase of convalescence provided control of infection could be maintained. To date, attempts at early removal of eschar have been of mechanical or enzymatic nature. Mechanical means have failed because of the blood loss associated with excision of eschar and the difficulty in accurately assessing depth of burn. Enzymatic means have also been unsuccessful because of the unavailability of a proteolytic enzyme that could distinguish between living and devitalized collagen, and the added threat of sepsis arising in the digested tissue.

Recently, several new compounds have been developed and are available for evaluation. It is the purpose of this study to screen these compounds on an animal model in an attempt to evaluate their effectiveness prior to establishing a protocol for clinical use.

I. Animal Model

A. Enzyme-- Sutilains*:

Methods and Results

The experiment was divided into two parts using 60 white, male, Sprague-Dawley (Holtzmann) rats, 30 rats to each part. All rats were anesthetized with barbiturates, shaved, and subjected to a 20% scald burn. The rats in Part I

* Enzyme supplied by Travenol Laboratories.

were treated daily for five days starting one hour postburn. Rats in Part II were treated in the same manner for five days starting 72 hours postburn. The test material was spread over the burn wound and surrounding shaved skin twice daily. Any loose, dry, or necrotic debris was scraped away with a wooden tongue depressor before each application. One animal from each group was sacrificed on day 3 and day 5.

The 30 animals in each part were divided into six groups which received the following therapy:

Group 1: Burn control--no therapy.
Group 2: Hydrophilic cream base alone.
Group 3: 10.22 g Sutilains and 290.88 g cream base.
Group 4: 12.77 g Sutilains and 287.23 g cream base.
Group 5: 7.66 g Sutilains and 292.34 g cream base.
Group 6: 10.22 g Sutilains and 290.88 g Sulfamylon acetate cream (Winthrop Laboratories).

Enzymatic debridement was scored subjectively on a 0-4+ scale by three people. At the conclusion of the 5-day treatment period, surviving animals were observed weekly until separation of the eschar was complete.

Part I. No evidence of eschar debridement was noted in either Group 1 (control) or Group 2 (base alone) during the 5-day treatment period. All groups receiving Sutilains showed evidence of eschar dissolution from the first day on, starting as small, dark holes which gradually enlarged and coalesced. Debridement was more rapid in areas where there was greater skin motion and eschar cracking (e.g., neck and between shoulders). By the third day of treatment, wounds of the treated rats were necrotic and foul smelling and many animals appeared sick. There were two deaths on day 4 in Group 6 (Sutilains and Sulfamylon) and one death in Group 3. At the conclusion of five days' treatment, it was our clinical impression that enzymatic debridement had occurred, but it was patchy and uneven in depth. In most areas there was a deep layer of dermis still intact. The most thoroughly debrided areas were about the neck and between the shoulders, where movement of the animal caused cracks in the eschar, allowing better enzyme penetration. In this area, debridement was complete to the neck muscles. Eschar separation occurred between the 14th-17th days in the treated rats and between the 21st-26th days in the control Groups 1 and 2.

Part II: No evidence of eschar separation was noted in Group 1 or 2 during the 5-day treatment period. There was no

evidence of enzymatic debridement in the treated groups until the third treatment day, and in general debridement was less active than in the comparable groups of Part I. By the last day of treatment, all eschar in Groups 4 and 6 was crusted and partially necrotic. Groups 3 and 5 showed relatively little debridement other than patchy dark spots which were slowly enlarging. There were no deaths in any animals treated in Part II. Group 6 showed the most active debridement. Final eschar separation in the treated groups occurred in the third week after treatment was started. The control Groups 1 and 2 separated 5-7 days later.

Microscopic examinations in Part I were generally in agreement with clinical impressions. Group 1 confirmed uniform third-degree burn in all animals. Enzymatic debridement through dermis and superficial subcutaneous tissue was unpredictable, and the barrier layer below which no specimens were debrided appeared to be the muscularis cutaneous trunci (panniculus carnosus). Bacterial colonization of eschar was found in all groups except Group 6 (Sutilains and Sulfamylon).

In Part II, debridement was found to be more superficial than in Part I and did not penetrate dermal collagen except in Groups 1 and 6.

Summary

In the doses tested, Sutilains did demonstrate the ability to debride eschar. Debridement was generally inconstant in depth and did not penetrate the panniculus carnosus in most specimens evaluated. There was no evidence of adverse effects of the enzyme on unburned skin or muscle. The relationship between enzyme and Sulfamylon is an interesting one and merits further investigation since Sulfamylon did appear to prevent bacterial colonization without retarding enzymatic activity.

B. Enzyme--Bromelain*:

Methods and Results

The method of evaluating Bromelain was the same as that for Sutilains.

---

* Enzyme supplied by Dow.

Forty-seven 200 g male, Sprague-Dawley rats were subjected to a 20% total body surface, third-degree scald burn and divided into three groups:

Group 1: 5 rats kept as controls.
Group 2: 21 rats divided into three subgroups to which Bromelain paste was applied under a Saran wrap and gauze dressing one hour postburn. Attempts were made to remove eschar by blunt dissection from each subgroup at 1,2 and 4 hours post-Bromelain application, respectively. Autograft was taken from the abdomen and applied to the debrided wound.
Group 3: 21 rats divided into three subgroups and above experiment repeated beginning treatment 48 hours postburn.

Group 1--Control Group: All rats survived burn and lived until sacrificed. Attempt to bluntly debride eschar one hour postburn without application of enzyme partially successful and compared histologically with the results of Bromelain-treated burn. Animals sacrificed on 2nd and 6th postburn day confirmed consistency of third-degree depth of burn.

Group 2--Application of Bromelain One Hour Postburn:

Subgroup A. Bromelain left on eschar one hour; 4/7 animals died within 48 hours of treatment. Autopsy revealed focal hemorrhages in heart, lung, liver, kidneys, spleen. Graft donor sites hemorrhagic. All grafts necrotic. Two animals sacrificed 48 hours post-treatment. No graft take noted. One animal sacrificed one hour post-treatment.

Subgroup B. Bromelain applied two hours; 4/7 animals died < 48 hours post-treatment with same autopsy findings. One rat sacrificed one hour, 2 days, 6 days post-treatment. No graft take noted.

Subgroup C. Bromelain applied four hours; 3/7 animals died < 48 hours post-treatment with same autopsy findings. Four rats sacrificed at one hour, 2 days, 4 days, 6 days post-treatment. No graft take noted.

Group 3--Bromelain Applied 48 Hours Postburn. After application of Bromelain for one, 2 and 4 hours to each respective subgroup, it was impossible to bluntly debride

eschar, and therefore no skin grafting was performed. Only two animals of the 21 died within 48 hours, and these showed petechiae of the heart, lungs, liver and spleen. The remaining animals were sacrificed on the day of the experiment (two animals from each group) and 4 days post-treatment.

Discussion

Clinical Impressions

Bromelain prepared easily with use of electric mixer.

Application of Bromelain satisfactory. No excessive dripping.

In Group 2 rats, eschar was bluntly debrided with a tongue depressor with much difficulty.

The length of time of application did not seem to effect ease of eschar removal.

After removal of eschar, a thick, white, bloodless base remained in the wound. Skin grafts applied over this base did not take.

In Group 3 rats, Bromelain appeared unable to penetrate eschar. Eschar could not be removed bluntly.

In an attempt to assess any toxic effect of Bromelain, a small amount of the mixed paste was applied under a dorsal back flap of three rats. All three animals died within 24 hours with grossly visible petechiae in the heart and liver.

Pathologic Findings

Part I. Rats receiving a 20% full-thickness scald burn and killed on the second and sixth postburn day confirm the full-thickness depth of injury histologically. When the burn eschar is scraped one hour postburn, there is limited removal of the eschar. In addition, there are numerous bacteria within and beneath the necrotic graft.

Animals killed between one and four hours following application of Bromelain demonstrate absence of epithelium and focal lysis of eschar collagen. Animals killed between 2 and 6 days postburn following a single application of Bromelain in the immediate postburn period demonstrate an inconsistent

absence of dermal eschar. Some sections show unchanged eschar, i.e., as might be expected in an untreated burn examined at this postburn interval. Other sections, however, demonstrate lysis of eschar. Spontaneous invasive bacterial burn wound infection is also noted in one instance. In summary, lysis of eschar is inconstant, and when it occurs, it is incomplete in the few animals examined.

The rats that died between 2 and 4 days postburn following a single application of Bromelain demonstrate an intact eschar for the most part. There are varying degrees of autolysis, but this degree of alteration might be expected in the untreated burn wound in this model system 4 days postburn. No invasive burn wound infection is present that would account for death in these animals. The focal visceral congestion and hemorrhage noted grossly is a finding of indeterminate significance.

Part II. Bromelain was applied for a duration of one to four hours in rats that received a 20% scald burn. Again, some lysis is noted in animals killed immediately after a single application of Bromelain. This effect is demonstrated by separation at the dermo-epidermal junction and tinctorial alterations in the dermal collagen. Animals killed 4 days following a single application of Bromelain, however, do not demonstrate any striking differences from what might be expected in an untreated burn wound.

No conclusions can be made with any certainty on the basis of the limited material available; however the findings suggest that a single application of Bromelain does increase lysis of the eschar. Examination of these burns 4 days following a single application, however, fails to demonstrate ary striking effects. The effects of repeated applications of Bromelain to the burn eschar are not available in this study.

A dorsal cutaneous flap was excised from three healthy rats, and Bromelain was applied and the skin flap returned to its original position. Sections of the autograft and wound bed are available to study. The autograft is necrotic. A fine layer of necrosis is present in the wound bed that involves a microscopic layer of fat and skeletal muscle. This degree of necrosis may be due to the trauma of original excision rather than Bromelain, and a specific statement in this regard is not warranted.

Conclusions

1. Bromelain applied to burn eschar produces epidermal lysis and partial dermal lysis in the rat scald model. However,

the ability of the enzyme to digest eschar is incomplete, sporadic and unpredictable.

2. Split-thickness autografts do not adhere to the Bromelain debrided burn wound in this model.

3. The high mortality rate in Group 2 animals suggests some toxic effect of Bromelain, probably due to absorption through the fresh burn wound. This is not seen in the 48-hour postburn animals, probably because the Bromelain does not penetrate into the hard, dry eschar as well as into the wet, edematous one.

4. The relation of petechial hemorrhages in vital organs to treatment with Bromelain is worthy of further examination if use of this enzyme is to be pursued. However, a significant cause-effect relationship cannot be proven from this experiment.

5. Clinical evaluation of this enzyme will not be undertaken until toxicity studies have been completed and a standardized method of effective application has been developed.

II. In Vitro Comparison of Enzymatic Agents for Debridement.

Methods

The rate of protein hydrolysis was followed with the ninhydrin method.[1] The standard incubation medium contained the following: 0.1 M potassium phosphate buffer pH 7.0; substrate concentration--bovine serum albumin (0.5%), microcrystalline collagen (0.5%), split-thickness pigskin (8 cm^2 in 10 ml of buffer or 16 cm^2 in 20 ml of buffer); enzyme concentration--for soluble substrates (0.25 mg/ml), for microcrystalline collagen and pigskin (1.5 mg/ml). Incubation was conducted at 37°. Aliquots of the sample (0.1 ml) were taken at timed intervals and subjected to ninhydrin analysis. Leucine was used as the standard.

Hydroxyproline Analysis. Hydroxyproline was measured according to the method of Miyada and Tappel.[2] Following a 60-minute incubation of enzyme with substrate, 1 ml of the sample was hydrolyzed by adding 1 ml of 12 N HCl and heating at 120° for 12 hours. The medium was then neutralized with NaOH, water added to 30 ml, and 1 ml of this sample subjected to hydroxyproline analysis.

Lipase Activity. Incubation conditions were identical to protease experiments. Concentration of enzyme: lipase (0.5 mg/ml), phospholipase A (0.2 mg/ml). Split-thickness pigskin (16 cm^2 in 20 ml of buffer) was used. Following a 60-minute incubation period at 37°, the skin was removed. The solution was acidified with 2 ml of conc. HCl and extracted twice with 3 ml of pentane and twice with 1.5 ml of pentane. Heptadecanoic acid was added as internal standard. The fractions were pooled, evaporated to dryness, and methyl esters of the fatty acids were prepared and analyzed by gas chromatography. Analysis of total skin fatty acids was accomplished by homogenizing 1 gm of skin in 50 ml of water with a Virtis homogenizer for approximately 15 minutes. The mixture was lyophilized, hydrolyzed for 12 hours in 5 ml of 2 N HCl under nitrogen, and extracted and submitted to gas chromatography as above.

Electrophoresis. Enzymes were subjected to polyacrylamide disc gel electrophoresis at pH 9 and the proteins stained with Coomassie Blue.

Materials

Sutilains was obtained from Travenol Laboratories and Bromelain (debridement grade) from the Dow Chemical Company. Collagenase (*Clostridium histolyticum* - Type I), lipase (steapsin), phospholipase A (*Naja naja* venom), and gelatin (swine skin - Type I) were purchased from Sigma Chemical Co. Collagen (Avitene H - Microcrystalline Collagen) was obtained from the FMC Corporation.

Results

A method was developed to determine the rate of protein hydrolysis by proteases currently being tested and evaluated as debriding agents. The ninhydrin method was found most suitable, since the assay method measures amino acid nitrogen and thus the release of amine groups following peptide bond hydrolysis. The assay was first conducted with the soluble substrate, bovine serum albumin. During the first 30 minutes, increase of ninhydrin positive substances was linear with time when either Sutilains or Bromelain was used. After 30 minutes, the rate decreased until no further reaction was detected. Proteolysis of pigskin was also followed as a function of time. The rate for each enzyme used was found to be linear with time for approximately 45 minutes, after which all rates decreased. Since eschar contains heat denatured proteins, proteolysis of pigskin boiled one minute was

followed with time. In a fashion similar to native pigskin, the rates of all enzymes exhibited linearity for the first 30 minutes, then decreased.

In order to assess the mechanism of proteolysis of skin preparations, skin size and enzyme concentration were varied. As expected, activity increased with increasing skin size until enzyme became limiting. Since barriers may exist to the immediate interaction of enzyme with protein in the inner layers of skin, an attempt was made to make the substrate the limiting factor by measuring activity at various enzyme concentrations. Within the wide range of enzyme concentration used, activity of all enzymes used increased linearly with increasing enzyme concentration.

The linearity of rates observed with all enzymes with respect to time when either soluble substrate or pigskin was used allowed for direct comparison of the specific activities of the enzymes tested with various substrates. Table I gives the comparison of rates of three enzymes tested with several substrates. With bovine serum albumin as substrate, Sutilains displayed highest activity followed by Bromelain with approximately one-half the Sutilains rate. Collagenase activity was almost insignificant with bovine serum albumin. This latter result was expected in light of the high degree of specificity of this enzyme for collagen. The small amount of activity observed may be explained by the contamination of the enzyme preparation with other proteases.

Pigskin gelatin and microcrystalline collagen represent purified preparations of collagen of two forms, heat denatured and native. It was of interest to determine the rates of hydrolysis of the proteolytic enzymes on these two forms of collagen. Collagenase showed maximum activity when pigskin gelatin was used as substrate (Table I). However, the rates of the other enzymes were not significantly different from the collagenase rate. The hydrolysis of microcrystalline collagen, on the other hand, was greatest when collagenase was used as enzyme. The Sutilains and Bromelain rates were 10-fold and 20-fold less respectively than the collagenase activity. When pigskin was used as substrate, the rate of protein hydrolysis was highest with Sutilains and Bromelain. Collagenase exhibited about one-half the rate of the other enzymes. Heat denaturation of skin proteins slightly increased the Sutilains rate and almost doubled the collagenase rate. The Bromelain rate remained approximately the same as with native pigskin.

Table 1. Rate of Protein Hydrolysis by Protease Action on Various Substrates

Specific Activity
(mμM Leucine/min./mg. Enzyme)

Substrate	Enzyme		
	Sutilains	Bromelain	Collagenase
Bovine Serum Albumin	267.0	117.0	2.1
Pigskin Gelatin	62.4	53.1	75.4
Microcrystalline Collagen (Avitene)	9.2	4.7	76.8
Pigskin	59.0	48.6	29.5
Boiled Pigskin	79.0	44.8	51.2

The ninhydrin method measures the appearance of amino nitrogen as a result of peptide bond cleavage, and the activities expressed above represent general protein hydrolysis. Since skin represents a complex substrate containing proteins of different chemical and physical properties, the rate of enzymatic hydrolysis toward two major groups of proteins, collagen and noncollagen protein, was followed. Since hydroxyproline is present exclusively in collagen, measurement of the release of this amino acid as a result of peptide bond hydrolysis indicates the degree to which the enzymes can hydrolyze skin collagen. These rates may be compared with the rates of general protein hydrolysis obtained by the ninhydrin method. Table 2 indicates that Sutilains and Bromelain are approximately equally effective in hydrolyzing collagen although the former shows greater protease activity. Collagenase, on the other hand, is approximately six times more effective as either Sutilains or Bromelain at digesting collagen in skin. However, the protease activity of collagenase is one-half that of the other enzymes.

Since collagenase is at least six times as effective as either Sutilains or Bromelain at digesting collagen in the skin, a study was conducted in which Sutilains was combined with collagenase. Table 3 shows the effect of simple combination of Sutilains and collagenase on protein breakdown. The ratio of the two enzymes was varied in order to find the most effective breakdown. In all instances, no enhancement of proteolysis was observable. The combination rate was only slightly greater than the rates of the individual enzymes measured separately and added together. In addition to combining a protease with collagenase, several lipolytic enzymes were combined with Sutilains (Table 4). When either phospholipase A or lipase was combined with Sutilains, no enhancement of proteolytic activity was observed, even though phospholipase A and Sutilains caused approximately 21% of the total skin fatty acid to be released.

As a measure of the purity of the enzyme preparations used in this study, electrophoretic patterns of these proteins following polyacrylamide disc gel electrophoresis at pH 9 were studied. Sutilains shows one prominent band and nine minor bands. Bromelain exhibits four equally dense bands. Collagenase contains two prominent bands and five minor bands. The trypsin pattern is shown as a control of two times recrystallized enzyme.

Discussion

Assessment of the eschar separating capability of the

Table 2. Rate of Peptide Hydrolysis vs. Rate of Hydroxyproline Release (Substrate - Pigskin)

Enzyme	Protease Activity (mμM Leucine/ml./min.)	Hydroxyproline Released (mμM/ml./min.)
Sutilains		
0.625 mg./cm.^{2}	36.5	1.5
0.940 " "	43.0	1.9
1.25 " "	59.3	1.6
Bromelain		
0.625 mg./cm.^{2}	22.6	1.2
0.940 " "	28.5	1.6
1.25 " "	38.0	2.1
Collagenase		
0.625 mg./cm.^{2}	12.5	8.6
0.940 " "	16.0	9.3
1.25 " "	19.0	12.2

Table 3. Effect of Combination of Enzymes on Protease Activity (Substrate - Pigskin)

mμM Leucine/ml./min.

A Sutilains	B Collagenase	C Sutilains Collagenase	A + B Calculated Activity
5 mg.	10 mg.	5 + 10 mg.	
28.8	5.8	39.4	34.6
7.5 mg.	7.5 mg.	7.5 + 7.5 mg.	
34.0	4.8	43.8	38.8
10 mg.	5 mg.	10 + 5 mg.	
50.2	2.8	55.6	53.0

Table 4. Effect of Lipid Breakdown on the Hydrolysis of Proteins by Protease Action (Substrate - Pigskin)

Enzyme	Protease Activity	Fatty Acid Released (mg.)	% of Total Fatty Acid Released
Control - No Enzyme	0.5	0.038	2.1
Sutilains (10 mg.)	49.3	0.236	13.0
Sutilains (10 mg.) + Phospholipase (2 mg.)	41.9	0.338	18.6
Sutilains (10 mg.) + Phospholipase (4 mg.)	46.8	0.382	21.0
Phospholipase (2 mg.)	0.7	--	--
Sutilains (10 mg.) + Lipase (10 mg.)	63.9	--	--
Lipase (10 mg.)	19.9	--	--

various hydrolytic enzymes must in the final instance be made in view of the results obtained by *in vivo* application. Highly desirable characteristics of any such agent include speed, thoroughness, and uniformity of the enzymatic process, non-toxicity of the enzyme preparation, and optimum preparation of the skin bed for grafting and early wound healing. However, information may be gained as to the actual mode of attack of these enzymes with various substrates under *in vitro* conditions similar to those found when these enzymes were applied topically. It is hoped that such experimentation may lead to more fruitful animal and clinical application.

One question frequently posed as a result of unsuccessful enzymatic debridement is why do enzymes fail as therapeutic agents. Although the answer may not be immediately discernable in most cases, several possible causes for failure can be considered. For instance, conditions of the immediate environment in which the enzyme must act may certainly determine the success or failure of a particular application. In this regard, unfavorable pH, lack of moisture, by-product formation, and generally poor contact with the substrate may be extremely important. How the enzyme is applied may also be very significant. Furthermore, the potential of the enzyme to catalyze particular reactions may be the limiting factor. For example, substrate specificity of a protease will determine whether proteolysis of a heterogenous group of proteins such as are found in skin will be complete. With proteases, self-destruction can also occur, which may limit the duration of effective proteolysis.

The results of these kinetic studies of several proteases on a diverse group of protein substrates indicated no unusual enzyme substrate interactions. Rates of hydrolysis for a soluble substrate such as bovine serum albumin as well as for a complex substrate such as pigskin were linear for an initial period of 30 to 45 minutes, followed by a decrease of rate. Most interesting were the results of the pigskin hydrolyses. Although protein and lipid barriers may exist to the interaction of enzyme with all substrate proteins initially, the rates of hydrolysis were zero order. It might be anticipated that as more protein substrate became available as a result of breakdown of these skin barriers, higher rates would be observed. Furthermore, when increasing levels of enzyme per unit area of skin were tried, no evidence was found that exposed skin protein could be saturated with enzyme initially, a result anticipated if true barriers to optimal enzymatic hydrolysis existed. These results would suggest that the

rate of hydrolysis of skin proteins is more a function of the nature of the enzyme used than the availability of substrate.

To assess the capability of particular enzymes to hydrolyze proteins of diverse nature, several categories of proteins were examined as substrates. These proteins are representative of general protein groups found in skin or burned skin. With the exception of collagen or its denatured form, gelatin, Sutilains was most effective in causing general protein hydrolysis. Collagenase was most effect with collagen or gelatin but less effective than Sutilains when either pigskin or boiled pigskin was used. Bromelain was consistenly lower in activity than Sutilains with all substrates used. Since these rates indicate general peptide hydrolysis, their values may not express the potential of the enzymes to catalyze the hydrolysis of particular skin proteins (i.e., collagen). For this reason, hydroxyproline release as a result of protease digestion was followed. The results of this study indicated that although Sutilains exhibited the best proteolytic activity, it was at least six times less effective than collagenase in breaking down collagen in pigskin. Since the deeper layers of skin contain collagen almost exclusively, effective collagen breakdown could be very important for optimal eschar removal. However, the high degree of specificity of collagenase may rule out its use as a successful debriding agent when used alone.

In view of the previous data on Sutilains and collagenase, a combination of Sutilains and collagenase was evaluated on pigskin. In simple combination, no enhancement of protein hydrolysis was observed. The rates observed in combination appeared to be the result of simple addition of rates of the individual enzymes. These results do not rule out the possibility that such enzyme combinations might be more effective with skin preparations containing more collagen than the split-thickness pigskin used or that sequential enzymatic treatment might produce more desirable results. Similarly, when Sutilains was combined with lipase or phospholipase A, no enhancement of protein hydrolysis was observed, as might be expected if lipid created a barrier against optimal protease interaction with skin proteins. However, as with the Sutilains-collagenase data, the split-thickness pigskin may not be the most suitable substrate for which to assess this parameter.

Summary

A spectrophotometrit method for protein hydrolysis was developed in order to study the potential of several proteo-

lytic enzymes as eschar removing agents. Proteolytic enzymes included in this study were Sutilains, Bromelain, and collagenase. A diverse group of protein substrates was studied as representative of the type of proteins found in native and denatured skin. Results indicate no unusual enzyme kinetics. Rates remained linear for the first 30 to 45 minutes, then decreased until no further reaction occurred. When the rates of protein hydrolysis of these enzymes were compared, Sutilains was found to be most active on all substrates except collagen and gelatin. In the case of collagen and gelatin, collagenase exhibited highest activity. When rates of protein hydrolysis were compared with rates of hydroxyproline released with pigskin as substrate, although Sutilains exhibited the highest proteolytic activity, this enzyme was approximately six times less effective as collagenase at releasing hydroxyproline. Combination of Sutilains with collagenase or Suitilains with lipase showed no enhanced effect of proteolysis. Polyacrylamide disc gel electrophoresis of the individual enzyme preparations showed all preparations containing one or two major protein bands and several minor protein bands, indicating preparations of moderate purity.

Publications and/or Presentations

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA 0D 6967	71 07 01	DD-DR&E(AR)636

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

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A061102B71R	01	184
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)* (U) Evaluation and Preparation of Formalin Fixed Cutaneous Grafts as a Temporary Wound Cover for Burned Soldiers (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*
003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
70 01	Cont	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 71	.50	13.3
	CURRENT 72	.72	21.0

19. RESPONSIBLE DOD ORGANIZATION
NAME:* US Army Institute of Surgical Research
ADDRESS:* Ft Sam Houston, Texas 78234
RESPONSIBLE INDIVIDUAL
NAME: PRUITT, B. A., JR, LTC, MC
TELEPHONE: 512-221-2720

20. PERFORMING ORGANIZATION
NAME:* US Army Institute of Surgical Research Clinical Division
ADDRESS:* Ft Sam Houston, Texas 78234
PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic institution)
NAME:* Paul Silverstein, MD, MAJ, MC
TELEPHONE: 512-221-4440
SOCIAL SECURITY ACCOUNT NUMBER:
ASSOCIATE INVESTIGATORS
NAME: Gilbert Raulston, Col, VC
NAME: Harrel Walker, MS DA

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Formalin; (U) Cutaneous Grafts; (U) Wound Cover

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

23. (U) The advantages of fresh allograft on granulating wounds have been well demonstrated. However, inadequate supply of cadaver donors has stimulated a search for allograft substitutes. Formalinized skin is an inexpensive, simple product to produce and has demonstrated previously its ability to function as an allograft substitute.

24. (U) Fresh allograft and porcine xenograft will be fixed with formalin and studied as to optimum time of fixation and adequacy of formalin washout by quantitative determinations of formaldehyde content in skin and wash water. Fixed skin will be stored in 0.5% formalin until ready for use. It will be evaluated in an animal model with a projected clinical trial, if sucessful.

25. (U) 70 01 - 71 06 Laboratory investigation has demonstrated that formalinized skin can supply adequate burn wound cover in the experimental animal model for up to 30 days. Formalin content of skin can be reduced by water washes to 0.016%. Sterility of the skin has been maintained for more than one year when stored in 0.5% formaline solution. A clinical trial is now in progress.

*Available to contractors upon originator's approval

DD FORM 1 MAR 66 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 65 AND 1498 1, 1 MAR 66 (FOR ARMY USE) ARE OBSOLETE

ANNUAL PROGRESS REPORT

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

REPORT TITLE: EVALUATION AND PREPARATION OF FORMALIN-FIXED CUTANEOUS GRAFTS AS A TEMPORARY WOUND COVER

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

1 July 1970 - 30 June 1971

Investigators:

Paul Silverstein, M.D., MAJ, MC
Gilbert L. Raulston, COL, VC
Harrel L. Walker, M.S.
Frank J. Ruzicka, CPT, MSC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

BLANK PAGE

ABSTRACT

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

REPORT TITLE: EVALUATION AND PREPARATION OF FORMALIN-FIXED CUTANEOUS GRAFTS AS A TEMPORARY WOUND COVER

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

Period covered in this report: 1 July 1970 - 30 June 1971

Investigators: Paul Silverstein, M.D., MAJ, MC
Gilbert L. Raulston, COL, VC
Harrel L. Walker, M.S.
Frank J. Ruzicka, CPT, MSC

Reports Control Symbol MEDDH-288(R1)

Formalin-fixed split-thickness heterograft or homograft has been found to provide adequate wound coverage for up to 30 days when applied to 30% total body surface area wounds on the backs of white male rats.

Freshly harvested split-thickness skin grafts are spread on fine-mesh gauze, immersed in 10% buffered formalin for 16 hours, washed in distilled water, and stored in 0.5% formalin. Prior to use, the skin is washed in physiologic saline to reduce formalin content to 0.016%.

Serial biopsies of 50 grafted wounds demonstrate excellent adherence of the formalinized graft to the wound bed with moderate granulation in-growth and proliferation of fibroblasts, minimal inflammatory reaction, and absence of suppuration. When the graft is stripped from the wound, the bed readily accepts autograft.

Repeated grafting with formalinized skin does not produce sensitization or accelerated rejection. Formalin-fixed skin stored for more than five years had no bacterial growth on

culture. No external dressing is required, and motion of the grafted part is not restricted. A clinical evaluation of formalinized skin as a substitute for fresh homograft is now in progress.

Formalin
Cutaneous Grafts
Wound Cover

EVALUATION AND PREPARATION OF FORMALIN-FIXED CUTANEOUS GRAFTS AS A TEMPORARY WOUND COVER

The attributes of viable cutaneous allografts used as physiologic dressings include decrease in wound pain, evaporative water and heat loss, stimulation and protection of granulating tissue, and suppression of bacterial flora.[1] Unfortunately, the limited viability and difficulty of matching supply and demand restrict the use of such allografts. Previous laboratory investigation [2] has indicated that the simple, inexpensive maneuver of formalinization decreased the antigenicity and increased the shelf life of cutaneous allograft or xenograft. The current study documents the effectiveness of such materials as temporary wound coverings and discloses the histologic basis for their non-reactivity.

Methods

Skin grafts 0.010-0.020 in. thick, harvested aseptically from recently deceased human donors or anesthetized laboratory animals, were spread on fine-mesh gauze, immersed for 16 hours in 10% buffered formalin, washed in distilled water for 24 hours, and then stored at room temperature in sealed containers filled with 0.5% formalin. Prior to use, the skin is washed in physiologic saline for 30 minutes to reduce extractable formalin content to 0.016% as determined by the method of Tanenbaum and Bricker.[3] Specimens produced by this method and stored for over 5 years have been proven sterile by cultures for bacteria and fungi.

Grafts were evaluated as temporary physiologic dressings on freshly excised 30% total body surface wounds on the backs of 200 g white, male Sprague-Dawley rats from which skin and panniculus carnosus had been removed. Grafts were subjectively scored daily for wound adherence. The end point was that day after application when 50% or more of the graft was no longer adherent. Comparisons were made between fresh skin and grafts fixed in formaldehyde, paraformaldehyde and glutaraldehyde. The latter two agents were subsequently discarded because they rendered the grafts too inflexible.

Results

Wound coverage by formalinized skin when compared in the same animal model to fresh human and porcine xenograft and

rat allograft (Long-Evans → Sprague-Dawley) revealed that satisfactory wound coverage was provided for 9.3 ± 0.6 days by fresh rat allograft, 8.2 ± 0.7 days by fresh porcine and human xenograft, and 30.0 ± 5 days by formalinized xenograft. All wounds readily accepted autograft from the rat's abdomen after removal of the physiologic temporary grafts, indicating that the test materials had adequately protected the granulating wound bed from desiccation and infection.

The histopathology of the graft-host wound interface was studied by injection of 5 cc of India ink into the rat tail vein 3 minutes prior to sacrific on day 3, 6, 9, 12 and 20 postapplication of the test graft. Autografted animals demonstrated 100% graft take with free circulation of carbon particles in both granulating wound bed and graft on all days biopsied. Viable homograft demonstrated initial graft take with free circulation of ink particles on day 3, but by day 9, carbon particles were no longer evident in the graft and a classic rejection sequence had begun, with thrombosis of graft vasculature and mononuclear round cell infiltration. Neither fresh porcine or human xenograft nor any of the formalinized grafts ever exhibited uptake of ink particles, despite excellent adherence to the wound and invasion of host fibroblasts with minimal polymorphonuclear or monocytic infiltration into the lower dermal collagen fibril networks of the grafts.

Second and third set application of viable xenograft or formalinized graft failed to manifest accelerated rejection customarily seen with immunologically active tissue. Absence of clinical immune response and lack of revascularization in the formalinized grafts resulted in their longer survival as effective wound covers and suggest that their physical properties are of critical importance in their function as "physiologic" dressings.

Summary

1. Formalinized split-thickness cutaneous grafts provide adequate wound coverage in an animal model for significantly longer periods than those seen with either viable homograft or xenograft.

2. Formalinized skin grafts are inexpensively and easily produced and stored with material readily available in any general hospital.

3. Formalinized skin grafts behave as if immunologically inert, and no vascular communications between host and graft were observed.

References

1. Artz CP, Becker JM, Sako Y, et al: Postmortem skin homografts in the treatment of extensive burns. Arch Surg, 71: 682-687, 1955.

2. Raulston GL, Teplitz C, Walker HL, et al: De-antigenization of skin of heterogenous origin. US Army Surg Res Unit Anl Prog Rpt, 30 Jun 1964, BAMC, Ft Sam Houston, Tx, Sect 55.

3. Tanenbaum M, Bricker CE: Microdetermination of free formaldehyde. Anal Chem 23:354-357, 1951.

Publications and/or Presentations

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0D 6395	71 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
70 07 01	D. CHANGE	U	U	NA	NL	[X] YES [ ] NO	A. WORK UNIT

10. NO. CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A061102B71R	01	082
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) Evaluation of Polyglycolic Acid Films as a Skin Substitute - A Possible "Synthetic Skin" for use in Burned Troops (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
70 01	Cont	DA	C. In-House

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

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 71	0.27	7.2
FISCAL YEAR CURRENT 72	0.27	7.9

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

20. PERFORMING ORGANIZATION
NAME: US Army Institute of Surgical Research Lab Division
ADDRESS: Ft Sam Houston, Texas 78234
PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME: Paul Silverstein, MAJ, MC
TELEPHONE: 512-221-2943
SOCIAL SECURITY ACCOUNT NUMBER:
ASSOCIATE INVESTIGATORS
NAME: Basil A. Pruitt, Jr., LTC, MC
NAME: A. D. Mason, Jr., M. D. DA

21. GENERAL USE: FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code): (U) Polyglycolic acid; (U) Skin

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

23. (U) Development of a temporary synthetic wound cover.

24. (U) This project is an attempt to develop a synthetic membrane that would function as a homograft substitute during treatment of the burn wound. Polyglycolic acid (PGA) membranes were chosen for investigation because they are easily degraded by the human body and can be technically woven, felted and knapped.

25. (U) 70 07 - 71 - 06 On the basis of results of previous experiments, attempts have been made to synthesize new forms of PGA laminated to a barrier membrane in an attempt to decrease evaporative losses and bacterial infiltration. Until satisfactory laminates can be synthetized, animal evaluation will be suspended.

*Available to contractors upon originator's approval

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

ANNUAL PROGRESS REPORT

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

REPORT TITLE: EVALUATION OF POLYGLYCOLIC ACID FILMS AS A SKIN SUBSTITUTE

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

1 July 1970 - 30 June 1971

Investigators:

Paul Silverstein, M.D., MAJ, MC
Basil A. Pruitt, Jr., M.D., LTC, MC
F. Daniel Foley, M.D.
Arthur D. Mason, Jr., M.D.

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

REPORT TITLE: EVALUATION OF POLYGLYCOLIC ACID FILMS AS A SKIN SUBSTITUTE

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

Period covered in this report: 1 July 1970 - 30 June 1971

Investigators: Paul Silverstein, M.D., MAJ, MC
Basil A. Pruitt, Jr., M.D., LTC, MC
F. Daniel Foley, M.D.
Arthur D. Mason, Jr., M.D.

Reports Control Symbol MEDDH-288(R1)

Previous animal experiments with knitted and woven forms of polyglycolic acid (PGA) films applied to excised wound beds elucidated several problems relating to the function of this product as a temporary wound cover. Objections to the tested specimens included: (1) stiffness and drying of the membrane and wound bed due to evaporation through the material, leading to sepsis and lack of adherence; (2) retention of large amounts of PGA fibers in the wound beds after stripping of the membranes from the recipient animal, with resulting multinucleated foreign body reaction.

It was, therefore, concluded that further technological development was necessary to overcome the above criticisms. Laminates of knitted PGA to semi-permeable membranes were recommended in an attempt to overcome evaporative losses leading to desiccation of the wound and bacterial invasion through open interstices in the knitted fibers. During this reporting year, no new specimens worthy of laboratory investigation were developed by the manufacturer.

Polyglycolic Acid
Skin

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OD 6969	71 07 01	DD-DR&E(AR)636

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

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A061102B71R	01	187
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) Hypertrophic Scarring - Etiology and Control of A Disabling Complication in Burned Soldiers (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
70 07	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 71	.82	21.8
	CURRENT 72	.82	23.9

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

RESPONSIBLE INDIVIDUAL
NAME: PRUITT, B. A., Jr, LTC, MC
TELEPHONE: 512-221-2720

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

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME: Paul Silverstein, MAJ, MC
TELEPHONE: 512-221-4440
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: Malcolm N Goodwin, Jr, MAJ, MC
NAME: Gilbert L Raulston, Col, VC DA

21. GENERAL USE: FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code): (U) Hypertrophic Scar; (U) Steroids; (U) Thermal Injury

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

23. (U) To create an experimental model of hypertrophic scarring in a laboratory animal in which new modes of therapy can be evaluated; to evaluate current modes of therapy in a clinical burn population.

24. (U) Attempts to induce hypertrophic scarring in an animal model by creating deep second degree burn wounds or partial-thickness skin loss with chronic healing have been applied to rats, dogs and pigs. Current clinical therapy includes evaluation of intradermal injection of steroids, topical steroid application, and chronic compression devices applied to hypertrophic burn scars.

25. (U) 70 07 - 71 06 Attempts at creating a hypertrophic scar have resulted in a successful prototype in the New Jersey red Duroc pig. Clinical evaluation is proceeding with good results from the use of compression devices and fair to poor results with the steroid applications. Statistical data is not available due to the limited number of patients in each study group.

*Available to contractors upon originator's approval.

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

ANNUAL PROGRESS REPORT

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

REPORT TITLE: HYPERTROPHIC SCARRING--ETIOLOGY AND CONTROL

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

1 July 1970 - 30 June 1971

Investigators:

Paul Silverstein, M.D., MAJ, MC
Malcolm N. Goodwin, Jr., M.D., MAJ, MC
Gilbert L. Raulston, COL, VC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

REPORT TITLE: HYPERTROPHIC SCARRING--ETIOLOGY AND CONTROL

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

Period covered in this report: 1 July 1970 to 30 June 1971

Investigators: Paul Silverstein, M.D., MAJ, MC
Malcolm N. Goodwin, Jr., M.D., MAJ, MC
Gilbert L. Raulston, COL, VC

Reports Control Symbol MEDDH-288(R1)

This project was created to study hypertrophic scarring with the purpose of developing an animal model in which scarring could be examined and to analyze human scar hypertrophy histologically and therapeutically.

Results of animal experimentation have been encouraging. A hypertrophic scar confirmed histologically has been created in one New Jersey Red Duroc pig. Attempts are now being made to reproduce this scarring in other pigs.

Histological examination of hypertrophic scar punch biopsies manifested no common characteristics by which hypertrophic scar may be identified. Whorling collagen patterns were not universally found, nor were foreign body tissue reactions. Hypertrophic scars from 10 patients were compared to biopsies of normal flat scars from other parts of the same patient's body.

Therapeutic trials of Dermajet infusion of triamcinolone and hyaluronidase were instituted, with limited success. Topical steroid application via Cordran tape is also being evaluated. The most promising therapy to date has been application of Jobst compression stockings for six months after completion of grafting. Twenty-eight patients have been studied during the past year.

Hypertrophic Scar
Steroids
Thermal Injury

BLANK PAGE

HYPERTROPHIC SCARRING--ETIOLOGY AND CONTROL

Since the advent of topical antimicrobial therapy, it has become apparent that hypertrophic scarring has increased in patients with healed nongrafted burn wounds. Several possible etiologies have been suggested, including: (1) incorporation of foreign bodies into the burn wound increasing and prolonging a local inflammatory reaction; (2) local irritation from topical therapy; (3) genetic predisposition. None of these theories has been proven to be uniformly valid. Hypertrophic scar differs clinically from keloid in that it is not genetically inherited and does not tend to expand progressively. Furthermore, it may not recur after excision. Over a long period of time, hypertrophic scars tend to regress and become more elastic; however in the interim there is severe deformity, pain, pruritus, and limitation of motion when areas around joints are involved. The purpose of this study is to elucidate the etiology of hypertrophic scarring in burn wounds and evaluate methods of treatment.

Methods

(1) Attempt to develop an animal model in which hypertrophic scarring can be induced in order to further study means of altering collagen synthesis.

(2) Serial biopsies of human burn wounds with and without hypertrophic scarring and examination of these tissues by electron and photomicroscopy for analysis of collagen bundle patterns, cross-linking, scar thickness, and presence of foreign bodies.

(3) Photography pre- and posttreatment.

(4) Measurements of circumference of involved extremities.

Discussion

In the past 70 years, physicians concerned with hypertrophic scarring have recorded pathologic and clinical characteristics, dermographic and racial predispositions, and innumerable methods of dealing with the problem--none of which has survived the test of time. Currently, three major types of therapy are generally employed: (1) radiotherapy, (2) surgical excision, (3) intralesional steroid injection--plus combinations of the three.

In recent years, new approaches have been developed and are in the process of evaluation: (1) control of collagen synthesis by directly influencing enzyme-controlled reactions involved with fibrillar construction and cross-linking, (2) renewed interest in control of the chronic edema seen characteristically in hypertrophic scar tissue by use of external pressure.

Currently, we are equipped to evaluate two methods of therapy on a clinical basis: (1) steroid therapy--intralesional Dermajet (intradermal) injections or application of Cordran tape, (2) pressure therapy using the Jobst pressure device, custom made to fit body segments of extremities. Since burn scars cover large body surface areas, surgical excision and radiotherapy are not usually feasible except for very small lesions. The two methods chosen for evaluation are more safely applicable to large wounds. Unfortunately, there are no drugs yet available to control collagen synthesis directly, although several are being tested in other laboratories.

Both Cordran tape and Jobst stockings can be used by the patient on convalescent leave, and the Dermajet treatments are given at 2- to 4-week intervals. Jobst pressure devices are not used about the chest and are limited to bilaterally burned extremities where one extremity can serve as the control for the other. Periodic photographs are the major means of evaluation. Biopsies are sparingly employed and confined to the limbs.

Results

A. Development of an Animal Model with Reproducible Hypertrophic Scars

Experimentation with scarification and introduction of foreign bodies into wounds has failed to produce hypertrophic scar in healthy rats, guinea pigs, and dogs. However, a histologically confirmed hypertrophic scar has been successfully created on the flank of a thermally injured New Jersey Red Duroc pig. The clinical history and scar maturation followed closely the course observed in human burn patients. Attempts are now being made to reproduce the scar in other pigs.

B. Histologic Examination of Human Scar Biopsies

Scar biopsies from 10 patients were examined for common histologic characteristics, specifically whorled collagen

patterns, size and numbers of collagen fibers, chronic inflammatory reaction, scar thickness, and presence of foreign bodies in the wound. Results have failed to demonstrate any common denominator characteristic of all hypertrophic scars. In general, small collagen bundles, frequently non-parallel to the skin surface, are interspersed with occasional small clusters of chronic inflammatory cells. No consistent whorl pattern was seen. Foreign body giant cells were present in the few specimens that contained foreign bodies--commonly particles of cotton fiber or inverted hair follicles.

C. Therapeutic Evaluation

1. Dermajet Injection of Steroid.

In nine selected patients, Dermajet treatments were undertaken at 2- to 4-week intervals. A mixture of triamcinolone (40 mg/cc) plus 150 units lyophilized hyaluronidase and 1% Xylocaine was used. Doses of 40-200 mg of triamcinolone were injected at each treatment, depending on the area covered. Specific attention was paid to scars of the face and hands. Two patients withdrew voluntarily from the study because of pain associated with the procedure. This treatment was moderately to poorly successful in producing cosmetic improvement judged by flattening and blanching of the scars. Symptomatic relief of pruritus and pain in the scar was commonly experienced. Softening and stretching of the scar was also achieved in several cases. No subcutaneous atrophy was encountered. Treatments were not undertaken in Negro patients to avoid the problems with depigmentation reported in the literature.

2. Topical Application of Steroid.

Three patients were treated with topical application of steroid via Cordran tape applied every 12 hours. Results were generally unremarkable, except for local erythema, probably indicative of vasodilation or increased capillary growth in the wound. Systemic absorbed steroid levels were not monitored.

3. Compression Elastic Devices.

Sixteen patients with bilaterally symmetrical wounds of the extremities were fitted with custom-made Jobst sleeves, gloves, stockings and leotards. Where possible, compression devices were applied to one extremity shortly after completion of graft healing. The treated extremity could then be compared with the

untreated one. Compression devices were worn 24 hours a day and removed only for washing. Best results were obtained when patients cooperated fully with the protocol and were treated for six months.

Reduction in circumference of the treated extremity, blanching and decreased pain in the scar were noticeable after one week of therapy. But edema reaccumulation within 4 hours of removal of the device was also encountered. Therefore, a therapy period of six months was advised. Patient acceptance of the device was excellent because of motivation and comfort.

At this time, statistical comparison of results is not possible because of insufficient numbers of patients in each group. However, results have been most encouraging, and compression devices are made routinely for patients with deep second-degree burns of the extremities to prevent, if possible, hypertrophy of scars in these highly susceptible individuals.

Summary

A successful prototype animal model for study of hypertrophic scars has been developed in a thermally injured pig. The reproducibility of this model is presently being assessed. Histologic examination of hypertrophic scar has failed to define any pathologic changes common to all scars. Biopsies will be continued to evaluate effect on the scars of various modes of therapy.

Of the three forms of clinical therapy for hypertrophic scars currently used at this institution, compression of the scars for six months from the date of graft healing has given the best results. Compression is achieved through custom-made Jobst garments and has been successfully applied to patients with burns of the extremities. The potential exists for extension of this mode of therapy to patients with chest, neck and head burns.

Steroid therapy, topically or intralesionally, has yielded disappointing results in terms of cosmetic improvement of small hypertrophic scars of the face and hands. No complications or side effects were noted with this form of therapy.

Publications and/or Presentations

None

ANNUAL PROGRESS REPORT

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

REPORT TITLE: ANESTHESIOLOGY

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

1 January - 31 December 1970

Investigators:

Joseph M. Garfield, M.D., MAJ, MC
Gary W. Allen, M.D., MAJ, MC
J. Gilbert Stone, M.D., MAJ, MC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

REPORT TITLE: ANESTHESIA

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

Period covered in this report: 1 January - 31 December 1970

Investigators: Joseph M. Garfield, M.D., MAJ, MC
Gary W. Allen, M.D., MAJ, MC
J. Gilbert Stone, M.D., MAJ, MC

Reports Control Symbol MEDDH-288(R1)

In 1970, 208 of 321 patients whose disposition was completed at the US Army Institute of Surgical Research were given 585 anesthetics, both at this Institute and elsewhere. Of the anesthetics given at this Institute (497 of the 585 anesthetics), 66.8% were nitrous oxide plus halothane, 15.7% were ketamine, with the remainder consisting of nitrous oxide, methoxyflurane, Innovar, regional nerve block, and spinal anesthesia. Of those patients receiving anesthesia, the mean number of anesthetics per patient was 2.81. Anesthetic complications during the year 1970 included airway obstruction secondary to the administration of ketamine in the prone position, and two cases of cardiac arrhythmia during and following administration of anesthesia. Two intraoperative deaths occurred in 1970, and are described in detail in the text of this report.

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 to age 4 are permitted clear liquids until 0400 hours. Using this regimen, we have had no vomiting or aspiration on induction due to unsuspected full stomachs in patients for elective surgery.

Seriously ill or dehydrated patients are given intravenous fluids preoperatively, including Ringer's lactate and 5% dextrose in either Ringer's lactate or saline solution. Special pediatric solutions are used for infants and children.

Hemodynamic and Respiratory Assessment

All acutely ill patients have arterial blood gases drawn daily until their status improves at which time the frequency of determinations is decreased. By knowing these values, preoperatively, in all seriously ill patients,we are able to adjust our anesthetic techniques accordingly. Patients who are hypoxemic and require ventilatory assistance are transported to and from the operating room with the administration of 100% oxygen given by positive pressure, utilizing either a Jackson-Rees modification of the Ayre's T-piece,[1] or a Bird respirator. Once in the operating room, patients requiring ventilatory assistance may be ventilated manually, with an Ohio anesthetic ventilator, or, if extreme pressures are required, by an Emerson postoperative ventilator or a Bird Mark XIV respirator. The latter two machines are capable of developing peak inspiratory pressures approaching 100 cm of water. Such extreme pressures are occasionally necessary when gross pulmonary edema and extensive pneumonitis drastically reduce lung compliance.

Circulatory status is assessed by hematocrit, serum electrolytes, serum osmolality, and urinary output, in addition to indirect or direct measurement of blood pressure. Central venous pressure measurements are taken on seriously ill patients.

Premedication

In general, no narcotics, barbiturates, or ataractics are given preoperatively to adult patients. Rather, atropine, 0.01 mg/kg, is given intravenously immediately prior to induction of general anesthesia. Patients receiving regional anesthesia (regional nerve blocks, spinal and epidural anesthesia), do receive premedication, consisting of a barbiturate, anticholinergic, and occasionally a narcotic (morphine or Demerol(R)), or ataractic (Valium(R)). Pediatric patients generally receive a narcotic plus an anticholinergic agent preoperatively, in order to allay anxiety and induce a state of quiescence.[1]

Types of Anesthesia Used (See Table 4)

A. General Anesthesia

1. N_2O-halothane: About two-thirds (66.8%) of the general anesthesias at our Institution are performed with this combination of agents, due to ease of administration, tranquil induction and emergence, relative lack of long-lasting cardiovascular depression, and nonflammability. We have followed SGOT values in a number of our patients who have received halothane, and in a control group of burn patients who did not receive this agent. Our data indicate, as detailed elsewhere in this report, that the SGOT by itself is of little or no value in predicting or assessing hepatotoxicity in the burn patient. From the standpoint of hepatotoxicity, it appears that multiple halothane administration is relatively safe. Since the incidence of this complication is approximately 1 in 10,000 patients, this seems to be an acceptable risk when it is weighed against the great advantages of the agent in the burn patient.[2] Thiopental (2- 4.5 mg/kg) is used in about a third of our patients for induction of general anesthesia, with no deleterious effects observed. We have not observed any significant incidence of prolonged emergence or postoperative grogginess in patients who received a total dose not exceeding 7 mg/kg of thiopental, provided that the last incremental dose is given at least 30 minutes before the case ends.

2. N_2O relaxant: This technique is often used in very seriously ill patients for laparotomies and other major procedures (amputations, etc.) due to its relative lack of cardiovascular depression. Since the technique requires controlled respiration, the trachea is intubated. Relaxants employed include d-tubocurarine (curare) and gallamine, both non-depolarizing relaxants. The latter has been shown not to raise serum potassium in burn patients.[3] Succinylcholine is rarely used except for acute emergencies, due to its tendency to cause severe rises in potassium

from about postburn day 15 through 90.[4]

3. Innovar(R): This is a combination of a tranquilizing agent (droperidol) and an ultra short-acting, extremely potent narcotic (fentanyl). We have used Innovar(R) with success for awake naso- and orotracheal intubations, and for various surgical procedures, with or without a muscle relaxant. Muscle relaxants are usually needed with this technique to prevent movement and permit relaxation, and, in this case, respiration is controlled with an endotracheal tube in place. We have observed that emergence from Innovar(R) is often prolonged when compared with halothane, which means that our patients may miss meals postoperatively when given Innovar(R), and lower their caloric intake for that day.

4. Ketamine:This is a new intravenous "dissociative" general anesthetic which has recently been released for routine clinical use. Prior to that time, we had been using and evaluating ketamine for several months on an investigational basis. The agent shows great promise for debridement, skin grafting, various orthopedic procedures, and for Hubbard tank treatments, especially since cardiovascular reflexes and tone are well preserved and a patent airway with good ventilation is usually maintained even in lateral and prone positions, without need of a mechanical airway. It must be emphasized, however, that on occasion significant airway obstruction can occur with ketamine. One such episode is described in the section on anesthetic complications. We have recently completed an investigation of certain psycho-pharmacologic properties of ketamine (see description elsewhere in this annual report).

B. Regional Anesthesia

Compared to 1968 (see Table 5), regional anesthesia, in particular, brachial plexus block, ulnar nerve block, and subarachnoid block, was used on a much greater scale. 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 anesthesias, and no incidence of infection or sepsis after nerve blocking was noted. We have found that supraclavicular brachial plexus blocks, coupled with block of the intercostal brachial and medial brachial cutaneous nerves, are especially suited for elbow surgery, a great deal of which is now being performed for myositis ossificans. We have had no

incidence of post-block pneumothorax following the supraclavicular approach.

Monitoring Techniques

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

A. Circulation

1. Precordial and/or esophageal stethoscope.

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

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

4. Central venous pressure (CVP) assessment.

5. EKG (major cases & d seriously ill patients).

6. Sponge-weighing; major cases.

7. Serial measurement of urine output during surgery.

B. Respiration

1. Counting of respiratory rate.

2. Observation of chest, rebreathing bag.

3. Auscultation of chest.

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

C. Temperature

1. Rectal or esophageal thermister probe - routine for cases lasting more than 45 minutes.

2. K-thermia heating-cooling blanket. This apparatus, recently acquired for the operating room, has proven 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.

Complications

Three noteworthy anesthetic complications occurred during 1970 and are described below.

Case Reports

Case 1. Upper Airway Obstruction During Ketamine Induction in Prone Position. This 23-year-old, white male with total body surface burns of 16%, 4.5% third degree, was scheduled for autografting in the prone position on the 37th postburn day. Premedication consisted of IV atropine, 0.01 mg/kg, 10 minutes prior to induction of anesthesia. Anesthesia was induced in the prone position with ketamine, 2 mg/kg IV, given over a 90 minute period. About two minutes after this dose was administered, the patient was noted to have poor air exchange, and had signs of upper airway obstruction. This became almost total over the next 30 seconds despite traction on the angle of the mandible, suctioning of the pharynx and nose, and administration of 100% O_2 by mask. At this time, the patient became mildly cyanotic and was immediately returned to the supine position, which enabled us to re-establish a patent airway almost instantaneously. The patient regained a pink appearance over the next 30-60 seconds of 100% oxygen administration by mask, and the procedure was carried out in the supine position. No postanesthetic sequelae of this hypoxemic episode ensued.

Comment. Ketamine is widely heralded as not interfering with the airway, even in the prone and other positions, where, during anesthesia, the airway is often compromised. Our experience, above, taught us that this is a dangerous assertion. Ketamine, like any other general anesthetic agent, carries no guarantee of a patent airway. Accordingly, when this agent is administered, all facilities for artificial ventilation must be immediately available, and, especially, if the patient is prone or in some other position where the airway is liable to be compromised; he should be turned supine if any airway obstruction, which cannot be satisfactorily overcome immediately, occurs.

Case 2. Transient Atrial Fibrillation Following a Ketamine Anesthetic. This 22-year-old, white male, 37.5% total body surface burns, 27% third degree, was scheduled on the 55th postburn day for decortication of several phalanges of the foot. Prior to this time, he had received five thiopental, nitrous oxide, halothane anesthesias without any intraoperative or postoperative complications. His sixth anesthetic consisted of atropine, 0.01 mg/kg IV immediately prior to induction, and ketamine

IV. A total of 1,750 mg of ketamine was given for this procedure, which took 115 minutes. Because the hematocrit at the beginning of the case was 27%, three units of blood were given intraoperatively. The blood pressure was stable and the pulse regular throughout the procedure, at 110-120/minute, apically.

Following surgery, the patient was taken to the recovery area, where he was noted to have an irregular pulse with an apical rate of 140-160/minute. An EKG demonstrated that acute atrial fibrillation had developed. The blood pressure remained stable, but the arrhythmia persisted over the next two hours, with the same apical rate. It then abruptly reverted to a mild sinus tachycardia with a rate of 120-130. No further episodes of atrial fibrillation occurred, and the patient received a subsequent nitrous oxide, halothane anesthetic on the 65th postburn day without incident.

Comment. Cardiac arrhythmias, including atrial fibrillation, occur, fairly commonly, both during and following the administration of general anesthetic agents. More evidence is needed before one can assume that a distinct causal relationship between the administration of ketamine and the occurrence of postoperative cardiac arrhythmias exists. In our experience to date, which covers well over 200 ketamine anesthesias, this is the only case in which an arrhythmia occurred postoperatively with this particular agent. We have noted other arrhythmias, such as A-V dissociation, nodal rhythms, and bigeminy during the administration of other anesthetic agents. (See the following case report.)

Case 3. Intraoperative Nodal Rhythm with Incomplete Atrio-Ventricular Dissociation During a Halothane Anesthetic. This 21-year-old, black, male, total body surface burns of 15%, 8% third degree, had undergone an open reduction of a right ankle fracture in RVN on the day of injury, under nitrous oxide, halothane anesthesia, without any apparent complications.

On the 24th postburn day, he was scheduled for an autografting procedure. The pre-anesthetic visit revealed that his chest was clear, that the heart rate was regular, and that A_2 was greater than P_2, with no obvious cardiomegaly and no murmurs.

Premedication consisted of atropine, 0.01 mg/kg IV immediately prior to induction of anesthesia. Heart rate was regular at this time. Anesthesia was induced with thiopental, 4.0 mg/kg IV and nitrous oxide, oxygen, halothane anesthesia was begun.

The trachea was intubated, uneventfully, under transient deep halothane anesthesia, in order to avoid the use of succinylcholine. About five minutes after intubation, the heart tones and peripheral pulse were noted to be irregular. An EKG was taken which revealed multiple junctional and nodal ectopic beats. A total of 100 mg of IV lidocaine was administered,without any observable effect on the arrhythmia. It was felt that until this arrhythmia was investigated further, it would be hazardous to proceed, and, accordingly, the procedure was terminated and the patient awakened. This arrhythmia persisted intermittently even while the patient was awake, and it was felt that the patient had had this for some time. He did, indeed,have periods of regular sinus rhythm and while unanesthetized had regular sinus rhythm most of the time. He subsequently had two autografting procedures, each performed under Innovar, N_2O, O_2, d-tubocurarine and fentanyl. Aside from infrequent periods of high nodal premature contractions, he did well and had an uncomplicated postoperative course. He was subsequently transferred to the orthopedic service for more definitive treatment of his orthopedic problems.

Comment. As in the preceding case history, arrhythmias during and after anesthesia do occur. Prompt recognition and proper therapeutic measures are essential. A precordial stethoscope, pulse monitor, and oscilloscopic EKG monitor will enable early detection to be made and permit on-line assessment of therapy in the operating room. In the case described above, termination of anesthesia was clearly indicated, especially since surgery had not yet begun.

Intraoperative Deaths

Two intraoperative deaths occurred during 1970,and are described below.

Case Reports

Case 1. This 30-year-old, white male telephone lineman, received burns estimated at 20½% total body surface with 14½% third degree when he came in contact with a 7,200 volt transformer. On admission to the Institute of Surgical Research, one hour postburn, he was hypoxic and hyperchloremic. With the administration of nasal oxygen and Kayexalate enemas, these conditions were rapidly reversed. Several hours later, when his overall condition improved somewhat, he was taken to the operating room, where exploration and debridement of the electrical burns of the leg

and debridement of the sternal area were carried out. His physical status at this time was 5-E. No premedication was given. Anesthesia consisted of nitrous oxide and d-tubocurarine, 30 mg; the airway consisted of a 9 mm orotracheal tube, inserted on the ward prior to surgery. The procedure lasted 70 minutes, and the patient tolerated the procedure satisfactorily. He was placed on a Bird respirator postoperatively for about 4 to 5 hours, and the action of the curare was allowed to dissipate spontaneously. No atropine or prostigmine were given at the termination of the case. Six hours postburn, the patient developed an elevated serum amylase and physical findings consistent with pancreatitis. At this same point, an acute coagulopathy was diagnosed with markedly elevated prothrombin time and decreased Factor V and Factor X levels. These deficiencies were controlled with the administration of multiple units of fresh frozen plasma. A progressive fall in the platelet count from 50,000 to 25,000 was likewise controlled with the administration of multiple units of platelets. By 24 hours postburn, the patient first displayed an inability to appropriately concentrate urine with specific gravity falling to less than 1.010 by 48 hours postburn. Associated with this was low osmolality and UUN compared to the progressively rising BUN and serum creatinine. The patient required hemodialysis on the fourth postburn day. Also, on the fourth postburn day, due to the deep electrical injury that the patient had incurred, disarticulation of the left knee was performed under general anesthesia, consisting of Innovar, nitrous oxide, and d-tubocurarine. An 8.5 mm orotracheal tube was used. The patient tolerated the procedure satisfactorily, and the muscle relaxant was reversed with appropriate doses of atropine and prostigmine. The patient was judged to have had satisfactory spontaneous respiration after this case. Physical status at this time was judged as ASA Class III. By the morning of the third postburn day, a left lower lobe infiltrate was noted, which spread to involve the left upper lobe and right lower lobe by the fifth postburn day. On the sixth postburn day, pulmonary edema, accompanied by mild elevation of the central venous pressure, was noted. In spite of the patient's poor overall status, which on postburn day 6 was judged as ASA 5, left hip disarticulation was deemed necessary. The patient was taken to the operating room, a tracheostomy was performed, and anesthesia was induced with nitrous oxide, oxygen, and d-tubocurarine, 18 mg,and controlled respiration was carried out via an Ohio operating room ventilator. However, because of the extremely high positive pressures required to adequately ventilate this patient, on the order of 50-60 cm of water, the patient became progressively hypoxic and acidotic with resultant cardiac arrest, from which he could not be successfully resuscitated. Thus, the patient succumbed before the planned surgical procedure

could be performed. At autopsy, he was found to have a severe bilateral bronchopneumonia, 20.5% total body surface electrical burn with extensive necrosis of both the anterior and posterior trunk and the lower extremity. Also present was focal coagulation necrosis of the left lobe of the liver, felt to have resulted from the electrical burn, hemorrhagic gastritis and an acute duodenal ulcer were also noted.

Case 2. This 9-year-old, white male sustained burns, second and third degree, involving the head, neck, anterior and posterior trunk, both upper extremities, both hands, both lower extremities, and both feet, 68.5% total body surface, with 64% third degree, when an explosion occurred in a store room while lighting a cigarette at the patient's home. Upon admission to the US Army Institute of Surgical Research, there was evidence of inhalational injury in that the nasal hairs were singed, the lips were swollen, and the teeth were stained with soot. The throat was red and edematous as was the uvula. Additional soot staining of the pharynx was also present. The patient was considered to be very seriously ill, and on the 14th postburn day, debridement and heterograft were performed under ketamine anesthesia. Physical status was considered to be ASA 4. Hematocrit was 31%. The procedure lasted 60 minutes. At the end of the case, the patient was considered to be in satisfactory condition. On the 17th postburn day, the patient was again taken to the operating room, and considered to be ASA physical status 5, with hematocrit of 36%. No premedication other than atropine, 0.01 mg/kg, intravenously, was given. Anesthesia was induced with intravenous ketamine, 0.7 mg/kg, and the surgery, consisting of debridement of the burn wounds and application of porcine heterograft, was begun. At the end of the procedure, which lasted approximately 35-40 minutes, the patient was noted to have shallow, inadequate respirations, and an endotracheal tube was inserted. Mechanical ventilation was begun with 100% oxygen. He was treated with rapid intravenous infusion of Ringer's lactate and blood. Despite these maneuvers, the patient sustained a cardiac arrest, from which he could not be resuscitated despite intravenous and intracardiac epinephrine and other resuscitative agents. At autopsy, the patient was found to have burns, 68.5% total body surface, 64% third degree, bilateral bronchopneumonia, and, most notably, an acute pulmonary embolism to one of the main branches of the pulmonary artery, originating in a mycotic suppurative thrombophlebitis of the left femoral vein. In retrospect, then, this acute pulmonary embolism was responsible for the patient's intraoperative demise. In addition, a mycotic myocarditis was present. Death in this patient was then attributed to the cardio-respiratory stress of pulmonary embolism and mycotic

myocarditis, superimposed upon a bilateral bronchopneumonia. All of these were considered to be complications of this patient's severe large burn.

Summary

When surgery is performed early in the hospital course of the burn patient (i.e., during the first 6 weeks), general anesthesia is usually necessary due to the nature of the procedure and to potential contamination. Although nitrous oxide-halothane is generally used, other agents (nitrous oxide, oxygen, muscle relaxant, Innovar(R)) are satisfactory. Ketamine shows great promise as a general anesthetic agent for debridement, chondrectomies, orthopedic procedures, and for the Hubbard tank.

For surgery during the reconstructive period of the burn (6 weeks and beyond), regional anesthesia can often be used, as long as the patient is not septicemic and does not have infected skin areas at or immediately adjacent to the site of nerve blocking. Ketamine is also indicated for certain procedures done at this time.

References

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

2. Klatskin G, Kimberg DV: Recurrent hepatitis attributable to halothane sensitization in an anesthetist. N.E.J.M. 280:512-522, 1969.

3. Carr J, Kitchings OE, Garfield JM, et al: Effect of flaxedil (Gallamine) on serum potassium in burn patients. Unpublished data; presented at annual meeting American Society of Anesthesiologists, San Francisco, Calif, October 1969.

4. Schaner PJ, et al: Succinylcholine-induced hyperkalemia in burned patients. Anesth Analg (Cleveland) 48:768-770,1969.

Publications

Garfield JM: Respiratory complications in the burned patient. Am Soc of Anesthesiologists Annual Refresher Course Lecture Outline, p. 206, 1970.

Presentations

Garfield JM: Comparative psychological study of ketamine. Combined Anesthesia conference, Univ of Tex Med School at San Antonio, San Antonio, Texas 21 September 1970.

Garfield JM: Respiratory complications in the burned patient. (Refresher course lecture) Am Soc of Anesthesiologists, New York, N.Y., 18 October 1970.

Garfield JM: The effects of varying preoperative information on psychologic responses to ketamine and halothane anesthesia. Am Soc of Anesthesiologists, New York, N.Y. 21 October 1970.

Garfield JM: Anesthetic management of the burned patient. Dept of Anesthesia, Peter Bent Brigham Hospital, Boston, Mass. 24 October 1970.

Garfield JM: Vasopressors in anesthesia. Dept of Anesthesia, BGH, BAMC, FSHTex, 28 November 1970.

TABLE 1. OVERALL PATIENT DATA, USAISR (1968-1970)

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

TABLE 2. NATURE OF SURGERY (PRIMARY) (1970)

	ISR		ELSEWHERE	
	NO.	%	NO.	%
DEBRIDE ONLY	16	3.2	68	77.3
D & H	74	14.9	0	0
D & A	282	56.7	1	1.1
ORTHO.	55	11.1	11	12.5
EAR	21	4.2	0	0
EYE	9	1.8	0	0
INTRA-ABDOMINAL	12	2.4	3	3.4
TRACH.	12	2.4	5	5.7
OTHER	16	3.2	0	0
TOTAL	497	100%	88	99.9

TABLE 3. NATURE OF SURGERY, ISR - % OF TOTAL OPERATIONS DONE

PROCEDURE	1965	1966	1970
DEBRIDEMENT AND HOMOGRAFT	60.8	55.6	18.1
AUTOGRAFT	31.7	35.7	56.7
CHONDRECTOMY	2.6	2.2	4.2
LAPAROTOMY	1.2	1.6	2.4
MISCELLANEOUS	3.7	4.9	18.5

TABLE 4. TECHNIQUES OF ANESTHESIA, 1970

	ANESTH ISR	% OF TOTAL	ANESTH ELSEWHERE	% OF TOTAL	TOTAL ANESTH	% OF TOTAL
TOTAL	497	100.0	88	100.0	585	100.0
GENERAL ANESTH.	471	94.8	87	98.9	558	95.4
HALOTHANE, N_2O, O_2	332	66.8	76	86.4	408	69.7
N_2O, O_2, relaxant	26	5.2	0	0.0	26	4.4
N_2O, O_2, other	16	3.2	2	2.3	18	3.1
METHOXYFLURANE	2	0.4	7	7.8	9	1.5
KETAMINE	93	18.7	2	2.3	95	16.2
INNOVAR	2	0.4	0	0.0	2	0.3
LOCAL ANESTHESIA	26	5.2	1	1.1	27	4.6
SPINAL	6	1.2	0	0.0	6	1.0
SUPRACL. BRACH. PL.	6	1.2	0	0.0	6	1.0
AXILLARY	4	0.8	0	0.0	4	0.7
ULNAR N.	2	0.4	0	0.0	2	0.3
OTHER REGIONAL BLOCK	2	0.4	1	1.1	3	0.5
LOCAL (TRACH. OR BRONCH)	6	1.2	0	0.0	6	1.0

TABLE 5. EMPLOYMENT OF ANESTHETIC AGENTS AT ISR, 1964-1970 (IN PER CENT)

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

TABLE 6. MULTIPLE HALOTHANE ADMINISTRATION

1970

No. of Halothane Anesthetics per Patient *	0	1	2	3	4	5	6	7	8	9	10	11
No. of Patients	160	67	43	20	5	9	7	2	3	2	1	2
Percent of Total No. of Patients	49.8	20.9	13.4	6.2	1.6	2.8	2.2	0.6	1.0	0.6	0.3	0.6

* Includes halothane anesthetics received prior to arrival at ISR

TABLE 7. GENERAL ANESTHETIC INDUCTION AGENTS
ISR - 1970

AGENT	NO. OF INDUCTIONS	PER CENT OF TOTAL
THIOPENTAL	132	28.0
INNOVAR®	12	2.5
KETAMINE	96	20.4
INHALATION	231	49.0
TOTAL	471	99.9

TABLE 8. TYPE OF AIRWAY DURING GENERAL ANESTHESIA
ISR-1970

AIRWAY	NO. OF ANESTHETICS	% OF TOTAL NO. OF GENERAL ANESTHETICS
MASK	231	49.1
ENDOTRACHEAL TUBE		
ORAL	109	23.2
NASAL	10	2.1
TRACHEOSTOMY TUBE	26	5.5
NATURAL AIRWAY	95	20.2
TOTAL	471	100.1

TABLE 9. USE OF MUSCLE RELAXANTS, ISR - 1970

TOTAL GENERAL ANESTHETICS	NO. OF ANESTHETICS WHERE MUSCLE RELAXANTS USED	dT CURARINE	GALLAMINE	SUCCINYLCHOLINE
471	34	16	15	3
% of Total Gen. Anesth.	7.2%	3.4%	3.2%	0.6%

TABLE 10. MORTALITY OF THOSE RECEIVING ANESTHESIA*

TOTAL PATIENTS GIVEN ANESTHESIA	208	INTRAOPERATIVE DEATHS	2
DEATHS	26	DIED WITHIN 24 HOURS	4
PER CENT	12.5	24 HOURS to 1 WEEK	5
		GREATER THAN 1 WEEK	15
		TOTAL	26

* INCLUDES ANESTHETICS GIVEN AT ISR AND ELSEWHERE.

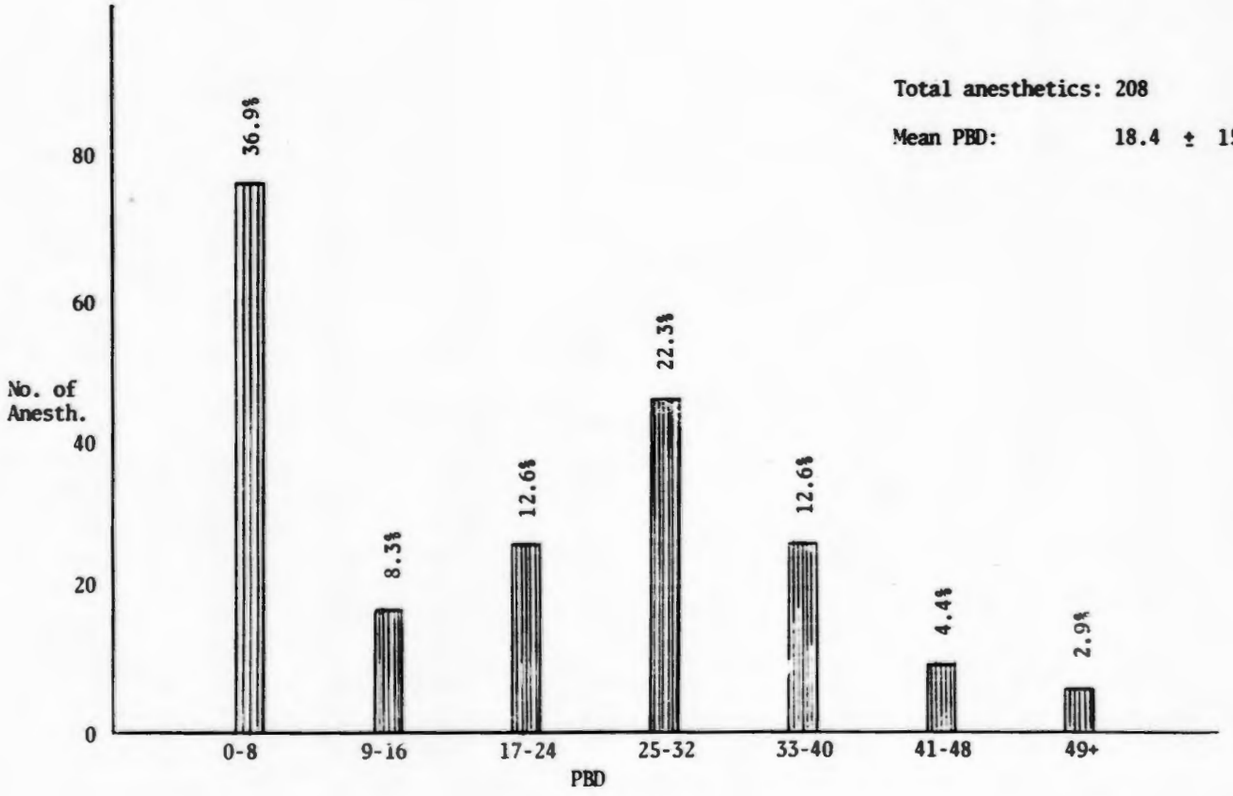
TABLE 11. DURATION OF ANESTHETICS ADMINISTERED AT ISR

YEAR	1964	1965	1966	1967	1968	1969	1970
NO. OF ANESTH.	332	495	713	670	794	601	497
MEAN DURATION (MINUTES)	82	76	78	68	68	74	75
STANDARD DEVIATION ±	51	40	42	41	33	42	40

FIGURE 1. POST-BURN DAY OF FIRST ANESTHETIC 1970 (ISR and Elsewhere)

Total anesthetics: 208

Mean PBD: 18.4 ± 15.9



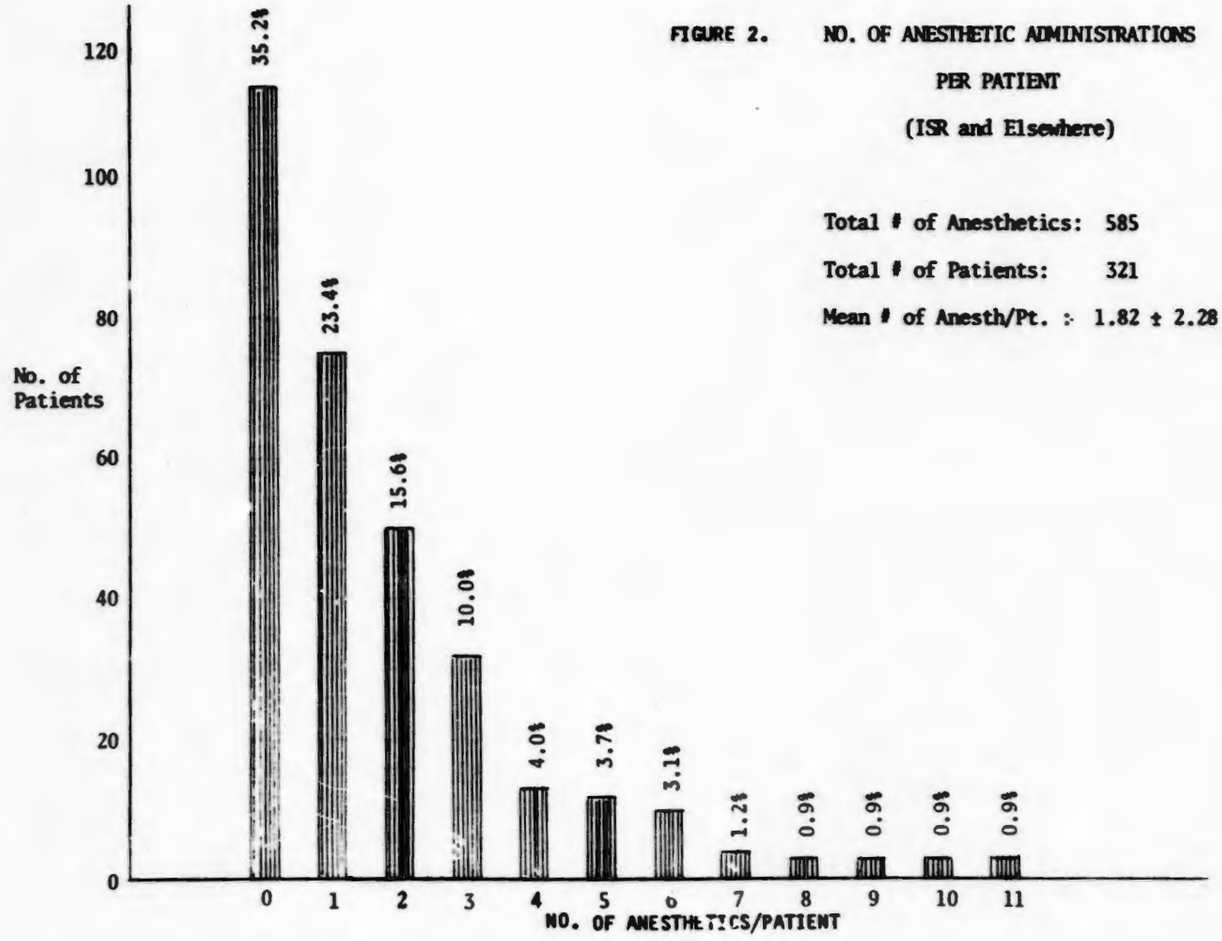


FIGURE 2. NO. OF ANESTHETIC ADMINISTRATIONS PER PATIENT (ISR and Elsewhere)

Total # of Anesthetics: 585

Total # of Patients: 321

Mean # of Anesth/Pt. : 1.82 ± 2.28

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA OC 6387	71 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
70 07 01	K. COMPLETION	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A061102B71R	01	234
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Procede with Security Classification Code)*
(U) Multiple Halothane Anesthesia 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
64 04	70 08	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 71	.17	4.5
	CURRENT 72	0	0

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

RESPONSIBLE INDIVIDUAL
NAME: PRUITT, B. A., JR, LTC, MC
TELEPHONE: 512-221-2720

20. PERFORMING ORGANIZATION
NAME:* US Army Institute of Surgical Research
Clinical Division
ADDRESS:* Ft Sam Houston, Texas 78234

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME:* Joseph M Garfield, MAJ, MC
TELEPHONE: 512-221-5712
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME:
NAME: DA

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Procede EACH with Security Classification Code)
(U) Halothane; (U) Hepatic necrosis; (U) Burns; (U) Anesthesia; (U) Hepatitis

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

23. (U) To determine whether the SGOT (an enxyme thought to reflect hepatocellular damage or dysfunction) is a useful index of hepatic status consequent to administration of halothane in the burn patient.

24. (U) During the study period, military burn patients when first brought to the ISR had an SGOT drawn and, thereafter, at weekly intervals throughout their hospital stay. The control group consisted of those patients who did not receive anesthesia. The patterns and time courses of SGOT fluctuations in the control group were compared with those in the group receiving one or more halothane anesthesias.

25. (U) 70 07 - 70 08 We have been unable to discern any discrete pattern of the SGOT levels in the burn patient with the exception that values tended to be high (greater than 200) during the acute and early convalescent phases of the burn and then decrease slowly. Fluctuations up to 500 units tended to occur in response to disease and nonspecific disease states, making the SGOT an essentially useless index of incipient hepatic dysfunction in the burn patient, unless grossly elevated values (greater than 1000) are encountered. Halothane is considered to be a relatively safe and effective general anesthetic agent in burn patients, but the risk of allergic hepatitis consequent to its use must be considered.

*Available to contractors upon originator's approval.

FINAL REPORT

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

REPORT TITLE: MULTIPLE HALOTHANE ANESTHESIA IN THE BURN PATIENT

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

1 July 1970 - 30 June 1971

Investigator:

Joseph M. Garfield, M.D., MAJ, MC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

REPORT TITLE: MULTIPLE HALOTHANE ANESTHESIA IN THE BURN PATIENT

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

Period covered in this report: 1 July 1970 - 30 June 1971

Investigator: Joseph M. Garfield, M.D., MAJ, MC

Reports Control Symbol MEDDH-288(R1)

Between March 1968 and August 1970, the SGOT was followed at weekly intervals in a group of approximately 200 adult patients who received halothane at our unit, and in a control group of 40 patients who did not receive any anesthesia. This study was undertaken in order to determine whether the SGOT was a useful index of hepatic dysfunction in the burn patient, and whether it would indicate hepatic damage secondary to halothane administration.

We concluded that the SGOT is not a useful index of hepatic dysfunction in burn patients, because great fluctuations of the SGOT occurred in response to a diverse number of disease states where hepatic damage was not present, on the basis of physical examination and other laboratory indices of hepatic function. A review of our records since 1964 indicated that in only one patient, out of more than 3000 halothane administrations, in over 1000 patients, was halothane hepatitis considered to have been a possibility. Accordingly, we consider halothane to be a relatively safe and effective general anesthetic agent in burn patients, for both single and multiple administration. However, the risk of allergic hepatitis consequent to its use, although small (1:10,000-25,000) is ever present, and must be carefully considered every time the agent is contemplated for use.

Halothane
Hepatitis
Hepatic necrosis
Burns
Anesthesia

MULTIPLE HALOTHANE ANESTHESIA IN THE BURN PATIENT

In 1969, a four-year retrospective study of multiple halothane anesthesia during 1964-1968 was published by Gunther, et al.,[1] and demonstrated the safety of halothane as a repetitive anesthetic for burn patients.

Beginning in March 1968, weekly SGOT determinations were begun on all patients receiving halothane anesthesia, and data collection was terminated as of August 1970, when SGOT values on 200 such patients were accumulated. In addition, through the acute and sub-acute burn phases, serial SGOT values were obtained on approximately 40 burn patients who never came to anesthesia, in order to generate a control population.

In evaluating the SGOT data, we posed the following questions: 1) Is there any predictable pattern or patterns to the time course of the SGOT levels in burns? 2) Is the SGOT level a function of burn size or severity? 3) Does a high SGOT level necessarily mean that there is hepatic dysfunction, or, instead, is it a nonspecific response to a severe systemic disease state?

We have been unable to discern any discrete patterns of SGOT level changes with time except that SGOT values tend to be high (> 200) during the acute and early convalescent phases of the burn and then decrease. We suspect that the SGOT level in burn patients, is, at best, a nonspecific response to a severe systemic disease state, rather than a specific index of hepatic dysfunction. Moreover, the SGOT level seems to vary with such disease states as muscle necrosis, sepsis, fluid and electrolyte disturbances, fever, myocardial, respiratory and renal failure, plus a host of other complicating variables which, taken together, make the SGOT level essentially useless as an index of hepatic dysfunction following anesthesia in the burn patient. Admittedly, we have encountered only one patient, since 1964 (see history below) with fulminant hepatic necrosis immediately following halothane anesthesia. The literature available on such cases indicates that the SGOT may exceed 5000, and is accompanied by gross changes in other parameters of hepatic function, such as bilirubin, BSP retention, LDH, etc.[1,2] The point to be made, however, is that in burn patients who have never received either blood or halothane, the SGOT level may range up to 800 or higher, without other evidence of hepatic dysfunction, and changes in serial SGOT values on the order of several hundred cannot be taken as conclusive evidence that hepatic dysfunction is present.

Accordingly, we conclude that a high SGOT value in a burn patient is not necessarily a contraindication to the administration of halothane, and that hepatic dysfunction must be ruled in or out on the basis of the total clinical picture, supplemented by a complete battery of liver function tests, rather than on the basis of the SGOT alone.

In reviewing our statistics covering the period from 1964 through 1970, there was only one case to date in which halothane hepatitis was a distinct possibility. This particular patient (47-68) had six operative procedures, including bilateral lower extremity amputations and an emergency gastrectomy and vagotomy for massive gastrointestinal hemorrhage. Nitrous oxide, halothane was used for all six anesthetics. He received a total of 37 units of blood, nine of these on the day of injury. He developed gastrointestinal hemorrhage and emesis on the 28th day postinjury. His mental status deteriorated markedly on the 29th day postinjury when he was bleeding massively and underwent gastrectomy. His SGOT was 2000 and SGPT 1800. He remained comatose postoperatively, and died on the 30th postburn day with an SGOT of 1100, and an SGPT of 1250. At autopsy, he had massive, diffuse, centrolobular, hepatic necrosis, acute tubular necrosis, and cerebral edema.

Serum hepatitis was considered the most likely cause of death, since nine units of blood had been given 30 days prior to death and signs and symptoms of serum hepatitis are not unusual this soon after transfusion.[1,2] However, halothane was used on multiple occasions, and it is impossible to completely rule it out as a possible cause.

In summary then, the SGOT level in the burn patient is considered <u>not</u> to be a useful index of either halothane-induced liver damage or of hepatic dysfunction in general, unless it reaches a value of at least several thousand units, and correlates with disturbance of other well-established liver function tests. Halothane, on the basis of this report and other data gathered from this institution, is considered to be a relatively safe and effective general anesthetic agent for use in burn patients. However, the possibility of halothane hypersensitivity is always present following multiple administrations of this agent, and this potentially fatal complication must be carefully considered. For this reason, it is our policy that halothane should not be used indiscriminately, and alternate techniques, such as nitrous oxide with muscle relaxants, ketamine, and regional anesthesia are used whenever feasible. Nonetheless, we

do not hesitate to use halothane, including multiple administrations, when its considerable advantages make it an agent of choice for a particular patient.

References

1. Klatskin G, Kimberg DV: Recurrent hepatitis attributable to halothane sensitization in an anesthetist. N.E.J.M. 280:512-522, 1969.

2. Klatskin G: Mechanisms of toxic and drug-induced hepatic injury, Toxicity of Anesthetics. Edited by BR Fink, Baltimore, Williams and Wilkins, 1969, pp. 159-172.

Publications and/or Presentations

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6974	71 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
70 07 01	K.COMPLETION	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A061102B71R	01	271
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Procede with Security Classification Code): (U) Comparison of Psychologic Response to Ketamine and Halothane Anesthesia Used 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
70 01	70 09	DA	C. In-House

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

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
FISCAL YEAR	PRECEDING 71	.44	11.7
	CURRENT 72	0	0

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

RESPONSIBLE INDIVIDUAL
NAME: PRUITT, B.A., JR, LTC, MC
TELEPHONE: 512-221-2720

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

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

ASSOCIATE INVESTIGATORS
NAME: F.B. Garfield, Ph.D.
NAME: L.A. Johns, LTC, ANC DA

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Procede EACH with Security Classification Code): (U) Ketamine; (U) Halothane; (U) Awareness during Anesthesia; (U) Psychologic Responses to Anesthesia

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

23. (U) To characterize and compare the incidences of perceptual alterations, anxiety, and acceptability of ketamine and halothane anesthesia in patients with minimal and detailed preoperative information.

24. (U) A total of 48 patients were selected for this preoperative, randomized, single-blind study. The patients were divided into four experimental groups, two of which received ketamine and two halothane. All patients were told that they were to receive ketamine. The entire patient group was further divided, according to the 2 x 2 experimental design, so that half received a minimal preoperative briefing and half received a detailed preoperative briefing outling what to expect from ketamine. All patients received the IPAT Trait Anxiety Scale preoperatively; 24 hours following surgery, all patients in the study were given questionnaire forms by a physician in the study, enabling us to document and quantitate specific perceptual phenomena and subjective feelings of the patients studied.

25. (U) 70 07 - 70 09 The study demonstrated that the incidence of perceptual alterations following the administration of ketamine was so high that a pharmacologic property of the drug can be inferred. Perceptual alterations also occurred with halothane, but their evidence was significantly lower than with ketamine. Ketamine was found acceptable for future administration by patients who received it unless their anesthetic depth was inadequate and these findings have been applied to the clinical usage of ketamine in the treatment of burned military patients.

*Available to contractors upon originator's approval.

FINAL REPORT

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

REPORT TITLE: THE INFLUENCE OF PREOPERATIVE INFORMATION ON PSYCHOLOGIC RESPONSES TO KETAMINE AND HALOTHANE ANESTHESIA IN BURN PATIENTS

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

1 January - 31 December 1970

Investigators:

Joseph M. Garfield, M.D., MAJ, MC
Frances B. Garfield, Ph.D.*
J. Gilbert Stone, M.D., MAJ, MC
David Hopkins, M.D., MAJ, MC**
Lois A. Johns, LTC, ANC

* Present address: Instructor in Psychiatry, University of Texas Medical School at San Antonio, San Antonio, Texas 78229.
** Present address: Dept. Anesthesiology, Brooke General Hospital, Brooke Army Medical Center, Fort Sam Houston, Texas 78234

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

REPORT TITLE: THE INFLUENCE OF PREOPERATIVE INFORMATION ON PSYCHOLOGIC RESPONSES TO KETAMINE AND HALOTHANE ANESTHESIA IN BURN PATIENTS

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

Period covered in this report: 1 January - 31 December 1970

Investigators: Joseph M. Garfield, M.D., MAJ, MC
Frances B. Garfield, Ph.D.*
J. Gilbert Stone, M.D., MAJ, MC
David Hopkins, M.D., MAJ, MC**
Lois A. Johns, LTC, ANC

Reports Control Symbol MEDDH(288)R1

Ketamine, a new dissociative anesthetic, may be accompanied by sensory and perceptual misinterpretations, dreaming and hallucinations. We hypothesized that a detailed preoperative description of the emergence reactions, known to occur after ketamine anesthesia, would reduce the patient's tendency to regard them as anxiety-provoking.

Our single blind prospective study contained four groups (A, B, C, D), each consisting of 12 young servicemen from our convalescent ward, scheduled for skin grafting or minor orthopedic procedures. Atropine was the only premedicant. Groups A and B received ketamine intravenously whereas Groups C and D received thiopental, followed by nitrous oxide, halothane, oxygen by mask. Every patient, however, was told that he would have ketamine anesthesia. Those in Groups B and D were furnished preoperatively with a detailed description of all emergence phenomena known to occur with ketamine. Groups A and C received a minimal preoperative briefing, mentioning only dreams and altered depth perception as possible emergence reactions.

Twenty-four hours after surgery, each patient was given a sentence completion form and a check list, by a physician unknown to the patient. By means of a coding system, subjective responses were tabulated and the incidence of specific psychic phenomena

determined for each group.

The incidence of perceptual alterations following ketamine was so high in both ketamine groups that a pharmacologic property of the drug can be inferred. The incidences of visual, auditory, proprioceptive distortions, and postoperative confusion were higher in the detailed as opposed to the minimal halothane group, reflecting a possible suggestion effect. Postoperative anxiety was lower in the detailed ketamine group than in the minimal. Patients found ketamine acceptable for future administration unless they were aware of the operative procedure during anesthesia. No patient,however, who received an induction dose of 1.7 mg/kg or more reported awareness during surgery.

Ketamine
Halothane
Awareness during anesthesia
Psychologic responses to anesthesia

---

* Present address: Instructor in Psychiatry, University of Texas Medical School at San Antonio, San Antonio, Texas 78229.

** Present address: Dept. of Anesthesiology, Brooke General Hospital, Brooke Army Medical Center, Fort Sam Houston, Texas 78234.

THE INFLUENCE OF PREOPERATIVE INFORMATION ON PSYCHOLOGIC RESPONSES TO KETAMINE AND HALOTHANE ANESTHESIA IN BURN PATIENTS

Ketamine, recently released for routine clinical use, has been found to induce dissociative states, sensory and perceptual misinterpretations, vivid dreaming, and hallucinations in some patients. Overall estimates of "emergence phenomena" range from 3% to over 30%. The investigators hypothesized that a detailed preoperative description of the emergence reactions known to occur after ketamine anesthesia would reduce the patient's tendency to regard them as unpleasant and anxiety provoking. A comparison of the incidence of perceptual misinterpretations following ketamine and halothane anesthesia was also made.

Methods

The study contained four groups, each consisting of 12 young servicemen from our convalescent burn ward in a single-blind, randomized, two by two, experimental design. No one who had received ketamine previously, or who was receiving major psychotropic agents or sedatives was included in this study. The patients were considered to be ASA Physical Status 2, and were to undergo skin grafting or minor orthopedic procedures. The 48 patients were randomly assigned to Groups A, B, C, or D, with Groups A and B receiving ketamine and Groups C and D receiving halothane anesthesia. Groups A and C received minimal preoperative briefing, while those patients in Groups B and D were furnished with a detailed description of all emergence phenomenon known to occur with ketamine.

Groups A and B received ketamine intravenously as the sole anesthetic agent. Induction dose was that necessary to result in cessation of vertical nystagmus and response to commands, and averaged 1.6 mg/kg, intravenously, over a 90second period. Reinforcing doses of 0.5 to 1.0 mg/kg were given as necessary to maintain this state. Groups C and D received thiopental, 4.5 mg/kg intravenously for the induction of anesthesia, and then halothane, nitrous oxide, and oxygen by mask in a semiclosed system in concentration sufficient to produce Plane I and II anesthetic depth. Atropine, 0.01 mg/kg, given intravenously immediately prior to induction of anesthesia, was the only premedicant, and was administered to all patients in the study.

Following surgery, all study patients were taken to a specially designated recovery area where they were cared for by

members of the nursing staff who were not told which anesthetic the patient had received. However, the staff was told specifically to treat the patients exactly like any other patients recovering from the general anesthetic. No particular efforts were made to reduce verbal or tactile stimulation. Staff members were instructed not to tease or banter with patients who appeared to be confused or incoherent. Patients were returned to the ward when they seemed rational and could take fluids.

Twenty-four hours after surgery, each patient in the study was given a sentence-completion form, and then a check list, by a physician unknown to the patient and not involved with his care. This physician knew neither the hypothesis of the study nor the group to which the patient had been assigned. This blinding on the part of the interviewer prevented bias in the questionnaire and sentence completion data. A coding system permitted grouping of sentence responses from the questionnaires and checklist items into mutually exclusive categories. Objective responses could then be tabulated across groups, and the incidence of specific psychic phenomena determined for each experimental group.

Perceptual alterations were differentiated from hallucinations in consideration of the data. A perceptual alteration was defined as a misperception of objective or true sensory data and a hallucination as a pseudoperceptual experience, i.e., the perception of something that had no basis in objective sensory stimulation. The data were analyzed by means of Chi-square analysis, which permitted comparison between the minimal and detailed groups within each drug, as well as comparisons between the entire ketamine group and the entire halothane group in order to assess overall drug effect.

Results

For ketamine, the incidence of perceptual alterations was uniformly high, except for auditory distortions, which even so had an incidence of 50% or greater. There were no significant differences between the two ketamine groups. For halothane,there was a significantly higher incidence in the detailed halothane group than in the minimal halothane group for auditory distortions and for postoperative confusion. When ketamine, overall, was compared against halothane overall, the difference between the two agents was significant in the case of visual distortions, proprioceptive distortions, and for postoperative confusion. It was interesting to note, in regard to patient awareness during anesthesia, that no patient who received thiopental, nitrous oxide, halothane reported awareness during the operative procedure. In

those patients who received ketamine, however, future preference for that anesthetic was closely correlated with awareness during anesthesia, i.e., those patients who were aware of their surgery while receiving the drug did not want ketamine again. In those patients receiving ketamine who were not aware of their surgery, an almost universal acceptance of ketamine was noted, despite a 33% incidence of dreams which tended to be unpleasant. No patient who received 1.7 mg/kg or more of ketamine as an induction dose reported awareness during surgery. On the other hand, there was no demonstrable relationship between incidence of awareness during ketamine anesthesia and total dose of ketamine, indicating that awareness during ketamine anesthesia seems to be related to induction dose rather than to total dose of the drug.

Conclusions

The following conclusions emerge from this study.

1) The incidence of perceptual alterations following the administration of ketamine was so high in both of the ketamine groups that a pharmacologic property of the drug can be inferred.

2) Perceptual alterations also occurred with the combination of thiopental, nitrous oxide, and halothane, but their incidence was significantly lower than with ketamine. The incidences of auditory distortions and of postoperative confusion were significantly higher in the detailed as opposed to the minimal halothane group. This may be attributable to a suggestion effect.

3) Postoperative anxiety was not significantly decreased in the detailed ketamine group as opposed to the minimal, indicating that our hypothesis in this regard was not upheld. However, there was also no significant difference in postoperative anxiety between ketamine overall and halothane overall in our patients, which indicates that ketamine as a drug is not any more anxiety-provoking than halothane.

4) With one exception, patients found ketamine acceptable for future administration unless they were aware of their surgery during anesthesia. No patient who had received an induction dose of 1.7 mg/kg or more reported awareness during surgery. In those whose induction dose was less, the incidence of awareness was 25%.

5) Great caution must be taken in extrapolating our re-

sults to other patient populations. All of our patients in this study were male servicemen from age 18 to 41. Additional studies to include women, different age ranges, and patients from different occupational socio-economic background should be undertaken to increase our understanding of the psychopharmacological properties of anesthetic agents.

Presentation

Garfield JM: The effects of varying preoperative information on psychologic responses to ketamine and halothane anesthesia. Am Soc of Anesthesiologists annual meeting, New York, N.Y. 17-21 October 1970.

Publications

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0A 6956	71 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
70 07 01	D. CHANGE	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A061102B71R	01	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 71	4.1	16.4
	CURRENT 72	4.1	17.2

19. RESPONSIBLE DOD ORGANIZATION

NAME: US Army Institute of Surgical Research

ADDRESS: Ft Sam Houston, Texas 78234

RESPONSIBLE INDIVIDUAL

NAME: PRUITT, B.A., Jr, LTC, MC

TELEPHONE: 512-221-2720

20. PERFORMING ORGANIZATION

NAME: US Army Institute of Surgical Research
Renal Section

ADDRESS: Ft Sam Houston, Texas 78234

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

NAME: Martin G White, MAJ, MC

TELEPHONE: 512-221-4307

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: Neil A Kurtzman, LTC, MC

NAME: DA

21. GENERAL USE

FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Renal Failure; (U) Hemodialysis; (U) Peritoneal Dialysis

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

23. (U) To care for acute renal failure of varied etiologies and to provide dialysis support for problems concerned with both endogenous and exogenous poisonings. To support clinical research activities, provide measurements of glomerular filtration rate, and to support the Renal Clinic.

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

25. (U) 70 01 70 12 Nine patients were treated for renal insufficiency during the re-porting period. Seven of these patients presented with acute renal failure, and two with chronic renal insufficiency. There were 339 patient days in the period covered by this report. In addition, the Renal Section performed 57 determinations of glomerular filtration rate, nine bicarbonate reabsorptive tests, and one glucose titration test. Metabolic balance studies covered an additional 284 patient days.

*Available to contractors upon originator's approval.

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

ANNUAL PROGRESS REPORT

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

REPORT TITLE: CLINICAL OPERATION, METABOLIC BRANCH - RENAL SECTION

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

1 January - 31 December 1970

Investigators:

Martin G. White, M.D., MAJ, MC
Neil A. Kurtzman, M.D., LTC, MC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

REPORT TITLE: CLINICAL OPERATION, METABOLIC BRANCH - RENAL SECTION

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

Period covered in this report: 1 January - 31 December 1970

Investigators: Martin G. White, M.D., MAJ, MC
Neil A. Kurtzman, M.D., LTC, MC

Reports Control Symbol MEDDH-288(R1)

Nine patients were treated in the Renal Section during the reporting period. This represents a total of 399 patient days. Seven patients presented with acute renal failure, with only one survivor. Two patients with chronic renal insufficiency were treated in the unit during this time. In addition, glomerular filtration rates, bicarbonate reabsorption tests, and glucose titration tests were performed. Patients on metabolic studies represented an additional 284 patient days.

Renal failure
Hemodialysis
Peritoneal dialysis

BLANK PAGE

CLINICAL OPERATION, METABOLIC BRANCH - RENAL SECTION

Nine patients were treated in the Renal Section from 1 January through 31 December 1970. Of the nine patients, seven were admitted with various forms of acute renal failure. Two patients were treated who had chronic renal insufficiency. Of these two patients, one was maintained on twice-weekly hemodialysis throughout the year's reporting period, and the other received four hemodialyses while an inpatient to undergo splenectomy and bilateral nephrectomy. These nine patients represent a total of 132 hemodialyses, covering 399 patient days. Each of the patients underwent hemodialysis, using the Travenol twin-coil No. 145 artificial kidney. Chronic dialyses were performed, using chronically indwelling arterial and venous cannulas, while acute dialysis were in main done using percutaneous insertion of catheters into appropriate arteries and veins. Anticoagulation of the hemodialysis coil was either with regional or total body heparinization, depending upon the individual circumstances. Of the seven patients with acute renal failure, one survived. Two patients with chronic renal failure continued to do well during the year. Table 1 represents the clinical characteristics of the patients treated for renal insufficiency in the Renal Section during 1970.

Patient No. 1 is a 38-year-old male who had been treated on chronic hemodialysis since 1964. His course during this past year included septicemia secondary to shunt infections, and the removal of a meningioma of the spinal cord. The patient tolerated these complications well, and at the conclusion of 1970 continued to do well on chronic, twice-weekly, hemodialysis.

Patient No. 2 is a 20-year-old female with chronic glomerulonephritis, who has been on intermittent hemodialysis since 1967. These hemodialyses had been done mainly in her home with assistance by either her parents or her husband. The patient was admitted to Brooke General Hospital for a bilateral nephrectomy and splenectomy in preparation for a subsequent renal homotransplantation. While in Brooke General Hospital, the patient received four hemodialyses. The patient was discharged and subsequently has done well; however, the transplant has thus far not been performed.

Patient No. 3 was admitted to the Medical Service of Brooke General Hospital with a history of the evolution of jaundice, fever, and abdominal pain. Subsequent workup demonstrated multiple

Table 1. Patients Hemodialyzed at ISR, 1970

No.	Age	Sex	Diagnosis	No. of Hemodialyses	No. of Hospital Days	Outcome
1	38	Male	Glomerulonephritis, chronic	104	365	Survived
2	20	Female	Glomerulonephritis, chronic	4	4	Survived
3	32	Male	Postop acute tubular necrosis	1	1	Survived
4	30	Male	Electrical burn	2	2	Expired
5	21	Male	Burn; acute renal failure	1	1	Expired
6	57	Female	Ingestion of Sani Flush	6	12	Expired
7	76	Male	Surgical trauma; acute renal failure	3	3	Expired
8	29	Male	Burn; acute renal failure	10	10	Expired
9	52	Female	Postop trauma; acute renal failure	10	10	Expired

hepatic abscesses thought to be due to pyelophlebitis secondary to appendicitis. The patient had a very stormy course, subsequently developing infected pulmonary emboli. In addition, the patient's renal function deteriorated, with gradual rise of his BUN to a range of 116-130. The renal insufficiency was thought to be secondary to his septicemia and the effect of nephrotoxic antibiotics. He was hemodialyzed on one occasion, using the percutaneous route. Subsequent to that dialysis, the patient's BUN gradually fell as renal function improved, and no further dialyses were necessary. The patient eventually recovered renal function and the underlying septic process resolved with long-term antibiotic therapy.

Patient No. 4 was admitted to the Institute of Surgical Research after sustaining burns due to contact with high-voltage electricity, with second or third degree involvement of 20% total body surface. The patient sustained extensive muscle damage as a consequence of his injury, and renal failure was evident from the outset of his hospital course. In addition, his course was complicated by thrombocytopenia and hyperkalemia. The renal failure was thought to be due to myoglobinuria resulting from the extensive muscle injury. The patient was hemodialyzed on two occasions, with good control of hyperkalemia and azotemia. The patient died of a cardiac arrest on the fifth postburn day.

Patient No. 5 was a 21-year-old male who was initially injured in the Republic of Vietnam on the 23rd of June 1970, sustaining a second and third degree burn, involving 28% of the total body surface. His early course was complicated by concussion, an episode of septicemia, and persistent diarrhea. Upon transfer to the Institute of Surgical Research, diarrhea continued to be somewhat of a problem, with an attendant poor nutritional intake. The patient developed a Herpes simplex infection on 11 August,with probable systemic involvement and subsequently the patient developed a picture of septicemia without positive blood cultures. Jaundice ensued, and the patient's BUN gradually rose to approximately 140 mg%. The patient's blood pressure fell during this time, and it was thought that his rising BUN was due to a decreased intravascular volume. Attempts at increasing his intake of intravenous fluids, however, did not result in any increase in his urine output, and a single six-hour hemodialysis was done on 24 August 1970. The patient continued to deteriorate, however, and died on 25 August, as a consequence of hypotension thought to be secondary to septicemia.

Patient No. 6 was a 57-year-old woman who was admitted to

the Surgical Service of Brooke General Hospital following the ingestion of an undertermined amount of "Sani-Flush" (hydrochloric acid and oxalic acid). These corrosive agents induced extensive destruction of the stomach and organs in the left upper quadrant of the abdomen. Renal failure occurred, presumably due to the deposition of calcium oxalate in the renal tubules as well as poor perfusion secondary to the hypovolemia induced by the loss of the fluid into the tissues of this patient. Resection of the necrotic tissue was performed on the fifth hospital day. The patient was maintained throughout this period on hemodialysis, being dialyzed six times in 12 days. This program sufficiently controlled azotemia and hyperkalemia. The patient had such extensive tissue damage, however, that gradual destruction of the pancreas, spleen, diaphragms, and pulmonary parenchyma on the left side occurred. As a consequence, she began to bleed massively and expired on the 13th hospital day.

Patient No. 7 was a 76-year-old man who developed an acute occlusion of the distal aorta one day prior to admission. An aortofemoral bypass was performed, and the patient subsequently developed acute renal failure. This was due to a period of hypotension that occurred after his surgery. The patient remained in moderate vascular collapse from the first postoperative day on until he died some three days later, and was dialyzed on each postoperative day in order to control his azotemia, hyperkalemia, and metabolic acidosis. Progressive vascular collapse occurred, and he died in shock on the third postoperative day.

Patient No. 8 was a 29-year-old male who was injured in an airforce refueling accident. He sustained 73% burns of the total body surface, and developed acute renal failure, presumably due to shock from hypovolemia shortly after his injury. The patient was hospitalized at the Institute of Surgical Research. The acute renal failure was first treated by hemodialysis on the third postinjury day. The patient was dialyzed daily for the next 10 days with good control of azotemia, serum potassium levels, and patient's fluid status. However, the patient developed a progressive pneumonia during the last three days of his hospital course and succumbed to progressive pulmonary insufficiency on the 14th postinjury day.

Patient No. 9 was a 52-year-old woman who developed peritonitis and gram negative septicemia two days after a selective superior mesenteric arteriogram was performed as part of the diagnostic workup of a splenic artery aneurysm. The patient developed thrombosis of the celiac axis and inferior mesenteric

artery with massive infarction of the liver, spleen, small bowel, and left kidney. She was hemodialyzed on one occasion because of progressive azotemia, using the percutaneous route. Because of progressive hypotension, presumably due to the septicemia, the patient died the following day.

In addition to the hemodialysis performed by the Renal Section, a seven-month female infant was peritoneally dialyzed for renal failure secondary to acute tubular necrosis from hypotension. This child sustained a 20% burn of the total body surface and developed a period of prolonged hypotension in her early postburn course. Renal failure ensued, and because of her small size and precarious hemodynamic status, peritoneal dialysis was performed as a means of controlling hyperkalemia; 146 exchanges of one liter of peritoneal dialysate were performed. The child, however, remained in moderate vascular collapse, which became progressive toward the end of her course, and the child died on the fifth postburn day with unresponsive hypotension.

The Renal Section evaluated glomerular filtration rates in 57 patients during the reporting period, using Glofil (iothalamate ^{125}I) excretion as an index of renal function. These studies were performed on patients within the Institute of Surgical Research as well as patients in Brooke General Hospital. Nine volunteer subjects were studied for their rate of bicarbonate reabsorption, and the report on these studies will appear in another section of this 1971 annual report. A glucose titration test was performed on one volunteer subject during this time as well. The staff and corpsmen of the Renal Section staffed the Renal Clinic of Brooke General Hospital in conjunction with Brooke General Hospital's nephrologists. This clinic meets weekly and has approximately 10 outpatient visits per clinic day per week. The staff of the Renal Section of the Metabolic Branch served as consultants to Brooke General Hospital on matters of acid base balance, hypertension and other nephrological problems.

Six patients were studied by balance techniques during the reporting period, involving a total of 284 patient days. Table 2 reveals the various balance studies done. Patient 1, a 32-year-old female with Addison's disease secondary to presumed adrenal tuberculosis, was studied in an attempt to demonstrate the renal defect leading to acidosis in Addison's disease. Patient 2, a 28-year-old male who had been previously burned and treated with large doses of parenteral vitamins, which included Vitamin D, was studied for the treatment of hypercalcemia due to hypervitaminosis D with potent diuretics. Patients 3, 4, 5, and 6 were all males

Table 2. Balance Studies Performed at ISR, 1970

Pt. No.	Age	Sex	Diagnosis	Purpose of Balance Study	Days
1	32	F	Addison's disease	Renal defect in Addison's disease	12
2	28	M	Hypercalcemia due to Hypervitaminosis D	Treatment of hypercalcemia with furosemide	51
3	35	M	Obesity, exogenous	Metabolic effect of starvation	40
4	53	M	Obesity, exogenous	Metabolic effect of starvation	61
5	41	M	Obesity, exogenous	Metabolic effect of starvation	80
6	42	M	Obesity, exogenous	Metabolic effect of starvation	40

with marked exogenous obesity who were studied for metabolic effects of starvation diets.

Publications

Asch MJ, White MG, Pruitt BA Jr: Acid base changes associated with topical Sulfamylon therapy: A retrospective study of 100 burn patients. Ann Surg 172: 946-950, 1970.

White MG: Bartter's syndrome: A manifestation of renal tubular defects. Arch Int Med (In press).

Rogers PW, Bunn SN Jr, Kurtzman NA, White MG: Schonlein-Henoch syndrome associated with exposure to cold. Arch Int Med (In press).

Presentations

White MG: Bartter's Syndrome. Univ of Texas Medical School at San Antonio, San Antonio, Texas, 4 March 1970.

White MG: Bartter's Syndrome. VA Hospital, Dallas, Texas, 13 March 1970.

White MG: Bartter's Syndrome. Ohio State Univ Medical School, Columbus, Ohio, 15 April 1970.

White MG: Diagnostic implications of hypokalemia. Santa Rosa Hospital, San Antonio, Texas, 2 September 1970.

White MG: Diagnostic implications of hypokalemia. Brooke General Hospital, Dept Med, BAMC, FSHTex, 10 September 1970.

Spitzer ME: Nutrition in burns. 91C Clinical Technician Course, BGH, BAMC, FSHTex, 19 September 1970.

Spitzer ME: Role of the research dietitian. Dietetic Interns, BGH, BAMC, FSHTex, 2 October 1970

White MG: Hypokalemia. Randolph AFB Hospital, Randolph AFB, Tex, 6 October 1970.

Spitzer ME: Rapid method of food analysis. Georgia Dietetic Assn., Atlanta, Ga, 21 October 1970.

Spitzer ME: Role of the research dietitian. Dietetic Staff,

Santa Rosa Hospital, San Antonio, Tex, 28 October 1970.

White MG: Bartter's Syndrome. US Army Surgeon General's Advisory Committee on the Metabolism of Trauma, FSHTex, 1 December 1970.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0C 6954	71 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
70 07 01	D. CHANGE	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A061102B71R	01	251
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U)Effect of Extracellular Volume on Renal Bicarbonate Reabsorption-A Laboratory Model of Renal Changes Observed in Injured Soldiers (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 Clinical Medicine

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

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

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 71	0.30	8.0
	CURRENT 72	0.30	8.8

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

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

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

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME: Neil A Kurtzman, LTC, MC
TELEPHONE: 512-221-5416
SOCIAL SECURITY ACCOUNT NUMBER

ASSOCIATE INVESTIGATORS
NAME: Martin G White, MAJ, MC
NAME: Philip W Rogers, MAJ,MC DA

21. GENERAL USE: FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Bicarbonate Reabsorption; (U) Sodium; (U) Extracellular Volume; (U) Potassium

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

23. (U) Disorders of acid-base homeostasis are extremely common in injured or ill troops. These disorders are perpetuated, compensated, or corrected by changes in renal bicarbonate reabsorption. This study was undertaken to examine the processes that regulate renal bicarbonate reabsorption.

24. (U) Thus far in this project, the role of effective extracellular volume, acute respiratory acidosis, potassium deficiency, potassium excess and aldosterone deficiency on renal bicarbonate reabsorption have been examined. Work is in progress to examine the role of cylic AMP parathormone acetyl-cholene, oxytocin, and prostaglandins on renal bicarbonate reabsorption.

25. (U) 70 07 - 71 06 - Thus far we have found that changes in extracellular volume markedly effect the response of renal bicarbonate reabsorption to the other variables mentioned above but when extracellular volume is controlled, acute respiratory acidosis, and potassium deficiency increase bicarbonate reabsorption, while potassium excess depresses bicarbonate reabsorption. Sufficient data concerning the effect of the agents listed above on bicarbonate reabsorption is not yet at hand to make any definitive statement.

*Available to contractors upon originator's approval

ANNUAL PROGRESS REPORT

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

REPORT TITLE: EFFECT OF EXTRACELLULAR VOLUME ON RENAL BICARBONATE REABSORPTION

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

1 July 1970 - 30 June 1971

Investigators:

Neil A. Kurtzman, M.D., LTC, MC
Martin G. White, M.D., MAJ, MC
Philip W. Rogers, M.D., MAJ, MC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

REPORT TITLE: EFFECT OF EXTRACELLULAR VOLUME ON RENAL BICARBONATE REABSORPTION

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

Period covered in this report: 1 July 1970 - 30 June 1971

Investigators: Neil A. Kurtzman, M.D., LTC, MC
Martin G. White, M.D., MAJ, MC
Philip W. Rogers, M.D., MAJ, MC

Reports Control Symbol MEDDH-288(R1)

We have previously shown that effective extracellular volume is such a potent regulator of renal bicarbonate reabsorption that the other principle regulators cannot be studied effectively if one does not precisely specify the level of extracellular volume at which one is working. By thus controlling volume we have defined the regulatory role of potassium, CO_2 tension, and carbonic anhydrase activity on bicarbonate reabsorption.

The emphasis of work currently being carried out is to define the mechanism by which volume expansion depresses bicarbonate reabsorption. The role of a variety of naturally occurring humoral agents on bicarbonate reabsorption is to be assessed.

Bicarbonate reabsorption
Sodium
Extracellular volume
Potassium

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0C 6399	71 07 01	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8A. DISP'N INSTR'N	8B. SPECIFIC DATA - CONTRACTOR ACCESS	9. LEVEL OF SUM
70 07 01	K.COMPLETION	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A061102B71R	01	246
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U)Volume Control of Bicarbonate Excretion in Human Subjects - A Clinical Analogue of Changes in Injured Patients (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
68 09	71 05	DA	C. In-House

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

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
FISCAL YEAR — PREVIOUS: 71	0.30	8.0
FISCAL YEAR — CURRENT: 72	0	0

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

20. PERFORMING ORGANIZATION
NAME: US Army Institute of Surgical Research
Renal Section
ADDRESS: Ft Sam Houston, Texas 78234
PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME: Martin G. White, MAJ, MC
TELEPHONE: 512-221-5703
SOCIAL SECURITY ACCOUNT NUMBER
ASSOCIATE INVESTIGATORS
NAME: Neil A Kurtzman, LTC, MC
NAME: DA

21. GENERAL USE: FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code): (U) Human Volunteer; (U) Bicarbonate excretion; (U) Volume expansion; (U)Volume contraction

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

23. (U) The purpose of this study is to see if the reabsorptive maximum for bicarbonate can be influenced by changes in vascular volume. Elucidation of the effects of acute vascular volume change will materially aid in improving resuscitation of the severely injured patient.

24. (U) The approach to this problem has been utilizing volunteer human subjects and infusing them with bicarbonate in order to determine the reabsorptive maximu. Some of these subjects have been maintained on a normal sodium-chloride intake prior to study, while some of them have been depleted by salt restriction.

25. (U) 70 06 - 71 05 - Seven volunteer subjects have been studied, and it appears that an inability to facilitate adequate intravascular volume expansion and contraction in the human subjects precludes the precise definition of the role of intravascular volume and bicarbonate reabsorption in this setting. This study has been completed.

*Available to contractors upon originator's approval

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

FINAL REPORT

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

REPORT TITLE: STUDY OF THE EFFECT OF VOLUME EXPANSION AND VOLUME CONTRACTION ON BICARBONATE EXCRETION IN HUMAN SUBJECTS. A CLINICAL ANALOGUE OF CHANGES IN INJURED PATIENTS

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

1 July 1970 - 30 June 1971

Investigators:

Martin G. White, M.D., MAJ, MC
Neil A. Kurtzman, M.D., LTC, MC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

REPORT TITLE: STUDY OF THE EFFECT OF VOLUME EXPANSION AND VOLUME CONTRACTION ON BICARBONATE EXCRETION IN HUMAN SUBJECTS. A CLINICAL ANALOGUE OF CHANGES IN INJURED PATIENTS

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

Period covered in this report: 1 July 1970 - 30 June 1971

Investigators: Martin G. White, M.D., MAJ, MC
Neil A. Kurtzman, M.D., LTC, MC

Reports Control Symbol MEDDH-288(R1)

The control of bicarbonate reabsorption in the human has been attributed to: (1) pCO_2 tension, (2) total body potassium stores, and (3) carbonic anhydrase activity. Recently, the role of effective arterial blood volume has been described as a major regulator of bicarbonate reabsorption in the dog. Studies in human volunteers prepared on high and low salt diets, with bicarbonate reabsorptive rates determined by standard techniques have been performed. Seven volunteer subjects have been studied, and it appears that an inability to facilitate adequate intravascular volume expansion and contraction in the human subjects precludes the precise definition of the role of intravascular volume and bicarbonate reabsorption in this setting.

Volume Expansion
Volume Contraction
Human Volunteer
Bicarbonate Excretion

STUDY OF THE EFFECT OF VOLUME EXPANSION AND VOLUME CONTRACTION ON BICARBONATE EXCRETION IN HUMAN SUBJECTS. A CLINICAL ANALOGUE OF CHANGES IN INJURED PATIENTS

The rate of renal bicarbonate reabsorption is controlled by total body potassium stores, carbonic anhydrase activity, pCO_2 tension, and the effective arterial blood volume. Studies from this laboratory have identified the effective arterial volume as a major regulator of bicarbonate reabsorption, presumably as a result of control of proximal tubular reabsorption. A recent report has suggested that the extracellular fluid volume in addition has an effect on bicarbonate reabsorption in man as well as the dog.[2] In these studies, the bicarbonate reabsorption rate in normal human subjects was studied before and after volume contraction, using dietary maneuvers. A small change in bicarbonate reabsorption was demonstrated by these techniques, so that volume expansion tended to decrease bicarbonate reabsorption and volume contraction tended to increase bicarbonate reabsorption.

Volunteer convalescent burn patients have been used as subjects in this study. They have been maintained on the following diets prior to study: (1) regular hospital diet with 35-150 mEq sodium intake; (2) salt-restricted diets, with 13 mEq of sodium; (3) high-salt diet containing approximately 500 mEq of sodium. At the time the bicarbonate reabsorptive studies were done, further attempts were made to decrease effective arterial volume by having the subject stand in the upright position with a partial occlusive wrap around the thighs to impede venous return or to expand the circulation by elevating and wrapping the legs from the feet to the thigh in order to decrease the volume of blood in the extremities, or by the rapid infusion of 33 ml/min of lactated Ringer's solution for 60-90 minutes. Standard clearance techniques were used to evaluate bicarbonate reabsorption. A total of seven subjects have been studied and the results are shown in the table.

These selected clearance periods on the seven subjects studied seemed to demonstrate a small but significant effect of changes in extracellular fluid volume on bicarbonate reabsorption in six of the seven subjects studied. These changes, however, are so small that it is difficult to be certain of their significance. The degree of change seen in these subjects, however, is no different than reported by Slatopolsky and his associates in a prior publication.[2] It appears that as chloride excretion

BICARBONATE REABSORPTION IN CONVALESCENT, HEALED BURN PATIENTS

	Patient	GFR ml/min	V ml/min	$U_{HCO_3}V$ mEq/min	Reabsorb. HCO_3 mEq/100 ml GFR	pCO_2 mmHg	HCO_3^- mEq/L	Na mEq/L	K mEq/L	C_{Cl} GFR x 100 (%)
1	→	162	22.8	1099	2.52	37	32.0	144.5	2.8	1.1
	↑ Occlusion	162	6.4	1055	3.18	44	38.0	141	2.8	0.4
2	→	112	12.2	616	2.84	44	33.9	142	3.2	0.1
	_/	128	13.9	924	2.34	38	30.6	142	3.1	0.14
	↑ Occlusion	116	4.3	817	2.90	38	36.0	146	2.8	0.06
3	→	101	16.9	659	3.25	44	39.0	140	2.9	0.9
	_/	106	21.8	872	3.08	44	39.0	143	3.0	2.4
	Infusion	108	13.6	1333	2.99	45	42.2	145	3.1	4.2
4	_/	156	33.2	2457	2.79	41	43.7	144	2.8	3.1
	+ Infusion	162	40.8	2489	2.58	41	41.2	149	2.6	5.4
5	Low salt	104	11.2	466	3.01	40	34.6	146	3.0	0.1
	High salt	116	16.1	369	2.86	43	31.8	139	3.3	1.1
	+ Infusion	132	17.6	998	2.83	44	36.0	142	3.1	0.6
6	Low salt	112	4.9	142	3.03	46	31.6	135	3.4	0.3
	Low salt	98	3.9	777	3.06	49	38.7	146	2.4	0.3
	High salt	135	21.2	488	2.80	46	31.6	138	3.3	2.3
	High salt	139	33.2	1560	2.93	49	40.5	141	3.0	3.3
7	→ Low salt	129	15.1	396	2.70	40	30.1	138	2.9	0.1
	↑ Occlusion	125	12.5	575	2.85	40	33.1	137	2.6	0

→ Supine

↑ Occlusion - Upright with partial occlusion of leg veins

_/ Legs wrapped, elevated, and relatively bloodless

Low salt diet = 13 mEq Na diet + 40 mg Lasix/day before study

High salt diet = 500 mEq Na diet

increases (chloride excretion being a marker of effective volume expansion), that bicarbonate reabsorption tends to decrease, and as fractional chloride excretion decreases, bicarbonate reabsorption tends to increase.

These studies suggest that in man bicarbonate reabsorption is mediated in part by changes in effective arterial blood volume. However, the changes seen in these studies are of such small degree that the conclusion cannot be definitely reached. The primary reason for the small changes seen in these subjects is the great difficulty in adequately volume expanding and volume contracting normal subjects. This only occurs effectively in disease states when the pathophysiologic situation is so abnormal that a major effect has been created on the effective arterial blood volume. It is not feasible to induce such major pathophysiologic abnormalities in these volunteer subjects; hence it will remain very difficult to satisfactorily document the effect of changes in the effective arterial volume in normal subjects.

References

1. Kurtzman NA: Regulation of renal bicarbonate reabsorption by extracellular volume. J Clin Invest 49:586, 1970.

2. Slatopolsky E, Hoffsten P, Purkerson M, Bricker NS: On the influence of extracellular fluid volume expansion and of uremia on bicarbonate reabsorption in man. J. Clin Invest 49:988-998, 1970.

Publications and/or Presentations

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6973	71 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
70 07 01	D.CHANGE	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A061102B71R	01	270
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U)Effect of Chloride and Extracellular Volume on Correction of Metabolic Alkalosis - A Common Problem in the Injured Troop (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
69 07	Cont	DA	C. In-House

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

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
FISCAL YEAR — PRECEDING 71	0.30	8.0
FISCAL YEAR — CURRENT 72	0.30	8.8

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

20. PERFORMING ORGANIZATION
NAME: US Army Institute of Surgical Research
Metabolic Branch
ADDRESS:
PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME: Neil A Kurtzman, LTC, MC
TELEPHONE: 512-221-5416
SOCIAL SECURITY ACCOUNT NUMBER:
ASSOCIATE INVESTIGATORS
NAME: Martin G. White, MAJ, MC
NAME: Philip W Rogers, MAJ, MC DA

21. GENERAL USE

22. KEYWORDS (Precede EACH with Security Classification Code): (U) Aldosterone; (U) Chloride; (U) Potassium; (U) Extracellular volume; (U) Metabolic alkalosis

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

23. (U) Metabolic alkalosis is a common acid-base disturbance seen in injred or severely ill troops. This study was designed to fully examine the pathophysiology of this disorder

24. (U) This disturbance of acid-base hemeostasis has been examined by studying the development and correction of hypokalemic metabolic alkalosis and contraction alkalosis in dogs. The effect on acid-base balance of selective aldosterone deficiency was also studied. Since it soon became apparent that aldosterone played a key role in this disorder, its effect on Na transport in the entire nephron was examined.

25. (U) 70 07 - 71 06- Results obtained thus far demonstrate that metabolic alkalosis cannot be generated by the kidney in the absence of hyperaldosteronism. Metabolic alkalosis can be maintained by the kidney in the absence of hyperaldosteronism provided a stimulus to proximal reabsorption exists. Potassium deficiency can resulc in metabolic alkalosis provided hyperaldosteronism exists. No effect of aldosterone on sodium transport at any site of the nephron save the distal exchange site has been demonstrated.

*Available to contractors upon originator's approval

DD FORM 1 MAR 66 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65 AND 1498-1 1 MAR 66 (FOR ARMY USE) ARE OBSOLETE

ANNUAL PROGRESS REPORT

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

REPORT TITLE: EFFECT OF CHLORIDE AND EXTRACELLULAR VOLUME ON CORRECTION OF METABOLIC ALKALOSIS

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

1 July 1970 - 30 June 1971

Investigators:

Neil A. Kurtzman, M.D., LTC, MC
Martin G. White, M.D., MAJ, MC
Philip W. Rogers, M.D., MAJ, MC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

REPORT TITLE: EFFECT OF CHLORIDE AND EXTRACELLULAR VOLUME ON CORRECTION OF METABOLIC ALKALOSIS

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

Period covered in this report: 1 July 1970 - 30 June 1971

Investigators: Neil A. Kurtzman, M.D., LTC, MC
Martin G. White, M.D., MAJ, MC
Philip W. Rogers, M.D., MAJ, MC

Reports Control Symbol MEDDH-288(R1)

Metabolic alkalosis is a common acid-base disturbance seen in injured or severely ill troops. This study was designed to fully examine the pathophysiology of this disorder. The development and correction of hypokalemic metabolic alkalosis and contraction alkalosis has been studied in dogs. The effect on acid-base balance of selective aldosterone deficiency has been also studied. Since it soon became apparent that aldosterone played a key role in this disorder, its effect on Na transport along the entire nephron has been examined. Metabolic alkalosis can not be generated by the kidney in the absence of hyperaldosteronism. Metabolic alkalosis can be maintained by the kidney, however, provided a stimulus to proximal reabsorption exists. Potassium deficiency can result in metabolic alkalosis provided hyperaldosteronism exists. No effect of aldosterone on sodium transport at any site of the nephron save the distal exchange site has been demonstrated.

Aldosterone
Chloride
Potassium
Extracellular Volume
Metabolic Alkalosis

EFFECT OF CHLORIDE AND EXTRACELLULAR VOLUME ON CORRECTION OF METABOLIC ALKALOSIS

This project has continued to explore the relationship of aldosterone, potassium, and extracellular volume to the genesis and maintenance of metabolic alkalosis.

We have now demonstrated that hypokalemic metabolic alkalosis requires both potassium deficiency and mineralocorticoid excess to be generated. New bicarbonate is generated almost entirely in the distal nephron. Once metabolic alkalosis has been generated, however, accelerated distal hydrogen for sodium exchange is not necessary to maintain it provided there is a stimulus to proximal reabsorption. Such a stimulus exists in contraction alkalosis where we have found that metabolic alkalosis once generated (in the presence of increased amounts of mineralocorticoid) may be maintained without excess mineralocorticoid. The model of hypokalemic metabolic alkalosis we use (DOCA, NaCl, and $NaHCO_3$ administration) does not have a strong stimulus to proximal reabsorption because the stimulating effect of potassium deficiency is counter-balanced by the inhibitory effect of volume expansion. Thus, this type of metabolic alkalosis requires enhanced distal hydrogen secretion to be maintained as well as to be generated. It may be corrected either by replacing the deficit of potassium or by removing excess mineralocorticoid hormone.

The role of aldosterone on sodium transport in the proximal tubule and the ascending limb of the loop of Henle has been examined by measuring bicarbonate reabsorption, steady state sodium excretion, and free water clearance and reabsorption during aldosterone deficiency. We have not been able to identify any net effect of aldosterone on sodium transport in any segment of the nephron save the distal exchange site.

Publications

Kurtzman NA, White MG, Rogers PW : The effect of aldosterone deficiency on the renal reabsorption of Na^+, Cl^-, and HCO_3^-. Clin Res 18: 507, 1970.

White MG, Kurtzman NA, Rogers PW: Aldosterone deficiency and renal bicarbonate reabsorption. Proc Amer Soc Nephrol 4:86, 1970.

Kurtzman NA, White MG, Rogers PW : Relationship of potassium deficiency, salt intake, and mineralocorticoid activity to metabolic alkalosis. Proc Amer Soc Nephrol 4:44, 1970.

Kurtzman NA, White MG, Rogers PW: Pathogenesis of hypokalemic metabolic alkalosis. Clin Res 19: 57, 1971.

Kurtzman NA, White MG, Rogers PW: Studies on the pathogenesis of metabolic alkalosis.Clin Res 19: 537, 1971.

Kurtzman NA, White MG, Rogers PW: Aldosterone deficiency and renal bicarbonate reabsorption. J Lab Clin Med 77:931, 1971.

Presentations

Kurtzman NA: Pathogenesis of metabolic alkalosis. Presented at Southern Salt, Water and Kidney Club meeting, Sarasota, Fla. October 1970.

Kurtzman NA: Pathogenesis of metabolic alkalosis. Presented at Univ of Maryland College of Medicine Grand Rounds, Baltimore, Md., November 1970.

Kurtzman NA: Pathogenesis of metabolic alkalosis. Presented at meeting of Amer Fed for Clin Res , New Orleans, La. January 1971.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA OC 6965	71 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
70 07 01	K. COMPLETION	U	U	NA	NL	☑ YES ☐ NO	A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A061102B71R	01	262
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)* (U) Effect of Exogenous Alkali on Body Weights, Chloride and Net Acid Balance in Military Patients with Chronic Renal Insufficiency (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*
003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
68 09	71 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 71	.30	8.0
FISCAL YEAR CURRENT 72	0	0

19. RESPONSIBLE DOD ORGANIZATION
NAME:* US Army Institute of Surgical Research
ADDRESS:* Ft Sam Houston, Texas 78234
RESPONSIBLE INDIVIDUAL
NAME: PRUITT, B.A., Jr, LTC, MC
TELEPHONE: 512-221-2720

20. PERFORMING ORGANIZATION
NAME:* US Army Institute of Surgical Research Clinical Division
ADDRESS:* Ft Sam Houston, Texas 78234
PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME:* Martin G White, MAJ, MC
TELEPHONE: 512-221-5703
SOCIAL SECURITY ACCOUNT NUMBER:
ASSOCIATE INVESTIGATORS
NAME: Neil A Kurtzman, LTC, MC
NAME: DA

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Sodium Balance; (U) Chronic Renal Insufficiency

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 the correction of metabolic acidosis associated with chronic renal insufficiency, by administration of exogenous alkali, results in expansion of the circulation. The relationship of sodium to intravascular volume is important in maintenance of renal function and in resuscitation therapy.

24. (U) This problem is being assessed by maintaining patients with chronic renal insufficiency, on a constant sodium chloride intake, and then for a given period of time administering exogenous alkali. Assessment of the retention of alkali and the net sodium balance will be by analysis of the urine sodium excretion, the patient's serum sodium, and changes in the patient's body weight.

25. (U) 70 07 - 71 05 One patient with chronic renal insufficiency has been studied. No other suitable patients have been available for study during this past year, and because of the lack of suitable patient material, this study is being terminated.

*Available to contractors upon originator's approval.

DD FORM 1 MAR 66 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A, 1 NOV 65 AND 1498-1, 1 MAR 65 (FOR ARMY USE) ARE OBSOLETE.

FINAL REPORT

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

REPORT TITLE: EFFECT OF EXOGENOUS ALKALI ON BODY WEIGHTS, CHLORIDE, AND NET ACID BALANCE IN PATIENTS WITH CHRONIC RENAL INSUFFICIENCY

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

1 July 1970 - 30 June 1971

Investigators:

Martin G. White, M.D., MAJ, MC
Neil A. Kurtzman, M.D., LTC, MC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

REPORT TITLE: EFFECT OF EXOGENOUS ALKALI ON BODY WEIGHTS, CHLORIDE, AND NET ACID BALANCE IN PATIENTS WITH CHRONIC RENAL INSUFFICIENCY

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

Period covered in this report: 1 July 1970 to 30 June 1971

Investigators: Martin G. White, M.D., MAJ, MC
Neil A. Kurtzman, M.D., LTC, MC

Reports Control Symbol MEDDH-288(R1)

Treatment of metabolic acidosis which accompanies chronic renal insufficiency by the administration of alkali has been regarded as hazardous. The criticism raised against the correction of metabolic acidosis by the administration of alkali is that sodium bicarbonate retention occurs, resulting in progressive extracellular volume expansion, leading to a venous congestive state. We acknowledge that this sequence of events is likely to occur when congestive heart failure, severe hypoalbuminemia, or liver disease with ascites coexist with chronic renal failure. Such a complication seems unlikely in the edema-free patient with chronic renal insufficiency. The capacity of the diseased kidney to excrete chloride and retain bicarbonate when the acidotic state is corrected by the administration of exogenous bicarbonate loads has not been explored in the chronic steady state situation.

It seems entirely possible that with the administration of alkali, and the correction of the metabolic acidosis, a retention of small to modest amounts of bicarbonate occurs. The kidney will excrete whatever amounts of bicarbonate is in

excess of what is necessary to correct the serum bicarbonate, leading to a bicarbonate diuresis, and no further bicarbonate retention will occur. Whereas, if sodium chloride were administered in the same situation, the degree of sodium chloride retention may be considerably greater than when the equivalent amount of sodium bicarbonate is administered. The reason for this is that sodium chloride will not be excreted in excess of the maximum reabsorptive rate as will bicarbonate, but rather a certain per cent of the filtered sodium chloride will be reabsorbed, depending upon the load administered and the state of the vascular volume. Unfortunately, suitable subjects for this study have not been available during this past reporting year. Because of the lack of availability of suitable patient material, this study is being terminated without any definite conclusions having been reached.

Sodium Balance
Chronic Renal Insufficiency

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0D 6965	71 07 01	DD-DR&E(AR)636

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

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A061102B71R	01	160
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) Relationship of Extracellular Volume and Urate Metabolism in Starvation Therapy of Grossly Overweight Troops (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS:
003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
70 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 71	.25	6.6
	CURRENT 72	.25	7.3

19. RESPONSIBLE DOD ORGANIZATION
NAME: US Army Institute of Surgical Research
ADDRESS: Ft Sam Houston, Texas 78234
RESPONSIBLE INDIVIDUAL
NAME: PRUITT, B.A., Jr, LTC, MC
TELEPHONE: 512-221-2720

20. PERFORMING ORGANIZATION
NAME: US Army Institute of Surgical Research
Metabolic Branch
ADDRESS: Ft Sam Houston, Texas 78234
PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME: Philip W Rogers, MAJ, MC
TELEPHONE: 512-221-5416
SOCIAL SECURITY ACCOUNT NUMBER:
ASSOCIATE INVESTIGATORS
NAME: Neil A Kurtzman, LTC, MC
NAME: Hershel Harter, MAJ, MC DA

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Uric Acid Study

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 role, if any, of extracellular volume on uric acid metabolism in starvation. Present explanations suggest that hyperuricemia associated with starvation may be secondary to impaired secretion by renal tubules due to effect of increased levels of the various ketones in the blood (B-hydroxylatyric acid, acetoacoticaid, ketones).

24. (U) Obese patients, on active duty, who are otherwise healthy are hospitalized and placed on a starvation diet until hyperuricemia develops; then given 100 Meq sodium diet for four weeks; then given a 100 gm carbohydrate diet. These three dietary blocks are given in random order with various subjects studied. The patient's weight serum and uric acid, renal function, electrolytes, ketonemia and ketonuria are monitored daily.

25. (U) 70 05 - 71 06 From initial studies, it appears that the hyperuricemia associated with starvation can be controlled by expansion of the extracellular volume. Further studies have not been accomplished because of the lack of suitable obese active duty servicemen who are willing to submit to such a study. Also measurement of B-hydroxybutryic acid (presently not available at our laboratory) is needed to make the data more meaningful.

*Available to contractors upon originator's approval.

ANNUAL PROGRESS REPORT

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

REPORT TITLE: RELATIONSHIP OF EXTRACELLULAR VOLUME AND URATE METABOLISM IN STARVATION THERAPY OF GROSSLY OVERWEIGHT TROOPS

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

1 July 1970 - 30 June 1971

Investigators:

Philip W. Rogers, M.D., MAJ, MC
Herschel R.Harter, M.D., MAJ, MC*
Neil A. Kurtzman, M.D., LTC, MC

* From the Department of Hospital Clinics, Brooke General Hospital, Brooke Army Medical Center, Fort Sam Houston, Texas 78234

Report Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT 3A061102B71R-01, RESEARCH IN BIOMEDICAL SCIENCES

REPORT TITLE: RELATIONSHIP OF EXTRACELLULAR VOLUME AND URATE METABOLISM IN STARVATION THERAPY OF GROSSLY OVERWEIGHT TROOPS

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

Period covered in this report: 1 July 1970 - 30 June 1971

Investigators: Philip W. Rogers, M.D., MAJ, MC
Herschel R.Harter, M.D., MAJ, MC *
Neil A. Kurtzman, M.D., LTC, MC

Reports Control Symbol MEDDH-288(R1)

Previous studies have clearly demonstrated an elevation in the serum uric acid during the starvation of obese individuals. Explanations for this phenomenon are varied. One of the previously unavoidable side effects of starvation was orthostatic hypotension, an indirect indication of reduced extracellular volume.

This study was designed to determine the effect of extracellular volume on uric acid metabolism. Three healthy, obese patients have been studied to date. From data obtained on these individuals, it would appear that the hyperuricemia associated with starvation can be controlled by expansion of the extracellular volume.

Obesity
Hyperuricemia
Starvation

---

* From the Dept. of Hospital Clinics, Brooke Gen. Hosp., Brooke Army Medical Center, Fort Sam Houston, Texas 78234

RELATIONSHIP OF EXTRACELLULAR VOLUME AND URATE METABOLISM IN STARVATION THERAPY OF GROSSLY OVERWEIGHT TROOPS

Studies by various investigators in the past have demonstrated a marked elevation in serum uric acid during starvation in obese patients. Past explanations for this phenomenon have included: (1) increased serum ketoacids; (2) decreased glomerular filtration rate, (3) altered enzymatic mechanisms of renal tubular cells, (4) altered serum lactate/pyruvate ratio, (5) decreased serum glucose and amino acid levels. These mechanisms do not fully explain the marked rise in serum uric acid levels noted. It is apparent that prolonged starvation is associated with orthostatic hypotension, an indirect indication of the reduction of extracellular volume. The purpose of this study is to determine the role of volume expansion on the uric acid clearance during starvation in healthy, obese, active duty servicemen.

Materials and Methods

All patients were placed on a regular hospital diet without added medications during the first three days of the study. During this time, baseline studies were obtained to include: CBC, electrolytes, serum uric acid, glucose, A/G ratio, calcium, phosphate, cholesterol, triglycerides, lipoprotein electrophoresis, creatinine clearance, 24-hour urine for calcium, phosphate, sodium potassium, uric acid. Other baseline studies include: PBI, T4, serum lactate and pyruvate, serum and urine osmolality, serum and urine ketones and arterial pH, pCO_2 and pO_2.

After three days of normal caloric intake, the patients were begun on a zero calorie diet with supplemental multivitamins and restricted salt intake. On day 3, and three times weekly thereafter, serum uric acid, electrolytes, glucose, and creatinine were obtained. Twenty-four hour urines for uric acid, creatinine, sodium and potassium and spot checks for urinary ketones were obtained on the same days. The hematocrit, serum and urine osmolality, serum pyruvate and lactate, ketones, calcium and phosphorus were obtained weekly.

The A/G ratio, cholesterol, triglycerides, and lipoprotein electrophoresis will be obtained every two weeks. Arterial pH, pCO_2 and pO_2 were obtained as indicated.

The zero calorie, salt restricted (10 mEq Na) diet was continued until all modalities stabilized except weight loss; 100 mEq Na was then added to the diet and the above laboratory procedures repeated as indicated. This salt intake was continued until all modalities again stabilized.

The starvation diet was continued on each patient until the desired weight reduction had been obtained. During the period of the study, water was not restricted. Activity about the ward was encouraged. Vital signs were obtained twice daily during the period of observation to include supine and erect blood pressure determinations.

Results and Comments

Three subjects have been studied. The data on one subject is inconclusive because of lack of cooperation in following the diet. The other two subjects had mean control serum uric acid levels of 6.0 mg%, mean serum uric acid of 12 mg% during starvation, 8 mg% during volume expansion with sodium chloride, and 6.0 mg% while receiving 100 g carbohydrate daily.

Although more subjects need to be studied, the present data suggest that expansion of the extracellular volume controls the hyperuricemia associated with starvation.

Publications and/or Presentations

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0D 6384	71 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
70 07 01	D. CHANGE	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3A061102B71R	01	312
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) Identification of Presence of Stress in Nursing Personnel Caring for Critically Ill Military Patients (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
70 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 71	.37	9.8
	CURRENT 72	.37	10.8

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

RESPONSIBLE INDIVIDUAL
NAME: PRUITT, B.A., Jr, LTC, MC
TELEPHONE: 512-221-2720

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

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic institution)
NAME: Lois A Johns, LTC, ANC
TELEPHONE: 512-221-5712
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME:
NAME:

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Stress; (U) Intensive care; (U) Nursing; (U) Social psychology

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

23. (U) To determine if prolonged exposure to critically ill individuals, such as patients with burns, in an intensive care situation leads to signs of increased stress in nursing personnel.

24. (U) The approach to this study will be to test each subject within the first 48 hours of his assignment to a work area in Brooke General Hospital or to Ward 14A. Each subject will be retested at the end of three months or six months.

25. (U) 70 07 - 71 06 Forty-five subjects, 16 Army Nurse Corps officers and 29 Clinical Specialists were tested during the reporting period. Seven Army Nurse Corps Officers and 15 Clinical Specialists were assigned to Ward 14A of the Institute of Surgical Research. The remaining subjects were assigned to Brooke General Hospital.

*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 66 AND 1498-1, 1 MAR 68 (FOR ARMY USE) ARE OBSOLETE

ANNUAL PROGRESS REPORT

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

REPORT TITLE: IDENTIFICATION OF PRESENCE OF STRESS IN NURSING PERSONNEL CARING FOR CRITICALLY ILL PATIENTS

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

1 July 1970 - 30 June 1971

Investigator:

Lois A. Johns, LTC, ANC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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

REPORT TITLE: IDENTIFICATION OF PRESENCE OF STRESS IN NURSING PERSONNEL CARING FOR CRITICALLY ILL PATIENTS

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

Period covered in this report: 1 July 1970 - 30 June 1971

Investigator: Lois A. Johns, LTC, ANC

Reports Control Symbol MEDDH-288(R1)

This study is designed to determine if prolonged exposure to critically ill individuals in an intensive care situation, such as patients with burns, leads to signs of increased stress in nursing personnel. Forty-five of the required 60 subjects have completed testing during the reporting period. Nine Army Nurse Corps officers and 14 Clinical Specialists (91C) were assigned to Brooke General Hospital. Seven Army Nurse Corps officers and 15 Clinical Specialists (91C) were assigned to Ward 14A of the Institute of Surgical Research.

Stress
Intensive care
Nursing
Social psychology

IDENTIFICATION OF PRESENCE OF STRESS IN NURSING PERSONNEL CARING FOR CRITICALLY ILL PATIENTS

A study is being carried out to determine if prolonged exposure to critically ill individuals in an intensive care situation, such as caring for burned patients, leads to signs of increased stress in nursing personnel.

Numerous studies have been done to measure physiological and psychological indicators of stress. Articles in nursing and medical journals have expressed concern about stress placed on nursing personnel. Publications concerning stress and nursing personnel have been philosophical and rarely refer to objective studies. Vreeland and Ellis have made observation of nursing personnel in an intensive care unit but did not carry out a planned study. Cleland,in a field study, showed that a little stress improved nursing performance, but mounting stress made performance drop dangerously.

Stress is some physiological, chemical, or emotional factor to which an individual fails to make a satisfactory adjustment which causes physiologic tensions. In this study, stress is defined as the work experiences of each subject from the time he is first tested until the time of his second testing.

Recommendations have been made that personnel working in intensive care units be relieved from this work area periodically. However, to develop nursing proficiency in a specialty care area, experience is necessary. Time and exposure are essential to acquire experience.

If indicators of stress in an intensive care area can be measured, steps may then be taken to decrease or to divert some of the causes of this stress. Hopefully, this would lead to improved personnel performance and improved nursing care. Guidelines for assignment could lead to an increased performance of nursing personnel and aid in the development of needed educational and staffing programs.

Two groups of 30 subjects each are to be used as the study sample. Each group will be divided into two sections of 15 subjects. Each section of 15 subjects will be comprised of five Army Nurse Corps (ANC) officers and 10 Clinical Specialists (91C). Subjects assigned to Ward 14A of the Institute of Surgical Research (ISR) will be the experimental group. The

control group will be .s assigned to other than cardiac care or inten: .ts in Brooke General Hospital (BGH).

Each subject will be tested immediately after assignment to a work area in BGH or to Ward 14A, ISR. He will be given the Mood Adjective Check List (MACL) and the group will be retested in six months. Analysis of Variance techniques will be used to test the subjects' scores.

It has been predicted that individuals assigned to the Intensive Care area of the ISR will demonstrate a significantly higher level of stress than will individuals assigned to the general care wards of BGH. Lack of significance will lead to an evaluation of unobtrusive measures such as number of times on sick call, days on quarters, and unplanned weight loss. Factor analysis of the eight factors in the MACL is planned and will be done by use of computer technique.

Summary

Forty-five of the required 60 subjects have completed testing in this study to determine if prolonged exposure to critically ill individuals in an intensive care situation leads to signs of increased stress in nursing personnel. After all test data have been compiled, they will be subjected to appropriate statistical tests. Although indications are that stress increases in intensive care units, data in the present study are incomplete and results are not measurable at this time. The intermittent pattern of assignment of new personnel into the two clinical situations will continue to affect the testing program.

References

Aasterud M: Defences against anxiety in the nurse-patient relationship. Nursing Forum 1(3): 35-59, 1962.

Cleland VS: The effects of stress on performance. Nursing Res. 14: 292-298, 1965.

Cleland VS: Effects of stress on thinking. Amer J Nursing 67: 108-111, 1967.

Gordan JED: Nursing stresses in the intensive care unit. J Amer Med Assn. 208: 2337-2338 (June 23), 1969.

Holsclaw PA: Nursing in high emotional risk areas. Nursing Forum 4: 36-45, 1965.

Nowlis V., Green RF: Factor analytic studies of the Mood Adjective Check List, Technical Report No. 11, 668 (12), Research Project NR 171-342.

Vreeland R, Ellis GL: Stresses on the nurse in an intensive care unit. J Amer Med Assn. 208: 332-334 (Apr 14), 1969.

Publications

None

Presentation

Johns LA: Identification of the presence of stress in nursing personnel caring for critically ill patients. Presented at 4th Annual Res Conf, Western Commission for Higher Education for Nurses, Las Vegas, Nevada, 28-30 April 1971.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0C 6972	71 07 01	DD-DR&E(AR)636

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8a. DISP'N INSTR'N	8b. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
70 07 01	K. COMPLETION	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A061101A91C	00	076
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Procede with Security Classification Code): (U) Evaluation of Frozen Electron-beam Irradiated Porcine Xenograft as a Skin Substitute for use 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
69 07	71 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 71	.1	2.6
FISCAL YEAR CURRENT 72	0	0

19. RESPONSIBLE DOD ORGANIZATION
NAME: US Army Institute of Surgical Research
ADDRESS: Fort Sam Houston, Texas 78234
RESPONSIBLE INDIVIDUAL
NAME: PRUITT, B. A., Jr, LTC, 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: Paul Silverstein, MAJ, MC
TELEPHONE: 512-221-4440
SOCIAL SECURITY ACCOUNT NUMBER:
ASSOCIATE INVESTIGATORS
NAME: P.W. Curreri, LTC, MC
NAME: A. M. Munster, LTC, MC DA

21. GENERAL USE: FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code): (U) Heterograft; (U) Burns; (U) Homograft substitute; (U) Xenograft; (U) Allograft Substitute

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

23. (U) Frozen, electron-beam irradiated, porcine cutaneous xenograft is being evaluated on the basis of clinical and immunological criteria for use as a temporary physiologic dressing in the treatment of full-thickness thermal burns. Development of a suitable skin sibstitute will facilitate care of extensively burned patients and eliminate the limitations associated with use of viable cutaneous allografts.

24. (U) One group of burned patients will have their burns covered only with porcine xenograft between eschar separation and autografting, and in another group procine xenograft will be compared with viable cutaneous allograft. Graft take and condition of the graft bed will be evaluated clinically and documented with photographs; autograft take and subsequent scar formation will be noted. Porcine skin cellular antigen will be produced, and both humoral and cellular antibodies will be assayed in recipients.

25. (U) 70 07 - 71 06 Clinical experience with the frozen, electron-beam irradiated porcine xenograft during the past year indicates that it is effective as a temporary biological dressing but that it is not, in its present form, as satisfactory as viable cutaneous allograft. The major disadvantages of the porcine material include early subgraft suppuration within 24 to 48 hours after application and desiccation at the edges of the graft. Major advantages include its availability, guaranteed sterility, and storage requirements.

*(Available to contractors upon originator's approval)

FINAL REPORT

PROJECT NO. 3A061101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: CLINICAL AND IMMUNOLOGICAL EVALUATION OF FROZEN, ELECTRON-BEAM IRRADIATED PORCINE XENOGRAFT AS A SKIN SUBSTITUTE

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

1 July 1970 - 30 June 1971

Investigators:

Paul Silverstein, M.D., MAJ, MC
P. William Curreri, M.D., LTC, MC
Andrew M. Munster, M.D., LTC, MC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A061101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: CLINICAL AND IMMUNOLOGICAL EVALUATION OF FROZEN, ELECTRON-BEAM IRRADIATED PORCINE XENOGRAFT AS A SKIN SUBSTITUTE

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

Period covered in this report: 1 July 1970 - 30 June 1971

Investigators: Paul Silverstein, M.D., MAJ, MC
P. William Curreri, M.D., LTC, MC
Andrew M. Munster, M.D., LTC, MC

Reports Control Symbol MEDDH-288(R1)

During the past 12 months, frozen, electron-beam irradiated porcine cutaneous xenograft was evaluated as a temporary physiologic dressing in the treatment of third-degree burn wounds during the period between eschar separation and autografting.

Results indicated that xenograft adequately prepared debrided granulating wound beds for autografting. Subcellular antigenic fractions of porcine skin did not elicit delayed hypersensitivity reactions when injected intradermally into 20 burn patients who had been previously treated with sequential applications of xenograft.

It was therefore concluded that frozen, irradiated porcine cutaneous xenograft functions is a satisfactory physiologic dressing in the treatment of healing burn wounds. A supply of this product will be maintained for clinical use at this institute.

Xenograft
Burns
Allograft Substitute
Heterograft
Homograft Substitute

CLINICAL AND IMMUNOLOGICAL EVALUATION OF FROZEN, ELECTRON-BEAM IRRADIATED PORCINE XENOGRAFT AS A SKIN SUBSTITUTE

This study was undertaken to evaluate potential substitutes for human cadaver allograft in the treatment of healing burn wounds. The benefits of allograft when used as a temporary physiologic dressing on granulating wounds has been well documented. However, insufficient supply of available cadaver donors necessitates a continuous search for adequate substitutes.

Method

Twenty-five burn patients with bilaterally symmetrical third-degree burns were treated with frozen irradiated porcine cutaneous xenograft during the period between eschar separation and autografting. Xenograft was applied to one-half of the wound and cadaver allograft to the other half. Grafts were removed and changed every 24 to 72 hours and graded subjectively according to graft adherence, appearance of the wound bed, and occurrence of subgraft suppuration. When the grafts were adherent to the entire wound and the wounds revealed granulation bleeding upon removal of the allo- or xenograft, autografting was performed.

Frozen, electron-beam irradiated porcine cutaneous xenograft was obtained from a commercial supplier and stored indefinitely in a deep freeze. The skin, packaged in sterile plastic bags, was thawed by submersion in a water bath at 37°C for 30 minutes prior to use.

A subcellular antigenic fraction extracted from the porcine skin was prepared and injected intradermally into 20 patients previously treated with porcine skin in an attempt to elicit evidence of an immunological response to xenograft.

Results

In all patients treated with porcine cutaneous xenograft, final autografting was accomplished at the same time in both halves of the wound. There was no significant difference in autograft take between wounds treated with porcine xenograft or human cadaver allograft.

Application of xenograft was somewhat more difficult than application of allograft because of decreased initial adhesion

to the wound bed. Wraps of wet saline dressings for 24 hours usually aided adherence when circumferential extremity burns were involved. Subgraft suppuration developed more frequently under xenograft than under allograft, resulting in an average of two more graft changes for wounds treated with xenograft. However, when all debris was gone and clean granulating wounds were encountered, xenograft adherence matched that of allograft, and brisk granulation bleeding resulted from stripping of the graft from the wound bed.

Attempts to elicit evidence of delayed hypersensitivity to porcine xenograft by intradermal injection of subcellular skin extracts were uniformly unsuccessful in 20 patients; 0.1 cc of extract was injected intradermally prior to xenografting and again after wound healing was complete. All skin tests were negative.

Conclusions

1. Frozen, electron-beam irradiated porcine cutaneous xenograft is a satisfactory substitute for fresh cadaver allograft in the treatment of healing burn wounds.

2. Porcine xenograft demonstrates no evidence of delayed hypersensitivity by the testing described in patients treated with serial applications of pig skin.

3. Frozen irradiated porcine xenograft is well preserved by deep freezing and is easily reconstituted for use by 30 minutes' submersion in 37°C water bath. After thawing, the skin may be stored for up to 2 weeks at 4°C in a standard refrigerator.

4. Fresh human allograft is still the preferred physiologic wound dressing, and porcine xenograft will be used only when homograft supply is inadequate because:

a. Porcine xenograft tends to desiccate on the wound and must be changed more often.

b. While porcine xenograft provides adequate temporary coverage to clean, granulating wounds, it does not adhere as well as allograft to incompletely debrided wounds due to subgraft suppuration.

5. On the basis of this report, a supply of frozen, electron-beam irradiated porcine cutaneous xenograft will be maintained for clinical use when fresh allograft is unavailable.

Presentations

Silverstein P: The graft-host relationship of split-thickness porcine xenograft to human and animal wounds. American Burn Assoc Anl Meeting, San Antonio, Texas, 16 Apr 1971.

Publications

None

51

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0D 6971	71 07 01	DD-DR&E(AR)636

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

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A061101A91C	00	081
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) Evaluation of Fresh Viable Porcine Cutaneous Xenograft as a Temporary Burn Wound Cover for use in Military Burn Patients (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
70 07	71 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 71	.15	6.8
	CURRENT 72	0	0

19. RESPONSIBLE DOD ORGANIZATION
NAME: US Army Institute of Surgical Research
ADDRESS: Fort Sam Houston, Texas 78234
RESPONSIBLE INDIVIDUAL
NAME: PRUITT, B. A., Jr, LTC, 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: Paul Silverstein, MAJ, MC
TELEPHONE: 512-221-4440
SOCIAL SECURITY ACCOUNT NUMBER:
ASSOCIATE INVESTIGATORS
NAME: P. W. Curreri, LTC, MC
NAME: A. M. Munster, LTC, MC DA

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Viable xenograft; (U) Burns; (U) Wound Cover

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

23. (U) Fresh viable porcine cutaneous xenograft is being evaluated on the basis of clinical, immunological and laboratory investigations for use as a temporary physiologic dressing in the treatment of full-thickness thermal burns. Development of a suitable skin substitute will facilitate care of the extensively burned patient and eliminate the supply limitations currently encountered with viable cutaneous allografts.

24. (U) Porcine skin will be evaluated in the laboratory for adequacy of wound coverage Investigation will also be made into itsimmune relationship to the host via first and second set reactions, India ink injections, and antigenic stimulation.

25. (U) 70 07 - 71 06 Clinical and laboratory investigation has shown viable porcine xenograft to provide adequate temporary burn wound coverage during the period from eschar separation to autografting. No clinical immune response has been noted to intradermal injection of subcellular skin antigen. First and second set applications of pigskin to our animal model also failed to demonstrate hyperimmune rejection. Clinical immune inertness is believed to be due to failure of vascularization of the xenograft.

*Available to contractors upon originator's approval

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

ANNUAL PROGRESS REPORT

PROJECT NO. 3A061101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: EVALUATION OF FRESH, VIABLE PORCINE CUTANEOUS XENOGRAFT AS A TEMPORARY BURN WOUND COVER

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

1 July 1970 - 30 June 1971

Investigators:

Paul Silverstein, M.D., MAJ, MC
P. William Curreri, M.D., LTC, MC
Andrew M. Munster, M.D., LTC, MC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

BLANK PAGE

ABSTRACT

PROJECT NO. 3A061101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: EVALUATION OF FRESH, VIABLE PORCINE CUTANEOUS XENOGRAFT AS A TEMPORARY BURN WOUND COVER

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

Period covered in this report: 1 July 1970 - 30 June 1971

Investigators: Paul Silverstein, M.D., MAJ, MC
P. William Curreri, M.D., LTC, MC
Andrew M. Munster, M.D., LTC, MC

Reports Control Symbol MEDDH-288(R1)

This study was undertaken to examine graft vascularization, graft rejection, and immediate and delayed immunologic sequelae following application of porcine xenograft to granulating wounds.

The wound interface was studied by (1) macroscopic observation of first, second, and third set grafts to excised wounds measuring 20% of the total body surface in 100 male rats; (2) serial histologic examination of the grafted wounds following in vivo intravenous India ink injections; and (3) clinical comparison of wounds treated with allograft and xenograft in 50 patients with major burns. Standard immunologic techniques were employed to evaluate antigenicity of porcine xenograft, elicitation of anti-pigskin antibodies (precipitins), and induction of delayed hypersensitivity in both patients and animals.

Porcine xenograft proved an acceptable substitute for allograft. It adhered to the wound and adequately prepared it for subsequent autografting. However, more frequent subgraft suppuration was noted under xenograft. Delayed hypersensitivity to pigskin antigen and precipitin-type antibodies were not observed. Xenografted rats showed neither typical

accelerated second set nor "white graft" rejection unless previously sensitized by parenteral administration of subcellular pigskin antigen. Graft vascularization was not observed; and it is probable that absence of vascularization prevents sensitization of the host and shortens duration of adequate wound coverage.

Viable Xenograft
Burns
Wound Cover

EVALUATION OF FRESH, VIABLE PORCINE CUTANEOUS XENOGRAFT AS A TEMPORARY BURN WOUND COVER

In recent years, surgeons have become familiar with the benefits of fresh cadaver allograft in the treatment of burn wounds. But because the supply of allograft seldom matches the demand, many physicians have been unable to avail themselves of this biologic dressing. Laboratory and clinical evaluation of viable porcine cutaneous xenograft as a substitute for cadaver allograft is the purpose of this study.

Since 1966 when Song and Bromberg published their experiences with porcine skin, many laboratories and burn centers have begun to use split-thickness xenograft both experimentally and in the hospital to fill the gap in supply of allograft. In general, xenograft is utilized in the same fashion as allograft. It is applied to full-thickness burns following eschar separation, amputation sites, or traumatic wounds in which large areas of denuded tissue are exposed with associated evaporative heat and water loss, exudative protein loss, and infection. Coverage of these wounds with temporary dressings of living xenograft is accompanied by alleviation of pain, reduction in evaporative losses, facilitation of physiotherapy and improvement in the quality of granulation tissue.

Although difficult to prove, an empirically seasoned impression is that xenograft and allograft facilitate wound healing by some unknown mechanism which encourages growth of granulation tissue and prepares the wound for earlier autografting than would have occurred with standard gauze dressings.

Xenografted wounds are usually left exposed, but they may be covered with moist gauze dressings in circumferentially burned extremities to prevent dislocation of the graft. After 24 to 48 hours, the grafts are usually well adherent to the wound, unless particles of retained eschar or bacterial infection have generated pockets of purulent exudate. In the latter case, grafts are changed daily until good adherence is noted. From that point, grafts are inspected daily but changed every two to five days until autograft coverage can be achieved. After several days on the wound, the graft edges begin to desiccate and the graft slowly separates from the wound bed unless removed and changed.

We have now treated over 250 patients with porcine xenograft. A controlled protocol was followed for the first 50 patients to

evaluate subjectively graft adherence and the appearance of healthy granulation tissue. After 48 hours on the wound, the graft was removed and graded on a 1 to 4 scale:

Grade 1. Poor adherence with subgraft suppuration.
Grade 2. Poor adherence with no subgraft suppuration.
Grade 3. Good adherence with no granulation bleeding.
Grade 4. Good adherence with granulation bleeding.

When a grade 4 was achieved, the wound was autografted with uniformly excellent results.

In the 50 patients studied, a grade 4 graft and granulating wound bed ready for autograft was achieved after:

1 application of porcine xenograft in 8 patients.
2 applications of porcine xenograft in 10 patients.
3 applications of porcine xenograft in 11 patients.
4 applications of porcine xenograft in 13 patients.
5 applications of porcine xenograft in 8 patients.

These results approach those achieved with fresh cadaver allograft which usually prepares the wound for autografting after two or three applications. Xenograft requires more frequent changes than allograft because of poorer adherence in the freshly debrided wound and increased subgraft suppuration. However, once all retained particles of eschar have been removed, porcine xenograft is as satisfactory a wound cover as allograft.

On histological examination, the xenografted wound demonstrates close coaptation at the graft-recipient junction. The epidermis of fresh, commercially supplied xenograft is intact but does not appear to undergo an epithelial regenerative cycle (in contrast to fresh xenograft harvested in our own laboratory). Granulation tissue from the host extends between the collagen bundles of donor dermis, thus anchoring the graft. Some bleeding occurs from these adherent foci when the xenograft is removed. Neutrophils infiltrate the graft from the recipient's vascular granulation tissue, although none of the classic histologic features described in immunopathologic rejection, such as hemorrhage, necrosis or mononuclear round cell infiltration occur.

Clinically, xenograft functions as an acceptable allograft substitute, and there has been no clinically significant

human sensitization to split-thickness cutaneous xenograft encountered. To learn more about xenograft adherence to wounds and the relatively inert behavior of xenograft without evidence of violent cross-species immune rejection, freshly harvested, viable porcine skin 0.010-0.020" thick was applied to excised 20% total body surface wounds on the backs of 200 g male Sprague-Dawley rats. First, second and third set grafts were studied, and an attempt was made to demonstrate graft vascularization by intravenous injection of carbon particle suspension at various intervals post grafting.

The application of split-thickness xenograft to excised rat wounds is performed under surgically clean conditions and barbiturate anesthesia. Grafts are secured by Michell clips and read daily by three observers. End of adequate wound coverage is arbitrarily selected as the day on which 50% or more epithelial slough is noted on the graft.

First set xenografts of pigskin or human skin applied to the Sprague-Dawley rat slough at an average of 8 to 10 days. Second and third set grafts applied 14 days later persist even longer--up to 13 days. The superaccelerated "white graft" rejection does not occur. Using the in vivo observation technique of Taylor and Lehrfeld, no circulation was ever seen in split-thickness xenografts viewed under a dissecting microscope. To achieve histologic confirmation of this phenomenon, 5 cc of carbon particle suspension were injected via the tail vein 3 minutes prior to sacrifice, and sections were made of the wounds at 3, 6, 9, 12 and 20 days post grafting. Appropriate controls of autograft and Long-Evans rat allograft were also studied for comparison. Carbon particles were seen to freely circulate to dermal vessels of rat autograft by the third postgraft day, and regenerating squamous epithelium in the pilo-sebaceous apparatus surrounding hair shaft was apparent. Xenograft vessels in contrast do not contain carbon particles, indicating that the graft is not vascularized by the recipient host. Nevertheless, neutrophils do infiltrate the graft from capillaries in the underlying granulation tissue, demonstrating that cellular interaction is possible regardless of actual vascularization. Fresh xenograft has a viable epidermis that undergoes regeneration at the surface of the graft and from the cut ends of hair follicles on the undersurface of the graft. However, because it is never vascularized, xenograft slowly becomes necrotic in the animal model, with superimposed bacterial infection. In contrast, autograft was fully taken in all sections studied at various postgraft intervals.

Allograft from Long-Evans to Sprague-Dawley rats underwent histologic rejection first noticeable at day 6, with necrosis and dissolution by ingrowth of granulation tissue.

Fresh human cadaver skin grafted onto the excised rat back demonstrates the same results as porcine xenograft. Viability of both porcine and human tissue is supported by respiratory measurements indicating that grafts consume 100-150 mu moles O_2/min/g tissue for up to two days post harvesting. Oxygen consumption thereafter decreases as cellular death occurs.

Thus, the xenograft may function as a "dressing" offering a semipermeable barrier layer to evaporation and a scaffolding layer at the cut surface for ingrowth of granulation tissue from the wound bed.

We have been unable to demonstrate any evidence of recipient sensitization. Subcellular extract prepared from both fresh and frozen porcine skin and fresh pig spleen fail to induce an accelerated rejection in grafted rats. Subcellular extract injected intradermally into 20 human patients before and after porcine xenografting elicited no evidence of delayed hypersensitivity.

Injection of extract intravenously into rabbits over a one-month period in an attempt to induce sensitization failed to reveal the presence of precipitin antibodies against the extract by the Ouchterlony technique.

If it is reasonable to presume that split-thickness xenograft is not vascularized and depends on diffusible material from the wound bed for nutrition, rather than a blood supply, then diffusible antigraft antibodies, if they are produced, should not materially hasten graft death, since these antibodies have previously been shown to be directed against the graft's vascular endothelium.

In summary, it may be said that split-thickness porcine cutaneous xenograft is an adequate substitute for fresh human allograft in the preparation of open granulating wounds for autografting or secondary closure. Xenograft does not appear to elicit strong antigenicity and dies by slough due to cellular ischemia rather than due to immunologic rejection. It

does not vascularize and is evidently incapable of inducing accelerated rejection by a host that has been sequentially grafted from the same or different donor animal. Should recipient sensitization be induced by other routes, such as intraperitoneal injection of splenic extract, subsequently applied split-thickness skin graft is not affected, probably because of its avascularly protected environment.

Future investigation is necessary to fully define the immunologic status of split-thickness cutaneous xenograft. Why is split-thickness allograft vascularized and rejected in predictable fashion when xenograft is not?

Aside from the question of immunity, there is also the logistic problem of supply and limited viability of fresh skin. New forms of xenograft must be developed which possess indefinite shelf life and sterility, thereby permitting inexpensive storage and worldwide distribution.

Presentations

Silverstein, P: The graft-host relationship of split-thickness porcine xenograft to human and animal wounds. Presented at meeting of the American Burn Assoc, San Antonio, Texas, 16 Apr 1971.

Publications

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6971	71 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
70 07 01	D. CHANGE	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A061101A91C	00	075
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Procode 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

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 71	.76	20.2
	CURRENT 72	1.09	31.8

19. RESPONSIBLE DOD ORGANIZATION

NAME: US Army Institute of Surgical Research

ADDRESS: Fort Sam Houston, Texas 78234

RESPONSIBLE INDIVIDUAL

NAME: PRUITT, B.A., Jr, LTC, 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: Alan H Morris, MAJ, MC

TELEPHONE: 512-221-4307

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: K. W. Spitzer, CPT, MSC

NAME: DA

21. GENERAL USE

FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Procode EACH with Security Classification Code) (U) Burns; (U) Lung Mechanics; (U) Pulmonary Diffusion; (U) Ventilation/Perfusion Abnormalities; (U) Blood Gases; (U) Shunt

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

23. (U) To define lung function in the thermally injured man in the early postburn period, in an effort to elucidate the pathogenesis of pulmonary complications in burned military patients.

24. (U) Young adult men are studied frequently during the early postburn period, and the following indices of lung function determined: vital capacity; static lung compliance, dynamic lung compliance, airways resistance, work of breathing, lung volumes (nitrogen washout), lung clearance index, carbon monoxide diffusing capacity, arterial blood gases (room air and 100% oxygen), minute ventilation, tidal volume, oxygen consumption, respiratory quotient.

25. (U) 70 07 - 71 06 Thirteen acutely burned patients have been studied in the early postburn period. A striking increase in minute ventilation and oxygen consumption was noted, beginning on the third postburn day, reaching a peak on the fifth postburn day, and remaining elevated until the 10th to 14th postburn day. During this time, blood gas analyses demonstrated no significant hypoxemia, static lung compliance was remarkably well preserved and revealed no significant decrease, diffusing capacity for carbon monoxide was within normal limits, the lung clearance index was within normal limits, the forced vital capacity was either normal or diminished but remained stable during the period of marked elevation of oxygen consumption and minute ventilation. These findings, coupled with the unremarkable x-ray and physical examination of the lungs of these patients at a time when minute ventilation and oxygen consumption are both strikingly increased, have led us to tentatively conclude that the driven respiration, so dramatically apparent at the bedside, is due to an extrapulmonary cause and not to a primary derangement of lung itself.

*Available to contractors upon originator's approval

ANNUAL PROGRESS REPORT

PROJECT NO. 3A061101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: PULMONARY PATHOPHYSIOLOGIC CHANGES FOLLOWING THERMAL INJURY

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

1 July 1970 - 30 June 1971

Investigators:

Alan H. Morris, M.D., MAJ, MC
Kenneth W. Spitzer, CPT, MSC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A061101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: PULMONARY PATHOPHYSIOLOGIC CHANGES FOLLOWING THERMAL INJURY

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

Period covered in this report: 1 July 1970 - 30 June 1971

Investigators: Alan H. Morris, M.D., MAJ, MC
Kenneth W. Spitzer, CPT, MSC

Reports Control Symbol MEDDH-288(R1)

Several indices of lung function in 13 patients, studied during the early postburn period, have been serially determined, in an effort to define the pattern of lung function following thermal injury. A dramatic tachypnea, with apparently labored respirations, is often noted at the bedside as the first suggestion of pulmonary difficulty, and precedes, by several days, the first appearance of abnormalities in the X-ray or physical examination of the lungs. During this period, the minute ventilation and oxygen consumption are both strikingly increased. Arterial oxygen tensions are very well preserved, and no significant hypoxemia is apparent. Measurements of the static lung compliance have been well preserved and fail to reveal any major change in mechanical properties of the lung. The diffusing capacity for carbon monoxide has been within normal limits. The lung clearance index has been within normal limits. The forced vital capacity has been normal in some patients, and depressed in other patients, but, in all patients studied, it has been stable during this period of increased minute ventilation and oxygen consumption. Arterial oxygen tensions, with the patient breathing 100% oxygen, have been normal or only slightly depressed. We have tentatively concluded that the driven respiration, observed in this early postburn period, is due to an extrapulmonary cause and not to a major derangement of the lung itself.

Burns	Shunt
Lung Mechanics	Ventilation
Pulmonary Diffusion	Blood Gases

PULMONARY PATHOPHYSIOLOGIC CHANGES FOLLOWING THERMAL INJURY

Because of the frequency and severity of pulmonary problems following thermal injury, and because of the paucity of information concerning lung function during this period, we have undertaken an investigation of lung function following severe non-pulmonary burns. At the Institute of Surgical Research, the first clinical suggestion of pulmonary difficulty has often been the dramatic development of tachypnea and labored respirations on the third to fifth postburn day, preceding by several days the first appearance of abnormalities of the physical or x-ray examination of the lungs.

We have, during the past year, studied 13 burn patients during the early postburn period. Their mean age, per cent burn, and full-thickness injury are indicated in Table 1. The diagnoses, made on a clinical basis, of inhalation injury were distributed among the five survivors and the eight patients who died as indicated. Two additional patients, without significant thermal injuries, were studied and their data included for comparison. One suffered an electrical injury and required amputation of both arms, and the other incurred a severe and well documented inhalation injury accompanied by an inconsequential 6% second-degree burn.

The results of studies of the indices of lung function listed in Table 2 were examined. The minute ventilation and oxygen consumption were determined by the open circuit technique, and the arterial blood gases measured while the patient was breathing room air.

Static lung compliance was calculated from the recorded static pressure volume deflation curve of the lung. The diffusing capacity for carbon monoxide was measured by the rebreathing technique. Lung clearance index is the number of liters of oxygen which must be breathed by the patient to dilute the lung nitrogen to 2% for each liter of air contained within his lungs.

In the figures, the postburn day is indicated on the horizontal axis and the function of interest on the vertical axis. In most, the upper and lower limits of normal are indicated by horizontal lines. The dots represent the mean values of the

Table 1

	Died	Survived
N	8	5
$\overline{\text{Age}}$ (years)	23	31
$\overline{\%}$ TBS	70	41
$\overline{\%}$ Third degree	33	11
Inhalation injury	4/8	2/5

Table 2

$\dot{V}_E$	- Minute ventilation
$\dot{V}_{O_2}$	- Oxygen consumption
P_{O_2}, P_{CO_2}, pH	- Arterial (room air)
C_L STAT	- Static lung compliance
$D_{L_{CO}}$	- CO diffusing capacity (rebreathing
LCI	- Lung clearance index
FVC	- Forced vital capacity

measurements made on N number of thermally injured patients, on any given day, and the bars, when present, represent the range of these measured values. The measurements made on the single patient with the severe and well documented inhalation injury, and those of the single patient with the electrical injury are included for comparison.

The magnitude of the increase in ventilation present in these tachypneic patients can be well appreciated from Figure 1. The minute ventilation attained a maximum of approximately 50 liters per minute on the fifth postburn day, and remained at or above 40 liters per minute for the next five days until the 10th postburn day. The patient with the electrical injury maintained a normal minute ventilation. The patient with the inhalation injury had a significantly elevated minute ventilation, but it appeared small when compared with the striking increase observed in the thermally injured patients.

The arrows indicate, in the following figures, the period of marked elevation of the minute ventilation, the middle arrows indicating the time of maximum increase in minute ventilation.

The oxygen consumption is also strikingly increased during the period of increased minute ventilation. The respiratory quotients are unremarkable (Figs. 2A and 2B).

The arterial oxygen tensions, during the period of striking increase in both minute ventilation and oxygen consumption, are unremarkable (Fig. 3). Significant arterial hypoxemia was absent in the thermally injured group. Moderate arterial hypoxemia was observed in the patient with the inhalation injury, but his minute ventilation, although increased, was much less strikingly increased than those of the thermally injured patients.

The static lung compliance was unremarkable and, for the most part, normal during the period of increased minute ventilation (Fig. 4). It was, in particular, normal at the time of maximal increase in minute ventilation. No obvious change in lung stiffness was present to explain the driven respirations observed in these patients.

The average lung clearance index of all thermally injured patients, the lung clearance index of those who died, and the lung clearance index of those who survived were all unremarkable and normal at the time when minute ventilation was maximally

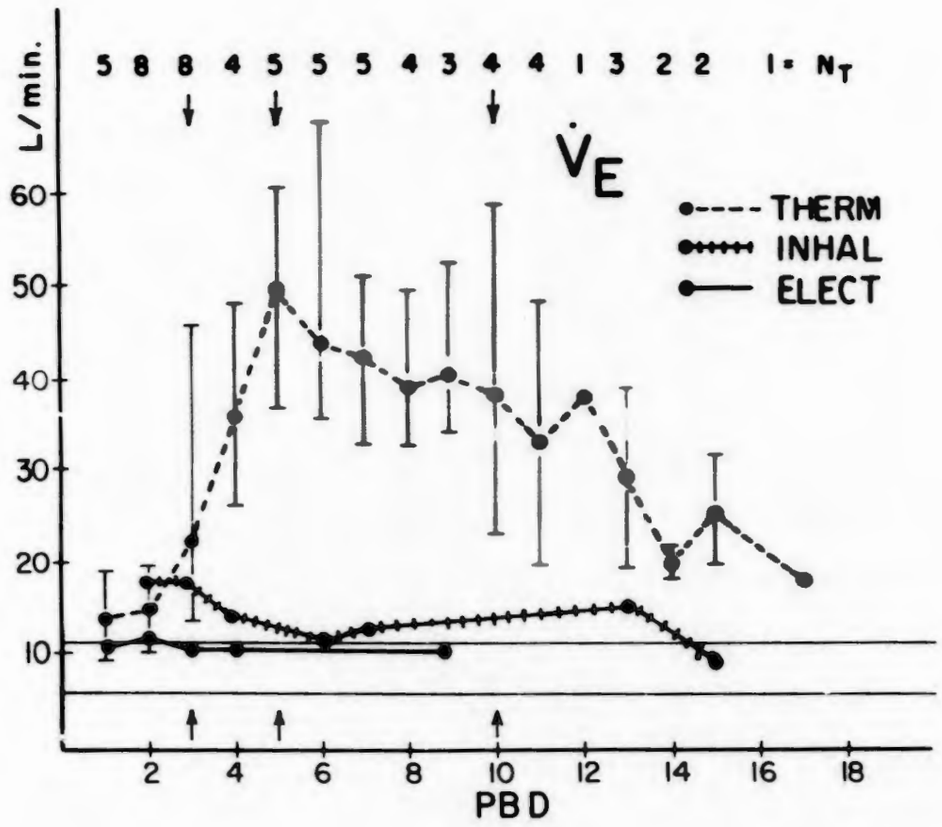

5 8 8 4 5 5 5 4 3 4 4 1 3 2 2 1= N_T
L/min.
$\dot{V}_E$
THERM
INHAL
ELECT
60
50
40
30
20
10
2
4
6
8
10
12
14
16
18
PBD

Figure 1

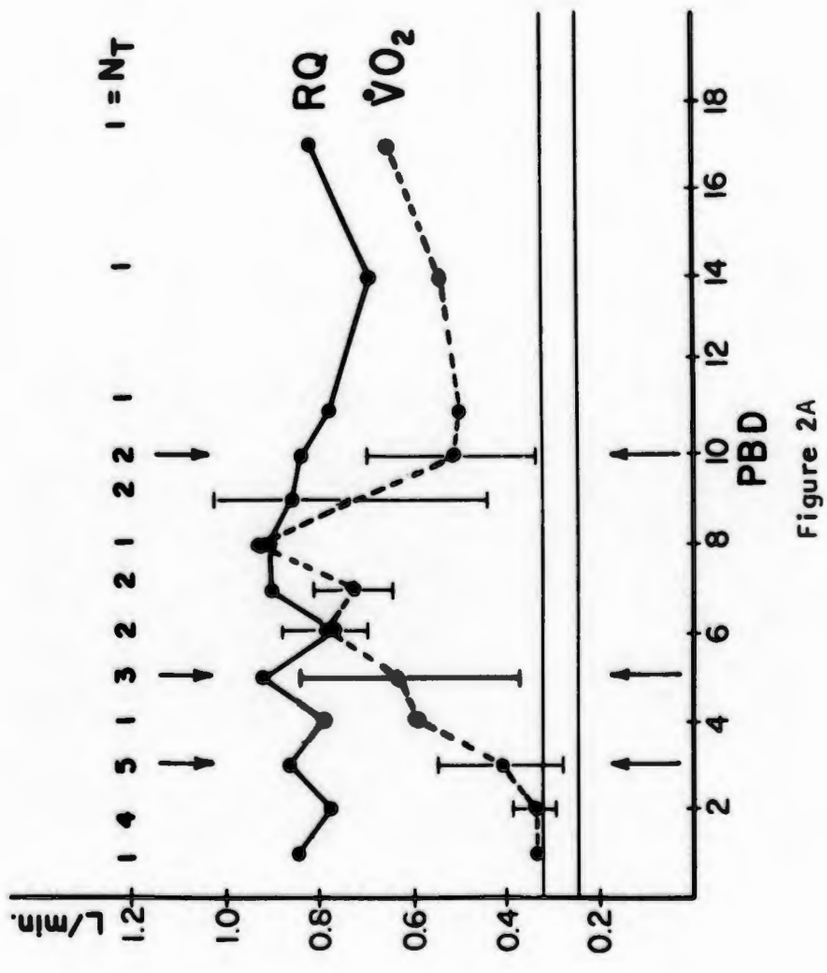

L/min.
1.2
1.0
0.8
0.6
0.4
0.2
1 4 5 1 3 2 2 1 2 2 1 1 1=NT
RQ
V̇O2
2
4
6
8
10
12
14
16
18
PBD

Figure 2A

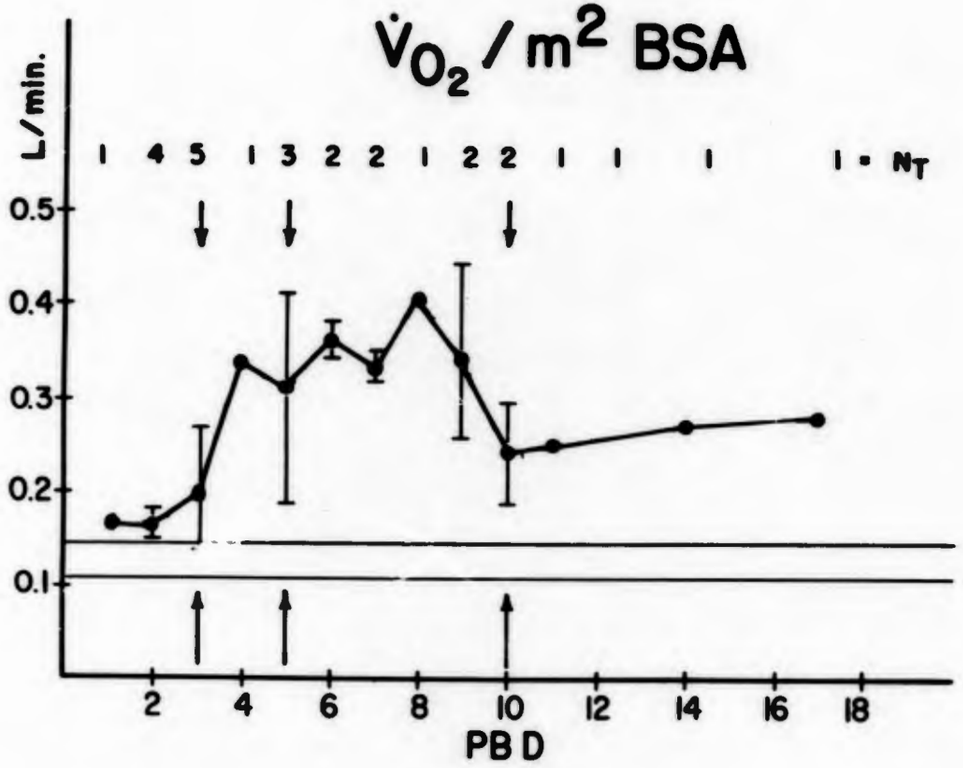

$\dot{V}_{O_2}$ / m² BSA
L/min.
1 4 5 1 3 2 2 1 2 2 1 1 1 1 = N_T
0.5
0.4
0.3
0.2
0.1
2 4 6 8 10 12 14 16 18
PBD

Figure 28

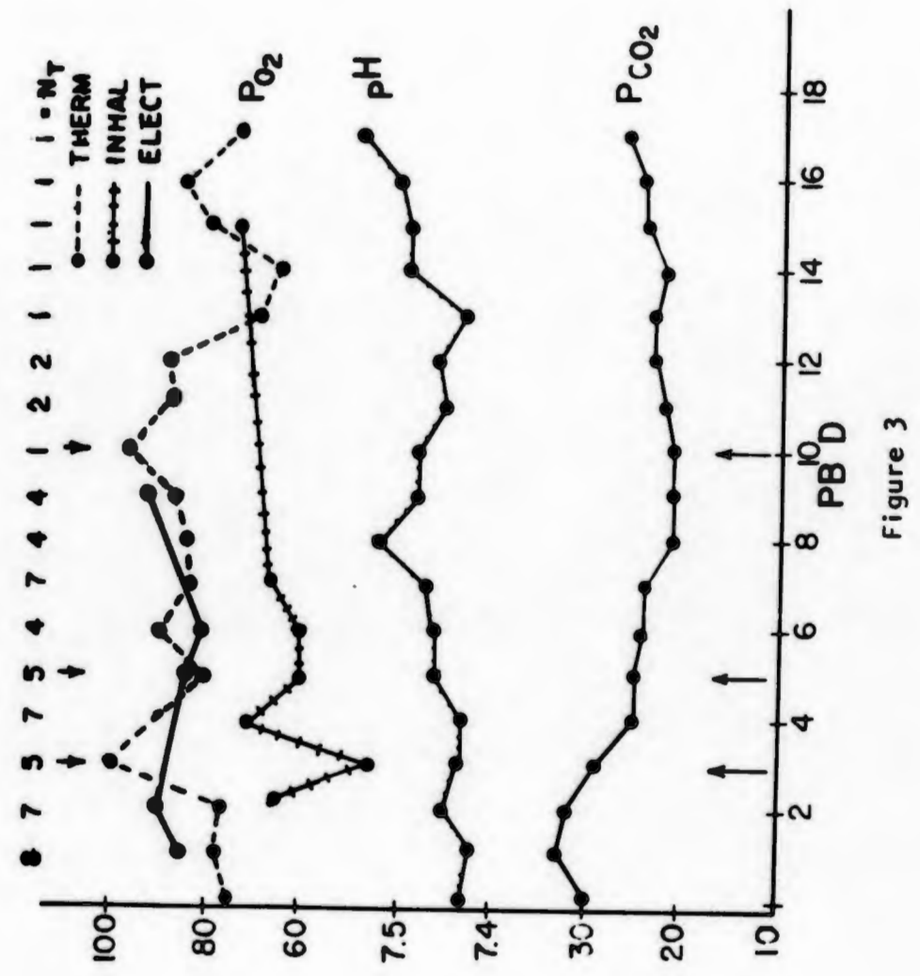

8 7 5 7 5 4 7 4 4 1 2 2 1 1 1 1 1 - N_T
THERM
INHAL
ELECT
P_{O_2}
pH
P_{CO_2}
100
80
60
7.5
7.4
30
20
10
2
4
6
8
10
12
14
16
18
PB D

Figure 3

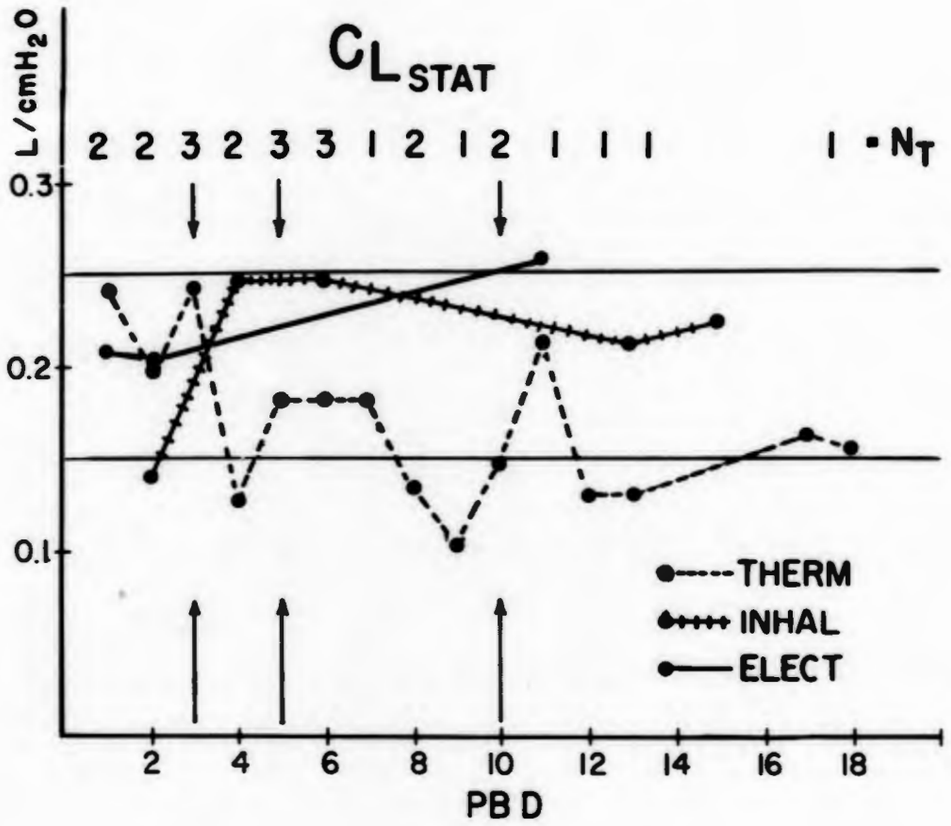

$C_{L_{STAT}}$
L/cmH_2O
2 2 3 2 3 3 1 2 1 2 1 1 1
1 • N_T
0.3
0.2
0.1
THERM
INHAL
ELECT
2
4
6
8
10
12
14
16
18
PB D

Figure 4

increased (Fig. 5). The increased values, which later followed in some patients, were obtained from patients near death at a time when X-ray and physical examination of the lung were abnormal. There was, during the early period, at a time when minute ventilation and oxygen consumption were both strikingly increased, no evidence of uneven distribution of inspired air.

The forced vital capacities of two burned patients measured in the upright position, those of three burned patients measured in the supine position, and those of the patient with the inhalation injury, measured in the semi-supine position, are depicted in Figure 6. Whether the forced vital capacity was normal or decreased, it remained unchanged before, during, and after the period of increased minute ventilation.

We have not yet completed calculation of all of the measured diffusing capacities, but do have these data available from a patient who survived a 59% total body surface burn (Fig. 7). His diffusing capacity was uniformly within normal limits before, during, and after the period of marked increase in minute ventilation. His static lung compliance decreased slightly from initially normal values, but remained stable during the period of marked minute ventilation.

These observations, coupled with the unremarkable physical and X-ray examinations of the lungs of these patients, during the period when the minute ventilation and oxygen consumption are so strikingly increased, have led us to tentatively conclude that this driven respiration, so dramatically apparent at the bedside, is due to an extrapulmonary cause and not to a primary derangement of the lung itself.

Publications and/or Presentations

None

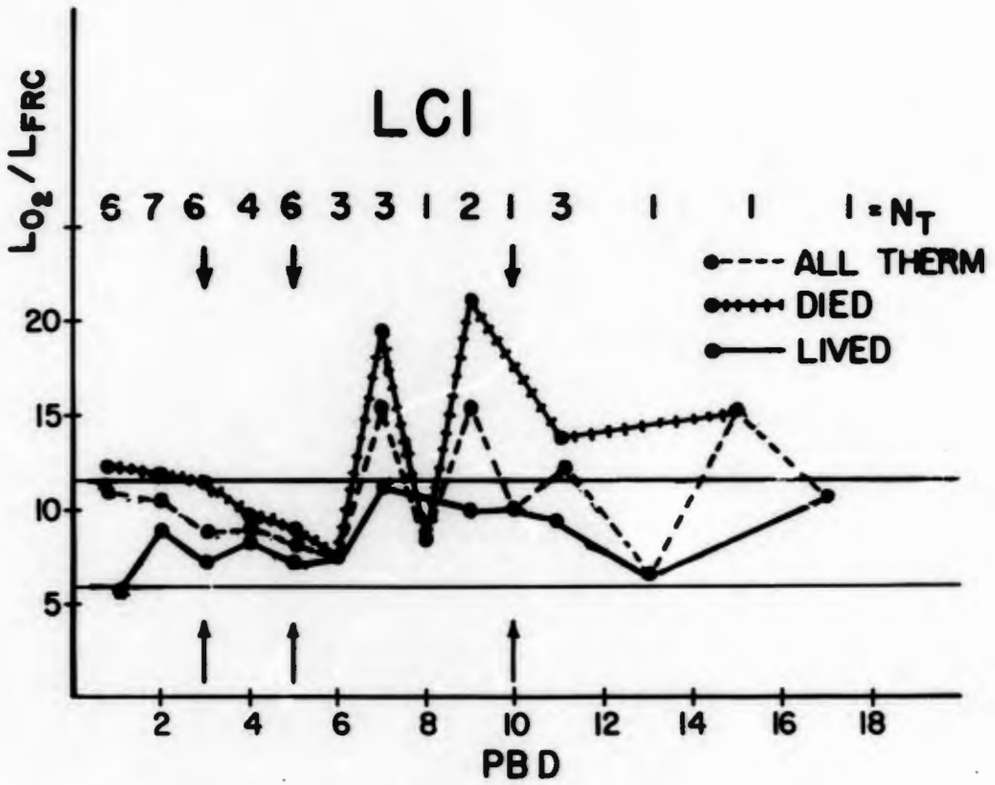

LCI
L_{O_2}/L_{FRC}
6 7 6 4 6 3 3 1 2 1 3 1 1 1 = N_T
ALL THERM
DIED
LIVED
20
15
10
5
2
4
6
8
10
12
14
16
18
PBD

Figure 5

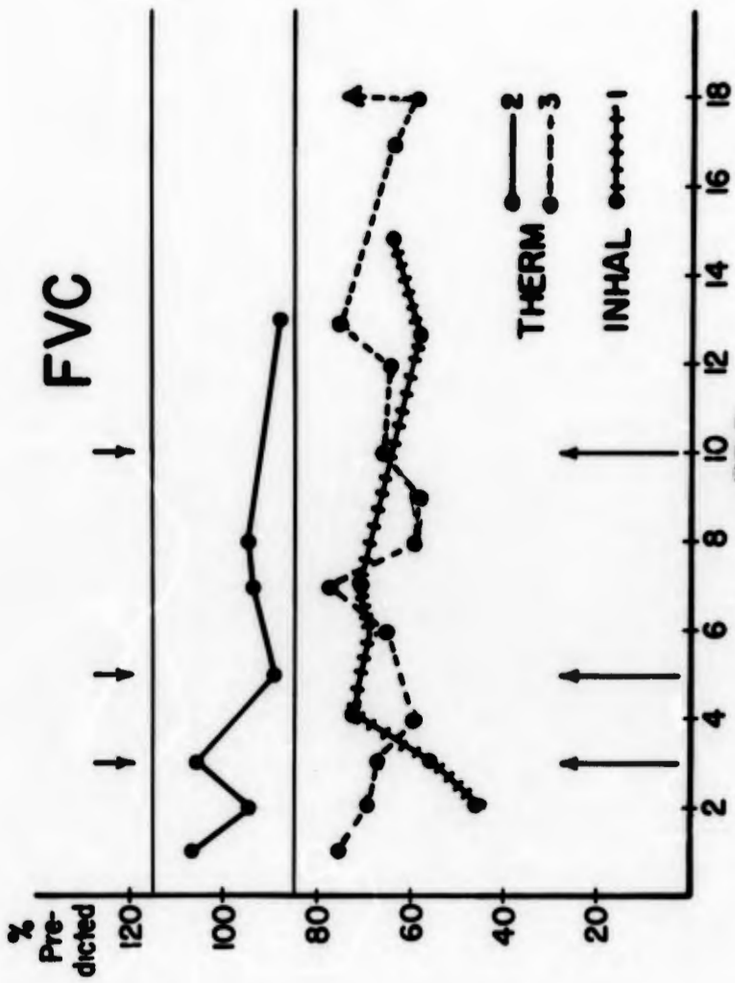

FVC
% Predicted
120
100
80
60
40
20
2
4
6
8
10
12
14
16
18
P8D
THERM 2
3
INHAL 1
A

Figure 6

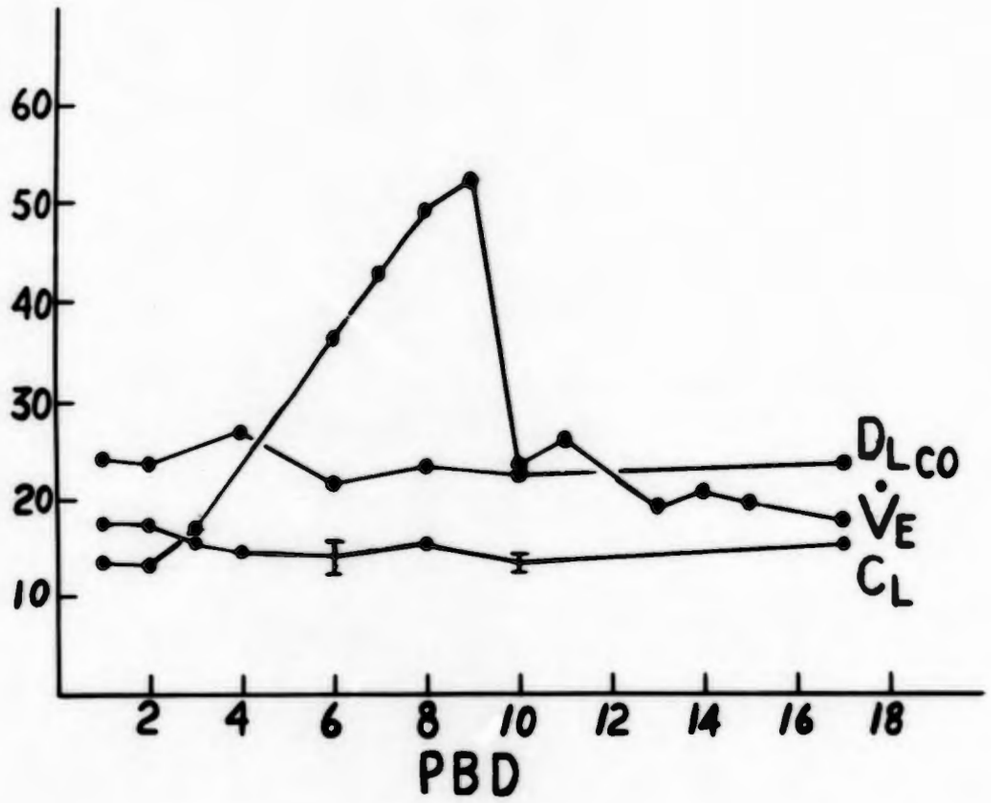


Figure 7

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0C 6980	71 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
70 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	3A062110A821	00	107
b. CONTRIBUTING	61102A	3A061102B71R	01	
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)

(U) Pulmonary Diffusing Capacity 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 11	Cont	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 71	.83	22.0
	CURRENT 72	.5	14.6

19. RESPONSIBLE DOD ORGANIZATION

NAME: US Army Institute of Surgical Research

ADDRESS: Fort Sam Houston, Texas 78234

RESPONSIBLE INDIVIDUAL

NAME: PRUITT, B. A., Jr, LTC, MC

TELEPHONE: 512-221-2720

20. PERFORMING ORGANIZATION

NAME: US Army Inst of Surgical Research
Clinical Division

ADDRESS: Fort Sam Houston, Texas 78234

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

NAME: K. W. Spitzer, CPT, MSC

TELEPHONE: 512-221-5712

SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS

NAME: A. H. Morris, MAJ, MC

NAME: J. M. Garfield, MAJ, MC DA

21. GENERAL USE

FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code)

(U) Pulmonary failure; (U) Burns; (U) Pulmonary diffusing capacity

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

23. (U) The purpose of this study is to determine the diffusing of the lung in burned patients.

24. (U) Five burned patients have been studied employing a rebreathing technique.

25. (U) The diffusing capacities of all patients studied were within normal limits.

*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. 3A062110A821-00, COMBAT SURGERY

REPORT TITLE: PULMONARY DIFFUSING CAPACITY FOLLOWING THERMAL INJURY

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

1 July 1970 - 30 June 1971

Investigators:

Kenneth W. Spitzer, CPT, AMSC
Alan H. Morris, M.D., MAJ, MC
Joseph M. Garfield, M.D., MAJ, MC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A062110A821-00, COMBAT SURGERY

REPORT TITLE: PULMONARY DIFFUSING CAPACITY FOLLOWING THERMAL INJURY

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

Period covered in this report: 1 July 1970 - 30 June 1971

Investigators: Kenneth W. Spitzer, CPT, AMSC
Alan H. Morris, M.D., MAJ, MC
Joseph M. Garfield, M.D., MAJ, MC

Reports Control Symbol MEDDH-288(R1)

Studies of the pulmonary diffusing capacity for carbon monoxide have been completed in five burned patients. Three of the patients were studied during the acute phase of their injury and two later during their course. One patient suffered a severe inhalation injury and one an electrical burn. All determinations of diffusing capacity were within normal limits.

Pulmonary Failure
Burns
Pulmonary Diffusing Capacity

PULMONARY DIFFUSING CAPACITY FOLLOWING THERMAL INJURY

Five acutely injured burn patients have been studied to date with burns ranging from 5 to 59% of the total body surface area (see Table) Patient 5 had a small surface injury but a well-documented severe inhalation injury. Patient 4 suffered a severe electric injury which necessitated disarticulation of the right shoulder and an above elbow amputation of the left arm on the 4th and 11th postburn days, respectively.

The pulmonary diffusing capacity was determined in duplicate according to the method of Lewis, et al.[1] Briefly, it involves the patient rebreathing for 20-25 seconds, at about 30 breaths/minute, a gas mixture containing 0.3% CO and 10.6% He in air. The contents of the bag were continuously analyzed for CO with a Beckman 215A CO analyzer. At the of the test, the concentration of helium, carbon dioxide, and oxygen in the bag was determined. The back pressure of carbon monoxide in the blood was measured[2] before the start of the first test only, and found to be within the normal limits of subjects studied in this laboratory. Previous studies on normals in the laboratory indicated the back pressure resulting from the rebreathing test itself had negligible effect on the calculated diffusing capacity.

Results and Discussion

The results in the table are the man value of two consecutive diffusion studies. All studies were with the "normal limits" of 15.6 to 40.4 with a mean value of 27.3, as reported by Lewis, et al.[1] These results suggest that diffusing capacity of carbon monoxide is well preserved following acute thermal injury. Additional studies are planned to clarify this preliminary finding.

References

1. Lewis BM, T-H Lin, FE Noe, EJ Hayford-Welsing: The measurement of pulmonary diffusing capacity for carbon monoxide by a rebreathing method. JCI 35:2073-2086, 1959.

Diffusing Capacity for Carbon Monoxide (Rebreathing Technique)

Patient	Age	Per Cent Body Surface Burned (Total/Full Thickness)	Postburn Day Studied	$D_{L_{CO}}$ (ml CO/min/mmHg)
1	26	59/7	1	24.3
			2	23.7
			4	27.0
			6	21.6
			8	23.2
			10	22.4
			17	23.4
2	30	46/30	2	35.5
			3	37.2
			5	41.2
			6	35.6
			11	28.1
3	27	36/6	27	18.1
			54	21.1
			88	21.0
4	17	18/0	1	23.4
			2	24.5
			10	28.9
5	25	5/0	15	20.2
			54	28.4

2. Henderson M, GH Apthorp: Rapid method of estimation of carbon monoxide in blood. Brit Med J 2:1853-1854, 1960.

Publications and/or Presentations

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OC 6976	71 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
70 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	3A062110A821	00	106
b. CONTRIBUTING	61101A	3A061101A91C	00	
c. CONTRIBUTING				

11. TITLE (Procede with Security Classification Code)

(U) Circulation in the Extremities of Burned Troops (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS

003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
69 07	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 71	.43	11.4
	CURRENT 72	.43	12.6

19. RESPONSIBLE DOD ORGANIZATION

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

RESPONSIBLE INDIVIDUAL
NAME: PRUITT, B. A., Jr, LTC, MC
TELEPHONE: 512-221-2720

20. PERFORMING ORGANIZATION

NAME: US Army Inst of Surgical Research
Burn Study Branch
ADDRESS: Fort Sam Houston, Texas 78234

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME: Joseph A Moylan, Jr, Maj, MC
TELEPHONE: 512-221-2943
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: Wellford W Inge, Jr, Maj, MC
NAME: Basil A Pruitt, Jr, LTC, MC DA

21. GENERAL USE

FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Procede EACH with Security Classification Code)

(U) Circulation; (U) Extremity; (U) Thermal Injury; (U) Escharotomy

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

23. (U) To study circulatory changes in all patients with extremity burns including those with escharotomies and to outline objective criteria for employment of escharotomy.

24. (U) All patients with third degree extremity burns were studied employing a doppler ultrasonic flowmeter. Changes in arterial and venous flow following escharotomy and/or resolution of edema were recorded.

25. (U) Twenty-four patients with circumferential extremity burns were studied. Selected patients with clinical indications for escharotomy but who had intact distal flow as determined with the ultrasonic flowmeter, were successfully treated nonoperatively with elevation and exercise with good results. Venous compression appeared to be the major factor in the development of circulatory insufficiency. The Doppler flowmeter has proved to be a valuable tool in evaluating extremity circulation and determining the need for escharotomy.

*Available to contractors upon originator's approval.

ANNUAL PROGRESS REPORT

PROJECT NO. 3A062110A821-00, COMBAT SURGERY

REPORT TITLE: CIRCULATION IN EXTREMITY BURNS

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

1 July 1970 - 30 June 1971

Investigators:

Joseph A. Moylan, Jr., M.D., MAJ, MC
Wellford W. Inge, Jr., M.D., LTC, MC
Basil A. Pruitt, Jr., M.D., LTC, MC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A062110A821-00, COMBAT SURGERY

REPORT TITLE: CIRCULATION IN EXTREMITY BURNS

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

Period covered in this report: 1 July 1970-30 June 1971

Investigators: Joseph A. Moylan, Jr., M.D., MAJ, MC
Wellford W. Inge, Jr., M.D., LTC, MC
Basil A. Pruitt, Jr., M.D., LTC, MC

Reports Control Symbol MEDDH-288(R1)

Following a circumferential thermal injury to an extremity, evaluation of distal circulation may be difficult because of the nature of the injury. A study using the ultrasonic flowmeter was undertaken to develop an objective method of measuring peripheral blood flow and to correlate clinical signs of vascular insufficiency with these measurements. In fifty limbs judged clinically to require an escharotomy, one-half were noted to have distal circulation using a Doppler* flowmeter.

Those limbs with distal flow were treated without escharotomy in all but three instances with no loss of viable tissue. Venous compression appears to be the major factor in the development of circulatory insufficiency following a circumferential extremity burn. The only reliable clinical sign was change in sensation to pain or touch.

Circulation
Extremity
Thermal Injury
Escharotomy

---

* Parks Electronics Laboratory, Beaverton, Oregon

BLANK PAGE

CIRCULATION IN EXTREMITY BURNS

Following circumferential thermal injury to an extremity, the vascular supply to the distal limb may be compromised because of the tourniquet-like effect of a rigid eschar with increasing underlying edema. The viability and function of the limb becomes dependent upon timely decompression. Without appropriate surgical therapy, distal ischemic necrosis and further tissue loss may result.

Decompressive escharotomy to relieve such vascular compromise was recommended in 1951 by Blocker and Moyer (1). Review of a two-year experience in the United States Army Institute of Surgical Research presented clinical indications for escharotomy including (a) cyanosis, (b) impaired capillary filling, and (c) progressive neurologic change (2). Other indications include loss of motion and inability to palpate pulses. Potential dangers of escharotomy have been reported by Monafo and others (3,4).

At this Institute we have attempted to accurately delineate the pathophysiology of vascular compromise after circumferential extremity burns and to correlate clinical signs of vascular insufficiency with objective measurements using an ultrasonic flowmeter. The use of an ultrasonic flowmeter in determining the need for an escharotomy in a thermally injured limb is also described.

Methods

Patients with recent circumferential extremity burns admitted to this Institute after January 1, 1970 were evaluated in this study. There were 93 patients admitted with recent burns of which 24 patients had deep circumferential extremity burns (Table I). Seven patients were 15 years old or less. A total of 60 limbs were involved, 39 being arms. The average time between burn and admission to the Institute was 14 hours, with a range of 5 to 31 hours.

In the cases with arm burns an average of 81% of the arm surface was burned while in those with leg burns an average of 85% of the surface area was involved.

Initial evaluation of the burned limb included surface temperature, color, capillary flush time, sensation, motor function and presence of palpable pulses. A clinical decision

Table I. CONUS Admissions
January 1- August 31,1970

Total Patients		93
Patients with Circumferential Extremity Burns		24
Children	7	
Adults	17	

A clinical decision concerning the need for an escharotomy was made and recorded prior to the flowmeter evaluation.

The ultrasonic flowmeter was then used to assess flow in the involved limb. Individual arterial patency was determined (Fig. 1) and segmental pressures at three levels were obtained in the involved limb. Digital pressures were recorded with a specially designed pressure cuff (Fig. 2). Extremity venous flow was evaluated on the basis of individual vein patency and flow dynamics. The venous augmentation test described by Sigel (5) was employed to show proximal venous obstruction. Following evaluation with the flowmeter, the burned limbs were either subjected to escharotomy (operative group), or treated with elevation and active exercise for five minutes each hour for the first 48 hours postburn (nonoperative group). Repeat studies were done post escharotomy in the operative group and each day postburn in both groups until the circulation was stable. Daily segmental circumferential measurements were made in the nonsurgical group.

Results

A summary of the clinical signs is contained in Table II. The color of the hands or feet varied from pale or cyanotic or red to normal. The temperature to touch in both groups was subnormal. The capillary flush time was greater than 5 seconds in all but five extremities. Motor function was absent in only seven limbs in which swelling mechanically limited motion. Palpation of pulses was variable. Sensation to touch was the most reliable sign, being absent in all but one of the limbs requiring an escharotomy.

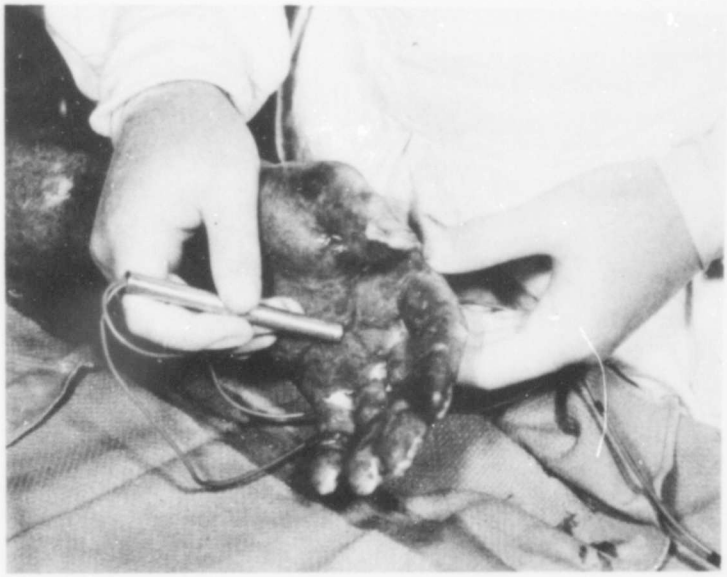


Fig. 1. Evaluation of arterial flow through the palmer arch.

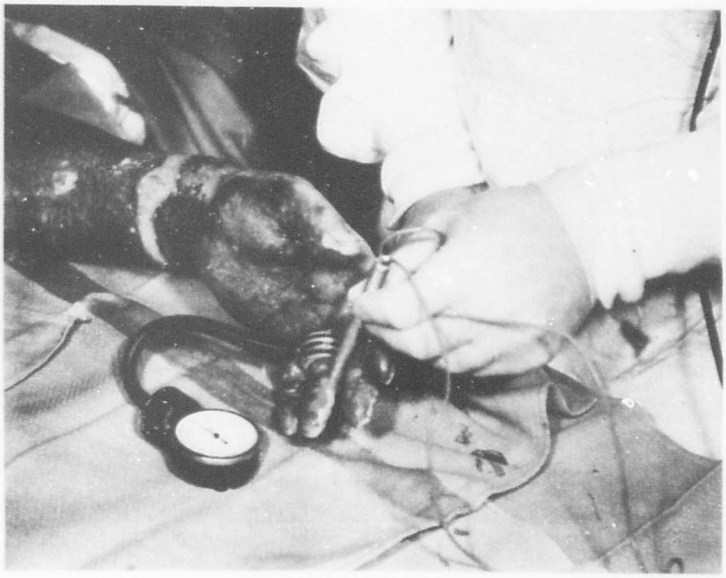


Fig. 2. Measurement of arterial pressure measure with Doppler flowmeter and digital blood pressure cuff.

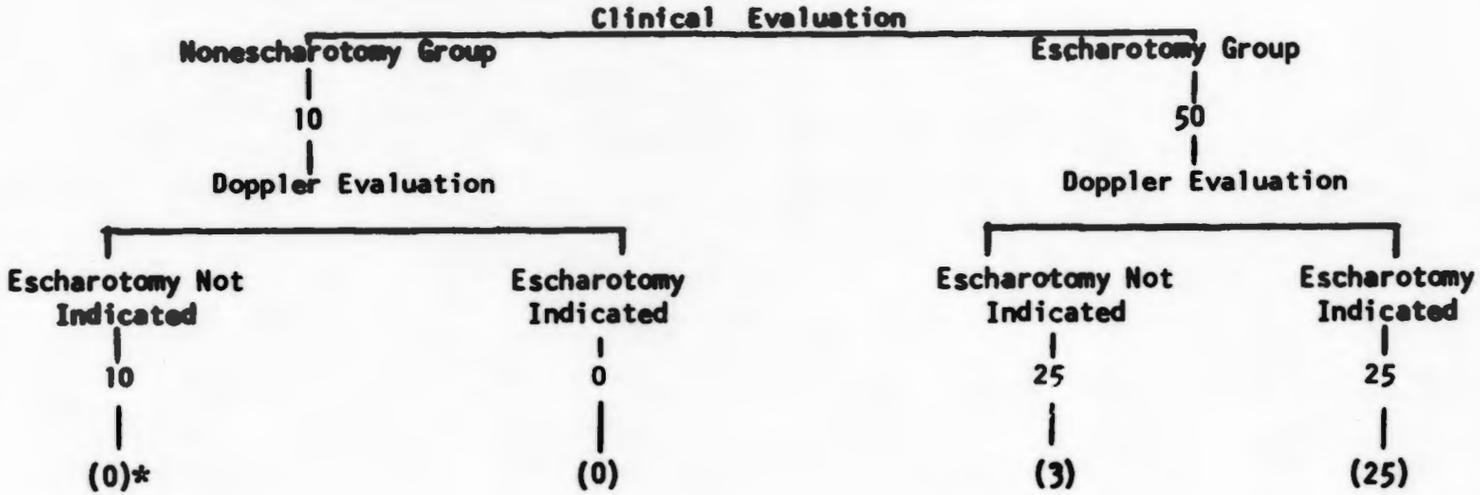
Table II. Summary of Clinical Signs

Clinical Signs	Escharotomized Group - 28	Nonescharotomized Group - 32
Color - Pale	18	18
Cyanotic	7	6
Red	3	3
Normal	0	5
Temperature - Cool	28	29
Normal	0	3
Capillary Flush Time		
< 5 seconds	0	5
> 5 seconds	28	27
Motor Function - Present	25	28
Absent	3	4
Sensation - Present	1	30
Absent	27	2

At admission, 10 of the 60 limbs with circumferential burns had adequate peripheral circulation by clinical parameters (Table III). These findings were corroborated by Doppler measurement revealing patent terminal arteries and essentially normal pressure gradients. The other 50 limbs had clinical findings indicating the need for escharotomy. All limbs in this clinical group had no distal palpable pulses, were pale or cyanotic and

Table III. Summary of Results

60 Limbs



* ( ) indicates number of escharotomies performed.

had prolonged capillary flush time. Approximately one-half had decreased sensation.

Of the 50 limbs, 25 had either audible palmer arch flow or posterior tibial artery flow upon testing with the ultrasonic flowmeter. All extremities had mild to severe venous obstruction. The 25 extremities with intact distal flow as detected by the ultrasonic flowmeter were elevated and a program of active motion carried out for 5 minutes each hour during the subsequent 48 hours. Of the 25 limbs treated in this manner, three arms required escharotomy after 24 hours because of arterial obstruction occurring at the radial level. These 3 escharotomies were necessary in two patients who were unable to exercise actively because of either a language barrier or coma.

In the limbs requiring operative decompression, the level of arterial obstruction is shown in Table IV. Following medial and lateral escharotomy (Fig. 3), palmer arch flow was audible in all 21 arms postoperatively and digital flow returned in nine of these limbs at that time. Digital flow returned to all remaining hands except one by the first postburn day. Posterior tibial flow as audible in all seven lower limbs following operation (Table V). No attempt was made to record toe flow. Arterial pressure measurements were made at three levels in both arms and legs. Prior to escharotomy, pressure gradients in the burned limbs were either unobtainable due to obstruction or were as great as a 90 mg Hg decrease from proximal to distal levels. Of the 15 upper limbs measured before and after escharotomy, 11 had normal pressure gradients by the second postburn day; the remaining four were normal by the third postburn day. Arterial pressure gradients were normal in all decompressed legs by the first postburn day.

Table IV. Level of Obstruction-Escharotomized Group

Arm:	Brachial	9
	Radial	6
	Palmer Arch	6
Legs:	Popliteal	5
	Posterior Tibial	2

Table V. Extremity Number - Total 60

Final Escharotomized Group	
Upper Extremity	21
Lower Extremity	7
Total	28
Final Nonescharotomized Group	
Upper Extremity	18
Lower Extremity	14
Total	32

Fig. 3. Preferred sites for escharotomy.

Prior to escharotomy, there was significant venous obstruction in both the burned arms and legs. This venous obstruction occurred mainly at the level of the lower forearm or midcalf. Venous dynamics returned to normal by the third postburn day in the limbs subjected to escharotomy. In the nonoperative group, return of normal arterial pressure gradients occurred by the third postburn day in the upper limbs and by the first postburn day in the lower extremities.

Discussion

It is often difficult to evaluate the circulatory status of a burned extremity. A hand or foot may be pale or cool due to inadequate perfusion or to marked edema secondary to the burn. Cyanosis in the 13 limbs in this study was due to venous obstruction. All had adequate arterial flow by flowmeter examination and did not require immediate escharotomy. All limbs in this study were cool irrespective of their circulatory status. The capillary flush time was prolonged in the majority of patients in both groups. Motor function was not a good sign for the need for escharotomy as it was limited in only 11% of the extremities and in these appeared related to the mechanical effects of edema. Sensation to pin prick and proprioception in the third degree burn areas proved to be the most reliable clinical sign giving a false negative in only one of the operative group and two false positives in the nonoperative group.

Clinical judgement favored escharotomy in 50 of the 60 burned limbs in the study. However, the finding of intact distal flow in 22 of these 50 limbs by ultrasonic flowmeter examination led to avoidance of escharotomy and there was no loss of viable tissue or function. The presence of flow in the palmer arch or posterior tibial artery indicates sufficient perfusion to preserve viability of the distal extremity. There were eight limbs in the operative group that had either an audible palmer arch or posterior tibial artery. Seven of these limbs were cyanotic. These patients were admitted early in the study and underwent an escharotomy before the present criteria of adequate flow required to prevent tissue loss were well defined. The pressure gradients are important only to document that normal flow returns completely by the second postburn day in the operative group and the third postburn day in the nonescharotomized group.

At the initial examination many of the upper extremities had no palmer arch flow and marked venous obstruction at the wrist.

Following "milking" of the venous bed by exercise, there was transient arterial flow in the hand of these cases. If the active motion was continued, arterial flow was preserved. The pathophysiology of circulatory insufficiency begins with progressive tissue edema under a nonelastic eschar. As tissue pressure rises, venous return from the distal extremity decreases (Fig. 4), producing increased capillary pressure and further transudation into the extravascular space (Fig. 5). When tissue pressure then exceeds venous pressure, venous return is completely obstructed, usually at the level of the wrist in the arm and lower calf in the leg. With return from the venous capillary bed blocked arterial flow ceases; however, the artery remains patent (Fig. 6). Active motion with elevation if used early in selected cases appears to prevent venous obstruction and maintain arterial flow.

The Doppler flowmeter affords the physician an easy, objective means of measuring extremity flow at the bedside. If flow in the palmer arch or posterior tibial artery is absent and fails to return after a brief period of active exercise, an immediate escharotomy is indicated. Extension of the escharotomy across an involved wrist joint appears important (Fig. 7). The radial artery and accompanying veins are most susceptible to compression at this level. The use of an escharotomy in the first web space or along the digits did not appear to increase flow distally.

It should be emphasized that if there is adequate flow on admission and the limb is elevated and exercised, close observation is necessary for the first 48 hours. Our patients were reexamined with the ultrasonic flowmeter every 6 hours during this interval.

Summary

Twenty-four patients who had circumferential extremity burns involving 60 limbs were included in the study. Correlation of clinical signs and ultrasonic flowmeter measurements is made. In all patients undergoing an escharotomy, there was return of either palmer arch or posterior tibial artery flow immediately postoperatively. Selected patients with clinical indications for escharotomy but who had intact distal flow as determined with the ultrasonic flowmeter, were successfully treated nonoperatively with elevation and exercise with good results. Venous compression appears to be the major factor in the development of circulatory insufficiency following circum-

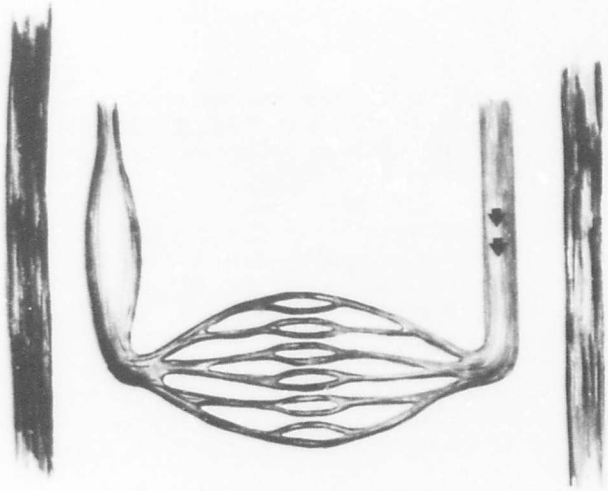


Fig. 4. Increase in tissue pressure produces venous obstruction and decreased venous return.

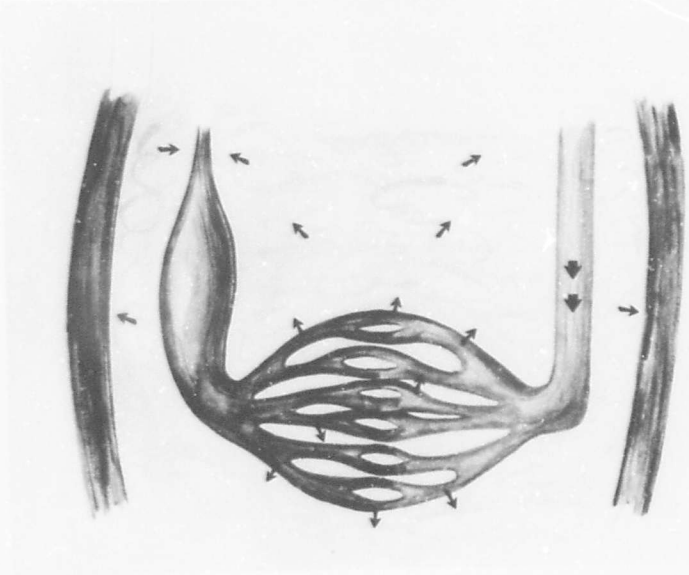


Fig. 5. Obstructed venous return produces increased capillary pressure and capillary transudation. Tissue pressure increases.

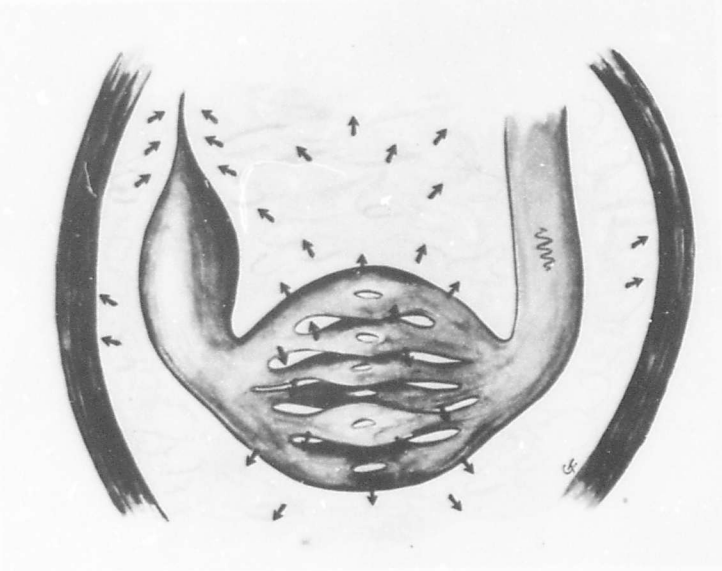


Fig. 6. Arterial inflow obstructed but artery remains patent.

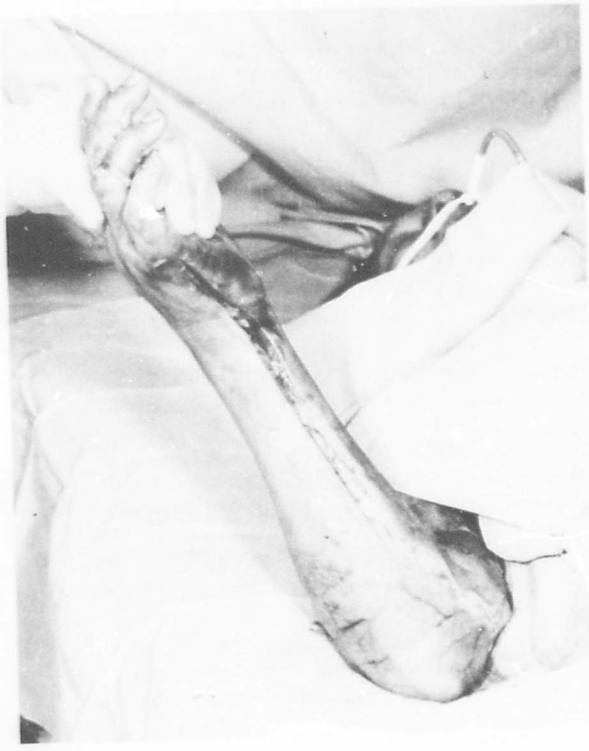


Fig. 7. Medial component of an escharotomy with extension across the wrist joint. Increased subeschar pressure produces wide separation of the incision.

ferential extremity burns. The Doppler flowmeter has proved to be a valuable tool in evaluating extremity circulation and determining the need for escharotomy.

References

1. Blocker TG, Moyer CA: 1953. In discussion, Brunner J. Delayed coverage of the burn wound and joint motion, p.172, In Womack NA (Editor). On Burns. Springfield, Ill., Chas. C. Thomas Publisher.

2. Pruitt BA Jr, Dowling JA, Moncrief JA: 1968. Escharotomy in early burn care. Arch Surg 96: 502-506.

3. Monafo WW, Brentano L, Gravens DL, Kempson R, Moyer CA. 1966. Gas gangrene and mixed clostridial infections of muscle complicating deep thermal burns. Arch Surg 92: 212-221.

4. Bennett JE. 1966. Decompression of constricting burns. A reappraisal. Surgery 60: 280-282.

5. Sigel B, Popky GL, Mapp EM, et al. 1970. Evaluation of Doppler ultrasound examination. Its use in diagnosis of lower extremity venous disease. Arch Surg 100: 535-540.

6. Stegall MF, Rushmer RF, Baker DW. 1966. A transcutaneous ultrasonic blood-velocity meter. J Appl Physiol 21: 707-711.

7. Strandness DE Jr, McCutchern EP, Rushmyer RF. 1966. Application of transcutaneous Doppler flowmeter in evaluation of occlusive arterial disease. Surg Gynec Obstet 122: 1039-1043.

Publications

Moylan JA Jr, Inge WW Jr, Pruitt BA Jr.: Circulatory changes following circumferential extremity burns evaluated by the ultrasonic flowmeter. An analysis of 60 thermally injured limbs. J Trauma (In press).

Presentations

Moylan JA Jr. Circulatory effects of thermal injuries on extremities with and without escharotomy. Amer Assn for the Surg of Trauma, Chicago, Ill. 9 October 1970.

Moylan JA Jr. Objective evaluation of peripheral circulation in thermally injured extremities employing the ultrasonic flowmeter. Amer Burn Assn mtg. San Antonio, Texas, 17 April 1971.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0D 6397	71 07 01	DD-DR&E(AR)636

3. DATE PREV SUM'RY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8A. DOD'N INSTR'N	8B. SPECIFIC DATA-CONTRACTOR ACCESS	9. LEVEL OF SUM
70 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	3A061102B71P	08	066
b. CONTRIBUTING	61101A	3A061101A91C	00	
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) Volume Control of Renal Glucose Reabsorption - Laboratory Study of Changes in Sick and Injured Troops (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS:
003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
70 07	Cont	DA	C. In-House

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

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 71	.30	8.0
	CURRENT 72	.30	8.8

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

RESPONSIBLE INDIVIDUAL
NAME: PRUITT, B.A., Jr, LTC, 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 SSAN if U.S. Academic Institution)
NAME: Neil A Kurtzman, LTC, MC
TELEPHONE: 512-221-5416
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: M. G. White, MAJ, MC
NAME: P. W. Rogers, MAJ, MC DA

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Glucose; (U) Extracellular Volume; (U) Sodium; (U) Glomerular Filtration Rate

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

23. (U) Disorders of glucose metabolism characterized by over production of glucose and under utilization of glucose are extremely common in troops subject to trauma or medical illness. These disorders of glucose metabolism would not result in symptomatic hyperglycemia if the kidney excreted the excess glucose. Since the kidney commonly does not excrete such excess amounts of glucose, this study was undertaken to determine if glucose is reabsorbed by the kidney in an independent rate limited transport mechanism or if glucose reabsorption is linked to that of sodium as has recently shown to be true for uric acid, calcium, phosphate, bicarbonate, etc. In addition, the effect of glomerular filtration rate on glucose reabsorption was examined.

24. (U) Since sodium reabsorption is controlled by extracellular volume, glucose reabsorption was measured in dogs with normal expanded and contracted extracellular volumes.

25. (U) 70 07 - 71 06 Results thus far obtained have shown that glomerular tubular balance for glucose exists and that this glomerular tubular balance may be disrupted when sodium reabsorption is changed. The therapeutic implication from these studies is that disorders of glucose metabolism which may result in hyperglycemia, can be ameliorated if proper attention to salt and water balance is paid and if extracellular volume contraction is avoided. Future studies will examine the effect of cylic AMP, prostaglandins, acetylcholene, parathormone, and oxytocin on renal glucose reabsorption.

*Available to contractors upon originator's approval.

ANNUAL PROGRESS REPORT

PROJECT NO. 3A061102B71P-08, BASIC RESEARCH IN SUPPORT OF MILITARY MEDICINE

REPORT TITLE: THE RELATIONSHIP OF EXTRACELLULAR VOLUME TO RENAL GLUCOSE REABSORPTION

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

1 July 1970 - 30 June 1971

Investigators:

Neil A. Kurtzman, M.D., LTC, MC
Philip W. Rogers, M.D., MAJ, MC
Martin G. White, M.D., MAJ, MC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A061102B71P-08, BASIC RESEARCH IN SUPPORT OF MILITARY MEDICINE

REPORT TITLE: THE RELATIONSHIP OF EXTRACELLULAR VOLUME TO RENAL GLUCOSE REABSORPTION

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

Period covered in this report: 1 July 1970 - 30 June 1971

Investigators: Neil A. Kurtzman, M.D., LTC, MC
Philip W. Rogers, M.D., MAJ, MC
Martin G. White, M.D., MAJ, MC

Reports Control Symbol MEDDH-288(R1)

Disorders of glucose metabolism characterized by overproduction of glucose and underutilization are common in troops subject to trauma or medical illness. These disorders of glucose metabolism would not result in symptomatic hyperglycemia if the kidney excreted this excess glucose. Since the kidney commonly does not excrete this excess glucose, this study was undertaken to determine if glucose is reabsorbed by the kidney by an independent rate limited transport mechanism or if glucose reabsorption is linked to that of sodium. Since sodium reabsorption is controlled by extracellular volume, glucose reabsorption was measured in dogs with normal, expanded, and contracted extracellular volumes. Our data show that glomerular tubular balance for glucose exists and that this balance is disrupted when sodium reabsorption is changed.

Glucose
Extracellular volume
Sodium
Glomerular filtration rate

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0C 6985	71 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
70 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	3A061102B71P	08	067
b. CONTRIBUTING	61101A	3A061101A91C	00	
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) Lymphocyte Corticosteroid Binding in the Rat after Thermal Injury - A Model of Changes Observed in Burned Soldiers (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
69 12	Cont	DA	C. In-House

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

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 71	.43	11.4
FISCAL YEAR CURRENT 72	.43	12.6

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

RESPONSIBLE INDIVIDUAL
NAME: PRUITT, B. A., Jr, LTC, MC
TELEPHONE: 512-221-2720

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

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME: Karl Eurenius, MAJ, MC
TELEPHONE:
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: R. F. Mortensen, SP5, MS
NAME: A. A. Johnson, BS DA

21. GENERAL USE: FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code): (U) Corticosteroids; (U) Steroid Binding; (U) Transcortin; (U) Lymphocytes

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

23. (U) (a) To measure postburn injury serum corticosteroid levels in a laboratory rat model and military burn patients. (b) To measure corticosteroid binding to lymphocytes from these subjects after a burn injury. (c) To measure serum transcortin (corticosteroid binding globulin) binding activity in these subjects after a burn injury. (d) To measure the competition between lymphocytes and transcortin for the available serum corticosteroid.

24. (U) (a) Have established fluorescene technique for measuring serum corticosteroids. (b) Have established tritiated corticosteroid binding measure to lymphocytes. (c) Have established tritiated corticosteroid absorption binding technique to measure transcortin binding capacity.

25. (U) 70 07 - 71 06 (a) Elevated serum corticosteroid levels postburn injury in both rats and patients have been observed. (b & d) Remaining studies are now in progress. (c) Elevated transcortin levels measured in rats but not in burn patients.

*Available to contractors upon originator's approval.

ANNUAL PROGRESS REPORT

PROJECT NO. 3A061102B71P-08, BASIC RESEARCH IN SUPPORT OF MILITARY MEDICINE

REPORT TITLE: LYMPHOCYTE CORTICOSTEROID BINDING IN THE RAT AFTER THERMAL INJURY

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

1 July 1970 - 30 June 1971

Investigators:

Karl Eurenius, M.D., MAJ, MC
Richard F. Mortensen, M.S., SP5
Avery A. Johnson, B.S.

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A061102B71P-08, BASIC RESEARCH IN SUPPORT OF MILITARY MEDICINE

REPORT TITLE: LYMPHOCYTE CORTICOSTEROID BINDING IN THE RAT AFTER THERMAL INJURY

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

Period covered in this report: 1 July 1970 - 30 June 1971

Investigators: Karl Eurenius, M.D., MAJ, MC
Richard F. Mortensen, M.S., SP5
Avery A. Johnson, B.S.

Reports Control Symbol MEDDH-288(R1)

There is some evidence for a defect in the defense mechanism of the body against infection following a burn injury. One of these defense mechanisms, delayed hypersensitivity, is though to be depressed. Since corticosteroids interfere with delayed hypersensitivity as well as the graft rejection process, serum levels of corticosteroids as well as the binding of corticosteroids to serum proteins and lymphocytes has been examined.

Elevated serum corticosteroid levels have been measured in both rats and man after burn injury. The total binding capacity or the corticosteroid-binding capacity (CBC) of serum and the fraction of the total binding due to corticosteroid-binding globulin (CBG) were increased in the rat following injury but were not significantly altered in burn patients. The level of serum cortisol in excess of the CBC in man was as much as five times normal in the early postburn period. It was concluded that the human, unlike the rat, does not respond with increased serum corticosteroid-binding activity following thermal injury.

Transcortin Steroid Binding
Corticosteroids Lymphocytes

LYMPHOCYTE CORTICOSTEROID BINDING IN THE RAT AFTER THERMAL INJURY

Since burned patients and animals exhibit heightened susceptibility to infection,[1] it has been suggested that a defect exists in cellular immune reactions.[2,3] An examination of serum corticosteroid levels and serum corticosteroid binding capacity after burn injury was undertaken because of the known inhibition of lymphocyte function by glucocorticoids.[4,5]

Only the unbound or albumin-bound serum corticosteroid is thought to be biologically active.[6] An increase in the level of corticosteroid-binding globulin (CBG) might protect the unburned rat or man from the effects of a high plasma corticosteroid concentration. Therefore, a simple adsorption method was employed to measure both the total corticosteroid-binding capacity (CBC) and the fraction of the total binding capacity due to corticosteroid-binding globulin (CBG) which has a high affinity for corticosteroids.[6]

Materials and Methods

Male, Sprague-Dawley rats (180-200 g) received a third-degree scald burn on the dorsum which covered 30% of their body surface area.[7] Sera was collected by cardiac puncture under mild ether anesthesia. Human sera was collected from 33 adult burn patients, five of which were serially monitored.

Cortisol (human) or corticosterone (rat)serum concentrations were determined by a standard fluorometric technique.[8] Serum albumin and total protein determinations were performed on the autoanalyzer using crystallized bovine serum albumin as a standard.

CBC and CBG capacity were determined by the method of Trapp and West[9] based on the separation of protein bound tritium-labelled cortisol from the unbound labelled cortisol by selective adsorption with dextran-coated florosil. It was found that the naturally occurring rat corticosteroid,

corticosterone and cortisol were bound in equal amounts by rat serum; therefore 1,2-^{3}H cortisol was used in all binding experiments. Each serum sample was examined in triplicate tubes. The heat-denaturable CBG capacity was calculated from the difference in binding capacity between unheated serum and serum heated at 60°C for 25 minutes.

Results

Increases in both CBC and CBG capacity were observed following the standardized scald-burn injury to rats. A maximum increase in the total binding capacity (CBC) was seen four days postburn (Fig. 1). This rise in total binding capacity is accounted for by the twofold increase in the CBG capacity. Control levels of binding were determined on rats receiving sodium barbital anesthesia without subsequent injury.

Corticosterone levels were determined on all rat serum samples since a small correction was necessary for the endogenous steroid level of the serum used in the binding assay. An immediate rise in the serum corticosterone level was observed in the burned rats (55.8 µg/100 ml) (Fig. 2). Following a drop in this initial level, the serum corticosterone levels peaked about the 6th day postburn and the level remained elevated even at 20 days postburn. No CBG rise was observed in unburned animals despite the high corticosterone level (38 µg/100 ml) seen one hour after i.p. sodium barbital. The rise in CBG seen in burned animals coincided with the rise in serum corticosterone levels.

Total protein and albumin concentrations (globulin by difference) were measured on all serum samples to determine any effects on the binding capacity by hemoconcentration and plasma protein evascularization following burn injury. All serum protein concentrations dropped significantly and immediately following injury (Table 1). Globulin concentrations returned to normal on the second postburn day and subsequently rose to elevated levels from the second to twentieth day postburn. To insure that the increase in binding capacity by CBG and alpha$_1$-globulin,[6] was not just a reflection of the increased synthesis of all globulins or the initial postburn concentration of serum proteins, a ratio of CBG capacity to globulin concentration was calculated. With this correction,

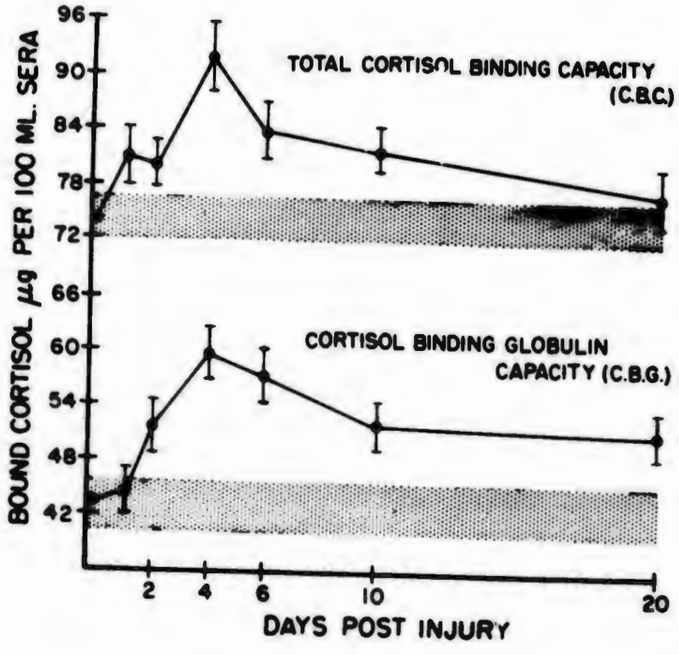


Figure 1. Binding capacities of the corticosteroid binding fractions of rat serum following thermal injury. The shaded area represents control values ± S.E.M. Vertical bars represent means ± S.E.M.

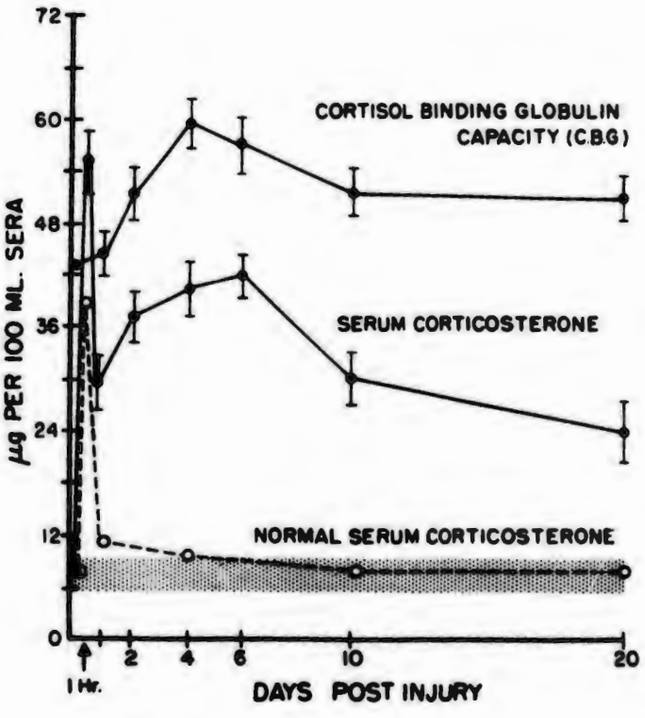


Figure 2. Serum corticosterone levels and CBG capacity in the rat following thermal injury. Dashed line represents the serum corticosterone levels in anesthetized, unburned rats. Shaded area represents normal serum corticosterone levels ± S.E.M. Vertical bars are means ± S.E.M.

Table 1. Serum Protein Levels and Specific CBG Capacity Following Thermal Injury in the Rat

	Days Postinjury						
	Control (16)	1 Hour (9)	1 (9)	2 (9)	4 (14)	10 (11)	20 (9)
Total protein g/100 ml sera	5.66 ± 0.12	4.62 ± 0.13	4.63 ± 0.11	4.18 ± 0.06	5.85 ± 0.20	6.05 ± 0.09	6.07 ± 0.13
Globulin g/100 ml sera	3.20 ± 0.08	2.95 ± 0.17	2.90 ± 0.11	3.38 ± 0.07	3.78 ± 0.17	3.80 ± 0.04	4.32 ± 0.12
CBG capacity/ g globulin	4.1		4.8	6.4	7.8	5.8	4.9

Data are mean ± SEM for albumin, globulin and total protein.

Numbers in parentheses are number of rats tested each day postburn.

a twofold increase in CBG activity was still seen by the fourth postburn day. The ratio of non-CBG binding/g albumin/100 ml sera remains relatively constant and correlates with serum albumin levels.

Human sera from burn patients was also assayed for both CBC and CBG activity. Both CBC and CBG capacity were slightly lower during the first 20 days following burn injury (Table 2). During the remaining course of the patient's recovery, these binding capacities returned to the levels of the unburned subjects with an increasing per cent of the CBC due to the CBG activity (Table 2). Individual patients were assayed at various times following their burn injury with no significant alterations in binding capacities noted; for example, in one patient, at 10,14,28, and 36 days following a 44% total body surface burn, CBC values of 32.0, 32.8, 38.8,and 30.7(µg cortisol bound/100 ml sera)and CBG levels of 9.3,13.0,16.0 and 11.9(µg cortisol bound/100 ml sera) were noted. Increased extent of burn was associated with both slightly lower CBC and CBG activities and an increasing fraction of the total binding due to CBG binding (Table 2).

Serum cortisol levels in these patients were measured and as expected were significantly elevated immediately following injury, and only returned to normal levels 40 plus days postburn (Table 2). The extent of the injury was correlated with increased levels of serum cortisol.[10]

Discussion

Estrogen treatment,[11,12] adrenalectomy,[13] and gonadectomy,[11,12] have been shown by others to increase CBG levels in male rats. We have now shown an increase in CBG activity following a scald burn injury (Fig. 1) to the levels seen in hypophysectomized male rats (65 µg/100 ml sera).[12] The time sequence for this CBG response (maximum at four days, Fig. 1) contrasted to a time period of 10 days for a maximum response in rats following either adrenalectomy or castration.[11,12,13]

In our experiments, the rise in serum corticosterone concentration paralleled the CBG rise (Fig. 2). This relationship of a serum steroid level and CBG binding is similar to that seen in pregnant [11,12] and estrogen-treated rats.[6,11] The daily administration of ACTH did not alter CBG binding

Table 2. Serum Cortisol Binding and Serum Cortisol Concentrations in Human Burn Patients

	Cortisol ug/100 ml sera	CBC	CBG
		ug cortisol bound/100 ml sera	
Control (4)	17.3 ± 1.1	36.3 ± 1.0	19.0 ± 0.8
5-20 days (14)	38.9 ± 2.7	28.4 ± 2.2	11.9 ± 1.5
21-30 days (9)	42.0 ± 4.4	35.6 ± 1.7	14.4 ± 1.9
31-40 days (11)	29.5 ± 1.8	30.5 ± 1.1	15.2 ± 1.0
41-90 days (13)	21.5 ± 2.4	28.6 ± 2.0	17.8 ± 2.0
6-30% TBS (15)	29.7 ± 2.4	35.7 ± 2.0	17.8 ± 2.1
31-50% TBS (19)	32.5 ± 3.0	32.8 ± 1.3	14.2 ± 1.2
51-70% TBS (13)	35.6 ± 3.9	29.6 ± 1.8	11.4 ± 1.1
All burn samples (47)	32.5 ± 1.8	32.8 ± 1.1	14.6 ± 1.0

All values are followed by ± S.E.M.
% TBS is the per cent total body surface area burned.
Numbers in parentheses are number of serum samples tested in each group.

which is in agreement with the results of others.[13]

Increased binding activity following any experimental manipulation of the rat may be due to the removal of a steroid that would normally compete for binding to CBG (e.g., progesterone) rather than increased synthesis of CBG itself. This is a distinct possibility following thermal injury. However, there is evidence for increased alpha-globulin (CBG is an α_1-globulin) synthesis in the rat following a burn.[14]

No significant changes in binding capacities were noted in the sera of the burn patients surveyed (Table 2). In contrast to the injured rat, the burn patients' serum cortisol concentration exceeded the total binding capacity (CBC) of that sera (Fig. 2, Table 2). This excess cortisol was seen up to 30 days postinjury (Table 2). This difference in response of man and rat to thermal injury may be related to the differences between these species in CBG changes after various endocrine gland resections.[6,13] The presence of cortisol in excess of the CBC of burn patients' sera implies either hypercorticism or weak binding of excess cortisol to other serum glucocorticoid components which have not yet been characterized.[6]

References

1. Verder E, Rosenthal SM: Role of infection in the delayed deaths of mice following extensive burn injury. Proc Soc Exper Biol Med 108:501-505, 1961.

2. Alexander JW, Moncrief JA: Alterations of the immune response following severe thermal injury. Arch Surg 93: 75-83, 1966.

3. Munster AM: Alterations of the host defense mechanism in burns. Surg Clin N Amer 50:1217-1225, 1970.

4. Nowell PC: Inhibition of human leukocyte mitosis by prednisolone in vitro. Cancer Res 21:1518-1521, 1961.

5. McIntyre OR, Eurenius K, Holland FC, Ebaugh FG Jr: Studies on the mechanism of hydrocortisone inhibition of the PHA response. In, Proceedings of the Third Annual Leukocyte Culture Conference, 1969, pp. 307-320.

6. Sanberg AA, Rosenthal H, Schneider SL, Slaunwhite WL: Protein-steroid interactions and their role in the transport and metabolism of steroids. In, Steroid Dynamics. Pincus, GT, Nakao, T, and Taid JF (eds). Academic Press, NY.

7. Walker HL, Mason AD Jr: A standard animal burn. J Trauma 8:1049-1051, 1968.

8. Guillemen R, Clayton GW, Libscomb HS, Smith JD: Fluorometric measurement of rat plasma and adrenal corticosterone concentration. J Lab Clin Med 53:830-832, 1959.

9. Trapp GA, West CD. Determination of corticosteroid binding proteins by an adsorption method. J Lab Clin Med 73:861-871, 1969.

10. Hume DM, Nelson DH, Miller DW. Blood and urinary 17-hydroxycorticosteroids in patients with severe burns. Ann Surg 143:316-329, 1956.

11. Gala RR, Westphal U: Further studies on the CBG in the rat: Proposed endocrine control. Endocrinology 79: 67-76, 1966.

12. Seal US, Doe RP: Vertebrate distribution of corticosteroid-binding globulin and some endocrine effects on concentration. Steroids 5:827-841, 1965.

13. Gala RR, Westphal U: Relationship between the pituitary gland and the corticosteroid-binding globulin in the rat. Endocrinology 78:277-285, 1966.

14. Brown WL, Bowler EG, Mason AD Jr: Studies of protein turnover in burn injury. US Army Inst of Surg Res Anl Prog Rpt, 30 Jun 1969, BAMC, Ft Sam Houston, Texas, Sect 50.

Presentations and/or Publications

None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA 0C 6983	71 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
70 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	3A061102B71P	08	068
b. CONTRIBUTING	61101A	3A061101A91C	00	
c. CONTRIBUTING				

11. TITLE (Procode with Security Classification Code): (U)Platelet and Megakaryocyte Kinetics in Burned Rat - Laboratory Study of Hematologic Changes occurring in Burned Soldiers (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
69 11	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 71	.27	7.2
	CURRENT 72	.27	7.9

19. RESPONSIBLE DOD ORGANIZATION
NAME: US Army Institute of Surgical Research
ADDRESS: Fort Sam Houston, Texas 78234
RESPONSIBLE INDIVIDUAL
NAME: PRUITT, B.A., Jr, LTC, 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: K. Eurenius, MAJ, MC
TELEPHONE: 512-221-5416
SOCIAL SECURITY ACCOUNT NUMBER:
ASSOCIATE INVESTIGATORS
NAME: P.W. Curreri, MAJ, MC
NAME: R.F. Mortensen, SP5, MS DA

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Procode EACH with Security Classification Code)
(U) Thrombocytosis; (U) Platelet survival; (U) Burn Thrombokinetics; (U) Megakaryocytes

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

23. (U) Document postburn thrombocytosis, platelet survival, and platelet and megakaryocyte kinetics.

24. (U) Platelet counts, chromium 51 tagged survival, bone marrow examination, label distribution and collection.

25. (U) 70 07 - 71 06 Thrombocytosis in burned animals, preceded by transient thrombocytopenia; shortened platelet survival; selective early increase in bone marrow megakaryocytopoiesis have been documented.

*Applicable to contractors upon originator's approval

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

ANNUAL PROGRESS REPORT

PROJECT NO. 3A061102B71P-08, BASIC RESEARCH IN SUPPORT OF MILITARY MEDICINE

REPORT TITLE: PLATELET AND MEGAKARYOCYTE KINETICS FOLLOWING THERMAL INJURY

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

1 July 1970 - 30 June 1971

Investigators:

Karl Eurenius, M.D., MAJ, MC
Richard Mortensen, M.S., SP-5

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A061102B71P-08, BASIC RESEARCH IN SUPPORT OF MILITARY MEDICINE

REPORT TITLE: PLATELET AND MEGAKARYOCYTE KINETICS FOLLOWING THERMAL INJURY

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

Period covered in this report: 1 July 1970 - 30 June 1971

Investigators: Karl Eurenius, M.D., MAJ, MC
Richard Mortensen, M.S., SP-5

Reports Control Symbol MEDDH-288(R1)

Significant thrombocythemia is a common event after burn injury. The following studies were performed to evaluate megakaryocyte kinetics (femur marrow chamber counts, nuclear maturation, cell diameter) and platelet kinetics (^{51}Chromium), in Sprague-Dawley rats inflicted with a 30% third-degree burn under pentobarbital anesthesia.

Following injury, there is a rapid increase in the number of rat femur megakaryocytes based upon total megakaryocytes/femur, or megakaryocytes/10^6 nucleated marrow cells, which reaches a maximum of four times normal by 48 hours, and remains twice normal by 30 days. A marked decrease in both cell diameter and lobe number is noted 24 hours post-injury indicating a new population of megakaryocytes which reach maturity in another 48 hours. Chromium labeled platelet survival is decreased by one-half during the immediate postburn period and rapidly returns toward normal. Platelet destruction is extrinsic and label deposition is increased only in the burned skin and urine.

Immediately following burn injury in the rat there is a significant drop in platelet survival, due, at least in part, to burn wound sequestration, with subsequent thrombocytopenia. During this same early period, there is a synchronous increase in new megakaryocytes followed by thrombocytosis. A sustained thrombopoietic stimulation is indicated in these studies by the

continuously high level of megakaryocyte and platelet concentration.

Thrombocytosis
Platelet survival
Burn thrombokinetics
Megakaryocytes

PLATELET AND MEGAKARYOCYTE KINETICS FOLLOWING THERMAL INJURY

Thrombocytosis is a common occurrence after thermal injury, and is associated with elevated fibrinogen concentration and factor V and VIII activity. Thrombocytopenia has also been observed in some burned patients and is thought to reflect platelet consumption. The dynamics of platelet production and survival are not well understood in burns. The present studies were undertaken to define these changes in a burned animal model.

Method

Sprague-Dawley rats were inflicted with 30%, third-degree scald burns. Daily platelet counts, ^{51}Cr blood volumes, and ^{51}Cr labeled platelet survival studies were performed using a phase microscopy and a gamma scintillation counter. Femoral bone marrow suspensions were examined for concentration, size and nuclear lobe number, using a standard hemacytometer, micrometer eye piece and staining with a modified May-Gruenwald-Giemsa technic.

Results

Blood volume corrected platelet concentration decreased to 50% of normal by 24 hours and then rose to 150% of normal during the next 30 days postburn.

^{51}Cr labeled platelet survival was significantly shortened (45% of normal) during the first five days postburn due to an extrinsic mechanism mediated, at least in part, by burn wound sequestration or consumption and increased urinary excretion of label. Survival had returned to normal by 30 days postburn, and no selective uptake above normal was ever noted in lung, liver, spleen or kidneys.

Bone marrow megakaryocyte concentration began to increase by one hour postburn, reaching a 5x normal concentration by 10 days and was still twice normal by 30 days postburn.

Megakaryocyte diameter and nuclear lobe number decreased during the first 24 hours, and then rose together to supernormal levels by four days and then returned to normal, suggesting the appearance of a cohort of new cells in response to the injury.

Discussion

These studies indicate that immediately following burn injury in the rat there is a significant, but brief, thrombocytopenia related to a drop in platelet survival due to burn wound sequestration. The bone marrow promptly responds by introducing a large new cohort of young megakaryocytes which synchronously enlarge and mature. The resultant thrombocytosis is sustained and reflects continued marrow megakaryocytopoeisis.

Conclusions

Platelet concentration in the burned patient is a reflection of the balance between production and survival, both of which are altered during the uncomplicated burn. The treatment of thrombocytopenia in burned patients should be directed toward the potential role of under-production (platelet transfusions) or over-destruction or consumption (? anticoagulants). The role of thrombocytosis in the potentiation of coagulation in burned subjects should be examined.

Presentations

Eurenius K.: Platelet and megakaryocyte kinetics following thermal injury. Presented at meeting of Amer. Fed. Clin. Res. in New Orleans, La., February 1971.

Eurenius K.: Platelet and megakaryocyte kinetics following thermal injury. Presented at meeting of Amer. Burn. Assn. in San Antonio, Tex., April 1971.

Publication

Eurenius K., Mortensen R.: Platelet kinetics after thermal injury. Clin Res, April 1971.(Abstr).

DISTRIBUTION LIST

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND, WASHINGTON, D.C. 20314 (5 copies)

DEFENSE DOCUMENTATION CENTER, ALEXANDRIA, VIRGINIA 22314 (12 copies)

U.S. ARMY COMBAT DEVELOPMENT COMMAND, MEDICAL DEPARTMENT AGENCY, BAMC, FORT SAM HOUSTON, TEXAS 78234 (1 copy)

PUBLICATIONS

1 July 1970 - 30 June 1971

Asch MJ, White MG, Pruitt BA, Jr.: Acid base changes associated with topical sulfamylon therapy: Retrospective study of 100 burn patients. Ann. Surg. 172: 946-950 (Dec) 1970.

White MG, Asch MJ: Acid-base effects of topical mafenide acetate in the burned patient. New Eng. J. Med. 284: 1281-1286 (June 10) 1971.

Bruck HM, Asch MJ, Pruitt BA, Jr.: Burns in children: A 10-year experience with 412 patients. J. Trauma 10: 658-662 (Aug) 1970.

Nash G, Foley FD, Pruitt BA, Jr.: Candida burn-wound invasion.A cause of systemic candidiasis. Arch. Path. 75-78 (July) 1970.

Curreri PW, Katz AJ, Dotin LN, Pruitt BA, Jr.: Coagulation abnormalities in the thermally injured patient. Current Topics in Surgical Research. Vol.2: 401-411, 1970.

Pruitt BA, Jr., Foley FD, Moncrief JA: Curling's ulcer: A clinical-pathology study of 323 cases. Ann.Surg. 172: 523-539 (Oct) 1970.

Pruitt BA,Jr.: Current treatment of thermal injury. Southern Med. J. 64: 657-662 (June) 1971.

Nash G, Asch MJ, Foley FD, Pruitt BA, Jr.: Disseminated cytomegalic inclusion disease in a burned adult. Jour. Amer. Med. Assn. 214: 587-589 (Oct 19),1970.

O'Neill JA, Jr., Ritchey CR, Mason AD, Jr., Villarreal Y: Effect of thermal burns on gastric mucous production. Surg. Gynec. Obstet. 131: 29-33 (July) 1970.

Zawacki BE, Pruitt BA,Jr.: Emergency treatment of burns. Amer. Family Phys/GP 2: 60-68 (July) 1970.

Moncrief JA, Pruitt BA, Jr.: Electric injury. Postgrad. Med. 48: 189-194 (Sep) 1970.

Curreri PW, Pruitt BA, Jr.: Evaluation and treatment of the burned patient. Amer. Jour. Occup. Therapy 24: 475-480 (Oct) 1970.

Munster AM, Hoagland HC, Pruitt BA, Jr.: Effect of thermal injury on serum immunoglobulins. Ann. Surg. 172: 965-969 (Dec) 1970.

Nash G,Foley FD,Goodwin MN,Jr.,Bruck HM, Greenwald KA,Pruitt BA, Jr.: Fungal burn wound infection. Jour. Amer. Med. Assn. 215: 1664-1666 (Mar 8) 1971.

Garfield JM, Allen GW, Silverstein P, Mendenhall MK: Flash fire in a reducing valve. Anesthesiology 34: 578-579 (June) 1971

Bowen JA, Nash G, Feldmann RJ: The goat as an artificial ventilation model. Lab. Animal Sci. 21: 252-255 (Apr) 1971.

Nash G, Foley FD: Herpetic infection of the middle and lower respiratory tract. Amer. Jour. Clin. Path. 54: 857-863 (Dec) 1970.

Pruitt BA, Jr., Mason AD, Jr., Moncrief JA: Hemodynamic changes in the early postburn patient: The influence of fluid administration and of a vasodilator (Hydralazine). Journ. Trauma 11: 36-46, 1971.

Curreri PW, Lindberg RB, DiVincenti FC, Pruitt BA, Jr.: Intravenous administration of carbenicillin for septicemia due to Pseudomonas aeruginosa following thermal injury. Jour. Infec. Dis. 122: Supplement: S40-S43 (Sep) 1970.

DiVincenti FC, Pruitt BA, Jr., Reckler JM: Inhalation injuries. Jour. Trauma 11: 109-117 (Feb) 1971.

Curreri PW, Wilmore DW, Mason AD, Jr., Newsome TW, Asch MJ, Pruitt BA, Jr.: Intracellular cation alterations following major trauma: Effect of supranormal caloric intake. Jour. Trauma 11: 390-396 (May) 1971.

Lindberg RB, Curreri PW, Pruitt BA,Jr.: Microbiology of burns treated with carbenicillin: Experimental and clinical observations. Jour. Infec. Dis. 122: Supplement: S34-S39 (Sep) 1970.

Pruitt BA, Jr.: Management of burns in the multiple injury patient. Surg. Clin. N. Amer. 50: 1283-1299 (Dec) 1970.

Kurtzman NA, Rogers PW, Harter HR: Neurotoxic reaction to penicillin and carbenicillin. Jour. Amer. Med. Assn. 214: 1320-1321 (Nov) 1970.

Pruitt BA, Jr., DiVincenti FC, Mason AD, Jr., Foley FD, Flemma, RJ: The occurrence and significance of pneumonia and other

pulmonary complications in burned patients:Comparison of conventional and topical treatments.Jour. Trauma 10:519-531 (Jul) 1970.

Bruck HM, Nash G, Foley FD, Pruitt BA, Jr.: Opportunistic fungal infection of the burn wound with Phycomycetes and Aspergillus: A clinical-pathologic review. Arch. Surg. 102: 476-482 (May) 1971.

Foley FD: Pathology of cutaneous burns. Surg. Clin. N. Amer. 50: 1201-1210 (Dec) 1970.

Nash G, Moylan JA, Jr.: Paradoxical catheter embolism. Arch. Surg. 102: 213 (Mar) 1971.

Pruitt BA, Jr.: Recent advances in burn treatment. Surg. 68: 412-418 (Aug) 1970.

Kurtzman NA: Relationship of extracellular volume and CO_2 tension to renal bicarbonate reabsorption. Amer. Jour. Physiol. 219:1299-1304 (Nov) 1970.

Nash G, Bowen JA, Langlinais PC: Respirator lung: A misnomer. Arch. Path. 91: 234-240 (Mar) 1971.

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

Wilmore DW, Curreri PW, Spitzer KW, Spitzer ME, Pruitt BA, Jr.: Supranormal dietary intake in thermally injured hypermetabolic patients. Surg. Gynec. Obstet. 132: 881-886 (May) 1971.

Curreri PW, Asch MJ, Pruitt BA, Jr.: The treatment of chemical burns: Specialized diagnostic, therapeutic and prognostic considerations. Jour. Trauma 10: 634-642 (Aug) 1970.

Kurtzman NA, White MG, Rogers PW: Aldosterone deficiency and renal bicarbonate reabsorptions. Jour.Lab. Clin. Med. 77: 931-940 (June) 1971.