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AD A088190

REPORT MEDDH-286(R1)

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ANNUAL RESEARCH PROGRESS REPORT

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

1 October 1978 - 30 September 1979

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Prepared for:
US ARMY MEDICAL RESEARCH & DEVELOPMENT COMMAND
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SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered) A057761

REPORT DOCUMENTATION PAGE

READ INSTRUCTIONS BEFORE COMPLETING FORM

1. REPORT NUMBER	2. GOVT ACCESSION NO. AD-A088 190
3. RECIPIENT'S CATALOG NUMBER	
4. TITLE (and Subtitle) Annual Research Progress Report, 1 Oct 78 - 30 Sep 1979	5. TYPE OF REPORT & PERIOD COVERED 1 Oct 78 - 30 Sep 79
	6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s)	8. CONTRACT OR GRANT NUMBER(s)
9. PERFORMING ORGANIZATION NAME AND ADDRESS US Army Institute of Surgical Research ✓ Fort Sam Houston, Texas 78234	10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS 3A161101A91C-00 3S161102BS05-00 3S162772A814-00
11. CONTROLLING OFFICE NAME AND ADDRESS US Army Medical Research & Development Command Fort Detrick Frederick, MD 21701	12. REPORT DATE 30 September 1979
	13. NUMBER OF PAGES 116
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)	15. SECURITY CLASS. (of this report)
	15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

Approved for public release. Distribution unlimited.

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

18. SUPPLEMENTARY NOTES

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

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

This report documents the clinical and laboratory activities of the US Army Institute of Surgical Research during the fiscal year 1979. These activities include patient care, clinical investigation and laboratory research in the areas of (1) burn injury, (2) acute renal failure, and (3) general trauma. Special emphasis is placed on the clinical management of burned patients and on studies related to prevention and treatment of burned wound infection.

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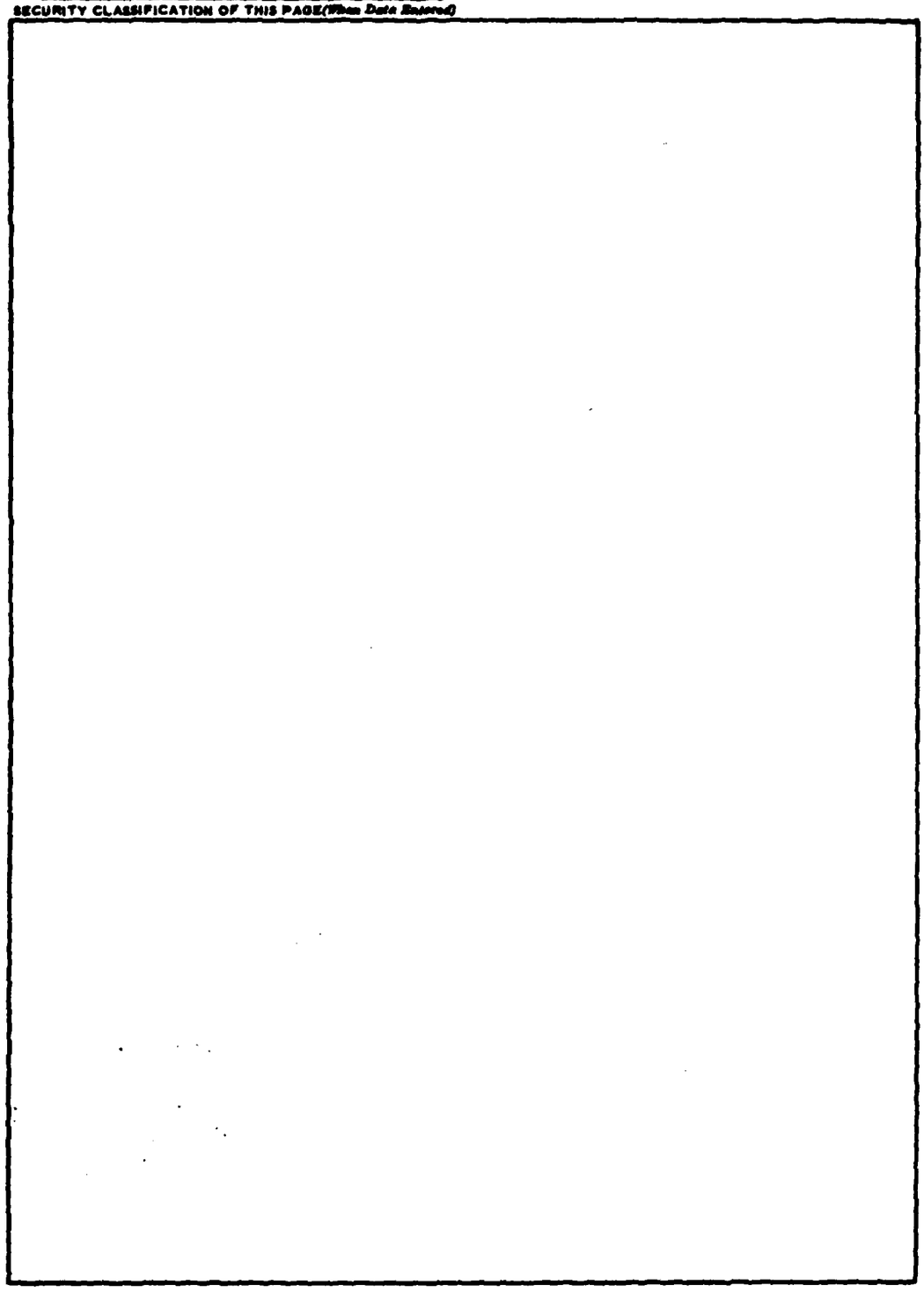
SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

4/04/82



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

SGRD-US

SUBJECT: Annual Research Report FY 1979

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Annual report(s) of the US Army Institute of Surgical Research for FY-79 are forwarded under provisions of the OTSG Regulation 70-31, dated 2 April 1969.

Basil A. Pruitt, Jr.

BASIL A. PRUITT, JR., M.D.
COLONEL, MC
COMMANDER & DIRECTOR

FOREWORD

"The essential wildness of science as a manifestation of human behavior is not generally perceived.....

It cannot be prearranged in any precise way; the minds cannot be lined up in tidy rows and given directions from printed sheets. You cannot get it done by instructing each mind to make this or that piece, for central committees to fit with the pieces made by the other instructed minds. It does not work that way.

What it needs is for the air to be made right..... If the air is right the science will come in its own season like pure honey."

Lewis Thomas "The Lives of a Cell"

The provision of resources is certainly a necessary, proper, and appreciated function of central committees and the Institute is certainly indebted to the Medical Department in general and the Medical R&D Command in particular for such support. It is obvious however, from Dr. Thomas' remarks, that the "air quality" can scarcely be influenced by a central committee and the very nature of science may vitiate the current emphasis and attempts of governmental bodies to direct research. Committees and management techniques to "direct" science would seem to contain their own inborn errors of metabolism which preordain functional ineffectiveness and a limited future. In fact one might anticipate that such central management groups might only promote the generation of scientific smog.

The studies reported in this volume have addressed clinically important problems of a categorical disease, thermal injury, but the data generated have direct application to the care and treatment of all injured soldiers. These data have been derived from efforts focused on problems of significance identified in the course of caring for burn patients. The "air" was "made right" by the combination of patient density and the enthusiasm, insight, and effective interplay between the Clinical and Laboratory staff of the Institute. As is also characteristic, solution of each problem has been quickly followed by the identification of new problems and questions and in that sense the process contains its own mechanism for amplification, guidance and expansion which could be termed the inertia or even acceleration

of scientific motion. The air quality of the Institute is well indexed by the progressive change over the past three decades from enumeration studies of cause of death through studies of whole body response to injury., to the reports herein of organ and even cell-specific effects of injury in man. Such progression has been rewarded by identification of universal characteristics of basic disease processes and by the development of physiologically based surgical and pharmacologic techniques to improve survival of the combat injured soldier.

Basil A. Pruitt, Jr.

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

The opinions expressed above are the private views of the author and are not to be construed as official or as reflecting the views of the US Army Medical Research & Development Command, the Department of The Army or the Department of Defense.

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RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA OA 6380	79 10 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
78 10 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	62772A	3S162772A814	00	118
b. COSATI///////	61102A	3S161102BS05	00	089
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)*

(U) Clinical Operation, Center For Treatment of Burned Soldiers (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*

003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
50 07	Cont	DA	C. In-House

17. CONTRACT/GRANT: Not Applicable

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:*

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 79	50	1182
FISCAL YEAR CURRENT 80	50	1186

19. RESPONSIBLE DOD ORGANIZATION

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ASSOCIATE INVESTIGATORS

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NAME:

21. GENERAL USE

FOREIGN INTELLIGENCE NOT CONSIDERED

DA

22. KEYWORDS (Precede EACH with Security Classification Code)

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

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

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

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

25. (U) 7810 - 7909 Two hundred sixty eight seriously burned patients were admitted and treated during 1978. Studies of effectiveness of prevention of massive upper gastrointestinal hemorrhage indicated that no patients required operative intervention for such complications. The treatment protocol developed at this Institute has thus eliminated Curling's ulcers as a significant cause of morbidity and mortality. Continued investigation of the metabolic response to and the nutritional support of acutely burned patients has further delineated basic disease mechanisms which serve as a guide for the support of acutely burned patients and as a model for injured man as well. Studies of pulmonary function following crystalloid and colloid intravenous fluid resuscitation are identifying advantages and disadvantages of such treatment.

*Available to contractors upon originator's approval.

ANNUAL PROGRESS REPORT

PROJECT NO. 3S162772A814-00, APPLIED RESEARCH

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

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

1 January 1978 - 31 December 1978

Investigators:

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Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3S162772A814-00, APPLIED RESEARCH

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

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

Period covered in this report: 1 January - 31 December 1978

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Two hundred and sixty eight patients were admitted to the Clinical Division of the United States Army Institute of Surgical Research during the calendar year of 1978. The emphasis of the Clinical Division remains a tripartite one to include optimal care for thermal, chemical and electrically injured patients, investigation of response to injury and education of health professionals. The iterative process of recognition of a clinical problem, subsequent laboratory and the patient based investigation with resultant recommendations for improved care of such patients has been and continues to be scientifically productive. Major areas of such investigation include documentation of pulmonary response to injury and resuscitation, metabolic and endocrine responses to injury, and infection control and treatment. This report summarizes the activities of the Clinical Division of the U.S. Army Institute of Surgical Research during the calendar year 1978 and catalogues the responses to treatment and complications which have contributed to morbidity and mortality of these critically injured patients.

CLINICAL OPERATION, CENTER FOR TREATMENT OF BURNED SOLDIERS

The Clinical Division of the United States Army Institute of Surgical Research continued throughout the calendar year 1978 to provide clinical care for the thermally injured soldier and other authorized patients.

Two hundred and sixty eight patients were admitted during the period of this report. There were 115 aeromedical evacuation flights for 139 patients of which 111 were within the Continental United States for a total of 133 patients. The OCONUS flights were to Santo Domingo, Hawaii, and Panama for a total of six patients. Fifty-two percent of all admissions were air evacuated by ISR burn teams. The first 100 air evacuation patients (78 males, 22 females, mean age 28 years) with an average 42% total body surface burn were studied. Of 94 patients in the Continental United States, 84 were seen by the burn team within an average of 10 hours post-injury, and 10 were evacuated at 3 to 8 days. Six patients received from outside the Continental United States were treated by the burn flight team within an average of 21 hours post-injury. Associated injury (long bone fracture, inhalation injury, concussion) was present in 45% of patients. When first seen by the burn team, 80% of patients were clinically stable, while 20% needed immediate treatment of: hypotension (6), oliguria (4), hypothermia (5), and unrecognized injury (5). Treatment prior to evacuation consisted of: catheter placement -- IV (14), Foley (3), NG (9); IV fluid push (10); and respiratory therapy -- intubation (3), institution of mechanical ventilation (5), tracheostomy (2), tube thoracostomy (1). In flight, all patients required routine monitoring and IV fluids, while 23% required additional treatment: IV catheter (2), respiratory therapy (11), escharotomy (3), IV fluid push (2), IV medications (8). On arrival at the burn unit, 95% of patients were considered to be clinically stable, while three were hypotensive ($<$ 90 mm Hg), and two were unconscious (carbon monoxide poisoning). These results demonstrate that critically ill patients can be safely transported when proper treatment has been administered both prior to and during air evacuation.

CLINICAL MANAGEMENT

The overall management of the thermal, chemical and electrically injured patient as practiced by this Institute has been adequately documented in previous Annual Reports and numerous scientific presentations and publications.

RESEARCH

Areas of continued investigation include the pulmonary response to injury and resuscitation, injury induced metabolic change, nutritional support of the compromised host, methods of infection control, and the endocrine response to injury and infection.

EDUCATION

Throughout the calendar year 1978, the staff of the Clinical Division of the Institute of Surgical Research conducted extensive educational activities for military and civilian professional and paraprofessional personnel. Sixteen resident physicians (13 Army and 2 Air Force) from military graduate training programs, 1 civilian resident, 8 medical students, 5 reserve and National Guard physicians were attached to the Institute of Surgical Research for education and experience in the care of thermally injured patients. Fourteen scientific publications appeared in refereed medical journals and 168 scientific presentations were conducted for military and civilian audiences. Formal educational rounds are conducted by the Institute staff for the Brooke Army Medical Center General Surgical house officers and staff. Numerous presentations at the Academy of Health Sciences and various military installations throughout the Continental United States were also conducted. In addition weekly professional conferences were conducted for and by Institute personnel.

MORBIDITY AND MORTALITY

Sixty nine of 268 patients for whom disposition was made during the calendar year 1978 died in the hospital for an overall mortality of 25.7%. Seventy four percent of hospital deaths had autopsies performed. The average total body surface injury of patients who died was 55.2% and the average third degree burn component was 33%. Thirteen patients who died (18.8%) were under 15 years of age with an average burn size of 67% of the total body surface. Eleven of these 13 patients (84.6%) had autopsies performed. Thirty five of the 69 patients who died (51%) had documented inhalation injury as a contributing or primary cause of death. This apparent increase in patients with acute inhalation injury is probably a reflection of an increased ability to diagnose inhalation injury rather than a real increase in the proportion of burn patients with inhalation injury.

COMPLICATIONS

Infection continues to be the most common problem following thermal, chemical or electric injury. One hundred and ten patients had positive blood cultures reported. The single most common organism recovered from blood cultures was Staphylococcus aureus coagulase positive in 95 patients, followed by Pseudomonas aeruginosa in 29 patients, E. coli in 14 patients, Klebsiella pneumoniae in 12 patients, Enterobacter species in 11 patients and Proteus species in 10 patients. Bacterial burn wound invasion was histologically and/or culturally identified in 41 patients, Candida burn wound infection in 2 patients, Aspergillus burn wound infection in 11 patients and Phycomycotic burn wound infection in 7 patients.

Ninety six patients (35.8%) had associated injuries. Seventy two

of these 96 patients had inhalation injury, three patients had major fractures, 13 patients had head injuries, 3 patients had corneal burns, while 2 additional patients had corneal abrasions and 1 patient had generalized blast injury. Gastrointestinal complications included minimal hemorrhage in 25 patients. However, again in the calendar year 1978 no patient has required operation for acute gastrointestinal hemorrhage or perforation which has been the experience since 1975 after the institution of prophylactic antacid/Cimetadine therapy to maintain the gastric pH above 5 and prevent stress ulceration. In addition two patients had acute cholecystitis, two patients had cholelithiasis and one patient required celiotomy, cholecystostomy and drainage for acute cholecystitis.

Two patients were pregnant when injured and both successfully completed their pregnancy without complication.

Forty two patients (15.7%) were diagnosed as having acute renal failure of which 18 patients had acute tubular necrosis, one patient had myoglobinuric nephrosis and two patients required hemodialysis. Most commonly the acute renal failure was a terminal event following sepsis/hypotension.

Cardiac complications during 1978 included two documented acute myocardial infarctions, three patients with symptomatic acute congestive heart failure, four patients with acute bacterial endocarditis and one patient with an acute pericardial effusion requiring pericardotomy.

Pulmonary complications included 72 patients (27% of admissions) who had documented inhalation injury, 56 patients who had bronchopneumonia, 2 patients had documented pulmonary emboli and 1 patient who had tracheal necrosis following heat injury of the larynx and trachea.

STATISTICAL RESUME DURING CALENDAR YEAR 1978: 268 thermally injured patients were admitted to the Institute of Surgical Research and there were 268 dispositions during the same period. Subsequent data will be based on dispositions. There were 198 males and 70 females with an average age of 26.8 years ranging from 4 months to 92 years of age. Seventy patients were under 15 years of age and 51 patients were over 45 years of age. The average total burn size was 32.3% with an average full thickness burn size of 14%. The average hospital stay for all patients was 37.7 days, however excluding convalescent leave for active duty military reduces the average hospital stay to 35.8 days. One hundred and one patients (37.7%) were admitted on the day of injury and the average post burn day of admission was 3.2 days.

During 1978 1,391 operative procedures were performed on 202 patients for an average of 5.2 operations per patient. Four hundred and thirty five anesthetic procedures were administered for an average of 1.6 per patients. One hundred and thirty nine patients had autografting performed and 105 patients had biologic dressings applied, a total of

343 allograft applications.

Table 1 identifies the source of admission of patients during calendar year 1978 and again the majority of patients were from the Continental United States. Table 2 summarizes the burn etiology and Table 3 summarizes the effect of age and total body surface injury on mortality. Table 4 lists mortality rate associated with increments of 10% total body surface burn involvement for the years 1975 through 1978. The overall mortality has continued to decline throughout this period. Of interest there were four surviving patients who had burns exceeding 80% of the total body surface which was an unprecedented number of survivors with such large injury.

SUMMARY

A total of 268 patients were admitted to the U.S. Army Institute of Surgical Research and 268 dispositions were made during calendary year 1978. Infection continued to be the most common cause of mortality. As in previous years no upper gastrointestinal hemorrhage required operation for control in 1978. The iterative process of clinical recognition of problem or complication which then can be examined in the laboratory and the solution developed then to be implemented in severely injured patients has continued to be a successful process at this Institute and has led to improved care of the injured patient.

Table 1. Source of Admission, 1978

Area	A	AD	AF	AFD	N	ND	VAB	Other	TOTAL
1st Army	3	2	1	0	0	0	3	1	10
3rd Army	4	3	2	1	3	1	7	13	34
5th Army	20	14	6	9	6	1	9	124	189
6th Army	3	3	0	4	1	0	1	3	15
Germany	1	3	0	0	0	0	0	0	4
Equador	0	0	0	0	0	0	0	1	1
Korea	0	2	0	0	0	0	0	0	2
Hawaii	0	0	0	0	0	1	0	1	2
Okinawa	0	0	0	0	1	0	0	0	1
Panama	0	0	0	0	1	0	0	3	4
Mexico	0	0	0	0	0	0	0	2	2
Turkey	0	0	0	2	0	0	0	0	2
M.D.W.	1	0	0	0	0	0	0	0	1
Dominican Republic	0	0	0	0	0	0	0	1	1
	32	27	9	16	12	3	20	149	268

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

Table 2. Burn Etiology, 1978 - 268 Dispositions

Causes	Number of Patients	% Disposition	Deaths	% Mortality
Gasoline & Kerosene	65	24.3%	17	26.2%
Structural Fires	25	9.3%	15	60.0%
Motor Vehicle Accidents	19	7.1%	6	31.6%
Aircraft Accidents	1	0.4%	0	0.0%
Open Flames	28	10.4%	10	35.7%
Electrical	17	6.3%	1	5.9%
Hot Liquid	41	15.3%	6	14.6%
Chemical	2	0.75%	0	0.0%
Others	26	9.7%	3	11.5%
Butane, Propane or Natural, Sewer Gas Exp.	22	8.2%	2	9.0%
Smoking Clothes Ignited	9	3.4%	7	77.8%
Bomb, Shell, Simulator Grenade, Gunpowder Exp.	5	1.9%	1	20.0%
Welding	4	1.5%	1	25.0%
Contact	3	1.1%	0	0.0%
Contact Glue caught fire	1	0.4%	0	0.0%
TOTAL	268		69	

Table 3. Age, Body Surface Involvement & Mortality, 1978

Age (Yrs)	Per Cent Burn										Total Cases	Total Deaths	% Mortality
	0-10	10-20	20-30	30-40	40-50	50-60	60-70	70-80	80-90	90-100			
0-1	1	2	1	0	0	0	1	0	0	0	5	0	0.0
1-2	1	3	6	3	2(1)	0	0	0	1(1)	1(1)	17	3	17.6
2-3	1	2	3	0	0	0	3(2)	2(2)	1(1)	0	12	5	41.7
3-4	0	3	3(1)	0	0	1	0	0	0	0	7	1	14.3
4-5	1	1	0	1(1)	0	0	0	0	0	0	3	1	33.3
5-10	3	2	4	1	1	0	0	0	0	1(1)	12	1	8.3
10-15	3	3	5	1	0	0	2(2)	0	0	0	14	2	14.3
15-20	3	6	3(1)	4(1)	4	2	4(3)	1(1)	3	0	30	6	20.0
20-30	21	14(1)	7	11(1)	5(1)	3	5(2)	3(3)	0	0	69	8	11.6
30-40	5	4	1	7	5	7(4)	4(2)	3(3)	1	0	37	9	24.3
40-50	3	4	4	2(1)	4(2)	0	0	2(2)	0	2(2)	21	7	33.3
50-60	3	3(2)	4	2(2)	3(2)	2(2)	1(1)	1(1)	0	1(1)	20	11	55.0
60-70	3	1	2(1)	3(2)	2(2)	1(1)	0	0	0	1(1)	13	7	53.8
70-80	0	1(1)	2(2)	0	0	1(1)	0	0	0	0	4	4	100
80-90	0	0	1(1)	2(2)	0	0	0	0	0	0	3	3	100
90-100	0	0	0	0	1(1)	0	0	0	0	0	1	1	100
Total	48	49	46	37	27	17	20	12	6	6	268		
Deaths	0	4	6	10	9	8	12	12	2	6		69	
% Mortality	0	8.2	13	27	33.3	47	60	100	33	100			25.7

Note: Deaths shown in parentheses.

Table 4. Per Cent Body Surface Involvement and Mortality, 1975 - 1978

% Burn	0-10	10-20	20-30	30-40	40-50	50-60	60-70	70-80	80-90	90-100	Total
					(1975)						
No. Burned	20	36	29	42	28	28	34	19	13	5	254
Deaths	0	1	4	11	8	14	23	16	12	5	94
% Mortality	0	2.8	13.8	26.2	28.6	50	67.6	84.2	92.3	100	37
					(1976)						
No. Burned	28	49	39	30	31	27	19	15	13	9	260
Deaths	0	2	3	4	10	13	12	13	13	9	79
% Mortality	0	4.1	7.7	13.3	32.3	48.1	63.2	86.7	100	100	30.4
					(1977)						
No. Burned	37	35	32	46	24	20	18	12	6	4	234
Deaths	0	1	5	10	9	11	14	11	5	4	70
% Mortality	0	2.9	15.6	21.7	37.5	55	77.8	91.7	83.3	100	29.9
					(1978)						
No. Burned	48	49	46	37	27	17	20	12	6	6	268
Deaths	0	4	6	10	9	8	12	12	2	6	69
% Mortality	0	8.2	13.0	27.0	33.3	47.0	60.0	100	33.3	100	25.8

Table 5. Survival and Death by Year for Patients With Extensive Burns, 1956-1978

Year	Survivors (burns over 30%)			Deaths		
	No. Cases	Average % Burn		No. Cases	Average % Burn	
		Total	3°		Total	3°
1956	22	41.0	17.3	20	57.8	37.8
1957	19	38.4	24.1	17	57.1	38.8
1958	15	42.3	21.6	23	56.5	35.3
1959	29	43.1	20.6	24	63.1	38.1
1960	17	44.2	20.1	30	57.8	37.3
1961	18	44.2	25.0	31	58.0	39.7
1962	18	42.7	21.4	54	59.1	46.2
1963	28	45.8	19.6	57	69.0	41.0
1964	40	41.8	14.8	37	65.0	42.4
1965	47	43.8	21.0	33	66.0	33.4
1966	68	41.5	14.9	59	59.9	31.3
1967	103	42.7	13.3	51	59.9	32.3
1968	143	44.2	12.6	38	54.6	24.6
1969	113	43.2	11.1	70	58.7	26.4
1970	92	39.4	10.7	70	51.9	32.6
1971	63	41.9	14.0	68	60.8	38.0
1972	62	42.0	17.2	103	56.7	35.9
1973	47	43.7	19.6	113	60.3	36.2
1974	55	43.9	12.2	97	60.8	35.9
1975	80	46.1	14.7	94	61.3	32.8
1976	69	45.5	15.0	79	64.2	31.1
1977	66	42.2	14.4	70	56.9	29.0
1978	67	45.7	14.8	69	55.2	33.0

Table 6. Comparison of Burn Mortality Rates, 1962-1963 and 1964-1978

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
1964-78	2051	71	3.5	618	115	18.6	505	162	32.0	339	169	49.9	634	534	84.2

Table 7. Cause of Death, 1978

Patient	Age	Sex	% Burn Total	% Burn 3°	PBD Death	Cause of Death
1	61	M	98	0	3	Bronchopneumonia and Inhalation Injury
2	43	F	95	95	!	*95% total body surface burn
3	1	M	94.5	94.5	0	94.5% total body surface burn and Inhalation injury
4	51	M	93	93	1	93% total body surface burn and Inhalation injury
5	41	M	93	72	1	93% total body surface burn
6	6	F	92	55	8	Staphylococcal Pneumonitis and septicemia
7	1 7/12	M	87	74	0	*87% total body surface burn
8	2	M	86	74	1	86% total body surface burn and inhalation injury
9	2	M	79	60.5	22	Staphylococcal septicemia secondary to bronchopneumonia
10	26	M	79	48.5	6	79% total body surface burn and Inhalation injury
11	21	M	76	46	2	Inhalation injury
12	40	F	75	39	16	*Septicemia, E. coli and Staphylococcus aureus; fungal burn wound sepsis
13	40	M	74	25	21	Acute myocardial infarction and bronchopneumonia
14	50	M	72	72	8	*Staphylococcus aureus septicemia and Inhalation injury
15	18	M	72	0	11	Bilateral bronchopneumonia
16	23	M	71	71	8	Bronchopneumonia and Phycomycotic burn wound invasion
17	37	M	71	38.5	46	Bronchopneumonia
18	39	M	70.5	24.5	20	*Inhalation injury and bronchopneumonia

* Autopsy not performed

Table 7. Cause of Death, 1978 (continued)

Patient	Age	Sex	% Burn Total	% Burn 3°	PBD Death	Cause of Death
19	35	F	70.5	17.5	3	Inhalation injury
20	2 6/12	M	70	65	29	Bronchopneumonia and hemothorax
21	23	M	68.5	16.5	7	Inhalation injury, Klebsiella, Enterobacter and Serratia septicemia
22	18	M	67.5	25.5	11	Inhalation injury and bronchopneumonia
23	13	M	66	43	37	Bronchopneumonia and acute bacterial endocarditis Staphylococcus aureus and Pseudomonas
24	11	F	66	37	29	Bronchopneumonia and Pseudomonas septicemia
25	30	M	66	19	15	Bronchopneumonia
26	50	M	65.5	61	37	Inhalation injury and Pseudomonas and Staph. aureus septicemia
27	32	M	65	32.5	7	Inhalation injury, bronchopneumonia and E. coli septicemia
28	2	M	65	0	22	Bronchopneumonia and herpes simplex cerebritis
29	28	F	64	31.5	53	Invasive burn wound sepsis, Pseudomonas and Pseudomonas septicemia
30	2	M	62.5	0	5	*Inhalation injury
31	18	M	62	61	11	Bronchopneumonia bilateral
32	19	M	61	47	50	Bronchopneumonia and inhalation injury
33	30	M	57	26	70	Bronchopneumonia and E. coli and Klebsiella septicemia
34	32	F	57	8	53	Airway obstruction, necrotic and hemorrhagic; Staphylococcus and Pseudomonas septicemia
35	38	F	55.5	36	7	Inhalation injury and bronchopneumonia

* Autopsy not performed

Table 7. Cause of Death, 1978 (continued)

Patient	Age	Sex	% Burn Total	% Burn 3°	PBD Death	Cause of Death
36	36	M	55	18	104	Inhalation injury and bronchopneumonia
37	58	F	53	51.5	22	Bronchopneumonia and Staphylococcus aureus septicemia
38	62	M	52.5	42	17	*Acute congestive heart failure and Staphylococcal septicemia
39	71	M	52.5	11.5	25	*Invasive burn wound sepsis and Pseudomonas septicemia
40	56	M	51	13.5	7	Acute inhalation injury
41	62	M	49	16.5	79	Inhalation injury, bronchopneumonia and Staphylococcus aureus, Proteus and Enterobacter cloacae septicemia
42	61	M	49	15	15	Bronchopneumonia and Pseudomonas septicemia
43	92	F	48	19	5	Acute myocardial infarction
44	54	M	47.5	43.5	19	Inhalation injury, bronchopneumonia and Pseudomonas septicemia
45	48	M	47	46.5	32	Bronchopneumonia and inhalation injury
46	1 10/12	M	45.5	21.5	3	Inhalation injury and bronchopneumonia
47	45	M	44	41.5	44	Bronchopneumonia
48	22	M	42	41.5	11	*Pseudomonas invasive burn wound sepsis
49	53	M	41	41	2	*Acute congestive heart failure
50	19	M	38.5	24	162	Inhalation injury and bronchopneumonia
51	52	M	38	30.5	44	Acute bronchopneumonia
52	80	F	38	30.5	9	Inhalation injury and bronchopneumonia

* Autopsy not performed

Table 7. Cause of Death, 1978 (continued)

Patient	Age	Sex	% Burn Total	% Burn 3°	PBD Death	Cause of Death
53	60	M	37.5	37.5	47	*Bronchopneumonia, Inhalation injury and Pseudomonas and Staphylococcal septicemia
54	57	F	37	32	1	Inhalation injury and alcoholic myocardopathy
55	4	F	37	0	15	Invasive burn wound sepsis and Pseudomonas septicemia
56	40	F	36.5	16	23	Inhalation injury and bronchopneumonia
57	29	M	35.5	13	1	Acute tubular necrosis secondary to extensive myonecrosis of electric injury
58	83	M	34.5	34.5	0	Inhalation injury
59	68	F	30.5	7.5	16	*Inhalation injury, bronchopneumonia and Staphylococcal septicemia
60	84	F	28	10	10	Bronchopneumonia
61	70	F	25	25	41	Bronchopneumonia and Pseudomonas septicemia
62	3	M	22.5	22.5	8	Inhalation injury and bronchopneumonia
63	15	F	22.5	20	12	Inhalation injury and bronchopneumonia
64	65	M	21.5	0	11	*ASHD and acute renal failure
65	72	M	21	21	27	*Bronchopneumonia and pulmonary embolus
66	22	M	19	0	8	*Inhalation injury and bronchopneumonia
67	52	M	19	0	4	*Inhalation injury and bronchopneumonia
68	78	M	12	12	40	Inhalation injury and multiple pulmonary fat emboli
69	56	M	12	6	58	*Acute hepatic failure secondary to [illegible] cirrhosis

* Autopsy not performed

PRESENTATIONS

Pruitt BA Jr: Treatment of Electrical Burns. Plastic Surg Conf. San Antonio Baptist Hospital, San Antonio, TX 6 Jan 78.

Treat RC: Treatment of Burns. Physical Therapy students, Academy of Health Sciences, Fort Sam Houston, TX 9 Jan 78.

Pruitt BA Jr: The Total Care for a Burn Patient. Houston-Galveston Area Society of Hospital Pharmacists. Houston, TX 12 Jan 78.

Pruitt BA Jr: Burns. Emergency Medical Care Seminar. Univ of Tennessee Center for the Health Sciences. Memphis, TN 13 Jan 78.

Treat RC: Care of the Burn Patient. 5501st Reserve Hospital Group, Fort Sam Houston, TX 16 Jan 78.

Treat RC: Modern Burn Therapy. Residents USAF Sch of Aerospace Med, Brooks AFB, TX 18 Jan 78.

Pruitt BA Jr: 1) Gastrointestinal Complication of Thermal Injury; 2) Metabolic Changes and Nutrition Support of the Severely Injured Patient; 3) Non-Bacterial Surgical Infections, 4) Fluid Resuscitation of the Extensively Burned Patient and 5) Treatment of Burns. Annual mtg Southeastern Surgical Congress. New Orleans, LA 20-24 Jan 78.

Treat RC: Treatment of Burns. Officers Basic Course. Academy of Health Sciences, Fort Sam Houston, TX 24 Jan 78.

Treat RC: Resuscitation of the Burn Patient. 5501st Reserve Hospital Group, Fort Sam Houston, TX 30 Jan 78.

Lescher TJ: Classification of burns. Intensive Care Nurse Clinician Course students, BAMC, Fort Sam Houston, TX 8 Feb 78.

Pieniadz C: Wound Therapy. Intensive Care Nurse Clinician Course students, BAMC, Fort Sam Houston, TX 9 Feb 78.

The following presentations were made at the Burn Seminar in Shreveport, LA on 9 and 10 Feb 78:

Treat RC: Treatment of the Burn Patient.
Jesse NF: The Physical Therapy Program for the Burn Patient
Ford DT: Occupational Therapy: Splinting Devices Utilized in the Treatment of the Burn Patient
Pesut DJ: Psychological Problems of the Burn Patient
Hunter EC: Nursing Considerations in the Burn Patient
Zitzka CA: Nutritional Support for the Burn Patient

Treat RC: Care of the Burn Patient. 5501st Reserve Hospital Group, Fort Sam Houston, TX 13 Feb 78.

Lescher TJ: Initial Care of the Burn Patient. BAMC interns AMIC-ER, Fort Sam Houston, TX 14 Feb 78.

Treat RC: Care of the Burn Patient at the Scene of the Accident. Airport Managers Conference, San Antonio, TX 14 Feb 78.

Pruitt BA Jr: Triage and Early Care of Patients Injured in Aircraft Accidents. Airport Managers Disaster Symposium, San Antonio, TX 15 Feb 78.

Treat RC: Complications of Burns. Intensive Care Nurse Clinician Course students, BAMC, Fort Sam Houston, TX 16 Feb 78.

Goodwin CW: The Role of Medicine Today. Science Fair, University of Texas at Austin, TX 17 Feb 78.

The following presentations were made at a two day seminar "Immediate Burn Care: For the Occupational Health Worker" sponsored by the School of Nursing, University of Michigan, Ann Arbor, MI 25 Feb 78.

Treat RC: Modern Burn Therapy
Pesut DJ: Psychological Problems of the Burn Patient

Treat RC: Complications of Burns. 5501st Reserve Hospital Group, Fort Sam Houston, TX 27 Feb 78.

Treat RC: Care of the Burn Patient. Safety Engineers, EMT's and personnel of the City and County Health Departments of Corpus Christi, TX 2 Mar 78.

Pruitt BA Jr: Recent Advances in Burn Care. Surgical Grand Rounds. University of Colorado, Denver, CO 4 Mar 78.

Pruitt BA Jr: Burn Care. Staff Conference. St. Mary-Corwin Hosp, Pueblo, CO 7 Mar 78.

Pruitt BA Jr: Care of the Burned Child. Staff Conference. Denver Childrens Hospital, Denver, CO 7 Mar 78.

Pruitt BA Jr: Treatment of Inhalation Injuries. Staff Conference. Weld County Hospital, Greeley, CO 8 Mar 78.

Treat RC: Treatment of Burns. In-Service Education mtg, Scott AFB Aeromedical Evacuation Squadron, Scott AFB, IL 6 and 8 Mar 78.

Pruitt BA Jr: 1) Acute Management of Massive Body Burns; 2) Stress Ulcers; 3) Nutritional Support of the Burn Injured Patient; 4) Early Diagnosis and Management of Inhalation Burn Injury; and 5) Techniques for Immediate Care of the Burn Patient. Critical Care Course. Las Vegas, NV 15-19 Mar 78.

The following presentations were made at the American Burn Assn Anl Mtg in Birmingham, AL 30 Mar - 1 Apr 1978:

Lescher TJ: Antennas, Electricity and Amputations
Dorethy JF: Evaluation of Left Ventricular Function During Early Postburn Resuscitation: Lack of Evidence for a Clinical Myocardial Depressant Factor
Aulick LH: Distribution and Control of Peripheral Blood Flow in Burn patients
Pieniadz CJ: A Conceptual Model for Burn Nursing
Pesut DJ: Relationship Between Psychological Symptoms, Stressful Life Events, and Depressed Mood in Burn Nursing Personnel

The following presentations were made at the Burns Workshop. ND State University Department of Nursing, Fargo ND 7-8 Apr 78:

Dyer A: Nursing Care of Burn Patients
Henderson N: Physical Therapy and the Burn Patient
Goodwin CW: 1) Pathophysiology of Burns; 2) Medical Management of Burns During Emergency; and 3) Management of Burn During the Acute Phase.

Lescher TJ: 1) Care of the Burn Patient - Acute Phase and 2) Gastrointestinal Complications in Thermally Injured Patients. Staff Broward General Hospital, Fort Lauderdale, FL 4 Apr 78.

Treat RC and Hunter LC: Modern Burn Therapy and Nursing Care of the Burn Patient. Burn Seminar sponsored by the University of Kansas Continuing Medical Education, Liberal, KS 7-8 Apr 78.

Pruitt BA Jr: Chemical Burn Therapy. Medical Mtg of Allied Chemical Co. Physicians. New Orleans, LA 9 Apr 78.

Goodwin CW: 1) Adaptive Activation of Succinic Dehydrogenase in Chronic Systemic Hypoxia, and 2) Workshop on Optical Monitoring of Metabolism. Federation of American Societies of Experimental Biology mtg, Atlantic City, NJ, 11-13 Apr 78.

Treat RC: Modern Burn Therapy. Trinity University students, San Antonio, TX 13 Apr 78.

Treat RC: Treatment of Burns. EMS technicians, San Antonio, TX 17 Apr 78.

Treat RC: Treatment of Burns. EMS technicians, San Antonio, TX 18 Apr 78

Treat RC: Treatment of Burns. BAMC Interns AMIC-ER, Fort Sam Houston, TX 18 Apr 78.

Treat RC: Initial Care of the Burn Patient. Staff of Hays Memorial Hospital, San Marcos, TX 18 Apr 78.

Treat RC: Treatment of Burns. Anesthesia Residents, Brooke Army Medical Center, Fort Sam Houston, TX 20 Apr 78.

Lescher TJ: Gastrointestinal Complications in Thermally Injured Patients. Lahey Clinic Foundation, Boston, MA 25 Apr 78.

Treat RC: The Burn Patient. Army Chaplains Clinical Pastoral Course, Brooke Army Medical Center, Fort Sam Houston, TX 4 May 78.

Pruitt BA Pr: 1) Use of Biologic Membranes; 2) Nutritional Support of the Burn Patient; 3) Management of the Upper Extremity Burn; and 4) The Burn Unit Mental Health Team. American Burn Assn Regional Burn Seminar. San Diego, CA 4-5 May 78.

Pruitt BA Jr: Current Treatment of Major Thermal Insury. Washington Hospital Center Surgical Grand Rounds, Washington, DC 9 May 78.

Pruitt BA Jr: Research in Thermal Injury. International Symposia on Trauma. Washington, DC 10 May 78.

Treat RC: Initial Care of the Burn Patient. EMS and physicians University of Texas at San Antonio, TX 11 May 78.

Pruitt BA Jr: 1) Fluid Resuscitation and Initial Care of the Seriously Burned Patient; 2) Burn Wound Care; 3) Pulmonary Complications of Thermal Injury. US Army European Medical Command, Medical and Surgical Training Conference, Garmisch West Germany 16-17 May 78.

Pruitt BA Jr: Burn Injury During War. Swedish Symposium on War Surgery, Gothenburg, Sweden. 19 May 78.

Pruitt BA Jr: 1) Fluid Resuscitation of the Extensively Burned Patient; 2) Metabolic Changes and Nutrition of Injured Man. Third Annual Clarence E. Stafford Memorial Surgical Symposium, Los Angeles,CA 28 May 78.

Treat RC: Modern Burn Therapy. Residents USAF Sch of Aerospace Med, Brooks AFB, TX 1 Jun 78.

Treat RC: Treatment of Burns. BAMC Interns AMIC-ER, Fort Sam Houston, TX 13 Jun 78.

Pruitt BA Jr: 1) Overview of Burn Care; 2) Fluid Resuscitation of the Extensively Burned Patient; and 3) Metabolic Changes and Nutrition in Burn Patients. French Plastic and Reconstructive Surgery Society Burn Symposium, Tours, France 14 Jun 78.

Pruitt BA Jr: Post Traumatic Pulmonary Insufficiency. Univ of Oslo Guest Lectureship, Oslo, Norway 17 Jun 78.

Pruitt BA Jr: 1) Epidemiology of Burn Injury; 2) Suppurative Thrombophlebitis in Burn Patients; 3) Overview of Burn Patient Resuscitation. 5th International Congress on Burn Injuries. Stockholm, Sweden 19, 20, 22 Jun 78.

Treat RC: Initial Care of the Burn Patient. Officers Basic Course Academy of Health Sciences, Fort Sam Houston, TX 28 Jun 78.

Pruitt BA Jr: History and Activities of the U.S. Army Institute of Surgical Research. San Antonio Downtown Rotary Club mtg, San Antonio TX 5 Jul 78.

Treat RC: The Burn Patient. Special education teachers, Trinity University, San Antonio, TX 6 Jul 78.

Treat RC: Initial Care of the Burn Patient. AF Reserves Kelly AFB, TX 8 Jul 78.

Treat RC: Disaster Planning Regarding Burn Victims. County Medical Society members, members of the Flying Physicians Association, Airport Managers, FAA Air Traffic Control Center chiefs. Memphis, TN 13 Jul 78

Treat RC: Modern Burn Therapy. Officers Basic Course, Academy of Health Sciences, Fort Sam Houston, TX 19 Jul 78.

Pruitt BA Jr: Burn Care in the United States. Univ of Texas Dental School at San Antonio, San Antonio, TX 27 Jul 78.

Treat RC: Seminar on Burns. USAF Reserves Westover AF Base, MA 27 Jul through 1 Aug 78.

Treat RC: Treatment of Burns. Officers Basic Course, Academy of Health Sciences, Fort Sam Houston, TX 9 Aug 78.

McManus WF: Classification of Burns. Intensive Care Nurse Clinician Course students, BAMC, Fort Sam Houston, TX 10 Aug 78.

Treat RC: Burn Wound Therapy. Intensive Care Nurse Clinician Course students, BAMC, Fort Sam Houston, TX 11 Aug 78.

Treat RC: Inhalation Injury. University of Texas at San Antonio, Grand Rounds. 11 Aug 78.

Treat RC: Burn Assessment and Early Management. BAMC Interns AMIC-ER, Fort Sam Houston, TX 22 Aug 78.

Pruitt BA Jr: Emergency Room Management of Burn Injury. Emergency Physicians Staff Conference, BAMC, Fort Sam Houston, TX 23 Aug 78.

Pruitt BA Jr: Care of the Severely Burned Patient. 102nd USAACOM Medical Symposium, St. Louis, MO 9 Sep 78.

Pruitt BA Jr: 1) Diagnoses and Treatment of Pulmonary Complication in Burn Patients; 2) Fluid Resuscitation of Burn Patients. Visiting Professor Univ Calif, Davis Medical School, Sacramento, CA 12 Sep 78.

Treat RC: Burn Therapy. Staff U.S. Military Academy Hospital, West Point, NY 15 Sep 78.

Treat RC: Initial Care of the Burn Patient. Staff U.S. Navy Hospital, Portsmouth, VA 19 Sep 78.

Sirinek KR: Treatment of Burns. Officers Basic Course, Academy of Health Sciences, Fort Sam Houston, TX 20 Sep 78.

Spebar MH: Basic Burn Care. Social Service In Service for relatives of burn patients. 20 Sep 78.

Pruitt BA Jr: Burn Care in Pediatric Patients. Grand Rounds. Univ of Texas Health Science Center at San Antonio. 22 Sep 78.

McManus WF: Emergency Care of Burns. Film videotape for the International Association of Fire Chiefs/International Association of Fire Fighters. University of N. Carolina, Chapel Hill, NC 26 Sep 78.

Goodwin CW: Burn Assessment. Emergency Room staff. AMIC Clinic, Brooke Army Medical Center, Fort Sam Houston, TX 28 Sep 78.

Treat RC: Treatment of Burns. 804th Hospital Centers Symposium, Cambridge, MA, 30 Sep 78.

The following presentations were made at the Burn Seminar at the Western Iowa Tech Community College, Sioux City, Iowa 5-7 Oct 78:

Treat RC: Modern Burn Therapy
Dunn MA: Nursing Care of the Burn Patient
Henderson NE: Physical Therapy for Burn Patients
Zitzka CA: Nutrition in Burn Patient Care

The following presentations were made at the Burn Seminar sponsored by the Wichita College of Pharmacists, Wichita, KS 7 Oct 78.

Goodwin CW: 1) Pathologic Processes in the Burned Patient and 2) Medical Management of the Acutely Burned Patient
Dyer A: Nursing Care of the Burn Patient.

Treat RC: Treatment of Burns. Surgical Staff, Louisiana State University Medical Center, Shreveport, LA 9 Oct 78.

Spebar MJ: Burn Treatment. Southern Safety Conference, San Antonio, TX 10 Oct 78.

Pruitt BA Jr: 1) Surgical Infections; 2) Multidisciplinary Symposium on Control/Prevention of Surgical Wound Infection. San Francisco, CA 14 Oct 78.

Pruitt BA Jr: 1) Burn Wound Management and 2) Thermal Injury of the Extremities 17 Oct 78 and 3) Practical Intravenous Feedings in Burns. 18 Oct 78. Clinical Congress American College of Surgeons, San Francisco, CA.

Pruitt BA Jr: Southwestern College Health Assn Convention. San Antonio, TX 20 Oct 78.

McManus WF: Treatment of Burns. Officers Basic Course, Academy of Health Sciences, Fort Sam Houston, TX 18 Oct 78.

The following presentations were made at the Burn Seminar sponsored by the Industrial Nurses, Merriville, IN on 20 Oct 78:

Spebar MJ: 1) Overall View of the Burn Patient; 2) Types of Occupational Health Burns and Their Emergent Care; 3) On the Scene Assessment and Treatment of the Burned Worker 4) Panel Discussion
Dyer A: 1) Physiologic and Psychologic Response to Burns; 2) Psychosocial Adjustment of the Burn Victim Returning to Work; 3) Burn Prevention in Occupational Health; and 4) Panel Discussion

Pruitt BA Jr: 1) Initial Management of the Burned Patient; 2) Kennedy Oration: Burn Injury as a Model of Systemic Trauma. Annual mtg of the New York State Medical Society. 24 Oct 78.

Goldfarb IW: Symposium on Clinical Nutrition. Department of Continuing Medical Education and the Department of Surgery of The Western Pennsylvania Hospital 28 Oct 78.

The following presentations were made at the Seminar on Rehabilitation of the Burn Patient. College of Medicine, College of Nursing and College of Allied Health Professions, University of Kentucky, Lexington, KY 3-4 Nov 78:

Treat RC: Treatment of Burns
Dunn MA: Nursing Care of the Burn Patient
Henderson NE: Physical Therapy for Burn Patients
Zitzka CA: Nutrition and the Burn Patient

Treat RC: Initial Management of Burns. Kingsville Naval Air Station, Kingsville, TX 9 Nov 78.

Pruitt BA Jr: Fluid Resuscitation of Burn Patients NIH Consensus Development Exercise on "Suppurative Therapy in Burn Care!' Bethesda, MD 10 Nov 78.

The following presentations were made at the Seminar on "Burns" at the University of Texas School of Nursing, Continuing Education Program, Austin, TX 16-17 Nov 78:

Treat RC: Modern Burn Therapy
Diaz HM: Occupational Therapy for the Burn Patient
Henderson NE: Physical Therapy Programs for Burn Victims
Hunter EC: Nursing Care in the Acute Phase
Zitzka CA: Nutritional Importance in Burn Patients

Sirinek KR: Moderate Session on GI Hormones. Assn for Academic Surgery anl mtg, Case Western University, Cleveland, OH 12-15 Nov 78.

Treat RC: Modern Burn Therapy. Graduate students UTSA School of Nursing, San Antonio, TX 8 Nov 78.

Pruitt BA Jr: 1) Topical Antibacterial Therapy; 2) Inhalation Injury; 3) Management of Burn Complications. American Burn Assn Regional Seminar. Philadelphia, PA 17 Nov 78.

Pruitt BA Jr: 1) Clinical Evaluation of Hypertonic and Hypotonic Solutions to Resuscitate Severely Burned Children: A Prospective Study; 2) Influence of [H+] on Bile Acid Induced Acute Gastric Mucosal Ulcerogenesis. Southern Surgical Association. 4-6 Dec 78.

Treat RC: Inhalation Injury. Hillcrest Hospital, Tulsa, OK 5 Dec 78.

Treat RC: Treatment of Burns. Harlingen Medical Group, Harlingen, TX 7 Dec 78.

Pruitt BA Jr: 1) Post Burn Immunological and Hormonal Alteration; 2) Topical Chemotherapy; 3) Planning a Burn Unit; 4) Electrical Injuries; 5) Respiratory Complications; 6) Nutritional Support and Hyperalimentation; 7) The Role of Antibiotics. Post Graduate Education Course. International Society for Burn Injuries. Denver, CO 14-16 Dec 78.

Treat RC: Burn Assessment. BAMC Interns AMIC-ER, Fort Sam Houston, TX 19 Dec 78.

PUBLICATIONS

Panke TW, McManus AT Jr, McLeod CG Jr: Fruiting Bodies of Aspergillus on the Skin of a Burned Patient. Amer J of Clin Path 69:188-189, Feb 78.

Levine BA, Sirinek KR, Teegarden DK, McLeod CG Jr, Pruitt BA Jr: Effect of Cimetidine on Gastric Secretory Function During Stress. J of Surg Res 24:178-181, Mar 78.

Levine BA, Petroff PA, Slade CL, Pruitt BA Jr: Prospective Trials of Dexamethasone and Aerosolized Gentamicin in the Treatment of Inhalation Injury in the Burned Patient. J Trauma 18:188-193, Mar 78.

Pruitt BA Jr: Advances in Fluid Therapy and the Early Care of the Burn Patient. World J Surg 2:139-150, Mar 78.

Levine BA, Sirinek KR, Pruitt BA Jr: Wound Excision to Fascia in Burn Patients. Arch Surg 113:403-407, Apr 78.

Panke TW, Teegarden DK and Lescher TD: Extensive Cutaneous Burn Complicated by Severe Necrotizing Amebic Enterocolitis. Am J Trop Med Hyg 27(4) 766-769, 78.

Heimburger SL, McDougal WS, Wilmore DW and Pruitt BA Jr: Correction of Hepatocellular Dysfunction During Endotoxemia. J of Surg Res 24: 442-448, May 78.

Levine BA, Schwesinger WH, Sirinek KR, Jones D and Pruitt BA Jr: Cimetidine Prevents Reduction in Gastric Mucosal Blood Flow During Shock. Surgery 84:113-119, Jul 78.

McDougal WS, Heimburger S, Wilmore DW, Pruitt BA Jr: The Effect of Exogenous Substrate on Hepatic Metabolism and Membrane Transport During Endotoxemia. Surgery 84:55-61, Jul 78.

Panke TW, Langlinais PC, Goyette RE: An "Abnormal" Lymphoid Proliferation Simulating Hodgkin's Disease in a Burn Patient. Human Pathology 9:716-723, Nov 78

Lescher TJ, Teegarden DK, Pruitt BA Jr: Acute Pseudo-obstruction of the Colon in Thermally Injured Patients. Diseases of the Colon and Rectum 21:618-622, Nov-Dec 78.

Wilmore DW, Aulick LH: Metabolic Changes in Burned Patients. Surg Clinics of North America 58:1173-1187, Dec 78.

Aulick LH, Wilmore DW, Mason AD, Pruitt BA Jr: Muscle Blood Flow Following Thermal Injury. Ann of Surg 188:778-782, Dec 78.

Pruitt BA Jr: Fluid and Electrolyte Replacement in the Burned Patient. Surg Clin of North America 58:1291-1312, Dec 78.

MOTION PICTURES

The following motion picture was shown during the year 1978:

"Burn Wound Management" at the American College of Surgeons, San Francisco, CA 16-20 Oct 78.

ANNUAL PROGRESS REPORT

PROJECT NO. 3S162772A814-00, APPLIED RESEARCH

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

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

1 January 1978 - 31 December 1978

Investigator:

Anton J. Jirka, MD, MPH, Lieutenant Colonel, MC

Reports Control Symbol MEDDH-288(R1)

Unclassified

ABSTRACT

PROJECT NO. 3S162772A814-00, APPLIED RESEARCH

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

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

Period covered in this report: 1 January 1978 - 31 December 1978

Investigator: Anton J. Jirka, MD, MPH, Lieutenant Colonel, MC

Reports Control Symbol MEDDH-288(R1)

In the period covered in this report, 435 anesthetics were administered to 151 patients, an average of 2.88 anesthetics per patient. The most commonly used anesthetic agent was Ethrane® (48.3%), followed by ketamine (21.6%), nitrous oxide (13.2%), and halothane (8.4%). Regional anesthesia was used for 6.7% of anesthetic administrations.

Anesthesia

ANESTHESIOLOGY

PREOPERATIVE EVALUATION

Most burn patients are several days postinjury when first seen by the anesthesiologist. In the immediate postburn period, the time is used to gain abundant physiologic data from routine monitoring of various indices: hematologic (hematocrit, electrolytes, liver and renal function tests), pulmonary (arterial blood gases, respiratory rate, daily chest roentgenograms), cardiovascular (blood pressure, central venous pressure, cardiac index measured by use of Swan-Ganz catheters), and renal (urine output, urine chemistry), in addition to the usual preoperative patient interview and physical examination.

All patients, regardless of age, who have electrical injuries have a preoperative electrocardiogram performed to rule out possible myocardial damage.

PREOPERATIVE PREPARATION

All patients are kept NPO after 2400 the day prior to surgery with the exception of patients with protected airways (intubation by some route plus tube feedings) and children, who may receive clear liquids up to five hours prior to surgery.

PREMEDICATION

Most burn patients require some pain relief during the trip to the operating room, and most receive a narcotic such as morphine sulfate, 0.1 mg/kg, to a maximal dose of 10 mg, one hour prior to surgery. Glycopyrrolate (Robinul^{R}), 0.005 mg/kg to a maximal dose of 0.4 mg, is used to dry secretions. Both of these medications are delivered intramuscularly.

Valium (PO) plus Robinul in the above dosage, are used as premedication prior to regional anesthesia.

FLUIDS

All fluids are changed to D_5RL or RL on arrival in the operating room.

TYPES OF ANESTHESIA

The pattern of anesthetic administration has changed from previous years and involves a greater use of enflurane and a lesser use of ketamine. The reasons for this change will be discussed under individual agent headings.

TABLE 1. PRIMARY AGENTS - 1978

	% of Total	Total Cases
Enflurane	48.5	211
Ketamine	21.8	95
Halothane	8.3	36
N_2O	13.3	58
Local	6.7	29
Other	1.6	7

1. Enflurane (Ethrane^R)

Enflurane is a halogenated ether which has been commercially available for approximately the past six years. It has a rapid induction with good muscle relaxation. Biotransformation amounts to less than 2% of an inhaled dose, a fact which perhaps accounts for the few clinical toxic effects observed in spite of the fact that increased plasma fluoride ion concentrations have been observed after administration to patients taking hepatic enzyme inducing drugs. Plasma fluoride levels in hypermetabolic burn patients during and after Ethrane administration have been measured and found not to be in the toxic range.

2. Halothane^R (Fluothane)

The use of halothane is avoided mostly for less than rational reasons related to descriptions of probable hepatotoxicity (incidence 0.7 per 1000) in the literature. Previous studies at the Institute of Surgical Research show its repeated use to be safe in the thermally injured patient, and the National Halothane Study showed halothane to be the anesthetic with the best overall mortality rate. It is a smooth anesthetic, unsurpassed as an agent for pediatric patients. This anesthetic is mainly used now for asthmatics, patients with digitalis toxicity, and children.

3. Nitrous oxide

This agent is used in concentrations of 50% or 60% with oxygen. It is used mainly in conjunction with other analgesic or anesthetic agents. Pancuronium is the only relaxant used in conjunction with

this agent. Succinylcholine has not been used for any purpose in this unit for more than four years.

4. Ketamine

This agent is used both IM and IV to produce its characteristic dissociative state, with preservation of basal functions (breathing) and laryngeal reflexes plus secondary catechol stimulation of the cardiovascular system.

Unfortunately, ketamine shares with its parent compound, phencyclidine, the production of a high incidence of unpleasant hallucinogenic side effects. There seems to have been a "batch" difference in ketamine, and that possessed by ISR in the past has had an almost 100% incidence of these effects. New methods of administering the drug, as well as various methods of premedication and patient preparation, appear to have reduced the unpleasant emergence reactions to less than 15%. Laryngospasm, airway obstruction, and regurgitation can occur with ketamine. Pronounced blepharospasm prevents its use in eye cases.

5. Subanesthetic Ketamine

Subanesthetic ketamine (single dose 1.5-2 mg/kg IM) has seldom been used during this reporting period. Several factors limit the usefulness of this agent. The Hubbard tank restricts the use of intravenous administration at times, and only procedures with low projected blood loss are scheduled for the tank room; repeated use of ketamine may lead to a buildup of tolerance to the drug's beneficial effects. Larger and larger doses must be used, with sequential administration leading to a greater incidence of undesirable side effects, and multiple anesthetic administrations, spaced closely together, interfere with the feeding schedule of patients, resulting in delayed wound healing.

It should be noted that large doses of narcotics are most definitely not used for "tank" procedures. The pain encountered is not of the type which is relieved by narcotics, and "tank" procedures are of short duration. Narcotic use provides little pain relief and may lead to postoperative respiratory depression.

Short, repeated painful procedures should be carried out with a minimum of sedation, to the limit of the patient's tolerance, and narcotic premedication should only be used in small doses. General anesthesia is required for procedures in which pain is not alleviated by minimum narcotic sedation.

6. Regional Anesthesia

Regional anesthesia is generally considered one of the safest methods available, but its use in the thermally injured patient is limited for several reasons: sepsis and infection of the skin over the site of injection are contraindications for use, and multiple-site operations also limit the practicality of this method.

MONITORING TECHNIQUES

A. CIRCULATION

1. Precordial and/or esophageal stethoscope

2. Peripheral pulse

3. Blood pressure, usually by Infrasonde,^{R} but also may be by Riva Rocci method. Direct arterial lines have been used when necessary. The Dinamap^{R} blood pressure measuring instrument has recently been placed into use.

4. CVP

5. Swan Ganz catheter

6. ECG

7. Sponge weight - rarely used

8. Urine output

B. RESPIRATION

1. Rate

2. Auscultation

3. Arterial blood gases

C. TEMPERATURE

In most cases a temperature monitor is now employed. Because of the greatly increased evaporative heat losses in burn patients, hypothermia is a serious problem. Several methods are employed to maintain body temperature during anesthesia:

1. Ambient temperature is maintained at 80-85^{o}F. This is probably the most important method to reduce heat loss.

2. The anesthetic gases may be heated and humidified.

3. A circle system may be used to minimize heat loss.

4. Radiant heat lamps.

5. A K-thermia heating blanket can also be used. It is probably used most effectively on children weighing less than 10 kg and for cooling febrile patients.

INTEROPERATIVE COMPLICATIONS

There was one intraoperative death in 1978. It occurred in a 27 year old male who sustained a 37% of total body surface electrical injury with 13% third degree burn, mainly involving his left lower extremity. The patient became progressively hyperkalemic, due to extensive myonecrosis during his second postburn day. The hyperkalemia did not respond to medical management and it was felt that surgical intervention was in order. The patient underwent an emergency left hip disarticulation and at the end of the procedure sustained a cardiac arrest from which he could not be resuscitated. An intraoperative serum potassium was 8.5.

TABLE 2. OVERALL PATIENT DATA, USAISR (1966-1978)

Year	No. of Patients	No. Patients Anesthetized (ISR Only)	No. Patients Anesthetized / No. Patients (x100)	Total Anesthetics (ISR Only)	Anesthetics / No. Patients Anesthetized (x100)
1966	311	181	58.2	713	3.94
1967	389	239	61.4	670	2.80
1968	389	259	66.6	794	3.07
1969	294	189	64.3	601	3.18
1970	321	198	61.7	497	2.51
1971	301	179	59.5	475	2.65
1972	301	183	60.8	575	3.14
1973	273	141	51.6	377	2.67
1974	226	123	54.4	380	3.09
1975	254	142	55.9	490	3.45
1976	277	139	50.2	476	3.43
1977	242	129	53.3	344	2.67
1978	268	151	56.3	435	2.88

TABLE 3. NATURE OF SURGERY, USAISR (PER CENT)

Procedure	1971	1972	1973	1974	1975	1976	1977	1978
Excision	15.5	19.7	21.5	22.60	25.0	23.0	19.7	19.3
Autograft	52.9	51.3	52.6	56.9	51.0	53.0	52.6	59.9
Orthopedics	13.0	8.9	8.0	8.1	8.0	5.0	6.2	7.1
Chondrectomy	4.0	3.1	2.6	1.60	1.0	2.0	1.9	0.9
Eye and Lid	3.8	0.7	1.8	1.60	2.0	3.0	2.7	1.3
Intra-abdominal	1.7	7.8	2.1	3.70	2.0	1.0	1.0	1.3
Plastic*							5.7	1.3
Other	4.4	1.9	4.8	3.70	11.0	13.0	10.9	10.8

*Accounting classification began for 1977 annual report

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA OG 6975	79 10 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
78 10 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	62772A	3S162772A814	00	120
b. [illegible]	61102A	3S161102BS05	00	085
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)*

(U) The Hemodynamic Response to 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
76 10	Cont	DA	C. In-House

17. CONTRACT/GRANT: Not Applicable

a. DATES/EFFECTIVE: EXPIRATION:

b. NUMBER:*

c. TYPE: d. AMOUNT:

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

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 79	2	47
FISCAL YEAR CURRENT 80	2	45

19. RESPONSIBLE DOD ORGANIZATION

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RESPONSIBLE INDIVIDUAL

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

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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) Humans; (U) Dogs; (U) Resuscitation fluids; (U) Echocardiography; (U) Burn injury; (U) Cardiac output; (U) Septic shock; (U) Cardiovascular hemodynamics

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

23. (U) To evaluate systemic and pulmonary hemodynamic changes in burned soldiers and the influence of fluid resuscitation. To study noninvasively myocardial function in burned and burned-infected patients. To assess use of vasoactive agents in burned soldiers.

24. (U) Hemodynamic flow and pressure changes are studied in burn patients during and after resuscitation. Myocardial is studied by echocardiography. The effect of treatment on hemodynamic changes in burn patients with septic shock is assessed. Changes will also be studied in animal models.

25. (U) 7810 - 7909 The efficacy of dopamine in the treatment of burn wound-induced septic shock was evaluated. Cardiac output (CO), ejection fraction (EF), and mean velocity of diameter shortening (VCF) were determined by echocardiography. Dopamine was without effect in hypotensive patients who maintained elevated cardiac outputs (CO: 11.8 ± 2.34 min. vs (0.7 ± 1.9; EF: 78 ± 4% vs 76 ± 2; and VCF 1.68 ± .48 circ/sec vs 1.76 ± .52). Hypodynamic septic patients, however, responded with an elevated CO (4.7 ± 1.2 L/min vs 6.5 ± 1.2, $p < 0.5$) but with no change in contractility (EF: 74 ± 6% vs 76 ± 4 and VCF 1.48 ± .26 circ/sec vs 1.60 ± .40). Non-invasive hemodynamic evaluation is an effective tool in the proper selection of appropriate treatment of septic shock.

*Available to contractors upon originator's approval

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

ANNUAL PROGRESS REPORT

PROJECT NO. 3S162772A814-00, APPLIED RESEARCH

REPORT TITLE: THE HEMODYNAMIC RESPONSE TO THERMAL INJURY IN BURNED SOLDIERS: THE HEMODYNAMIC RESPONSE TO DOPAMINE IN PATIENTS WITH SEPTIC SHOCK

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

1 October 1978 - 30 September 1979

Investigators:

Cleon W. Goodwin, M.D., Major, MC
James F. Dorethy, M.D.
Richard C. Treat, M.D.

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3S162772A814-00, APPLIED RESEARCH

REPORT TITLE: THE HEMODYNAMIC RESPONSE TO THERMAL INJURY IN BURNED SOLDIERS: THE HEMODYNAMIC RESPONSE TO DOPAMINE IN PATIENTS WITH SEPTIC SHOCK

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

Period covered in this report: 1 October 1978 - 30 September 1979

Investigators: Cleon W. Goodwin, M.D., Major, MC
James F. Dorethy, M.D.
Richard C. Treat, M.D.

Reports Control Symbol MEDDH-288(R1)

Hemodynamic flow and pressure changes are studied in burn patients during and after resuscitation. Myocardial contractility is studied by echocardiography. The effect of dopamine treatment on hemodynamic changes in burn patients with septic shock is assessed.

The efficacy of dopamine in the treatment of burn wound-induced septic shock was evaluated. Cardiac output (CO), ejection fraction (EF), and mean velocity of diameter shortening (VCF) were determined by echocardiography. Dopamine was without effect in hypotensive patients who maintained elevated cardiac outputs. Hypodynamic septic patients, however, responded with an elevated cardiac output but with no change in contractility. Non-invasive hemodynamic evaluation is an effective tool in the proper selection of appropriate treatment of septic shock.

Septic shock
Dopamine
Echocardiography
Ejection fraction
Cardiac output

THE HEMODYNAMIC RESPONSE TO THERMAL INJURY IN BURNED SOLDIERS: THE HEMODYNAMIC RESPONSE TO DOPAMINE IN PATIENTS WITH SEPTIC SHOCK

Even with modern topical antimicrobial chemotherapy, sepsis and related complications are the major identifiable causes of death of thermally injured patients. Sustained bacteremia usually terminates with cardiovascular collapse and eventually death. Although the basic tenets of septic shock treatment dictate identification and eradication of the septic focus, the manifestations of hemodynamic collapse must simultaneously be reversed in an effort to restore adequate organ and tissue perfusion. If restoration of an adequate intravascular volume fails to restore tissue perfusion, cardiotonic agents are instituted to increase total body blood flow [1-7]. The ability of dopamine to increase cardiac output and organ perfusion is related to the infusion dose and is limited by vasoconstrictive effects at higher dosages [8].

METHODS

Twelve thermally injured patients were studied at the onset of clinical sepsis (see Table 1) [9] and serially thereafter until recovery or death.

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1. MacCannell KL, McNay JL, Meyer MB, Goldberg LI: Dopamine in the treatment of hypotension and shock. N Engl J Med 275:1389-1398, 1966.

2. Carvalho M, Vyden JK, Bernstein H, Gold H, Corday E: Hemodynamic effects of 3-hydroxytyramine (dopamine) in experimentally induced shock. Am J Cardiol 23:217-223, 1969.

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6. Goldberg LI: Cardiovascular and renal actions of dopamine: Potential clinical applications. Pharmacol Rev 24:1-29, 1972.

7. Goldberg LI: Dopamine - Clinical uses of an endogenous catecholamine. N Engl J Med 291:707-710, 1974.

8. Beregovich J, Bianchi C, Rubler S, Lomnitz E, Cagin N, Levitt B: Dose-related hemodynamic and renal effects of dopamine in congestive heart failure. Am Heart J 87:550-557, 1974.

9. Dorethy JF, Treat RC: Echocardiographic left ventricular volume and ejection indices during treatment of septic shock with dopamine. USAISR Annual Report FY 1978, BAMC, Ft Sam Houston, TX, pp 93-103.

Table 1. Clinical Criteria for the Diagnosis of Septic Shock

1. An absolute criterion was the presence of bacterial sepsis defined as two consecutive positive venous blood cultures.

2. Hypotensive episode and/or persistent hypotension (systolic < 100 mm Hg).

3. In addition, one of the following was present:

   A. Mental confusion, obtundation, seizures, and/or coma.

   B. Marked urinary output changes: oliguria (< 20 cc/hr) or anuria.

   C. Unexplained respiratory failure (< 60 PaO_2 on 50% FIO_2)

The hemodynamic effects of septic shock and subsequent infusion of dopamine on cardiac function were evaluated by echocardiography (Echoline 20A) and by measurement of cardiac output by thermodilution (Edwards Model 6582). Ultrasonic transducers included 3.5 and 2.25 MHz sizes focused at 5.0, 7.5 and 10.0 cm.

RESULTS

The efficacy of dopamine in the treatment of burn wound-induced septic shock was evaluated. Cardiac output (CO), ejection fraction (EF), and mean velocity of diameter shortening (VCF) were determined by echocardiography. Dopamine was without effect in hypotensive patients who maintained elevated cardiac outputs (CO: 11.8 ± 2.34 min vs 0.7 ± 1.9; EF: 78 ± 4% vs 76 ± 2; and VCF: 1.68 ± .48 circ/sec vs 1.76 ± .52). Hypodynamic septic patients, however, responded with an elevated CO (4.7 ± 1.2 L/min vs 6.5 ± 1.2, $p < 0.5$) but with no change in contractility (EF: 74 ± 6% vs 76 ± 4, and VCF: 1.48 ± .26 circ/sec vs 1.60 ± .40). Non-invasive hemodynamic evaluation is an effective tool in the proper selection of appropriate treatment of septic shock.

DISCUSSION

With the onset of septic shock, blood pressure falls and is accompanied by biochemical evidence of impaired tissue perfusion (hyperlactatemia and acidosis). Classically, the toxic bacterial breakdown products initially produce a hyperdynamic circulation with an increased cardiac output, vasodilatation, and decreased

arteriovenous extraction of oxygen [10-12]. If the source of sepsis remains undiscovered and septicemia persists, the hyperdynamic state is succeeded by one characterized by a low cardiac output and vasoconstriction. Septic patients exhibiting a low output state generally respond poorly to therapeutic interventions and die of their infections.

The response of burned patients with septic shock to dopamine depended on the pretreatment level of cardiac output. In those patients whose cardiac outputs were elevated above normal levels (the usual hemodynamic response of hypermetabolic burned patients), dopamine had no effect on cardiac output. In those patients who manifested low cardiac outputs, dopamine was moderately effective in producing an increase in flow, but not to the levels of those in the hyperdynamic patients with septic shock. No patients in either the hyperdynamic or the hypodynamic groups demonstrated any evidence of decreased myocardial contractility (as measured by ejection fraction or by mean velocity of circumferential fiber shortening). As such, digitalis would not be expected to produce any beneficial effect in these patients and in fact may be quite dangerous in this situation.

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PUBLICATIONS/PRESENTATIONS - None.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY	1. AGENCY ACCESSION* DA OG 6971	2. DATE OF SUMMARY* 79 10 01	REPORT CONTROL SYMBOL 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
78 10 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	62772A	3S162772A814	00	119
b. ~~CONTRIBUTING~~	61102A	3S161102BS05	00	091
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)*
(U) Evaluation of Burn Wound Care in Troops With Burn Injury (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*
003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
76 10	Cont	DA	C. In-House

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

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 79	3	95
	CURRENT 80	2	69

19. RESPONSIBLE DOD ORGANIZATION
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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) Burn injury; (U) Topical therapy; (U) Sulfamylon; (U) Wound excision; (U) 5% Sulfamylon acetate solution; (U) Humans; (U) Autografts

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

23. (U) The military relevance of improving burn wound care will be realized in increased troop survival following thermal injury. Newer methods under current investigation include the use of 5% aqueous Sulfamylon soaks, and excision of eschar from burned soldiers. Our objective is to further define the use of these methods.

24. (U) Patients admitted to the Institute of Surgical Research for care of thermal injuries receive burn wound care based on the specific injury. The 5% aqueous Sulfamylon soaks, excision of the eschar, and other modalities of wound care may be used.

25. (U) 7810 - 7909 Treatment with the Sulfamylon soaks was utilized in 176 patients, and 13 patients exhibited some form of allergic reaction. This 7.3% incidence of reactions noted was the only adverse reaction to this medication. These results support the continued use of 5% Sulfamylon soaks on a routine basis. Burn wound excision continued to be extensively utilized in an attempt to decrease the burn wound size and reduce the infectious complications. Tangential excision, full-thickness excision to viable fat, and excision to fascia were all utilized and are undergoing continued evaluation. Of the 78 patients (29% of total admissions) who underwent some type of operative excision in 1978, excision to fascia was performed in 32 patients (11%), escharectomy was performed in 24 patients (9%), and tangential excision of burn wounds of hands was performed in 46 patients (17%).

*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. 3S162772A814-00, APPLIED RESEARCH

REPORT TITLE: EVALUATION OF BURN WOUND CARE IN TROOPS WITH BURN INJURY: 5% AQUEOUS SULFAMYLON SOAKS USED IN TOPICAL TREATMENT OF BURNED SOLDIERS

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

1 October 1978 - 30 September 1979

Investigators:

William F. McManus, M.D., Lieutenant Colonel, MC
Basil A. Pruitt, Jr., M.D., Colonel, MC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3S162772A814-00, APPLIED RESEARCH

REPORT TITLE: EVALUATION OF BURN WOUND CARE IN TROOPS WITH BURN INJURY: 5% AQUEOUS SULFAMYLON SOAKS USED IN TOPICAL TREATMENT OF BURNED SOLDIERS

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

Period covered in this report: 1 October 1978 - 30 September 1979

Investigators: William F. McManus, M.D., LTC, MC
Basil A. Pruitt, Jr., M.D., Colonel, MC

Reports Control Symbol MEDDH-288(R1)

Improvement of wound care continues to be a major goal of the Institute of Surgical Research. Methods under current investigation include the use of 5% aqueous Sulfamylon dressings. During this reporting period, 176 patients required 5% Sulfamylon soaked dressings for the care of their burn wounds. These dressings were employed either in final debridement of the wound or following a mesh cutaneous autograft procedure to prevent desiccation of the fresh skin. A 7.3% incidence of significant skin rash (atopy) was noted as the only adverse reaction. These results support continued use of 5% Sulfamylon solution.

Burn injury
Topical therapy
5% Sulfamylon acetate solution
Humans

EVALUATION OF BURN WOUND CARE IN TROOPS WITH BURN INJURY: 5% AQUEOUS SULFAMYLON SOAKS USED IN TOPICAL TREATMENT OF BURNED SOLDIERS

The evaluation of 5% Sulfamylon acetate solution in topical treatment of burn wounds has continued at this Institute. During the reporting period of 1 Oct 78 - 30 Sep 79, 268 patients were admitted to the U.S. Army Institute of Surgical Research. Of these 268 patients, 176 had 5% aqueous Sulfamylon dressings employed for burn wound care. During this period, 352 split thickness skin autograft procedures were performed on 139 patients. Five percent aqueous Sulfamylon soaked dressings were used in conjunction with these skin autografting procedures in most patients. The 5% Sulfamylon acetate soaked dressings are used either as continuous wet dressings or as wet to dry dressings to debride burn wounds. When meshed cutaneous autografts are applied, dressings soaked with 5% Sulfamylon acetate solution are utilized to decrease the rate of bacterial growth and to keep the mesh cutaneous autograf moist until vascular ingrowth occurs.

Allergic reactions to the 5% Sulfamylon solution were noted in 13 of the 176 patients. This represents an incidence of 7.3% allergic reactions (atopy). In the 13 patients who developed allergic reactions rapid resolution of the reaction most often followed the administration of an antihistamine, however if atopic manifestations continued following the antihistamine administration, the 5% Sulfamylon soaked dressings were discontinued and saline or other agents were employed. No other adverse reactions were noted in this group of patients.

The use of 5% Sulfamylon acetate dressings continued to be an important agent for the treatment of patients both in the preparation of the burn wound for cutaneous autografting or in the prevention of desiccation of freshly placed meshed cutaneous autograft. Its efficacy and low incidence of adverse side effects speak for its continued use.

ANNUAL PROGRESS REPORT

PROJECT NO. 3S162772A814-00, APPLIED RESEARCH

REPORT TITLE: EVALUATION OF BURN WOUND CARE IN TROOPS WITH BURN INJURY: EXCISION OF ESCHAR IN BURNED SOLDIERS

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

1 October 1978 - 30 September 1979

Investigators:

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Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3S162772A814-00, APPLIED RESEARCH

REPORT TITLE: EVALUATION OF BURN WOUND CARE IN TROOPS WITH BURN INJURY: EXCISION OF ESCHAR IN BURNED SOLDIERS

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

Period covered in this report: 1 October 1978 - 30 September 1979

Investigators: William F. McManus, M.D., Lieutenant Colonel, MC
Basil A. Pruitt, Jr., M.D., Colonel, MC

Reports Control Symbol MEDDH-288(R1)

Improvement in the care of the burn wound continues to be a major goal of the US Army Institute of Surgical Research. Newer methods of treatment under current investigation include excision of eschar from burned soldiers. Patients admitted to the Institute of Surgical Research for treatment of thermal injuries receive burn wound care based upon specific injury needs. Under appropriate conditions, excision of eschar is utilized. Tangential, full thickness excision to viable fat and excision to the level of investing fascia continue to be evaluated.

Burn injury
Wound excision
Humans

EVALUATION OF BURN WOUND CARE IN TROOPS WITH BURN INJURY: EXCISION OF ESCHAR IN BURNED SOLDIERS

Excision of the burn wound and subcutaneous tissue to the level of the investing fascia, escharectomy, and tangential excision with immediate split-thickness autografting continue to be utilized at the US Army Institute of Surgical Research. Seventy eight patients (29% of admissions) underwent some type of excision during calendar year 1978.

Excision of burns to the layer of investing fascia was an operative procedure reserved primarily for patients with burns in the 40% to 60% total body surface range. Excision of this type was not used in burns under 40% of the total body surface except in instances of proven invasive burn wound infection.

Following excisional therapy, wound coverage with cutaneous autograft, allograft, or xenograft is performed. The type of biologic material chosen for coverage depends on the indication for the operative procedure, the appearance of the fascia following excision, and the condition of the patient. In excess of 80% "take" of autograft can be anticipated if the fascia has been completely and atraumatically cleared of fat. Either cutaneous allograft or xenograft is used as a biologic dressing for fascia, a bed of granulation tissue suitable for accepting autograft skin usually develops within 5 to 10 days. Thirty two patients underwent excision to fascia of their burn wounds during the past year.

Escharectomy has been utilized both in early and late burn wound care. In the early post burn period, this type of excision has been used principally for treatment of small burn wounds. We have preferentially used excision to fascia for treatment of larger wounds because of the lesser blood loss associated with such excision and a more acceptable take of the autograft on fascia than on fat. Later in the post burn course, this type of excision is used to remove tenacious eschar and achieve rapid burn wound coverage with autograft skin. In both of these situations, excisions of the entire eschar with a guarded knife to freely bleeding fat, followed by application of cutaneous allografts, or xenografts to the excised area is most commonly performed. Cutaneous allograft dressings are changed as appropriate but not allowed to vascularize until a lush bed of granulation tissue develops and autografting can be accomplished. Blood loss which may be massive may limit the extent of any excision. The use of tourniquets on extremities undergoing escharectomy significantly reduces this blood loss. In the previous year, 24 patients underwent escharectomy.

Tangential excision as described by Janzekovic and Douglas Jackson (1) has been used at our unit primarily for the treatment of deep partial thickness burns of the hands. More superficial burns of the hands which are characterized by a moist surface sensitive to pin-prick, may be treated conservatively and be expected to heal in less than three weeks with a satisfactory epithelial covering. Deeper partial thickness burns,

which are usually white and insensate will take longer to heal but more importantly may heal with an unsatisfactory epithelium that is too thin to withstand minor trauma and develop hypertrophic scarring. Such hands are best treated by tangential excision with immediate autograft placement. (2) Nine patients underwent tangential excision of burns of one or both hands during the past year.

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PRESENTATIONS AND/OR PUBLICATIONS

None

ANNUAL PROGRESS REPORT

PROJECT NO. 3S162772A814-00, APPLIED RESEARCH

REPORT TITLE: EVALUATION OF BURN WOUND CARE IN TROOPS WITH BURN INJURY-INHIBITION OF ALKALINE PHOSPHATASE BY SEVERAL DIURETICS

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

1 October 1978 - 30 September 1979

Investigators:

Gary H. Price, PhD, Major, MSC
John Dubois, B.S.
Sharon E. Kirk, SSG

Reports Control Symbol MEDDH-288(R1)

Unclassified

ABSTRACT

PROJECT NO. 3S162772A814-00, APPLIED RESEARCH

REPORT TITLE: EVALUATION OF BURN WOUND CARE IN TROOPS WITH BURN INJURY-INHIBITION OF ALKALINE PHOSPHATASE BY SEVERAL DIURETICS

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

Period covered in this report: 1 October 1978 - 30 September 1979

Investigators: Gary H. Price, PhD, Major, MSC
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Sharon E. Kirk, SSG

Reports Control Symbol MEDDH-288(R1)

Acetazolamide, furosemide, ethacrynic acid and chlorothiazide, diuretics of considerable structural diversity, inhibit alkaline phosphatase. The inhibition is reversible and the mechanism is of the mixed type, having both competitive and noncompetitive characteristics. Ki is calculated to be 8.4, 7.0, 2.8, and 0.1 mmol/l for acetazolamide, furosemide, ethacrynic acid and chlorothiazide, respectively. Chlorothiazide is a much more potent inhibitor of alkaline phosphatase than the other three diuretics. The combination of ethacrynic acid and cysteine, itself an alkaline phosphatase inhibitor, is less inhibitory than ethacrynic acid alone. Rat and human kidney alkaline phosphatase are equally sensitive to chlorothiazide, ethacrynic acid and furosemide.

EVALUATION OF BURN WOUND CARE IN TROOPS WITH BURN INJURY-INHIBITION OF ALKALINE PHOSPHATASE BY SEVERAL DIURETICS

We have recently described (1) the inhibition of human alkaline phosphatase (AP) by a group of sulfonamides which includes the diuretics acetazolamide (Diamox^R) and furosemide (Lasix^R). The methylated xanthine diuretic, theophylline, has been shown to inhibit AP in vitro (2-4). Although dissimilar in structure, furosemide and ethacrynic acid are considered to be much alike in their pharmacologic properties (5). Ethacrynic acid depresses calcium ion accumulation in rat kidney cortex mitochondria, and it is thought that this process is involved in the physiologic regulation of calcium and active transcellular transport in renal tubules (6). Chlorothiazide (Diuril^R) also affects calcium metabolism, causing hypercalcemia as well as inhibiting phosphodiesterase (7). We studied the effects of furosemide, ethacrynic acid, acetazolamide and chlorothiazide on the kinetics of AP activity to elucidate the mechanisms of inhibition. Because of the reported (8,9) difference in diuretic effect of ethacrynic acid in rats and humans, we compared

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the effects of ethacrynic acid, furosemide and chlorothiazide on activity from rat and human kidney.

A synergistic interaction between cysteine and ethacrynic acid in the inhbition of Na-K-ATPase in the thick ascending limb of Henle's loop has been reported (10). Burg attributed the amelioration of other toxic effects of ethacrynic acid to the formation of a less toxic or nontoxic cysteine-ethacrynic acid adduct with enhanced ability to block chloride transport. Because cysteine is a potent AP inhibitor, we tested the interaction of both agents to determine whether the effects on AP are synergistic, additive, or antagonistic.

MATERIALS AND METHODS

Chlorothiazide was manufactured by the research laboratories of Merck, Sharp and Dohme, West Point, Pa.; furosemide was procured from Hoechst-Roussel, Somerville, N.J.; ethacrynic acid was purchased from Sigma, St. Louis, Mo; acetazolamide was obtained from Lederle Laboratories, Pearl River, NY. All other reagents were reagent grade.

Specimens of human liver, burn wound granulation tissue, bone, kidney and intestine were taken at autopsy of patients who had succumbed to severe thermal injury. A placenta was obtained from a normal live birth. Adult, male, Sprague-Dawley rats were killed by heart puncture and exsanguination and the kidneys removed. The tissues were homogenized and the homogenates butanol extracted and dialyzed as described previously (1).

AP was assayed by a modification of the method of Bessey, et al. (11), using 0.5 ml of 2-methyl-2-amino-1-propanol (AMP) buffer (Sigma 325-3), 0.75 mol/l, pH 9.4 at 37°, containing 1 mmol/l $MgCl_2$. The final reaction volume of 2 ml included 0.5 ml of 15.2 mM p-nitrophenyl phosphate (pNPP, Sigma 104). Final concentrations of pNPP in the kinetic experiments were 0.25, 0.5, 1.0, 2.0, 4.0 and 8.0 mmol/l. The reaction was stopped after incubation for 30 minutes by addition of 3 ml of 1 N NaOH and the absorbance read at 410 nanometers using a Gilford 240 spectrophotometer with 1 cm light path. Sample absorbance divided by .1176 yielded activity in mIU. In the kinetic experiments, approximately a half milligram of total protein with a total activity of about ten mIU was employed in each assay.

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Inhibition studies were carried out by addition of aqueous solutions of the inhibitor to the amount of substrate added. Inhibitor concentrations were calculated with respect to final assay mixture.

RESULTS AND DISCUSSION

The plot of I/V vs I/S yielded straight lines at several concentrations of acetazolamide ranging from 1 to 20 mmol/l (Fig 1). The mechanism of inhibition is of the mixed (competitive and non-competitive components) type similar to that ascribed to mafenide inhibition of AP (1) and the Lineweaver-Burk plot resembles those of furosemide (Fig 2), ethacrynic acid (Fig 3) and chlorothiazide (Fig 4). There appears to be no difference in mechanism of inhibition between bone and kidney, or between rat and human. From these plots, we calculated inhibition constant (Ki) values of 8.4, 7.0, 2.8 and 0.1 mmol/l for acetazolamide, furosemide, ethacrynic acid and chlorothiazide, respectively.

The placental isoenzyme was relatively resistant to acetazolamide, while those of bone, intestine, liver, kidney and granulation tissue were all inhibited about 63 per cent at 20 mmol/l acetazolamide (Table 1).

Table 1. Acetazolamide Inhibition (%) of Alkaline Phosphatase From Human Tissues

Tissue	Acetazolamide final concentration mmol/l			
	1.0	5.0	10.0	20.0
Liver	8.0 ± 4.0	31.7 ± 2.0	44.7 ± 0.6	63.4 ± 2.3
Bone	5.9 ± 2.8	30.0 ± 2.6	44.4 ± 3.2	64.1 ± 1.7
Granulation tissue	7.5 ± 3.7	29.6 ± 3.8	46.9 ± 0.7	63.3 ± 1.0
Kidney	0.6 ± 0.8	28.3 ± 2.3	44.8 ± 1.5	61.6 ± 0.0
Intestine	2.4 ± 2.2	30.5 ± 2.8	45.2 ± 3.3	63.3 ± 1.0
Placenta	7.5 ± 2.1	9.0 ± 1.9	13.4 ± 1.9	21.1 ± 2.0

Data represent mean ± standard deviation, n=3

There is no species difference in response of human and rat kidney AP to inhibition by chlorothiazide, ethacrynic acid or furosemide at several concentrations (Table 2). Ethacrynic acid and L-cysteine separately inhibited AP activity, while the combination

at equimolar concentrations was less inhibitory than L-cysteine alone (Table 3).

Table 2. Comparison of Inhibition (%) of Human and Rat Kidney AP By Three Diuretics

Species	Chlorothiazide, final concentration mmol/l			
	0.25	0.5	1.0	2.0
Human	16.6 ± 1.2	25.4 ± 1.4	39.1 ± 0.6	55.4 ± 1.6
Rat	19.8 ± 1.2	28.1 ± 1.4	45.2 ± 1.1	62.7 ± 1.1
	Ethacrynic acid, final concentration mmol/l			
	5.0	10.0	15.0	
Human	21.6 ± 0.6	52.2 ± 0.5	62.4 ± 0.7	
Rat	35.1 ± 0.9	54.4 ± 1.0	64.6 ± 0.3	
	Furosemide, final concentration 3.0 mmol/l			
Human	20.2 ± 3.5			
Rat	22.5 ± 2.1			

Data represent mean ± standard deviation, n=3

Table 3. Inhibition (%) of Human Kidney AP By Ethacrynic Acid, Cysteine or Both

Inhibitor	Concentration of inhibitor, mmol/l		
	0.01	0.2	1.0
Ethacrynic acid(EA)	0.6 ± 0.7	4.0 ± 2.6	11.1 ± 3.0
L-cysteine (Cys)	4.3 ± 1.5	65.8 ± 3.3	92.9 ± 0.2
EA + Cys	3.5 ± 0.8	36.1 ± 1.1	74.3 ± 1.7

Data represent mean ± standard deviation, n=3

The inhibition of human kidney AP by ethacrynic acid, furosemide and chlorothiazide is shown in Table 4.

Table 4. Inhibition (%) of Human Kidney Alkaline Phosphatase By Three Diuretics

Drug	Final concentration, mmol/l			
	1	2	5	10
Ethacrynic acid	4.3 ± 1.6	15.6 ± 0.7	34.2 ± 0.1	49.3 ± 0.8
	1.9	3.8	7.5	15
Furosemide	10.5 ± 0.5	17.7 ± 0.5	36.4 ± 1.2	57.3 ± 1.5
	0.25	0.5	1.0	2.0
Chlorothiazide	16.6 ± 1.2	25.4 ± 1.4	39.1 ± 0.6	55.4 ± 1.6

n=3, data represent mean ± standard deviation.

Final pNPP concentration = 4 mmol/l

Interpretation of the significance of inhibition of an enzyme whose physiologic role is poorly understood is obviously difficult. Although the compounds tested are diuretics of diverse chemical structure, several arguments can be made against a conclusion that alkaline phosphatase plays a significant role in diuresis.

The kidney isoenzyme is no more sensitive to the action of these diuretics than are those from liver or bone. It has been reported that furosemide and ethacrynic acid were equally effective diuretics in man (10), but that rats were relatively insensitive to ethacrynic acid (11). If the inhibition of AP played a major role in diuresis, one might expect a difference in the response of rat and human AP to ethacrynic acid. But our data show no species difference in inhibitory responses. The doses of diuretics which significantly inhibit AP in vitro are markedly higher than those which are effective in vivo. It should be remembered, however, that the test conditions are nonphysiologic, in terms of substrate, pH and

ionic strength and the enzyme has been solubilized from the cell membrane. The same caveat applied to comparisons of Ki or Km values, derived from these experiments, to those reported in other systems.

The role of AP in normal physiologic processes, development or healing has not been defined. Perhaps studies of its response to inhibitors or stimulators may help to elucidate its function. It may be useful, after observing inhibition by drugs such as the diuretics employed in this study, to investigate the effects of endogenous analogues of known inhibitors.

PRESENTATIONS AND/OR PUBLICATIONS.

Submitted to Clinical Chimica Acta for consideration.

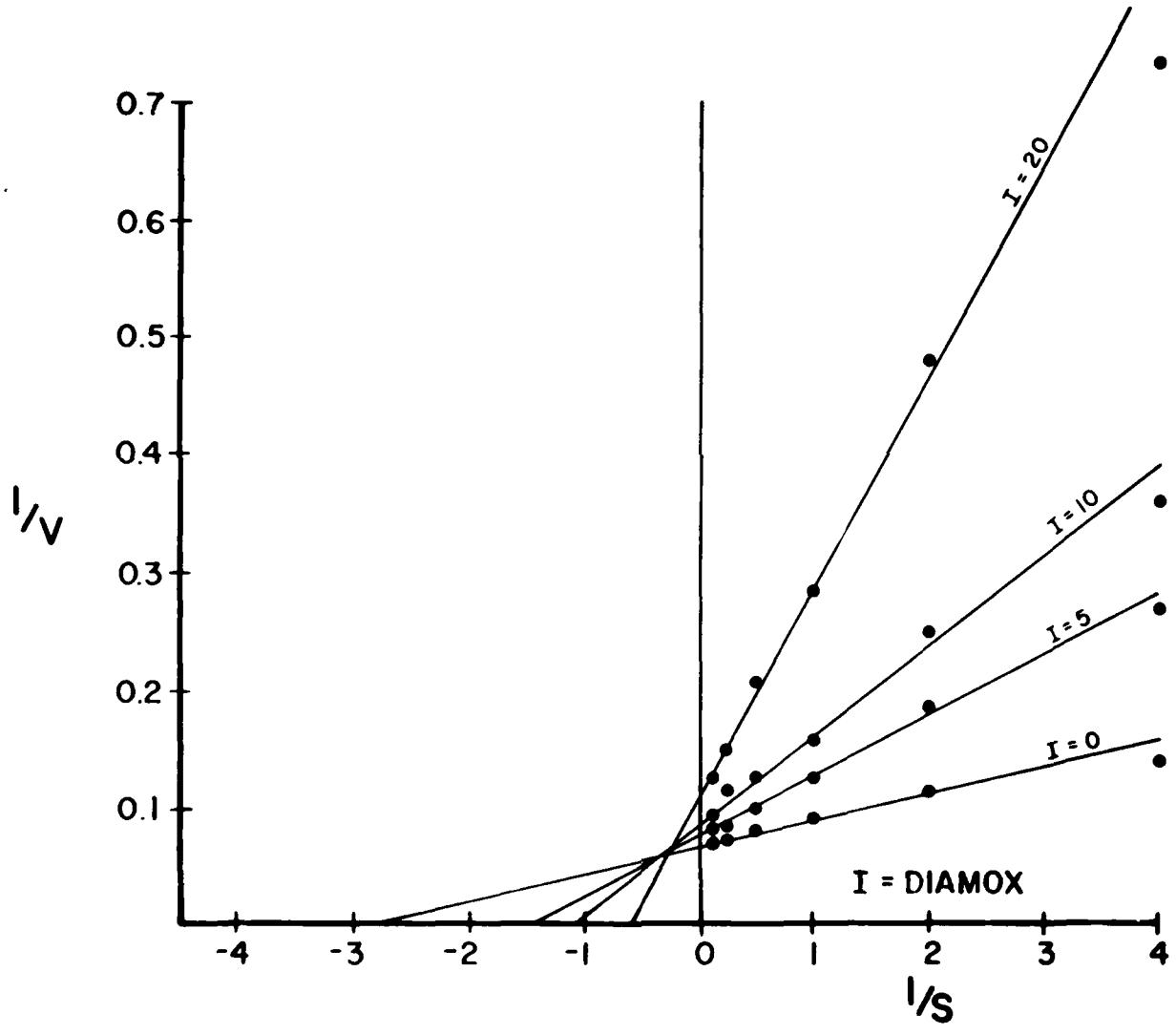

Fig. 1. Lineweaver-Burk plot (min·micromole^{-1} vs 1·millimole^{-1}) of acetazolamide inhibition of human bone AP. Inhibitor concentrations in millimole/liter.

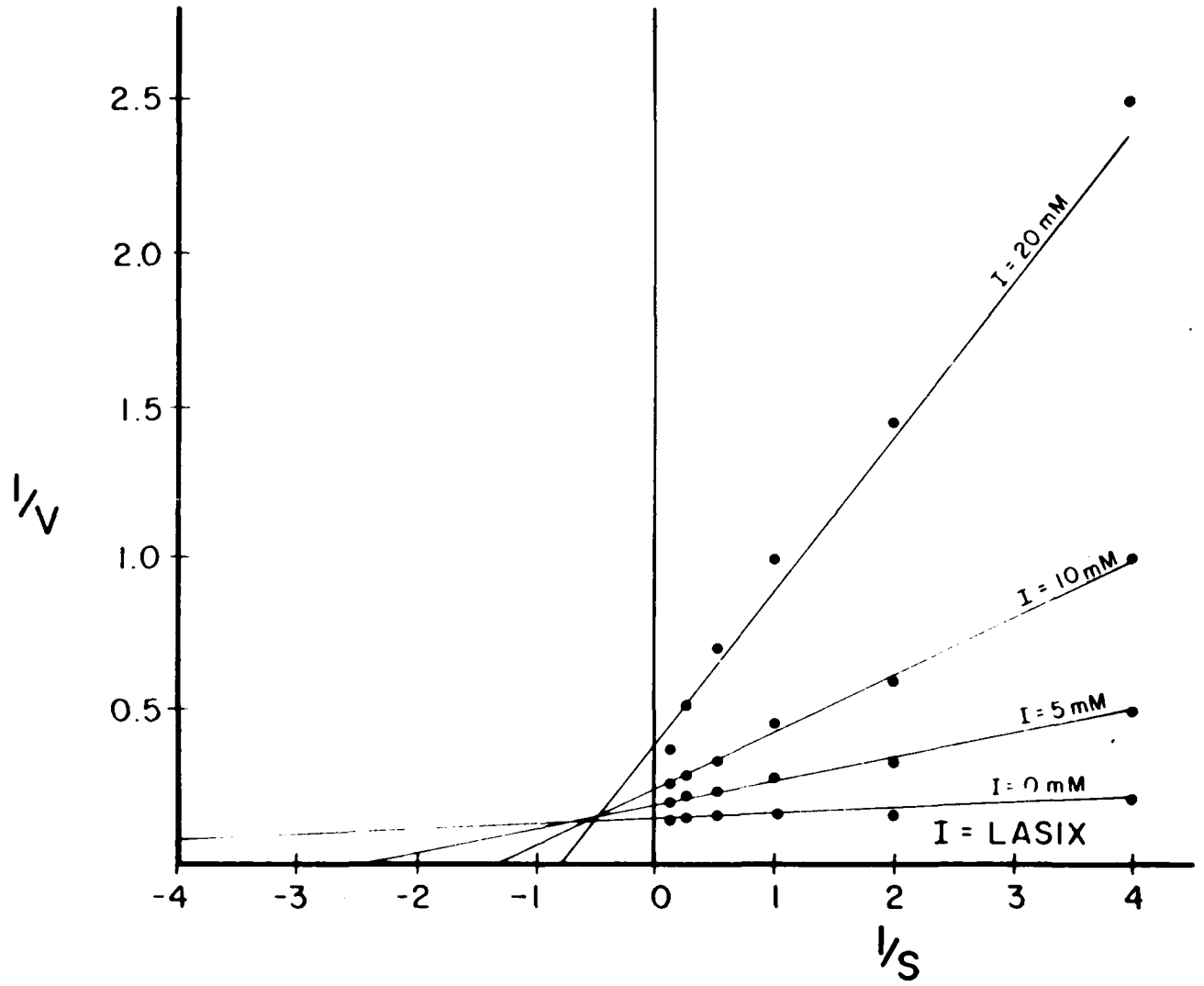

Fig. 2. Lineweaver-Burk plot (min·micromole^{-1} vs l·millimole^{-1}) of furosemide inhibition of human kidney AP. Inhibitor concentrations in millimole/liter.

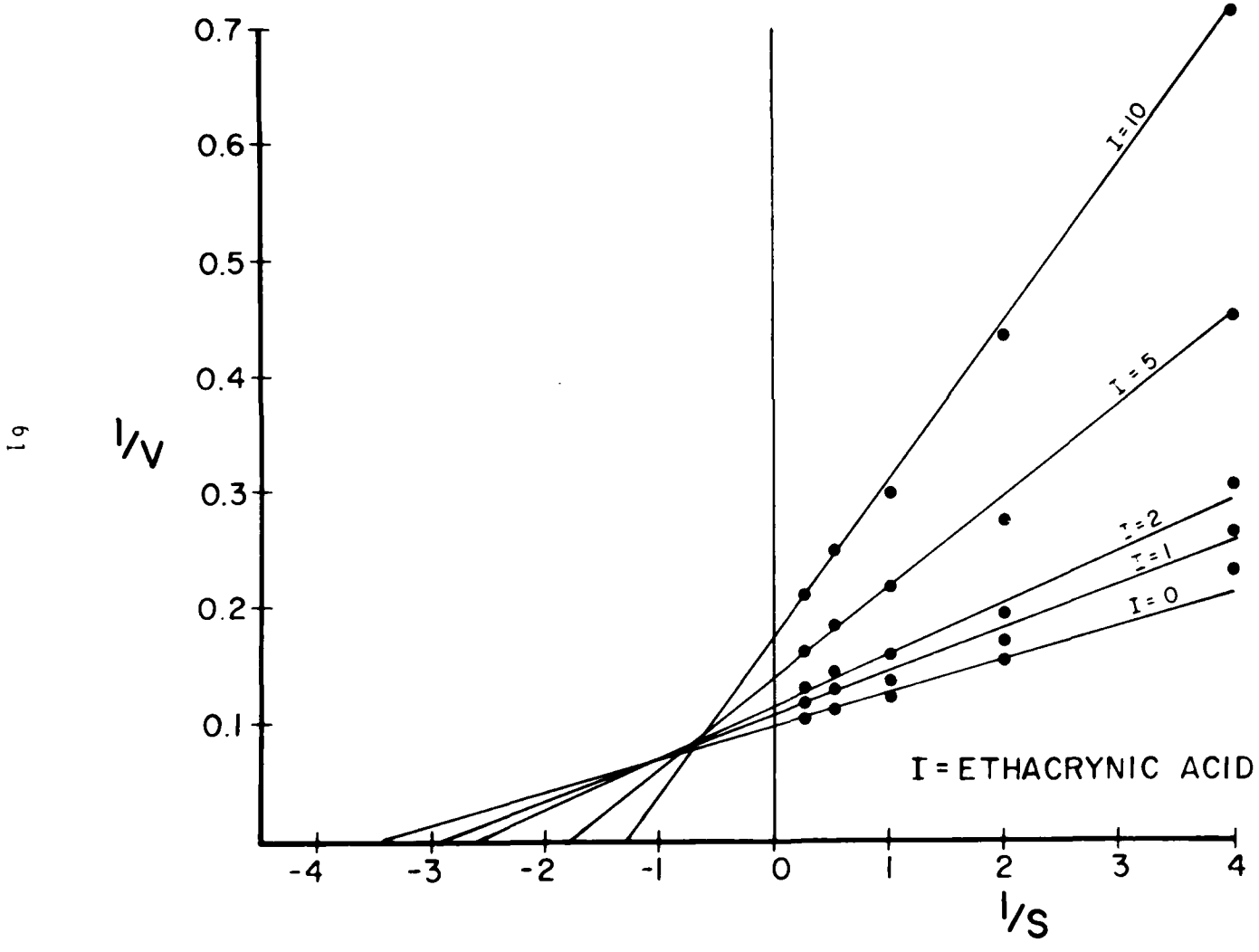

Fig. 3. Lineweaver-Burk plot of ethacrynic acid inhibition of human kidney AP. Inhibitor concentrations in millimolar.

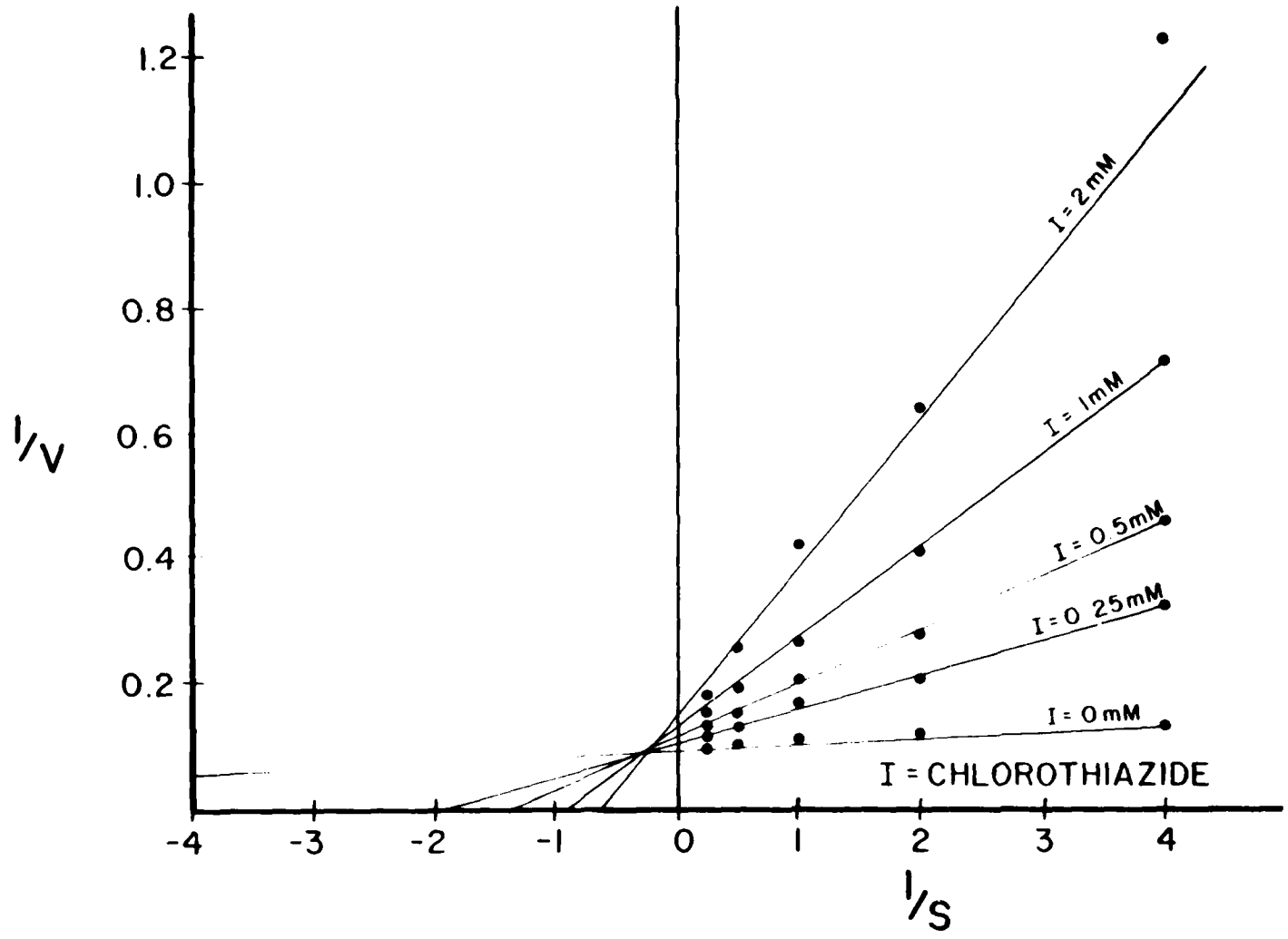

Fig. 4. Lineweaver-Burk plot (min·micromole^{-1} vs 1·millimole^{-1}) of chlorothiazide inhibition of rat kidney AP. Inhibitor concentrations in millimole/liter.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OG 6970	79 10 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
78 10 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	62772A	3S162772A814	00	114
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) Studies of Acute Renal Insufficiency and Renal Function Changes 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
76 10	Cont	DA	C. In-House

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

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
PRECEDING FISCAL YEAR 79	2	57
CURRENT FISCAL YEAR 80	2	100

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

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

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

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME: Richard H. Merrill, LTC, MC
TELEPHONE: 512-221-5416
SOCIAL SECURITY ACCOUNT NUMBER:

ASSOCIATE INVESTIGATORS
NAME: George Vaughan, MAJ, MC
NAME: DA

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Depressed immune response; (U) Calcium; (U) Renin; (U) Angiotensin; (U) Dog

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

23. (U) To determine the effect of calcium on the generation of renin in a chronic, awake, intact dog model. To assess the frequency, duration and pathophysiology of the depressed immune response in patients with end-stage renal disease.

24. (U) A chronic dog model is to be created with catheters in the renal artery, both renal veins, the abdominal aorta, and both ureters in such a fashion as to allow the animal to be studied in the awake mode. Calcium will be infused into the renal artery with assessment of its effect on the renin-angiotensin system. Immune studies including T and B cell enumeration, ingestion and killing of bacteria by leukocytes, chemotaxis, and lymphocyte blastogenesis will be performed on patients with end-stage renal disease.

25. (U) 7810 - 7909 Although the surgery on the dog has been perfected, the ability to prevent the intravascular catheters from being encased in a fibrous cocoon has not been achieved for a period longer than two weeks. Therefore, no physiologic studies have been done to date. Depression in T and B cell numbers, blastogenesis, and chemotaxis have been noted in patients with end-stage renal disease and are not improved by dialysis. Ingestion and killing of leukocytes from uremic patients is not abnormal.

*Available to contractors upon originator's approval

ANNUAL PROGRESS REPORT

PROJECT NO. 3S162772A814-00, APPLIED RESEARCH

REPORT TITLE: STUDIES OF ACUTE RENAL INSUFFICIENCY AND RENAL FUNCTION CHANGES IN INJURED SOLDIERS: CLINICAL OPERATION, METABOLIC BRANCH, RENAL SECTION, FOR TREATMENT OF SOLDIERS WITH RENAL FAILURE

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

1 October 1978 - 30 September 1979

Investigator:

Richard H. Merrill, Lieutenant Colonel, Medical Corps

ABSTRACT

PROJECT NO. 3S162772A814-00, APPLIED RESEARCH

REPORT TITLE: STUDIES OF ACUTE RENAL INSUFFICIENCY AND RENAL FUNCTION CHANGES IN INJURED SOLDIERS

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

Investigator: Richard H. Merrill, Lieutenant Colonel, Medical Corps

Reports Control Symbol, MEDDH-288(R1)

The nephrology presence in the US Army Institute of Surgical Research has been phased down over the reporting period, and the remaining nephrology slots are unfilled as of 30 September 1979. The dialysis mission has been turned over to Brooke Army Medical Center, and they will continue to provide dialysis and nephrology support to the Unit

STUDIES OF ACUTE RENAL INSUFFICIENCY AND RENAL FUNCTION CHANGES IN INJURED SOLDIERS - CLINICAL OPERATION, METABOLIC BRANCH, RENAL SECTION, FOR TREATMENT OF SOLDIERS WITH RENAL FAILURE

The Renal Section included in the Internal Medicine Branch as a part of the Clinical Division had as its principal mission the support of patients with thermal injury who had developed acute renal failure or developed significant acid-base or electrolyte disturbances. The Chief of the Renal Section has also been on the staff of the hospital, and the Chief of the Internal Medicine Branch, also a Nephrologist, has served as Chief of Nephrology at Brooke Army Medical Center. Both of these individuals have been active in clinical and laboratory research in addition to their patient care responsibilities. In June of 1978, the Chief of the Internal Medicine Branch resigned as Chief of Nephrology at the hospital to pursue full-time laboratory research at the institute. At the same time, the Chief of the Renal Section left the service, and by direct verbal order, the Chief of the Internal Medicine Branch assumed control of the Renal Section and was placed under the Laboratory Division. As such, he had no clinical responsibilities, although he did from time-to-time supervise some of the hemodialysis on the Burn Ward.

Over the period ending in June, 1979, the dialysis capabilities of the Institute were phased out, and that mission was assumed by the Nephrology Service at Brooke Army Medical Center. Currently the Institute has no dialysis technicians, and all dialysis equipment is in the process of being transferred to other clinical hemodialysis units. In addition, the remaining Nephrologist leaves at the end of this reporting period and has not been replaced. Coincidental with the departure of the Chief of the Renal Section, all renal research has been phased out and/or terminated.

In October of 1978, the Fourth Annual Army Nephrology Seminar entitled "Hypertension Update" was held, and in April, 1979, the Fifth Annual Army Nephrology Seminar entitled "Complications of End-Stage Renal Disease" was held. The April course was sponsored by the American College of Physicians and received excellent ratings from the College. The Chief of the Renal Section served as Course Director for both seminars. With the departure of this individual, the Annual Army Nephrology Seminars will not be continued.

PROGRESS REPORT

PROJECT NO. 3S162772A814-00, APPLIED RESEARCH

REPORT TITLE: STUDIES OF ACUTE RENAL INSUFFICIENCY AND RENAL FUNCTION CHANGES IN INJURED SOLDIERS: IMMUNE DEFICIENCY IN DIALYSIS PATIENTS: A STUDY OF ACUTE RENAL INSUFFICIENCY AND RENAL FUNCTION CHANGES IN INJURED SOLDIERS HEMATOLOGIC IMPAIRMENT IN END-STAGE RENAL DISEASE

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

1 October 1978 - 30 September 1979

Investigator:

Richard H. Merrill, Lieutenant Colonel, Medical Corps

Reports Control Symbol MEDDH-288(R1)

Unclassified

ABSTRACT

PROJECT NO. 3S162772A814-00, APPLIED RESEARCH

REPORT TITLE: IMMUNE DEFICIENCY IN DIALYSIS PATIENTS: A STUDY OF ACUTE RENAL INSUFFICIENCY AND RENAL FUNCTION CHANGES IN INJURED SOLDIERS HEMATOLOGIC IMPAIRMENT IN END-STAGE RENAL DISEASE

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

Period covered in this report: 1 October 1978 - 30 September 1979

Investigator: Richard H. Merrill, LTC, MC FACP

Reports Control Symbol MEDDH-288(R1)

STUDY OF ACUTE RENAL INSUFFICIENCY AND RENAL FUNCTION CHANGES IN INJURED SOLDIERS - HEPATOLOGIC IMPAIRMENT IN END-STAGE RENAL DISEASE

It was recognized in the late 1950's that hematologic function was not normal in patient's with end-stage renal disease and, in fact, this hematologic depression was sited as a significant factor in the early success in renal transplant patients (1, 2). The accumulating evidence lead Dr. H. Sherwood Lawrence to call uremia "nature's immunosuppressive device" in an editorial in the Annals of Internal Medicine in 1965 (3). Montgomerie (4), in an exhaustive review of renal failure and infection in 1968, concluded that infection was an important problem in acute renal failure but that the demonstrable alterations in various immune mechanisms had an uncertain relationship to infection in chronic renal failure. Despite intensive investigation of the immune system in patients with end-stage renal disease, very little has been added to elucidate this relationship in the ensuing decade. Although it is intuitively satisfying to link a laboratory abnormality with a clinical correlate, it is difficult to find data supporting the generally held concept that infection is more common in well managed end-stage renal failure patients on dialysis than would be expected.

In culling the literature for adequate studies investigating the immune system, one can conclude that the humoral and reticuloendothelial systems are probably intact or only modestly perturbated in patients with end-stage renal disease. The major defect is in the so called cellular immune system (5). Since the manifestations of uremia are protean, it may be fortuitous that the immune system is altered in uremia, since its investigation may provide some insight into the more generalized uremic state. We have studied a large number of patients with end-stage renal disease eventually requiring dialysis and have sufficient data on 19 to include them in this report.

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4. Montgomerie JZ, Kalmanson GM, Guze LB: Renal failure and infection. Medicine 47:1-32, 1968.

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MATERIALS AND METHODS

The patients were all studied longitudinally with data in some extending up to 108 months after the onset of dialysis. In addition, several patients were studied prior to the onset of hemodialysis. T lymphocytes were enumerated by the E rosetting technique, and B lymphocytes were enumerated by the EAC rosetting technique with adjustment for rosetting EAC monocytes. Chemotaxis was assayed using the modified Boyden technique with a standard endotoxin as the chemo-attractant. Blastogenesis was studied by measuring the tritiated thymidine uptake in the cell culture and applying phytohemagglutinin as the mitogen. Bacterial ingestion and killing was measured by the Acridine Orange technique as an index bacterium..

RESULTS

The average lymphocyte count in patients on chronic hemodialysis averaged 1,619 with an average total white count of 6,782 for a 24% lymphocyte population. These results are essentially normal although slightly lower than the population of patients with end-stage renal disease just prior to the initiation of dialysis where the same figures were 2,039, 8,860 and 26%. The percentage of B lymphocytes in the dialysis population was significantly lower than the normal control individuals although the average level was barely outside the normal range for our laboratory. The percentage of T lymphocytes was also significantly depressed, and all of the patients, save one, fell outside the normal range. An assessment of lymphocyte function by blastogenesis revealed a statistically significant difference between dialysis patients and normals at two concentrations of PHA, although at the higher concentration of PHA, the scatter produced considerable overlap. Analysis of neutrophil function by chemotactic measurement revealed significant differences between the normal group and the dialysis patients with almost all of the patients in the dialysis group falling outside the normal range. The unstimulated movement of neutrophils was slightly higher in the dialysis group but does not explain the major reduction in the chemotactic index. When the chemotactic index is plotted as a function of time that the patient has been dialyzed, one can clearly see that there is no improvement in chemotactic index and, in fact, there is a slight downward progression.

DISCUSSION

From this data the following observations may be cautiously made The defect in blastogenesis cannot be described to lymphopenia, since

the cell count was adjusted in each experiment to a standard number. The suggestion that the lymphocytes had been previously stimulated by uremic serum is not a viable explanation, since at the higher concentration of PHA they were capable of an incremental stimulation not unlike that of normals. The percentage of B lymphocytes being more normal than the T lymphocytes is consistent with the relatively normal humoral immune system observed in most studies whereas the more marked depression of T lymphocytes is consistent with a delayed skin graft rejection seen in experimental animals. The percentage of monocytes in our dialysis population was 10% as opposed to 3% in normals and the percentage in null cells is increased, but the significance of these observations is unknown. The defect in polymorphonuclear cell function does not seem to be due to an impairment of leukocyte mobility, since randomal movement as measured in our assay was not impaired. Therefore, the ability of white cells to respond to a chemotactic stimulus is abnormal, but whether this reflects a toxin, a defect in production of chemotactic lymphokine, alterations of the intracellular cyclic AMP/cyclic GMP ratios, altered transport of calcium and/or magnesium into the leukocytes, or any of a number of other factors known to be important in normal chemotactic response is unknown. The fact that chemotaxis declines with increasing longevity of dialysis suggests that a toxin is not at fault but, of course, does not exclude a higher molecular weight non dialyzable toxin. Although not felt to play a major role, the effect of increasing patient age has not been considered in this study.

In summary, the contribution of impaired cellular immune response, as well as non-specific immune response that has been manifested by defects in the leukocyte function, to the susceptibility of infection in end-stage renal failure patients is unknown. The data purporting to show increased incidence of infection in these patients is not convincing. However the easily measured defects in the immune system, presumably caused by some aspect of the uremic state, may be of value in the further investigation of pathophysiology of end-stage renal disease.

PROGRESS REPORT

PROJECT NO. 3S162772A814-00, APPLIED RESEARCH

REPORT TITLE: STUDIES OF ACUTE RENAL INSUFFICIENCY AND RENAL FUNCTION CHANGES IN INJURED SOLDIERS - THE EFFECT OF CALCIUM ON THE RENIN-ANGIOTENSIN SYSTEM-USE OF AN ANIMAL MODEL OF HYPERTENSION

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

1 October 1978 - 30 September 1979

Investigator:

Richard H. Merrill, Lieutenant Colonel, Medical Corps

Reports Control Symbol MEDDH-288(R1)

Unclassified

ABSTRACT

PROJECT NO. 3S162772A814-00, APPLIED RESEARCH

REPORT TITLE: STUDIES OF ACUTE RENAL INSUFFICIENCY AND RENAL FUNCTION CHANGES IN INJURED SOLDIERS - THE EFFECT OF CALCIUM ON THE RENIN-ANGIOTENSIN SYSTEM-USE OF AN ANIMAL MODEL OF HYPERTENSION

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

Period covered in this report: 1 October 1978 - 30 September 1979

Investigator: Richard H. Merrill, Lieutenant Colonel, Medical Corps

Reports Control Symbol MEDDH-288(R1)

The success in implanting catheters into the renal artery and both renal veins plus either ureteral interposition grafts or bilateral ureterostomies has been uniformly successful in the last six months. However, using polyethylene catheter material, a cocoon-like formation of fibrous tissue has gradually encroached upon the free end of the intravascular catheters and prevented their use. New catheter material has been tried, but the results are inconclusive, and since the principal investigator is leaving the service, the protocol will be concluded. No useable data have been derived from this chronic model.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OG 6968	79 10 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
78 10 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	3S161102BS05	00	090
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)
(U) Alteration of Host Resistance 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
76 10	Cont	DA	C. In-House

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

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In Thousands)
FISCAL YEAR	PRECEDING 79	4	93
	CURRENT 80	4	125

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NAME: DA

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede each with Security Classification Code)
(U) Microbial virulence; (U) Tissue spreading factors; (U) Rat model; (U) Infection; (U) Neutrophil function; (U) Metabolism; (U) Immunostimulants

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 basis of infection susceptibility in burned soldiers. To identify specific white blood cell dysfunction and establish the metabolic basis for such. To determine the effect of antibiotics on neutrophil function. Establish a burned rat model of granulocyte dysfunction. Examine the effect of immunostimulant drugs and metabolism altering drugs on rat granulocyte function. Examine tissue spreading factors of opportunistic pathogens that may accentuate defects in host phagocyte mobilization.

24. (U) The high susceptibility of burned rats to Pseudomonas infection will be investigated. Rat granulocyte function will be examined. The in vitro effects of antibiotics and stimulants will be examined in cells from burned humans.

25. (U) 7810 - 7909 The previously reported defective inflammatory responses in 60% burned rats have been further examined. Animals preimmunized with Pseudomonas aeruginosa were found to have readily demonstrable antibody titers and normal hemolytic complement levels (C_{50}). These animals, however, still demonstrated defective intraperitoneal inflammatory responses following injection of Pseudomonas organisms. Levamisole therapy (1 mg/K/day) improved the inflammatory response when compared to burned controls. The Levamisole treatment did not correct the response to the level seen in unburned controls.

*Available to contractors upon originator's approval.

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ANNUAL PROGRESS REPORT

PROJECT NO. 3S161102BS05-00, BASIC RESEARCH

REPORT TITLE: ALTERATION OF HOST RESISTANCE IN BURNED SOLDIERS -- BACTERIAL MOTILITY: A COMPONENT IN EXPERIMENTAL PROTEUS MIRABILIS BURN WOUND SEPSIS

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

1 October 1978 - 30 September 1979

Investigators:

Albert T. McManus, Major, MSC
Arthur D. Mason, Jr., M.D.
William J. Northam, SP5

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3S161102BS05-00, BASIC RESEARCH

REPORT TITLE: ALTERATION OF HOST RESISTANCE IN BURNED SOLDIERS -- BACTERIAL MOTILITY: A COMPONENT IN EXPERIMENTAL PROTEUS MIRABILIS BURN WOUND SEPSIS

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

Period covered in this report: 1 October 1978 - 30 September 1979

Investigators: Albert T. McManus, Major, MSC
Arthur D. Mason, Jr., M.D.
William J. Northam, SP5

Reports Control Symbol MEDDH-288(R1)

A burn wound isolate of *Proteus mirabilis* has been found to cause a rapidly invasive infection in burned rats. The LD_{50} of the strain is less than 1,000 organisms per square centimeter of burn surface. Serial sacrifice experiments demonstrated an infection progressing from initial colonization of nonviable burn tissue to invasion of underlying viable tissue and bacteremia. The burn wound infection was histopathologically distinct from experimental Pseudomonas infection, i.e., perivascular and perineural lesions were not obvious. The association of soft agar (0.5%) subsurface spreading and virulence was tested by comparing the mortalities following inoculation with (1) nonspreading mutants, (2) the parent strain, or (3) spreading clone isolated from the parent. Nonspreading clones displayed marked reduction in virulence ($P < 0.001$). These results suggest that motility and/or swarming and/or chemotaxis may be a virulence factor in this model of invasive burn surface infection by an opportunistic *Enterobacteriaceae*.

Rat model
Endotoxemia
Invasion

ALTERATION IN HOST RESISTANCE IN BURNED SOLDIERS -- BACTERIAL MOTILITY: A COMPONENT IN EXPERIMENTAL PROTEUS MIRABILIS BURN WOUND SEPSIS

Experimental Pseudomonas infection of the burned rat (1) was a major advance in the understanding of host-pathogen relationships in burn infections. This model has been extensively used to develop topical antimicrobial agents that have proven effective clinically (2). Additionally, the model remains the standard for evaluating Pseudomonas vaccines, toxoids (3), parenteral antibiotics (4) and proposed virulence factors (5). With the increasing incidence of non-Pseudomonas isolates, however, the utility of the model may be waning.

We would like to report a potentially useful model of fatal experimental burn wound infection with an Enterobacteriaceae. A human burn wound isolate of Proteus mirabilis has been found to be highly virulent for the 30% burned rat. The strain's virulence is related to inoculation dose (Fig. 1). An observed disadvantage of the model has been the failure of decreasing inoculation dose to proportionally increase time to death. All fatally infected rats died within 96 hours of inoculation. This fact may limit the utility of the model for the evaluation of chemotherapy. No prophylactic modalities have been tested to date.

Morphologically, the infection follows a progression from colonization of nonviable tissue to invasion of viable tissue with terminal bacteremia (6).

The possible role of motility in invasion with this agar swarming strain was examined as previously described for Pseudomonas. Soft agar immobile strains were found to have markedly reduced virulence when surface inoculated. These non-motile strains when

---

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

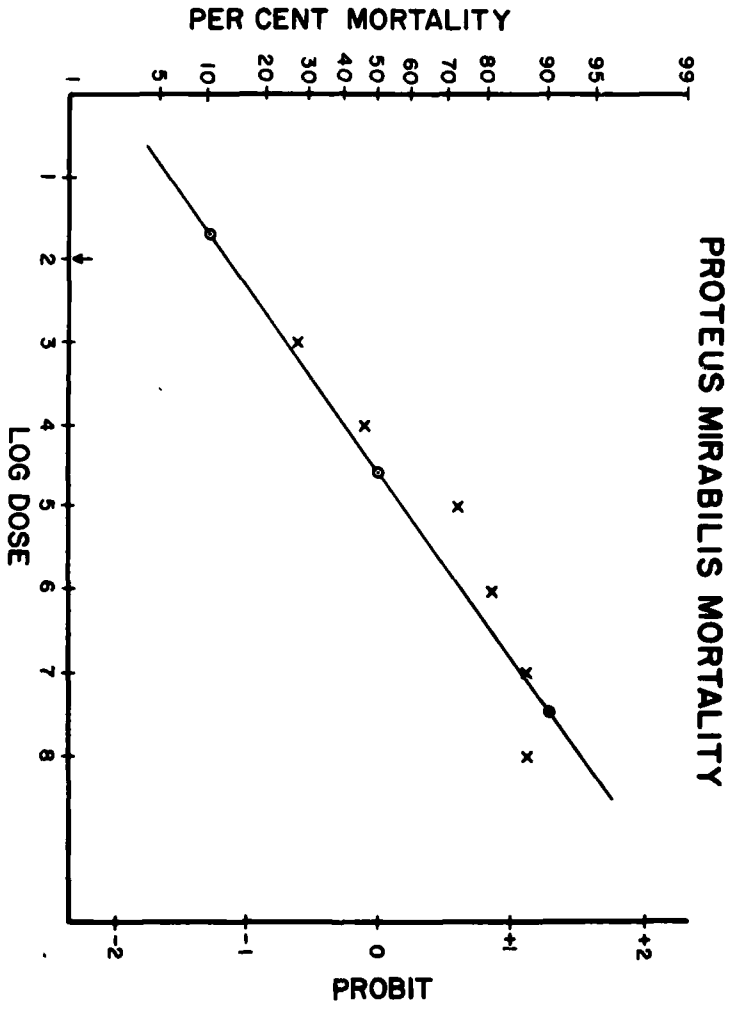
2. Lindberg RB, Moncrief JA, Switzer WE, Order SE, Mills W Jr: The successful control of burn wound sepsis. J Trauma 5:601-616, 1965.

3. Walker HL, McLeod CG Jr, Leppla SH, Mason AD Jr: Evaluation of Pseudomonas aeruginosa toxin A in experimental rat burn wound sepsis. Infect Immun 25:828-830, 1979.

4. Lindberg RB: Personal communication.

5. McManus AT Jr, Mason AD Jr: Alterations of host resistance in burned soldiers. Bacterial motility: A component in experimental Pseudomonas aeruginosa burn wound sepsis. USAISR Annual Report FY 1978, BAMC, Ft Sam Houston, TX, pp 57-65.

6. McLeod CG Jr: Personal communication.


PER CENT MORTALITY
1
5
10
20
30
40
50
60
70
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95
99
PROTEUS MIRABILIS MORTALITY
LOG DOSE
1
2
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PROBIT
-2
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0
+1
+2

Figure 1

injected below the eschar were virulent (Table 1). This fact adds to the possibility that bacterial motility may be a significant factor to consider in the development of future burn wound chemotherapy.

Table 1. Relative Virulence of *Proteus mirabilis** Strain 8-22-34 Motility Mutants

	Mortality
Parent	20/20
Non-motile Clones	13/80**
Non-motile Clones (injected)	36/40**
Motile Controls	25/30

* Inocula = 10^8 CFU
** $P < 0.001$

PUBLICATIONS/PRESENTATIONS - None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OG 6973	79 10 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
78 10 01	K. COMP.	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO. CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3S161102BS05	00	083
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)
(U) Gastrointestinal Alterations and Complications 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
76 10	79 07	DA	C. In-House

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

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 79	1	27
	CURRENT		

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NAME: DA

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Burn injury; (U) Infection; (U) Curling's ulcer; (U) Gastritis; (U) Pepsinogen; (U) Small bowel; (U) Intestinal flora; (U) Intestinal absorption

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

23. (U) To document and evaluate the gastrointestinal changes in burned soldiers. To study the role of pepsinogen in the etiology of Curling's ulcer and the effectiveness of cimetidine in ulcer prophylaxis. To assess the role of infection on stress ulcers and changes in small bowel morphology and function. To evaluate etiologies of stress ulcers.

24. (U) Esophageal, gastric and duodenal mucosal lesions will be verified endoscopically and with biopsies in burn patients. Mucosal lesions will be related to serum pepsinogen levels and the effect of cimetidine determined. Small bowel structure and function will be evaluated and the effect of bacterial flora assessed. A laboratory animal model of stress ulcer will be develeped.

25. (U) 7810 - 7907 A prospective, randomized, double-blind endoscopic study has been carried out in which the efficacy of cimetidine (13 patients) was compared to that of antacids (14 patients) in the prevention of stress ulceration following severe thermal injury. Each treatment modality was equally effective in the prevention of acute gastroduodenal disease and its associated complications. Both cimetidine and antacids produced a near elimination of duodenal disease and markedly reduced the severity of gastric disease when compared to that of untreated historical controls. Lack of major side effects and ease of administration make cimetidine an attractive alternative to antacid therapy in the prophylaxis of stress-induced gastroduodenal disease in the thermally injured patient. All studies are now complete.

*Available to contractors upon originator's approval

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

ANNUAL PROGRESS REPORT

PROJECT NO. 3S161102BS05-00, BASIC RESEARCH

REPORT TITLE: GASTROINTESTINAL ALTERATIONS AND COMPLICATIONS IN BURNED TROOPS-ULCERATIVE GASTRIC DISEASE IN THE SEPTIC BURNED RAT: A MODEL FOR THE STUDY OF NON-HEALING STRESS ULCERS IN THE MILITARY POPULATION

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

1 October 1978 - 30 September 1979

Investigators:

Charles G. McLeod, Jr., Major, VC
Albert T. McManus, Captain, MSC
Thomas W. Panke, M.D., Major, MC
Arthur D. Mason, Jr., M.D.

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3S161102BS05-00, BASIC RESEARCH

REPORT TITLE: GASTROINTESTINAL ALTERATIONS AND COMPLICATIONS IN BURNED TROOPS-ULCERATIVE GASTRIC DISEASE IN THE SEPTIC BURNED RAT: A MODEL FOR THE STUDY OF NON-HEALING STRESS ULCERS IN THE MILITARY POPULATION

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

Period covered in this report: 1 October 1978 - 30 September 1979

Investigators: Charles G. McLeod, Jr., Major, VC
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Arthur D. Mason, Jr., M.D.

Reports Control Symbol MEDDH-288(R1)

The burned rat infected topically with Pseudomonas aeruginosa develops a spectrum of gastrointestinal lesions similar to that which occurs in human burn patients and other victims of severe trauma. Morphological studies in this model have identified two distinctly different ulcerative processes. The first type is apparently a typical "stress" erosive or ulcerative process which quickly heals in rats that do not develop sepsis. The second type of ulcerative lesion represents a gastrointestinal hematogenous bacterial infection.

Ulcerations
Sepsis
Stress

GASTROINTESTINAL ALTERATIONS AND COMPLICATIONS IN BURNED TROOPS -- ULCERATIVE GASTRIC DISEASE IN THE SEPTIC BURNED RAT: A MODEL FOR THE STUDY OF NON-HEALING STRESS ULCERS IN THE MILITARY POPULATION

Currently, gastric ulcerations of clinical importance are rare in the burned patient. The apparent success of antacid therapy has practically eliminated deep gastric mucosal ulcerations and serious bleeding in burn patients (1). This, the final report for this project will summarize the findings of morphologic studies of ulcerative gastric disease which were conducted in the burned rat.

METHODS

The standard burn rat model is utilized (2). Pentobarbital anesthetized rats receive a 60% total body surface scald burn and are inoculated topically with Pseudomonas aeruginosa strain 1244. Non-infected burned rats serve as controls.

RESULTS

A final test was conducted to determine the incidence of gastrointestinal ulceration in burned rats without sepsis. In a group of 18 non-septic 60% total body surface burned rats examined 14 days following injury, no gastric or intestinal ulcerations were observed. This supports the results of all other experiments which indicate:

1. Ulcerative "stress" lesions occur primarily in the immediate post burn period. These lesions heal promptly unless other stresses (primarily sepsis) are incurred.

2. The deep ulcerative mucosal defects which contain gram-negative rod-shaped bacteria apparently develop only in septic rats. The spectrum of lesions observed in all previous experiments support the concept that these lesions result from hematogenous submucosal infections.

DISCUSSION

Septic burn patients may occasionally have gastrointestinal lesions

1. McAlhany JC Jr, Czaja AJ, Pruitt BA Jr: Antacid control of complications from acute gastrointestinal disease after burns. J Trauma 16:645-649, 1976.
2. Walker HL, Mason AD Jr: A standard animal burn. J Trauma 8:1049-1051, 1968.

which are thought to be caused by hematogenous Pseudomonas aeruginosa (3-7). Review of autopsy material at this Institute has revealed additional cases which are morphologically similar to the hematogenous lesions observed in the burned rat. Metastatic ulcerative pseudomonas lesions in unburned skin may resolve following antibiotic therapy (8). Similarly, it may be anticipated that some of the metastatic ulcerative gastrointestinal lesions may heal spontaneously or with systemic treatment. In our opinion, a healing metastatic pseudomonas lesion of the gastrointestinal tract may lose the characteristic histologic features of infection and be indistinguishable from the so-called "Curling's ulcer."

PRESENTATIONS AND/OR PUBLICATIONS.

A summary of this information entitled, "Gastrointestinal Ulceration in the Septic Burned Rat" has been accepted for presentation at the American College of Veterinary Pathologist annual meeting, December 1980.

3. Fraenkel E: Uber allgemeininfektionendurch den Bacillus pyocyaneus. Virchows Arch Path Anat 183:405-440, 1906.

4. Markley K, Gurmendi G, Chavez PM, Bazan A: Fatal Pseudomonas septicemias in burned patients. Ann Surg 145:175-181, 1957.

5. Sevitt S: Duodenal and gastric ulcerations after burning. Br J Surg 54:32-41, 1967.

6. Rubin ER, Graber CD, Vogel EH, Finkelstein RA, Tumbusch WA: Fatal Pseudomonas infections in burned patients. New Engl J Med 265:1225-1231, 1961.

7. Teplitz C: Pathology of Burns, p 78. In The Treatment of Burns, Artz CP and Moncrief JA, eds, WB Saunders Co, Phila, 1969.

8. Loebl EC, Marvin JA, Curreri PW, Baxter CR: Survival with ecthyma gangrenosum, a previously fatal complication of burns. J Trauma 14:370-377, 1974.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OG 6974	79 10 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
78 10 01	K. COMP.	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3S161102BS05	00	084
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) Alterations in Pulmonary Function and Pulmonary Complications 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
76 10	79 07	DA	C. In-House

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

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING	79	2	63
FISCAL YEAR CURRENT			

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21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED
DA

22. KEYWORDS (Precede EACH with Security Classification Code): (U) Burn injury; (U) Pulmonary tests; (U) Humans; (U) Goats; (U) Total thoracic resistance; (U) Arterial blood gas tension

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 mechanisms of pulmonary dysfunction in the thermally injured to document the site of inhalation injury early in the clinical course and to follow its progression quantitatively; to determine the extent and significance of temperature for arterial blood gas values in the thermally injured population.

24. (U) Pulmonary function status of the burned patient will be measured by various techniques including oscillometrics, pulmonary extravascular water studies, arterial blood gas measurements (corrected for temperature by the nomogram of Nunn and Kelman), and pulmonary mechanics determinations.

25. (U) 7810 - 7907 Total thoracic pulmonary resistance measurements have been completed in extrinsic asthmatic and allergic rhinitis patients during routine intradermal skin testing. It is concluded that total thoracic resistance measurement during skin testing can replace inhalation bronchial challenges for extrinsic asthmatics. Lack of computing facilities in the hospital ward has prevented measurements in the thermally injured soldier. On-line computer acquisition of blood gas values has allowed rapid computation of corrected values; 791 studies were analyzed in 17 patients with a mean body temperature of 101.1° F. Corrections resulted in changes in PaO_2 of +7 torr, $PaCO_2$ of +2, pH of -.02 and $P(A-a)O_2$ gradient of -10. All studies are now 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 66 AND 1498-1 1 MAR 68 FOR ARMY USE) ARE OBSOLETE.

TERMINATION

PROJECT NO. 3S161102BS05-00, MILITARY BURN RESEARCH

REPORT TITLE: ALTERATIONS IN PULMONARY FUNCTION AND PULMONARY COMPLICATIONS IN BURNED SOLDIERS -- BODY TEMPERATURE CORRECTION OF ARTERIAL BLOOD GAS STUDIES

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

1 October 1978 - 30 September 1979

Investigators:

Victor Lam, M.D., Major, MC
Douglas E. Mills, First Lieutenant, MSC
SP5 Dianne L. Martin

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3S161102BS05-00, MILITARY BURN RESEARCH

REPORT TITLE: ALTERATIONS IN PULMONARY FUNCTION AND PULMONARY COMPLICATIONS IN BURNED SOLDIERS -- BODY TEMPERATURE CORRECTION OF ARTERIAL BLOOD GAS STUDIES

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

Period covered in this report: 1 October 1978 - 30 September 1979

Investigators: Victor Lam, M.D., Major, MC
Douglas E. Mills, First Lieutenant, MSC
SP5 Dianne L. Martin

Reports Control Symbol MEDDH-288(R1)

Arterial blood gas [ABG] tensions are widely utilized to monitor pulmonary function in the thermally injured patient. Although methods to correct pH and partial pressures of gases in blood for body temperature have been available for over 10 years, they are not universally employed.

This study prospectively evaluated 791 serial ABG determinations in 77 burn patients to determine the mean correction for body temperature of measured pH, $PaCO_2$, PaO_2, calculated alveolar arterial oxygen gradient [$P(A-a)O_2$] and computer derived primary acid-base disturbance diagnosis. Data for all consecutive ABG studies were entered into a PDP 11-40 computer. A Fortran computer program employed the temperature correction of Nunn and Kelman and the acid-base mapping technique of Goldberg. The $P(A-a)O_2$ was computed from the alveolar air equation with a temperature-dependent water vapor pressure and assumed respiratory quotient of 0.8. The acid-base map contained the area of 95% probability of a primary acid-base process as determined by $PaCO_2$ and pH.

The results of this study clearly demonstrate the significance of correcting arterial blood gas measurements for body temperature in the thermally injured patient.

Burn injury
Pulmonary function tests
Humans
Arterial blood gas tensions

ALTERATIONS IN PULMONARY FUNCTION AND PULMONARY COMPLICATIONS IN BURNED SOLDIERS -- BODY TEMPERATURE CORRECTION OF ARTERIAL BLOOD GAS STUDIES

Arterial blood gas measurements are frequently utilized in critical situations to characterize both the efficiency of the oxygen transfer process and the acid-base status of the patient. In the clinical laboratory, the arterial blood sample is brought to a constant temperature (generally 37° C) by the measurement apparatus prior to determination of pH, PCO_2 and PO_2. A difference between the patient's body temperature and arterial blood sample temperature during measurement is known to affect blood gas values; however, the appropriate corrections are not universally applied. Although the effects of temperature on partial pressure of gases in blood have been published and correction factors have been programmed into computers, the corrections are perceived to be small and possibly of little consequence.

This study sought to determine the magnitude of alterations of blood gas tension values and the effect on clinical acid-base disturbance diagnosis in an intensive care facility. Since patients with thermal injury frequently have a febrile course, all arterial blood gas studies from the United States Army Institute of Surgical Research critical care unit during the study period were evaluated.

METHODS

All arterial blood gas studies from the United States Army Institute of Surgical Research critical care unit during a 6-month period were entered on-line into a DEC PDP 11/40 computing facility. Data entered included the subject's name, date, time of study, body temperature (oral or rectal), hemoglobin, oxygen saturation, pH, PaO_2 and $PaCO_2$. Results computed by a Fortran program were alveolar-arterial oxygen gradient, total oxygen content, temperature corrected PCO_2, PO_2, pH and acid-base disturbance.

Alveolar-arterial oxygen gradient is calculated from ideal air equation with an assumed respiratory quotient of 0.8 (1). Oxygen content of the blood is determined with a value of 1.34 volume percent and a solubility of .003 volume percent dissolved in plasma. The nomogram of Kelman and Nunn (2) is used to correct PaO_2 and $PaCO_2$ (Figure 1). The PaO_2 correction is dependent upon oxygen saturation,

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1. Bates DV. Respiratory Function in Disease. Philadelphia, W.B. Saunders, 1971, p 66.
2. Kelman GR, Nunn JF: Nomograms for correction of blood PO_2, PCO_2, pH and base excess for time and temperature. J Appl Physiol 21:1484-1490, 1966.

TEMPERATURE CORRECTION FACTORS FOR PO_2 AND PCO_2

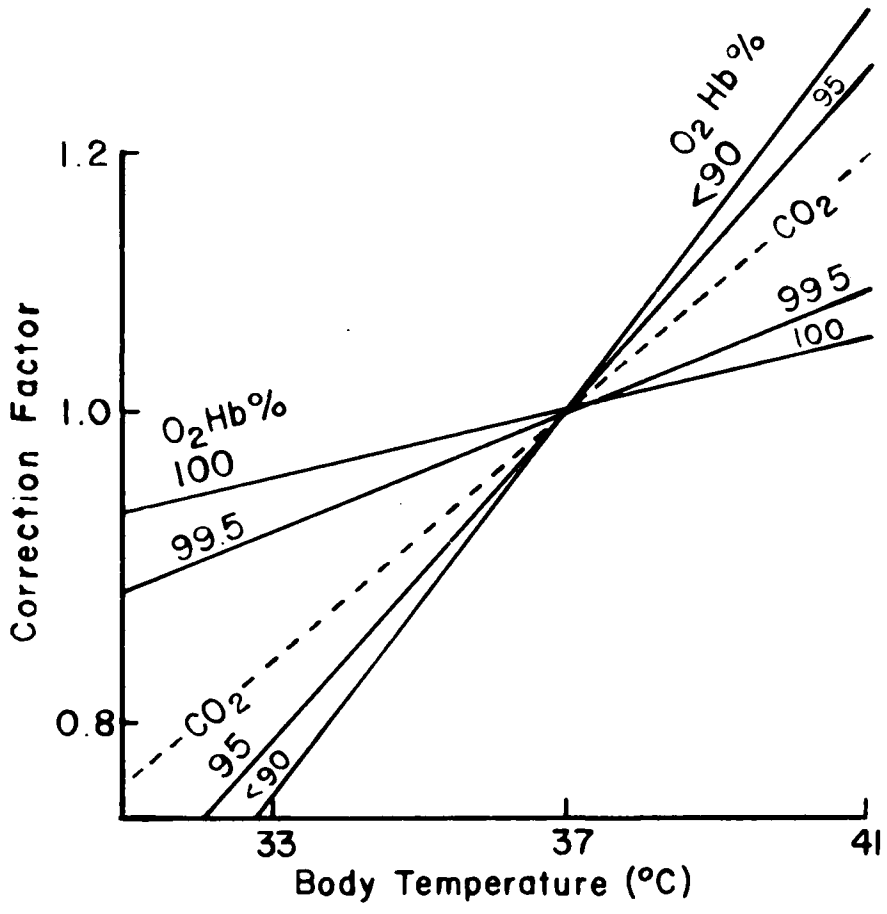

Fig. 1

for which a calculated value was used. The diagnosis of acid-base disturbance is computer derived using the method of Goldberg et al (3). A mapping technique with pH and PCO_2 as variables allows the determination of a primary acid-base diagnosis at the 95% confidence level (Figure 2). The labeled areas include:

Normal
Acute respiratory acidosis
Acute respiratory alkalosis
Chronic respiratory acidosis
Chronic respiratory alkalosis
Metabolic acidosis
Metabolic alkalosis
Acute-chronic respiratory acidosis
Acute-chronic respiratory alkalosis
Mixed disturbance

RESULTS

The data base consisted of 791 arterial studies from 77 patients. The mean age of the patients was 38 years (range 1 to 92 years), the mean total body surface area burn 47% ± 19 S.D., and the mean body temperature 101.1° F (range 96° to 105.6° F).

For arterial PO_2, the uncorrected value was 105 torr ± 2 and was increased to 112 ± 2 ($P < 0.01$). Alveolar-arterial oxygen was improved from 121 to 111 torr. This improvement was due in part to PaO_2 correction and in part to the correction for increasing water vapor saturation pressure with temperature. The mean pH was decreased from 7.44 to 7.42, while mean $PaCO_2$ increased from 32 to 34 with temperature correction.

Figure 3 shows the distribution of all pH and PCO_2 data plotted on the computer derived acid-base map. Since the computer program has 10 diagnostic compartments, there are 90 permutations for change with temperature correction. Table 1 lists the number for each diagnosis before temperature correction, Table 2 lists the number for each diagnosis after temperature correction, and Table 3 lists the changes of acid-base diagnosis with temperature correction of pH and $PaCO_2$.

Initial diagnoses were altered in 32% of all cases with temperature corrections, including 36% of patients with the initial diagnosis of respiratory alkalosis and 39% with mixed disturbances.

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3. Goldberg M, Green SB, Moss ML, Marbach CB, Garfinkel D: Computer-based instruction and diagnosis of acid-base disorders. JAMA 223:269-275, 1973.

Table 1. Non-temperature Corrected Blood Gases

Diagnosis	Number	% of Total
Mixed disturbance	179	22.62
Metabolic acidosis	93	11.75
Chronic respiratory alkalosis	177	22.37
Acute respiratory alkalosis	207	26.16
Metabolic alkalosis	44	5.56
Normal	63	7.96
Acute-chronic respiratory alkalosis	1	0.12
Acute respiratory acidosis	18	2.27
Chronic respiratory acidosis	3	0.37
Acute-chronic respiratory acidosis	6	0.75

Table 2. Temperature Corrected Blood Gases

Diagnosis	Number	% of Total
Mixed disturbance	195	24.65
Metabolic acidosis	112	14.15
Chronic respiratory alkalosis	153	19.34
Acute respiratory alkalosis	150	18.56
Metabolic alkalosis	51	6.44
Normal	90	11.37
Acute-chronic respiratory alkalosis	1	0.12
Acute respiratory acidosis	28	3.53
Chronic respiratory acidosis	4	0.50
Acute-chronic respiratory acidosis	7	0.88

Table 3. Nontemperature Corrected to Temperature Corrected

Diagnosis Change	Number	Diagnosis Change	Number
1 → 2	34	5 → 1	1
1 → 3	21	5 → 4	1
1 → 4	1	5 → 6	10
1 → 5	8	5 → 7	1
1 → 6	4	5 → 8	3
1 → 10	1	6 → 1	4
2 → 1	32	6 → 2	1
2 → 6	1	6 → 8	7
3 → 1	34	6 → 10	2
3 → 2	18	7 → 8	1
3 → 6	4	8 → 9	1
4 → 1	11	8 → 10	1
4 → 3	14	10 → 1	1
4 → 5	14	10 → 6	1
4 → 6	21	10 → 8	1
TOTAL CHANGES = 254		% CHANGE = $\frac{254}{791}$ = 32%	

Code:
1. Mixed disturbance
2. Metabolic acidosis
3. Chronic respiratory alkalosis
4. Acute respiratory alkalosis
5. Metabolic alkalosis
6. Normal
7. Acute-chronic respiratory alkalosis
8. Acute respiratory acidosis
9. Chronic respiratory acidosis
10. Acute and chronic respiratory acidosis

Figures 4 and 5 show the distribution of acid-base diagnosis before and after temperature correction.

DISCUSSION

Computed temperature correction for oxygen tension resulted in a clinically significant decline of 11 torr in the mean alveolar-arterial oxygen gradient. Current laboratory apparatus is designed for calibrations of gas tensions to less than 1 torr. In effect, noncorrection of data for temperature would negate the accuracy results assured by careful calibration of laboratory equipment.

In actual clinical practice, the deterioration in daily serial alveolar-arterial gradient may reflect an increase in patient temperature rather than a deterioration in oxygen transfer. Appropriate temperature correction may reduce unnecessary pulmonary therapy such as intubation or supplemental oxygen.

The clinical significance of pH and PCO_2 corrections with temperature can be estimated by computer-derived diagnosis. This is an unbiased method to sort out changes in acid-base diagnosis. It is arbitrary, and changes in diagnosis may be the result of only small changes in the values of PCO_2 and pH. However, the large incidence of altered acid-base disorders suggests that in a certain patient population, it is of clinical importance.

Although the study population is comprised of thermally injured patients, the results of this investigation can be applied to all critically ill patients. However, burned patients often present with unique respiratory alterations:

(1) A wide range of respiratory quotients (0.7 to > 1.0) depending on source and quantity of caloric intake.

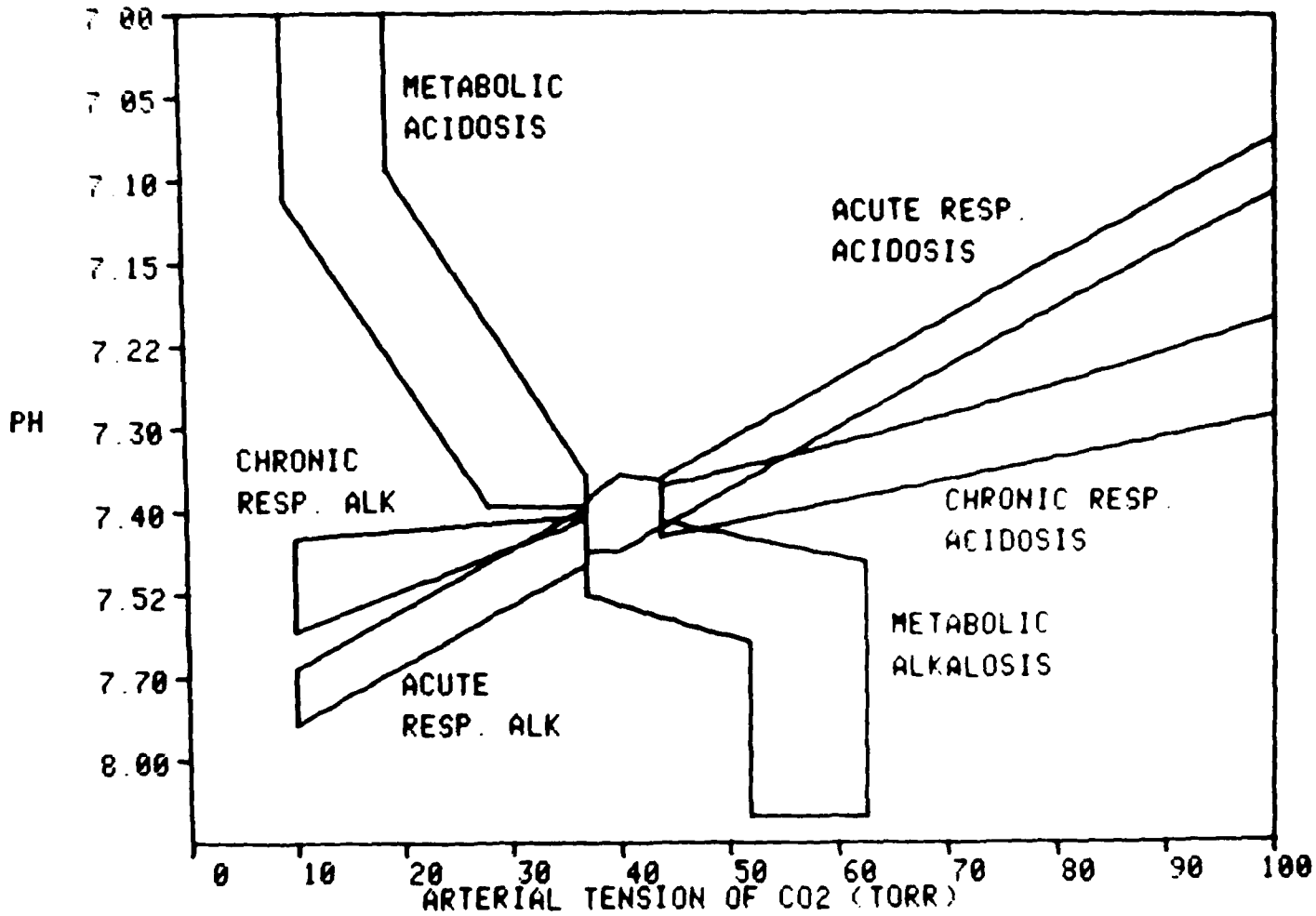
(2) An average arterial oxygen tension higher than in most intensive care patients, so that the percentage correction for PaO_2 will be greater.

(3) A chronic respiratory alkalosis in most cases, in spite of greatly increased CO_2 production with thermal injury.

PUBLICATIONS: None

PRESENTATIONS:

Lam V: Body Temperature Correction of Arterial Blood Gas Studies Necessary in the Burned Patient. Presented at the Annual Meeting of the American Burn Association, New Orleans, Louisiana, 16 March 1979.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CHRONIC RESP. ACIDOSIS
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ACUTE RESP. ALK
METABOLIC ALKALOSIS
0
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50
60
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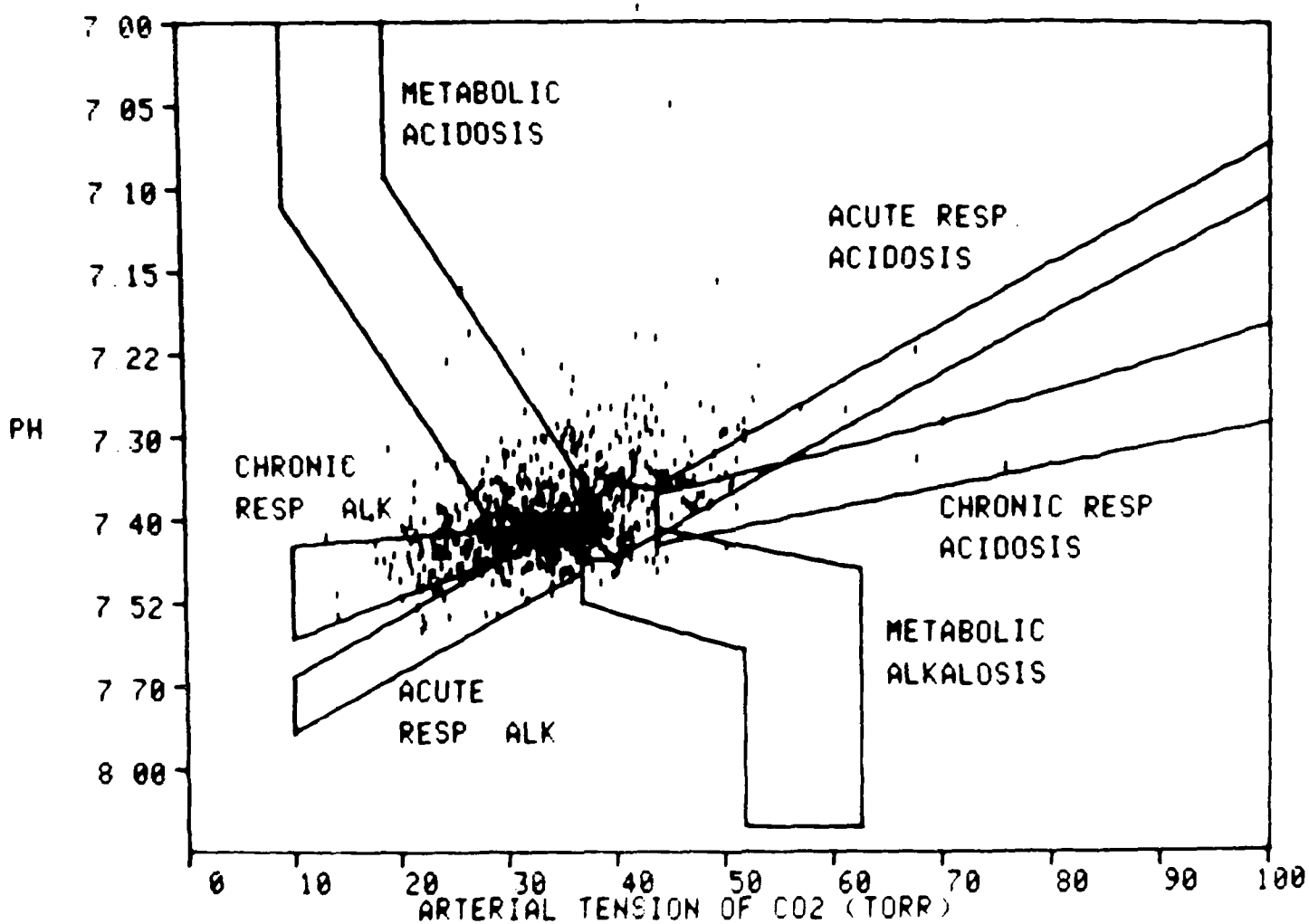
100
ARTERIAL TENSION OF CO2 (TORR)

Fig. 2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METABOLIC
ACIDOSIS
ACUTE RESP
ACIDOSIS
CHRONIC
RESP ALK
CHRONIC RESP
ACIDOSIS
METABOLIC
ALKALOSIS
ACUTE
RESP ALK
0
10
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30
40
50
60
70
80
90
100
ARTERIAL TENSION OF CO2 (TORR)

Fig. 3

FREQUENCY OF ACID-BASE DISTURBANCE IN THERMAL INJURY

(NON-TEMPERATURE CORRECTED DATA)

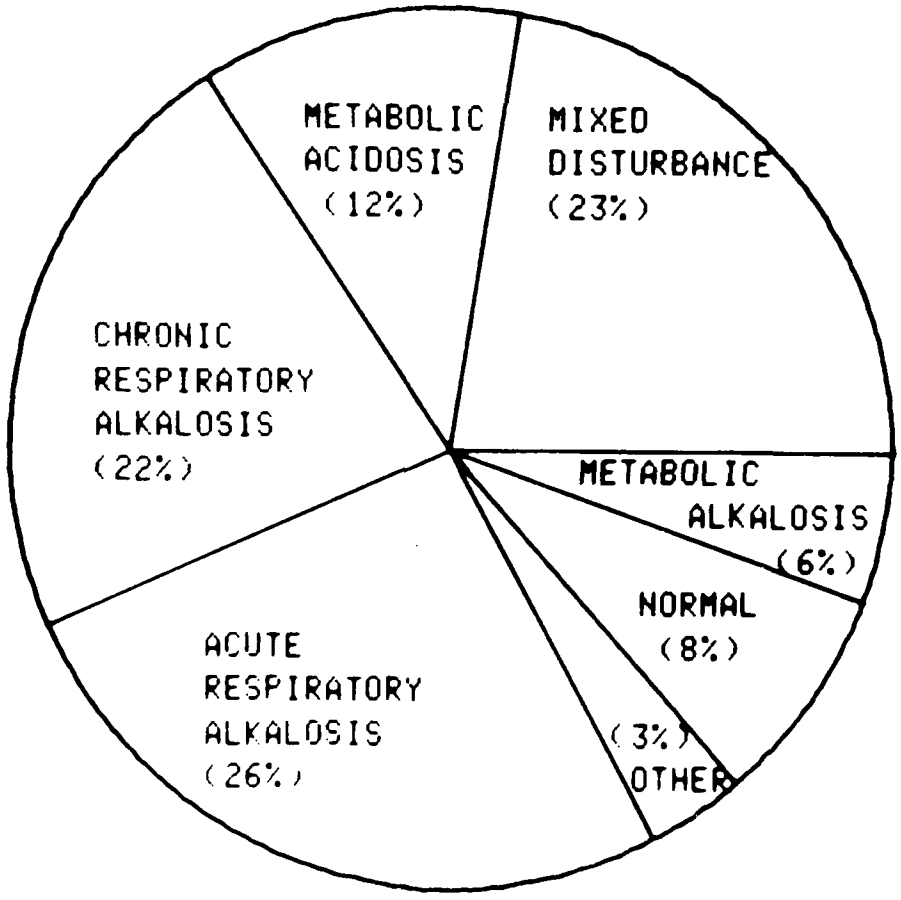

Fig. 4

FREQUENCY OF ACID-BASE DISTURBANCE IN THERMAL INJURY

(TEMPERATURE CORRECTED DATA)

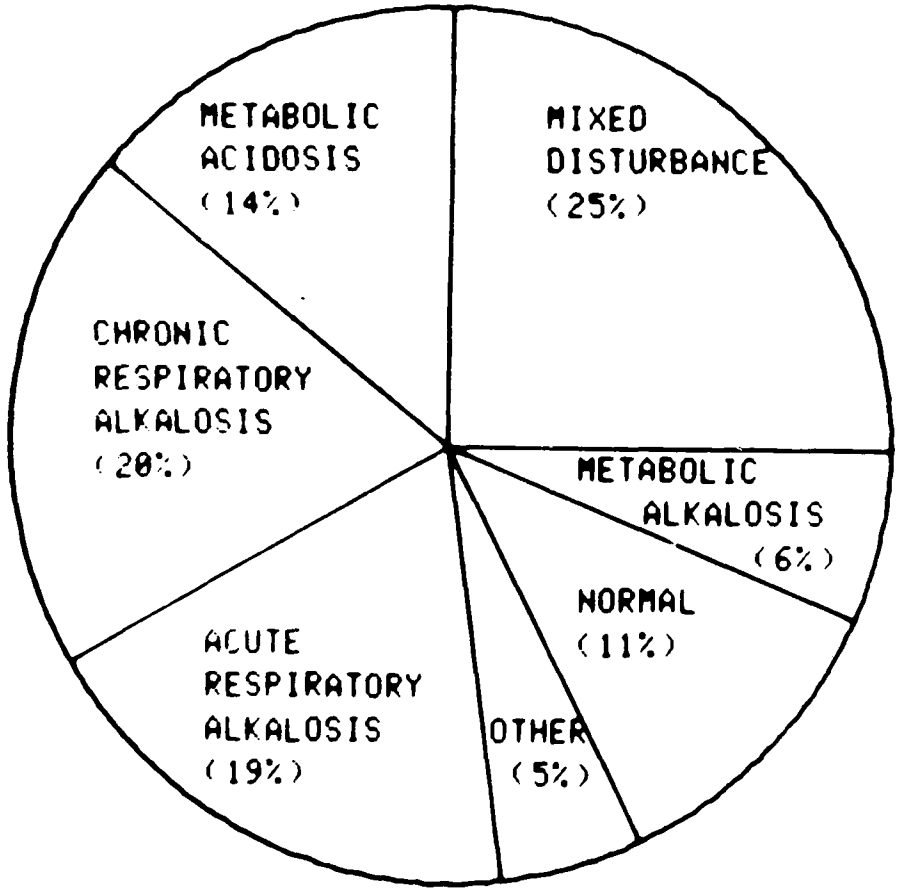

Fig. 5

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION*	2. DATE OF SUMMARY*	REPORT CONTROL SYMBOL
DA OD 6978	79 10 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
78 10 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	3S161102BS05	00	081
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)* (U) Evaluation of Synthetic Sheeting as Operating Room Drape Material For Use in a Military Burn Unit (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS*
003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
70 07	Cont	DA	C. In-House

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

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING	79	.2	7
FISCAL YEAR CURRENT	80	.2	10

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

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SOCIAL SECURITY ACCOUNT NUMBER:
ASSOCIATE INVESTIGATORS
NAME: Robert B. Lindberg, PhD
NAME: DA

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Military burn unit; (U) Operating room based infections; (U) Surgical drapes; (U) Surgical gowns

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

23. (U) Evaluation in terms of draping characteristics, absorbency, physician acceptance, and bacterial barrier qualities of a Spunbonded Olefin-cellulosic Laminated sheeting as surgical drapes and gowns. A decrease in bacterial seeding of operative wounds via drapes will minimize postoperative wound infections decreasing subsequent morbidity and mortality in injured troops.

24. (U) Laboratory assessment of bacterial barrier properties 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) 7810 - 7909 The bacterial barrier property of six newly fabricated synthetic drape materials has been assessed using the testing method developed in this laboratory. The penetration of the six materials by Ps. aeruginosa, Staphylococcus aureus, E. coli, Klebsiella pneumoniae and Serratia marcescens was determined with identification of differences in penetration apparently related to both organism and drape characteristics. Drape characteristics were the predominant determinant of penetration with two materials showing clearly superior barrier properties.

*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. 3S161102BS05, MILITARY BURN RESEARCH

REPORT TITLE: EVALUATION OF SYNTHETIC SHEETING AS OPERATING ROOM DRAPE MATERIAL FOR USE IN A MILITARY BURN UNIT

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

1 October 1978 - 30 September 1979

Investigators:

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

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3S161102BS05, MILITARY BURN RESEARCH

REPORT TITLE: EVALUATION OF SYNTHETIC SHEETING AS OPERATING ROOM DRAPE MATERIAL FOR USE IN A MILITARY BURN UNIT

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

Period covered in this report: 1 October 1978 - 30 September 1979

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

Reports Control Symbol MEDDH-288(R1)

The bacterial barrier properties of six types of synthetic surgical drape material were assessed using test methods developed at this Institute. The transmission of each of five different bacteria suspended in liquid culture media and inoculated on samples of each of the six drape materials was determined in seven replicate trials of each material.

Penetration rates differed significantly between drape materials. Penetration of individual materials also showed some organism variability. Two of the six materials possessed clearly superior barrier properties with penetration identified at only 1.9 per cent and 2.4 per cent of all inoculation sites respectively. Penetration of the other four materials ranged from 22.4 to 41.9 per cent of all inoculation sites indicating that they were ineffective barriers to the passage of bacteria. Material composition thus appeared to be the critical factor determining the rate of microbial penetration in these tests.

Military burn unit
Operating room based infections
Surgical drapes
Surgical gowns

EVALUATION OF SYNTHETIC SHEETING AS OPERATING ROOM DRAPE MATERIAL FOR USE IN A MILITARY BURN UNIT

Surgical drapes made of muslin or cotton, when they become moistened in the course of an operation, permit ready passage of bacteria and serve as inadequate barriers to microbial migration into the surgical field and operative wound. Synthetic drape materials possessing superior barrier properties have been increasingly accepted and are now used in more than 50 per cent of all operations. First generation synthetic materials had poor draping characteristics and permitted quantitative runoff of liquids from the operative field onto the surgeon. The processes required to soften the synthetic materials to improve their draping characteristics and to bond cellulosic materials to the synthetics to improve absorbency and limit fluid runoff have compromised the barrier properties of the synthetic materials. Testing of newer forms of non-woven spun-bonded Olefin drape material, both treated and untreated with water-repellency compounds, has been carried out to evaluate their adequacy as microbial barriers.

Methods

Discs of the synthetic drape material 90 mm in diameter were cut, gas-sterilized and placed on the surface of blood agar culture plates. The top-bottom orientation designated for each drape material sample was observed with the upper surface of the disc corresponding to the side of the drape which would be away from the patient when used. Six drops of an overnight TSB broth culture of each test strain were placed at equidistant intervals on each disc. The spacing permitted differentiation of individual areas of growth of the test organism if it had penetrated the disc material. The discs of drape material were left in position for four hours at room temperature following which any remaining culture liquid was removed using a micro pippette. The discs of draping material were then removed and the plates incubated overnight at 35^{o}C. Penetration of the drape material was considered to have occurred if growth of the test strain was apparent at any site where it had rested on the material. If confluent growth occurred which encompassed two inoculation sites, each of the sites involved was considered as a site of penetration although it would be theoretically possible for organisms from a single site of penetration to spread over adjacent sites under the disc of drape material. Each of six drape materials was tested for penetration by Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, and Serratia marcescans. Seven replicate trials of each drape material for each organism were carried out - a total of 1,254 inoculation sites.

Results

The percentage of penetration by all test strains of each drape material is shown in the table. Although there is some microbial strain-specific

variation in penetrability within drape materials a greater and significant difference is observed between materials. The percentage of inoculation sites at which microbial penetration occurred was least in material No. 77-92-2 (1.9%) and material No. 77-92-4 (2.4%). The per cent of inoculation sites penetrated in the other materials ranged from 22.4 per cent to 41.9 per cent indicative of limited effectiveness as bacterial barriers.

TABLE I

PENETRATION OF INOCULATION SITES BY TEST STRAINS

Organism and Fraction of Test Sites Penetrated

Sample Number	Pseudomonas aerug	Staph aureus	E. coli	Klebsiella pneumoniae	Serratia marces.	Totals	Percentage of Sites Penetrated
77-92-1	9/42	19/42	8/42	8/42	18/42	62/210	29.5
77-92-2	0/42	0/42	0/42	1/42	3/42	4/210	1.9
77-92-3	9/42	11/42	5/42	10/42	12/42	47/210	22.4
77-92-4	2/42	2/42	0/42	1/42	0/42	5/210	2.4
77-92-5	11/42	31/42	11/42	31/42	4/42	88/210	41.9
77-92-6	1/42	8/42	1/42	8/36	36/42	54/210	26.5
TOTALS	32/252	71/252	25/252	59/246	73/252		

Discussion

The six drape materials which were tested showed considerable variation in microbial penetration which ranged from only 1.9 per cent for material No. 77-92-2 to 41.9 per cent for material No. 77-92-5. Four of the drape materials tested possessed ineffective barrier properties with penetration of bacteria at over one-fifth of all inoculation sites. Two materials appeared to possess highly effective barrier properties with penetration at only 4 of 210 or 1.9 per cent and 5 of 210 or 2.4 per cent of inoculation sites respectively. All of the drape materials which were tested in this study were composed of non-woven spun-bonded Olefin and all were of similar appearance, of similar density, and had similar gross physical properties. The two materials which served as effective barriers had each received a post-fabrication water-repellency treatment.

In summary, the results of these studies confirm earlier tests indicating that drape composition is of critical importance in terms of bacterial barrier

properties, but identify for the first time that inherent drape penetrability can be modified by post-fabrication treatment with compounds which influence the water-repellency of the material. The barrier function reliability of these two drape materials exceeds that of any material tested in the past several years except that of the original unsoftened Tivek supplied by the fabricator. Further testing is planned of these softened drape materials during the forthcoming year to assess in greater detail the effect of water-repellency treatments and other techniques by which bacterial penetrability can be modified.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY				1. AGENCY ACCESSION* DA OG 6972	2. DATE OF SUMMARY* 79 10 01	REPORT CONTROL SYMBOL DD-DR&E(AR)636
3. DATE PREV SUM'RY 78 10 01	4. KIND OF SUMMARY D. CHANGE	5. SUMMARY SCTY* U	6. WORK SECURITY* U	7. REGRADING* NA	8. DISP'N INSTR'N NL	8b. SPECIFIC DATA-CONTRACTOR ACCESS ☐ YES ☐ NO	9. LEVEL OF SUM A. WORK UNIT

10. NO./CODES:*	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61102A	3S161102BS05	00	088
b. CONTRIBUTING	62774A	3S162774A820	00	116
c. CONTRIBUTING				

11. TITLE (Procede with Security Classification Code)* (U) Studies of Infection and Microbiologic Surveillance of Troops With Thermal Injury (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS* 003500 Clinical Medicine

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
76 10	Cont	DA	C. In-House

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

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 79	4	154
FISCAL YEAR CURRENT 80	4	221

19. RESPONSIBLE DOD ORGANIZATION
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TELEPHONE: 512-221-2018
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) Pseudomonas; (U) Klebsiella; (U) Staphylococci; (U) Wound Infection; (U) Sepsis; (U) Endotoxin; (U) Topical chemotherapy; (U) Humans

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

23. (U) Burns constitute a large component of military injuries in combat. The military relevance of this research lies in the fact that infection and resultant sepsis are major problems in burned soldiers. Control of surface infection has been pursued empirically. At the same time, mechanisms of infection, epidemiology, topical chemotherapy and antibiotic use are assessed in relation to causative bacterial species.
24. (U) Culture of burn wound surfaces, sputum, blood, biopsies, urine and other sites related to infection are done. Detailed strain differentiation by multiple technics is done. Endotoxin is assayed. Virulence is assessed on experimental burn wound models, and new drugs are assessed on standardized infection models in burned animals.
25. (U) 7810 - 7909 Control of epidemic Ps. aeruginosa episodes in burn patients appeared to be increasingly effective with continuation of alternate therapy regimens with Sulfamylon and Silvadene. Continued increase in silver resistance was noted. Linkage with antibiotic resistance via plasmid mediation was sought unsuccessfully, but this study is being continued. Methicillin-group antibiotic resistance was shown to fluctuate rapidly, with Nafcillin-resistance being prominent in methicillin sensitive groups of Staphylococci. A new pattern of moderate Cleocin sensitivity appeared in these strains. Effective experimental metal-sulfonamides included Cr, Cu, and Mn-sulfadiazenes; a common factor not related to antibacterial anions was indicated. Antibiogram-differentiation of epidemic strains of Pseudomonas and Staphylococcus was applied as an effective epidemiologic guideline in assessing such outbreaks, thus enlarging the potential for rapid delineation of nosocomial outbreaks of infection.

*Available to contractors upon requestor's approval.

ANNUAL PROGRESS REPORT

PROJECT NO. 3S161102BS05-00, BASIC RESEARCH

REPORT TITLE: STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE OF TROOPS WITH THERMAL INJURY -- ANTIBIOTIC SENSITIVITY OF CURRENT MILITARY BURN PATIENT FLORA

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

1 October 1978 - 30 September 1979

Investigators:

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Susan J. Constable, SSG
Loralie R. Sanders, SP4

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3S161102BS05-00, BASIC RESEARCH

REPORT TITLE: STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE OF TROOPS WITH THERMAL INJURY -- ANTIBIOTIC SENSITIVITY OF CURRENT MILITARY BURN PATIENT FLORA

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

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Reports Control Symbol MEDDH-288(R1)

Five hundred and six strains of bacteria, recovered from blood cultures, biopsies, and other clinical sources, were assessed for antibiotic sensitivity by a tube-dilution MIC procedure. Staphylococcus aureus and Pseudomonas aeruginosa were, as was the case in 1977-1978, the principal causes of sepsis. S. aureus was resistant to aminoglycosides, sensitive to tetracyclines, and sensitive to some but not all methicillins. Vancomycin was highly effective. Keflin was also active against most strains, and was especially active in very low dilutions. Pseudomonas strains were resistant to aminoglycosides except for amikacin, and were Minocin and Vibramycin sensitive. As has previously been noted, the therapeutic response with antibiotics in the presence of gram-negative sepsis is not encouraging.

Antibiotic sensitivity
Pseudomonas
Burn wounds
Staphylococci

STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE OF TROOPS WITH THERMAL INJURY -- ANTIBIOTIC SENSITIVITY OF CURRENT MILITARY BURN PATIENT FLORA

The delineation of antibiotic sensitivity is a paramount consideration in infection in burn injuries. Although control of surface colonization by bacteria is primarily achieved by topical antibacterial agents, systemic infection or invasion of the burn wound requires prompt and systemic antibiotic therapy if control of such infection is to be achieved. Antibiograms of bacteria from burn patients are carried out in the Institute of Surgical Research by use of a broth dilution MIC procedure. All blood stream isolates are tested, as well as isolates from biopsies and indicated strains from other sources. Five hundred and six strains were tested in 1978-1979. Of these, 83 were from sources other than blood, primarily biopsy isolates. When a given species is recovered on an epidemic scale from a series of burn patients, antibiograms can be of real value in delineating the presence of an epidemic strain. This information is to be backed up with more definitive strain differentiating technics, but still, the antibiogram data has proven to be very useful. Epidemic strains have shown shifts from sensitive to resistant, but reversals to sensitive have also occurred and are actually more significant. Although it is widely assumed that the trend is invariably from sensitive to resistant among gram-negative bacteria, even in those populations re-emergence of sensitivity in burn patient flora has been observed.

STRAINS TESTED AND ANTIBIOTIC TEST BATTERY

There were 14 species of bacteria and a total of 506 strains tested. The comparative incidence of these species for the past 4 years is shown in Table 1. Three species, Staphylococcus saprophyticus, Acinetobacter spp., and Enterobacter aerogenes, were not previously observed. Further, two species previously tested in significant numbers in previous years, Entero. cloacae and Citrobacter spp., were not encountered in any significant site in 1978-1979. S. aureus was the predominant species, as it was in the previous year. Pseudomonas aeruginosa involved a number of patients each year that varied but little. S. epidermidis was not associated with serious clinical disease, although it occurred in 18 patients' blood cultures. Enteric species formerly numerically important included Klebsiella pneumoniae and Entero. cloacae. The former was of little significance and the latter was not recovered from blood in 1978-1979. The remaining species listed were numerically insignificant.

SENSITIVITY OF BURN PATIENT FLORA TO ANTIBIOTICS

The test battery of antibiotics used is shown in Table 2. This battery is selected on the basis of recent developments in the field of

Table 1. Species of Bacteria Tested for Antibiotic MIC: Number of Patients Positive and Number of Strains Recovered, 1 October 1978 - 30 September 1979

Species	Year, No. of Patients, and No. of Strains ( ) 1976	1977	1978	1979
Staphylococcus aureus	43 (125)	50 (75)	86 (345)	85 (245)
Staphylococcus epidermidis	21 (36)	28 (66)	39 (35)	18 (25)
Staphylococcus saprophyticus	.....	.....	.....	2 (2)
Streptococcus spp. (a)	9 (13)	15 (31)	19 (31)	11 (13)
Corynebacterium spp.	1 (1)	0	4 (4)	3 (3)
Pseudomonas aeruginosa	42 (94)	42 (90)	31 (71)	30 (166)
Acinetobacter spp. (b)	.....	.....	.....	4 (4)
Escherichia coli	10 (11)	15 (23)	19 (45)	8 (16)
Klebsiella pneumoniae	60 (145)	19 (32)	14 (25)	7 (15)
Enterobacter cloacae	2 (2)	28 (41)	9 (19)	0
Enterobacter aerogenes	.....	.....	.....	3 (4)
Serratia marcescens	2 (4)	7 (10)	2 (4)	2 (5)
Citrobacter spp.	1 (2)	8 (15)	2 (3)	0
Proteus spp. (c)	6 (11)	10 (19)	12 (20)	5 (8)

(a) Streptococcus spp. included Streptococcus viridans (3 strains), Group D Streptococcus, not enterococcus (1 strain), Streptococcus pneumoniae (2 strains), and Streptococcus faecalis (7 strains).
(b) Acinetobacter spp. included Acinetobacter anitratus (2 strains), and Acinetobacter lwoffii (2 strains).
(c) Proteus spp. included Proteus mirabilis (6 strains), Proteus morganii [Morganella morganii] (1 strain), Proteus rettgeri [Providencia rettgeri Bio 5 B] (2 strains).

Table 2. Antibiotics Used in MIC Assessment of Sensitivity
1 October 1978 - 30 September 1979

GRAM-POSITIVE ORGANISMS			GRAM-NEGATIVE ORGANISMS		
Gentamicin	:	G	Gentamicin	:	G
Tobramycin	:	To	Tobramycin	:	To
Oxacillin	:	Ps	Kanamycin	:	K
Methicillin	:	Sc	Amikacin	:	Ak
Nafcillin	:	U	Minocin	:	M
Minocin	:	M	Vibramycin	:	Vb
Vibramycin	:	Vb	Keflin	:	Kf
Keflin	:	Kf	Colistin	:	Co
Vancomycin	:	Va			
Clindamycin	:	Cl			

Pseudomonas aeruginosa: Add Carbenicillin : Cb
Ticarcillin : Ti

antibiotics and clinical experience. It is subject to change on short notice, but in 1978-1979, the only alteration from the preceding year was the addition of ticarcillin to the antibiotics used in testing P. aeruginosa. As has been established in previous years, sensitivity levels are expressed as MIC. For summation purposes, the upper level of sensitivity is designated as 6.25 mcg/ml for gram-positive organisms and 12.5 mcg/ml for gram-negative organisms. In the case of carbenicillin and ticarcillin, this sensitivity cutoff is set at 156 to 312 mcg/ml. In each instance, the ultimate decision as to the appropriateness of use of a given antibiotic depends on clinical decision, supported by the in vitro data derived by the laboratory.

Staphylococcus aureus: The major pathogenic species in burn patients for 1978-1979 was S. aureus. In past years, fluctuation in sensitivity of this species to methicillir analogues has been noteworthy and made the species of special interest. Table 3 presents the sensitivity of 245 strains tested. Minocin, its analogue Vibramycin, and the glycopeptide vancomycin were active with virtually all strains tested. Among methicillins, oxacillin was the most effective, with 75.9% of strains sensitive; methicillin inhibited 34.6%, and nafcillin was virtually ineffective. This distribution of sensitivity resembled that observed in 1977-1978. It continued to negate the concept of the methicillin group of antibiotics as exhibiting comparable degrees of effectiveness. Aminoglycosides were of little effect against this group of strains. Keflin was relatively active, as was clindamycin. The latter antibiotic had become much more effective during the year, in comparison to 1977-1978.

Table 3. Staphylococcus aureus: Cumulative Inhibitory Levels for 245 Strains
1 October 1978 - 30 September 1979

Antibiotic Level mcg/ml	Antibiotic and % Inhibited									
	G	To	Ps	Sc	U	M	Vb	Kf	Va	Cl
25.0	100	100	100	100	100	100	100	100	100	100
25.0	21.3	7.0	91.8	93.4	20.0	100	100	95.2	100	84.7
12.5	20.5	6.6	85.7	72.2	5.7	98.7	99.2	91.5	100	84.3
6.2	17.2	6.2	75.9	34.6	0.4	96.3	98.0	78.4	98.8	73.1
3.1	11.9	4.2	57.1	3.6	0.0	72.2	42.5	69.4	75.9	8.8
1.5	8.2	3.4	36.3	1.2	0.0	46.5	12.3	62.9	4.9	3.3
.78	4.5	2.2	10.2	0.0	0.0	22.4	9.0	53.1	0.0	0.0

G: Gentamicin; To: Tobramycin; Ps: Oxacillin; Sc: Methicillin; U: Nafcillin; M: Minocin; Vb: Vibramycin; Kf: Keflin; Va: Vancomycin; Cl: Clindamycin

A retrospective comparison of activity of these antibiotics against S. aureus for the past eight years is shown in Table 4. The emergence of aminoglycoside resistance was conspicuous, starting in 1977-1978. The methicillin group has shown a similar spectacular degree of change. Starting in 1972, the three drugs rose in effectiveness for two years. Methicillin resistance then increased, and sensitivity of Staphylococcus strains has fluctuated markedly since. Oxacillin has been more effective except for a sharp drop in 1977-1978. Nafcillin, highly effective in 1974 and 1975, fell in 1976 to a 50% resistant level; since then, Staphylococcus strains have been essentially resistant. The tetracyclines have been consistently effective. However, Minocin in 1975 and Vibramycin in 1978 exhibited reduced effectiveness, but the drop was transitory.

Table 4. Comparison of Sensitivity of Staphylococcus aureus to Antibiotics, 1972 - 1979

	Year and % of Strains Inhibited by 6.25 mcg/ml							
Antibiotic	1972	1973	1974	1975	1976	1977	1978	1979
Gentamicin	35.0	67.9	92.2	38.3	50.0	30.6	7.4	17.2
Tobramycin	....	....	....	88.0	100.0	65.4	16.7	6.2
Oxacillin	18.8	69.7	82.6	73.6	70.5	65.1	31.0	75.9
Methicillin	13.1	50.0	65.2	21.8	23.5	35.7	77.9	34.6
Nafcillin	26.0	62.3	83.3	85.6	49.5	1.8	0.5	0.4
Minocin	....	84.1	96.0	46.5	92.8	93.9	95.3	96.3
Vibramycin	....	....	....	78.3	94.2	96.9	42.2	98.0
Keflin	22.6	72.1	90.4	97.2	94.0	97.1	96.9	78.4
Vancomycin	....	....	....	100.0	100.0	100.0	99.6	98.8
Clindamycin	....	40.7	95.8	98.0	95.6	97.1	14.4	73.1

Staphylococcus epidermidis: This species has been recovered in blood cultures soon after injury in a large number of patients. These bacteremias have been transient and do not appear to represent a significant clinical problem. The sensitivity of 25 strains is shown in Table 5. The strains were highly sensitive to gentamicin, oxacillin, methicillin, Minocin, Vibramycin, Keflin, vancomycin and clindamycin. Sixty-eight per cent were tobramycin-sensitive. Only 20% were nafcillin-sensitive. The antibiograms were heterogeneous, which would be expected if the isolates were heterogeneous and not part of a monotype epidemic.

Table 5. Staphylococcus epidermidis: Cumulative Inhibitory Levels for 25 Strains
1 October 1978 - 30 September 1979

Antibiotic Level mcg/ml	Antibiotic and % Inhibited									
	G	To	Ps	Sc	U	M	Vb	Kf	Va	Cl
> 25.0	100	100	100	100	100	100	100	100	100	100
25.0	92.0	80.0	100	100	72.0	100	100	100	100	92.0
12.5	92.0	76.0	96.0	100	72.0	100	100	100	100	80.0
6.2	92.0	68.0	96.0	80.0	20.0	100	100	96.0	100	80.0
3.1	88.0	56.0	88.0	44.0	8.0	100	96.0	88.0	92.0	56.0
1.5	76.0	52.0	80.0	12.0	4.0	96.0	72.0	84.0	40.0	16.0
.78	60.0	44.0	48.0	8.0	4.0	92.0	64.0	76.0	12.0	8.0

G: Gentamicin; To: Tobramycin; Ps: Oxacillin; Sc: Methicillin; U: Nafcillin; M: Minocin; Vb: Vibramycin; Kf: Keflin; Va: Vancomycin; Cl: Clindamycin

Streptococci: The incidence of streptococcal bacteremia was low; only 13 strains were collected from 12 patients. The blood stream strains were heterogeneous, with seven strains of S. faecalis, three of S. viridans, two of S. pneumoniae and one Group D streptococcus.

Sensitivities are shown in Table 6. All but three antibiotics inhibited more than half of the strains. The most effective was vancomycin, which inhibited 92% of strains. The group was heterogeneous, as would be expected among the several species. There was no indication of epidemic transmission among these strains.

Pseudomonas aeruginosa: During 1978 and 1979, P. aeruginosa has been the principal gram-negative bacterial species causing sepsis in severely burned patients. There were unquestionably epidemic episodes among groups of patients. Forty-four patients yielded a total of 166 strains for testing during the observation period. Out of the 10 antibiotics used in testing, shown in Table 7, carbenicillin and ticarcillin were active against all strains; 73.5% of the strains were sensitive to amikacin, 86.7% to Minocin, and 60.8% to its analogue, Vibramycin; 94.6% were sensitive to colistin. The aminoglycosides gentamicin, Kantrex, and tobramycin were relatively ineffective.

A comparison of the sensitivity of the major antibiotics effective against P. aeruginosa over recent years shows more clearly the patterns of resistance and of re-emerging sensitivity that have evolved. Table 8 compares this information since 1973. The initial high level of sensitivity to gentamicin, in 1973, fell progressively to a level of 20% by 1976. Tobramycin was minimally effective in 1975, when it was first tested; 61.6% of strains tested the next year were sensitive, but thereafter resistance to tobramycin increased rapidly to the past year, when only 4% of strains were sensitive. Amikacin, first tested in 1976, was inhibitory for 98.3% of strains, but the following year only 60% were sensitive. In contrast, only a small proportion of strains were sensitive to Minocin in 1973; but since 1966, more sensitive strains have appeared, and 86.7% of strains were sensitive. Vibramycin has also increased in effectiveness, but not to the same degree. The level of sensitivity to colistin has remained high. Carbenicillin has been effective at levels from 58.6% of strains in 1976-1977 to 86% of strains in 1978-1979. Ticarcillin was only tested in the last observation period; its degree of effectiveness was in the same range as that of carbenicillin.

Klebsiella pneumoniae: The incidence of this opportunistic pathogen fell to a low level; only 15 strains were recovered from blood or biopsy in the year. Sensitivity is summarized in Table 9. Amikacin, Minocin, Vibramycin and colistin were highly effective; the other aminoglycosides and Keflin were active against only one-third to one-half of the strains. The strains were less frequently sensitive to gentamicin and tobramycin, but the sensitivity to tetracyclines had increased.

Table 6. Streptococci, Heterogeneous: Cumulative Inhibitory Levels for 13 Bacteremic Strains, 1 October 1978 - 30 September 1979

Antibiotic Level mcg/ml	Antibiotic and % Inhibited									
	G	To	Ps	Sc	U	M	Vb	Kf	Va	Cl
> 25.0	100	100	100	100	100	100	100	100	100	100
25.0	38.5	38.5	92.4	53.9	53.9	92.3	100	77.0	100	53.9
12.5	38.5	30.8	84.7	53.9	46.2	84.6	84.6	61.6	92.4	53.9
6.2	38.5	30.8	53.9	53.9	46.2	61.5	69.3	61.6	92.0	53.9
3.1	38.5	30.8	46.2	46.2	30.8	53.9	53.9	53.9	53.9	53.9
1.5	38.5	30.8	38.5	38.5	30.8	53.9	46.2	38.5	38.5	38.5
< .78	30.8	30.8	38.5	30.8	23.1	53.9	46.2	38.5	38.5	30.8

G: Gentamicin; To: Tobramycin; Ps: Oxacillin; Sc: Methicillin; U: Nafcillin; M: Minocin; Vb: Vibramycin; Kf: Keflin; Va: Vancomycin; Cl: Clindamycin

Strains include: Streptococcus viridans (3 strains); Group D Streptococcus not enterococcus (1 strain), Streptococcus faecalis (7 strains), Streptococcus pneumoniae (2 strains)

Table 7. Pseudomonas aeruginosa: Cumulative Inhibitory Levels for 166 Strains
1 October 1978 - 30 September 1979

Antibiotic Level mcg/ml	Antibiotic and % Inhibited								Conc. mcg/ml	Cb	Ti
	G	To	K	Ak	M	Vb	Kf	Co			
> 25.0	100	100	100	100	100	100	100	100	> 1250	100	100
25.0	39.8	11.3	9.0	94.6	94.6	90.9	2.4	95.2	1250	95.0	100
									625	94.4	100
12.5	25.9	4.0	3.6	73.5	86.7	60.8	1.2	94.6			
6.2	16.3	1.8	0.6	25.9	46.9	31.3	0.6	91.6	156	90.2	100
3.1	3.0	1.8	0.6	7.8	10.2	8.4	0.6	89.8	78	83.0	86.8
1.5	1.8	1.8	0.0	4.2	1.8	0.6	0.6	76.5	39	62.4	69.7
< .78	0.6	0.0	0.0	0.0	0.6	0.0	0.0	41.0	19	22.4	52.8
									9	9.7	28.9
									< 4.5	1.8	11.8
Total Tested	166	165	166	166	166	166	166	166		165	76

G: Gentamicin; To: Tobramycin; K: Kanamycin; Ak: Amikacin; M: Minocin; Vb: Vibramycin; Kf: Keflin; Co: Colistin; Cb: Carbenicillin; Ti: Ticarcillin

Table 8. Sensitivity of Pseudomonas aeruginosa to 3 Aminoglycosides, 2 Tetracyclines, Colistin and 2 Semi-synthetic Penicillins

Antibiotic	Year and % of Strains Inhibited by 12.5 mcg/ml					
	1973	1974	1975	1976-1977	1978	1979
Gentamicin	84.3	61.8	40.0	19.1	19.7	25.9
Tobramycin	....	....	18.5	61.6	17.0	4.0
Amikacin	....	....	....	98.3	60.0	73.5
Minocin	31.3	15.7	16.8	58.9	72.2	86.7
Vibramycin	....	....	20.0	43.6	63.6	60.8
Colistin	86.2	93.3	86.3	89.3	91.3	94.6
Carbenicillin*	80.4	70.8	68.8	58.6	62.0	86.0
Ticarcillin*	....	....	....	....	....	89.4

* Sensitivity cutoff: 156 mcg/ml; .... Antibiotic not in use

Escherichia coli: This organism was recovered in the same frequency as K. pneumoniae. It was more frequently encountered than it had been in recent years. The strains were 100% sensitive to gentamicin and colistin (Table 10). Tobramycin was inhibitory only for 18.8% of strains. The remaining antibiotics were effective for approximately half of the strains tested. Minocin was significantly more effective than Vibramycin.

Proteus mirabilis: Proteus septicemia became less common than in previous years; only six strains were recovered from blood. Two other species of Proteus were each recovered once. The strains were highly sensitive to all antibiotics tested except for Vibramycin and colistin (Table 11). This contrasted with 1977-1978, when amikacin was virtually ineffective. Keflin was more effective in 1978-1979 than previously. The variations in antibiogram and low frequency of systemic invasion suggest that the strains are heterogeneous as to virulence and invasiveness.

The less frequently encountered gram-negative bacilli are not charted. Two strains of S. saprophyticus were sensitive to all antibiotics. Three strains of Corynebacterium spp. were sensitive at 6.2 mcg/ml to all antibiotics except clindamycin. Four strains of Entero. aerogenes varied in sensitivity; all were tobramycin-resistant. Five strains of Serratia marcescens exhibited complete sensitivity to gentamicin, Minocin, and Vibramycin. Two-thirds were sensitive to Kantrex, one-half to amikacin, and none were sensitive to tobramycin, Keflin or colistin. Acinetobacter spp. were completely sensitive to Kantrex, Minocin, Vibramycin, and colistin. One strain was sensitive

Table 9. Klebsiella pneumoniae: Cumulative Inhibitory Levels for 15 Strains from Blood Cultures, 1 October 1978 - 30 September 1979

Antibiotic Level mcg/ml	Antibiotic and % Inhibited							
	G	To	K	Ak	M	Vb	Kf	Co
> 25.0	100	100	100	100	100	100	100	100
25.0	46.6	40.0	46.7	100	100	100	53.3	93.3
12.5	46.6	33.3	40.0	100	93.3	93.3	46.7	93.3
6.2	46.6	33.3	40.0	93.3	93.3	86.7	40.0	93.3
3.1	46.6	33.3	33.3	66.6	86.7	80.0	33.3	93.3
1.5	33.3	13.3	13.3	0.0	40.0	60.0	33.3	93.3
< .78	0.0	0.0	0.0	0.0	0.0	6.7	33.3	73.3

G: Gentamicin; To: Tobramycin; K: Kanamycin; Ak: Amikacin; M: Minocin; Vb: Vibramycin; Kf: Keflin; Co: Colistin

Table 10. *Escherichia coli*: Cumulative Inhibitory Levels for 16 Strains from Blood Cultures, 1 October 1978 - 30 September 1979

Antibiotic Level mcg/ml	Antibiotic and % Inhibited							
	G	To	K	Ak	M	Vb	Kf	Co
> 25.0	100	100	100	100	100	100	100	100
25.0	100	18.8	56.3	75.0	100	62.5	62.5	100
12.5	100	18.8	56.3	43.8	62.5	47.5	43.8	100
6.2	62.6	18.8	43.8	18.8	37.6	47.5	12.5	100
3.1	37.6	12.5	18.8	0.0	37.6	47.5	0.0	100
1.5	6.3	0.0	0.0	0.0	18.8	47.5	0.0	92.7
< .78	0.0	0.0	0.0	0.0	18.8	12.5	0.0	75.0

G: Gentamicin; To: Tobramycin; K: Kanamycin; Ak: Amikacin; M: Minocin; Vb: Vibramycin: Kf: Keflin; Co: Colistin

Table 11. Proteus spp: Cumulative Inhibitory Levels for 8 Strains from Blood Cultures, 1 October 1978 - 30 September 1979

Antibiotic Level mcg/ml	Antibiotic and % Inhibited							
	G	To	K	Ak	M	Vb	Kf	Co
> 25.0	100	100	100	100	100	100	100	100
25.0	100	62.5	100	100	87.5	0.0	62.5	0.0
12.5	87.5	62.5	100	100	87.5	0.0	62.5	0.0
6.2	87.5	62.5	100	82.5	0.0	0.0	50.0	0.0
3.1	72.5	37.5	100	50.0	0.0	0.0	50.0	0.0
1.5	12.5	0.0	37.5	12.5	0.0	0.0	0.0	0.0
< .78	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

G: Gentamicin; To: Tobramycin; K: Kanamycin; Ak: Amikacin; M: Minocin; Vb: Vibramycin: Kf: Keflin; Co: Colistin

Proteus spp. include Proteus mirabilis (6 strains), Proteus morganii [Morganella morganii] (1 strain), Proteus rettgeri [Providencia rettgeri Bio 5 B] (2 strains)

to tobramycin, two to Keflin and three to amikacin. Two strains of Aeromonas hydrophila were recovered. Amikacin, Vibramycin, Keflin, and colistin each inhibited both strains.

DISCUSSION

During the 1978-1979 period, there were only two species, S. aureus and P. aeruginosa, that could be regarded as of major importance in causing septicemia. Klebsiella and Enterobacter septicemia, when it occurred, was as potentially catastrophic as was the case when these genera appeared on an epidemic scale, but at the present time their incidence is more episodic. Fluctuations in sensitivity to methicillin-group antibiotics by S. aureus, and to tetracycline-group antibiotics by P. aeruginosa, emphasize the tentative state of knowledge concerning natural changes in sensitivity on the part of nosocomial bacterial populations. It is usually assumed that clinical use of antibiotics selects resistant strains, which may arise by a variety of mechanisms. However, the reappearance and persistence of sensitive strains in this population does not bear out that assumption.

PRESENTATIONS/PUBLICATIONS - None

ANNUAL PROGRESS REPORT

PROJECT NO. 3S161102BS05-00, BASIC RESEARCH

REPORT TITLE: STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE OF TROOPS WITH THERMAL INJURY -- SEROLOGIC TYPES OF PSEUDOMONAS AERUGINOSA FOUND IN BURNED SOLDIERS

US ARMY INSTITUTE OF SURGICAL RESEARCH
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1 October 1978 - 30 September 1979

Investigators:

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Arthur D. Mason, Jr., M.D.
Basil A. Pruitt, Jr., M.D., Colonel, MC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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Serotyping of Pseudomonas aeruginosa was carried out on 405 isolates from clinical sources, primarily blood, wound and sputum. Twenty-four types were distinguished. Epidemic outbreaks on the burn ward due to monotype infection occurred with five types. Major episodes were caused by types 4, 15, and 11, with type 4,10 causing a brief but significant episode. The occurrence of strain specific micro-epidemics in burn wards can be detected by typing, and improved control measures should be possible.

Pseudomonas
Serotype
Phage type
Infection
Humans

STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE OF TROOPS WITH THERMAL INJURY -- SEROLOGIC TYPES OF PSEUDOMONAS AERUGINOSA FOUND IN BURNED SOLDIERS

Pseudomonas aeruginosa has continued to exhibit a singular capability for colonizing burn wounds and inciting septicemia and invasive burn wound sepsis (BWS). Topical agents effective in controlling BWS and antibiotics which control sepsis from various sources have become increasingly available in recent years, but this availability has not hastened the elimination of this ubiquitous opportunistic microorgaism. Its incidence in burn wards has shown an episodic pattern consistent with the hypothesis that localized epidemics due to individual strains occur, and such outbreaks have been identified in the Institute of Surgical Research. For 15 years the patterns of identity were traced with a definitive bacteriophage typing system which was developed in this laboratory. The epidemiologic value of such typing data in delineating epidemic outbreaks was evident, but the technical manipulation involved in phage typing is great, and when an international serotyping set became available, it was tested for its capability to derive comparable epidemiologic data.

It has become evident, after 3 years of experience with serotyping, that the typing set is more crude in differentiating strains than is the bacteriophage system, but the essential information derived from the serotype data parallels closely that obtained with phage typing. It is now apparent that a rapid recognition of epidemic strain presence is possible with serotyping. Refinement of this information, by phage or pyocin typing of a collection of epidemic strains, can be done. This is the objective sought in current strain differentiating studies.

METHODS

Previous studies had made use of test suspensions prepared by suspending growth from a trypticase soy agar (TSA) plate in merthiolated saline, washing the suspension and resuspending in more concentrated form before typing on glass slides. Autoclaved suspensions were used in parallel with raw washed suspensions to achieve typing of otherwise non-reactive strains. In the current series, a simplified test procedure was used, with a marked saving in time and manipulation. The organism was collected from the surface of a blood agar isolation plate. In cases where a predominance of Pseudomonas occurred, the typing was done from the primary culture plate. Growth was collected with a flat toothpick and was streaked on an appropriate area of a glass test plate. A drop of serum was added from each serotype and the growth emulsified. Agglutination was rapid and legible.

The typing set was divided for efficiency into a major and a

minor series. Types 3, 4, 7, 8, 9, 10, 11, 15 and 16 made up the major set, based on most probable current distribution. Types 1, 2, 5, 6, 12, 13, 14 and 17 composed the minor set. They were used on all strains not reacting in the major set, and at intervals on major set reactors to screen for the presence of additional factors. Autoclaved antigens were kept in the system but were seldom needed. This system probably results in more non-typable reports, but it does not risk missing a numerically significant reactive strain.

RESULTS

Four hundred and five strains of P. aeruginosa were typed. Selection was made; at least one strain from each patient with a Pseudomonas-positive culture was typed, but when one patient recorded a score or more of positive cultures, a representative sample, both chronologically and by source, was collected. Special emphasis was placed on bloodstream isolates. The type distribution observed is summarized in Table 1, where the strains are compared with types recovered in 1976-77 and 1977-78. Aside from non-reacting strains, there were 10 types recognized in 1976-77, 20 in the following year, and 24 in the 1978-79 series. The increase in numbers of types recognized is most probably a reflection of increased technical skill in typing. In the past year, the use of direct colony fishing appeared to enhance the sensitivity of the reaction. In a manner similar to the course of events recognized with phage typing. there were three serotypes (1,9; 2,3,6,15; and 14) which were seen only in 1976-77. In the next year, unique types included 1,2,3,4,9,10; 3,8,9,14; 3,4,9,10; 4,6,8,11,12,14; 4,10,13; 7; 8,9; and 8,12. These strains are rare, but the identities are real; their presence is comparable to the hundreds of rarely encountered types recognized by phage typing. The major types by incidence included 4; 4,10; 11; 15; and 16. Of these types, only 4 and 15 have consistently been involved as epidemic types over the past 3 years. There have been nine different types that have occurred in epidemic incidence over this 3-year period. Types 6; 8; 10; 16; and 4,10 have thus far each appeared in epidemic intensity only during a single year.

During the 1978-79 period, the comparative incidence of strains from blood, wounds and sputum was assessed. If there were a preponderance of any type in septicemia, in wound infection or in pneumonia, such a pattern might be adduced from the types recovered. Table 2 sets down this comparison. Although the proportions of predominant types varied from the total percentages of strains, the differences were not significant. It does not appear that there was any type that was disproportionally represented in the three categories set forth.

The behavior of P. aeruginosa strains which were recovered from at least three patients on the burn ward during 1978-79 are summarized in Table 3. There were six types which, in at least 1 month of the

Table 1. Serotypes of Pseudomonas aeruginosa from Burn Patients 1976 - 1979

Type	No. of Isolates (Percent of Total, Major Types) 1976-77	1977-78	1978-79
1,9	2		
1,2,3,4,9,10		7	
2,3,6,15	3		
3		9	2
3,8,9,14		2	
3,4,9,10		1	
3,10			2
3,15			2
4,8,9,11,12,14		2	
4	110 (27.9%)	183 (38.3%)	42 (10.3%)
4,9,10	20 (5.0%)	12 (2.5%)	
4,10			21 (5.2%)
4,10,13		2	
4,10,15			1
4,11		1	1
4,11,15,16			1
4,15		2	2
5		1	1
6	38 (7.9%)		5
7		3	
8	2	17 (4.2%)	1
8,9		2	
8,11			1
8,12		1	
9	2	2	
9,10		10	2
10	3	16 (3.3%)	6
10,15			1
10,15,16			1
11		35 (7.3%)	89 (21.9%)
11,15			3
11,15,16			1
12		1	2
14	7		
15	239 (60.6%)	119 (24.9%)	158 (39.0%)
15,16			1
16		4	11 (2.7%)
NT*	3	21	43
Total	429	453	400

* NT: Non-typable; non-reactive with 17 serotypes.

Table 2. Predominant Serotypes of Pseudomonas aeruginosa Recovered from Blood, Wound and Respiratory Tract of Burn Patients* 1978-1979

Serotype	Source and No. of Isolates Recovered		
	Blood	Wound	Sputum
4	15	15	9
4,10	9	11	2
15	31	78	49
11	12	44	34
16	1	5	4
10		3	
12			3
6			6
3,15			2
4,15			2
8,11			3
11,15	3		
NT	9	17	13

* No strains represented by only one isolate were included.

year, were recovered from three or more patients. The major epidemic types were 4, 15 and 11. Type 15 constituted the predominant epidemic type during the first 4 months of the year; during December, a sudden rise of type 11 occurred, but neither of these types appeared again in significant numbers until the following spring. Meanwhile, a rise in type 4 occurred in February and extended through April, at which time type 15 reappeared in the ward population in significant numbers. It remained prominent until the end of August 1979, then diminished abruptly in numbers. At the time of the type 15 resurgence, a marked rise in type 11 occurred, in a prolonged epidemic outbreak. Types 10 and 16 each occasioned a small 1-month outbreak.

A resumé of epidemic outbreaks during the past 3 years is presented in Table 4. The incidence is expressed in terms of total incidence of individual types. Eight types have thus far occasioned epidemic outbreaks. The most consistent occurrence of this phenomenon occurred with types 4; 4,9,10; and 15. Each of the other six types established an epidemic episode in only 1 year.

DISCUSSION

Further confirmation of the effectiveness of the internationally proposed serotyping system has been collected, and the epidemiology

Table 3. Pseudomonas aeruginosa Strains Involving Three or More Patients in 1978-1979

Type	No. of Patients* - No. of Isolates by Month												
	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep
4	1-1	1-2	1-1	1-1	2-4	4-7	5-12	3-4	1-2	1-2	2-4		1-2
4,10					1-1	2-4	3-5	1-4		1-1	1-2		1-4
15	8-23	7-16	9-23	7-20	2-2	1-1	2-2	7-14	9-15	9-14	4-8	9-17	1-1
11		2-2	1-1	5-6				1-1	4-8	7-12	5-24	9-22	5-13
10						1-1				1-1	3-3	1-1	
16		2-4	1-1				3-5			1-1			
NT**	4-8	4-5	2-2	4-5			3-3	2-2	2-4	3-4	3-4	1-1	1-3

* Only types which occurred in at least three patients in a single month are included. There were six such types; 19 more were found in lower incidence.

** NT: Non-typable.

Table 4. Epidemic Outbreaks of Monotype Infection with *Pseudomonas aeruginosa* in Burn Patients

Type	Incidence and % of Annual Total Isolates 1976-77	1977-78	1978-79
4	110 (27.9%)	183 (38.3%)	42 (10.3%)
4,9,10	20 (5.0%)	12 (2.5%)	
4,10			21 (5.2%)
6	38 (7.9%)		
8		17 (4.2%)	
10		16 (3.3%)	
11		35 (7.3%)	89 (21.9%)
15	239 (60.6%)	119 (24.9%)	158 (39.0%)

of Pseudomonas infection in the Institute of Surgical Research burn wards has been delineated. A further streamlining of the typing process has been evolved, and currently the serotyping is approaching a level where it can be carried out expeditiously and probably as a routine determination. This procedure may improve the control of epidemic outbreaks of *P. aeruginosa* infection in the burn ward.

PRESENTATIONS/PUBLICATIONS - None

ANNUAL PROGRESS REPORT

PROJECT NO. 3S161102BS05-00, BASIC RESEARCH

REPORT TITLE: STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE OF TROOPS WITH THERMAL INJURY -- PATHOGENESIS OF BURN WOUND INFECTION: BACTERIAL FLORA OF BURN WOUNDS OF MILITARY PERSONNEL RECEIVING SULFAMYLON OR SILVER SULFADIAZINE TREATMENT

US ARMY INSTITUTE OF SURGICAL RESEARCH
BROOKE ARMY MEDICAL CENTER
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1 October 1978 - 30 September 1979

Investigators:

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Loralie R. Sanders, SP4

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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Bacterial infection remains the major cause of morbidity and death in severe burn injury. Monitoring on an annual basis has revealed wide differences in the same burn wards over several years' time. In the 1978-79 period, principal invasive species for burns were Staphylococcus aureus and Pseudomonas aeruginosa. In contrast to recent years, in which epidemics due to Klebsiella and Enterobacter species occurred, the Enterobacteriaceae played a minor role in causing sepsis in burns. Forty-three species of bacteria and nine of yeasts were differentiated in 1978-79.

Burns
Staphylococci
Pseudomonas
Sepsis
Yeasts
Humans

STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE OF TROOPS WITH THERMAL INJURY -- PATHOGENESIS OF BURN WOUND INFECTION: BACTERIAL FLORA OF BURN WOUNDS OF MILITARY PERSONNEL RECEIVING SULFAMYLON OR SILVER SULFADIAZINE TREATMENT

Bacterial infection in burned patients is represented by a wide spectrum of species. Most of the strains involved have not been shown to cause invasive infection; but with the passage of time, the list of species which can cause life-threatening wound invasion continues to grow. Epidemics of specific invasive sepsis have been less frequent since the development of effective topical therapy, but lethal invasive infection remains a real threat. Prior to 1978, epidemic episodes of septic infection due to several species of Enterobacteriaceae occurred. This phenomenon has not been evident, however, since mid-1978. No specific explanation of this difference has been forthcoming. During the past year, the microbial flora of burn patients has resembled that which was last seen in the late 1960's, with Staphylococcus aureus and Pseudomonas aeruginosa the species most frequently recovered in septicemia. The summation of species recovered from all sources is greater than it was prior to 1978; this extension of species recognized is explained by the increasingly effective determinative bacteriology which is being carried out in the Clinical Microbiology Section.

ANTEMORTEM BACTERIOLOGY OF BURN PATIENTS, 1978-1979

Bacteria and yeast strains recovered during this year are summarized in Table 1. The isolates recovered are listed by source. This reflects the relative incidence of species more clearly than is the case when incidence is expressed as number of patients positive for a given species. There were 267 patients admitted during the reporting period. Of these, only 221, or 82%, had any cultures at all taken. Blood cultures were collected from 82.3% of all patients cultured, wound cultures from 73.7%, urine cultures from 69.2% and sputum cultures from 51.1% of all patients who had culture samples collected. Four hundred and sixty strains of microorganisms were recovered from blood cultures. The most frequently observed bacterial species were S. aureus and P. aeruginosa. The overall most commonly encountered species were S. aureus (935 strains), P. aeruginosa (913), Klebsiella pneumoniae (243) and Escherichia coli (159). The total of all groups of streptococci was 516; half of these were non-Group D alpha-streptococci. Five hundred and eight strains of yeasts, including 162 of Candida albicans and 211 of C. rugosa were recovered. This represented an apparent increase in the incidence of yeasts in burn patients over previous years. Serratia marcescens recoveries increased from 10 to 53 isolates in this year. This is still a small number of strains in the overall picture. No strains of Providencia stuartii, formerly a major epidemic problem, were recovered. There were 43 species of bacteria and 9 of yeast differentiated in 1978-79.

Table 1. Antemortem Bacteriology of Burn Patients, 1 October 1978 - 30 September 1979

Organism	Source and Number of Isolates									
	Wound Surface	Blood	Sputum Luken's	Urine	IV Cath Tips	Foley Cath Tips	Biopsy	Xeno-graft	CSF	Total
S. aureus	265	206	329	73	15	1	42	2	2	935
S. epidermidis	27	21	44	15	2	0	6	0	0	115
S. saprophyticus	0	2	0	1	0	0	0	0	0	3
Alpha-hemol. Strep. not Gp D	25	1	217	7	0	0	0	0	0	250
Non-hemol. Strep. not Gp D	9	0	57	6	0	1	1	0	0	74
Beta-hemol. Strep. not Gp A, B or D	3	1	33	0	0	0	0	0	0	37
Gp D not enterococcus	4	1	43	2	0	0	0	0	0	50
Strep. pneumoniae	1	2	26	1	0	0	0	0	0	30
Strep. faecalis	7	6	17	19	2	1	1	0	0	50
Strep. durans	3	0	6	0	0	0	0	0	0	9
Gp A Beta Strep.	0	0	4	0	0	0	0	0	0	4
Gp B Beta Strep.	0	0	1	0	0	0	0	0	0	1
Bacillus spp.	3	3	4	0	0	0	1	0	0	11
Corynebacterium spp.	4	3	1	0	0	0	1	0	0	9
Neisseria spp.	2	0	32	0	0	0	0	0	0	34
P. aeruginosa	281	114	284	146	7	4	76	1	1	913
P. fluorescens	0	0	1	0	0	0	0	0	0	1
P. cepacia	0	0	2	0	0	0	0	0	0	2
P. putida	0	1	0	0	0	0	0	0	0	1
P. alcaligenes	1	0	0	0	0	0	0	0	0	1
P. maltophilia	0	0	2	0	0	0	0	0	0	2
Acineto. anitratus	2	2	5	0	0	0	1	0	0	10
Acineto. lwoffii	1	2	2	0	0	0	0	0	0	5
Group 5E-2	1	0	1	0	0	0	0	0	0	2
Escherichia coli	46	10	35	59	0	5	4	0	0	159
Escherichia coli AD	3	0	0	0	0	0	0	0	0	3
Citro. freundii	4	0	1	0	0	0	0	0	0	5
Citro. diversus	11	0	3	0	1	0	0	0	0	15

Table 1. Antemortem Bacteriology of Burn Patients (continued)

Organism	Source and Number of Isolates									
	Wound Surface	Blood	Sputum Luken's	Urine	IV Cath Tips	Foley Cath Tips	Biopsy	Xeno-graft	CSF	Total
Klebsiella pneumoniae	45	9	130	38	2	2	7	0	0	243
Klebsiella oxytoca	3	0	7	1	1	1	0	0	0	13
Klebsiella ozaenae	6	1	25	4	1	0	0	0	0	37
Enterobacter cloacae	26	0	25	9	2	0	5	0	0	67
Enterobacter aerogenes	4	4	51	5	0	0	0	0	0	64
Enterobacter hafnia	1	0	3	0	0	0	0	0	0	4
Enterobacter agglomerans	2	0	8	1	0	0	0	0	0	11
Serratia marcescens	9	5	33	4	0	0	2	0	0	53
Serratia liquefaciens	1	0	0	0	0	0	0	0	0	1
Proteus vulgaris	3	0	0	0	0	0	1	0	0	4
Proteus mirabilis	32	6	40	32	2	2	1	0	0	115
Proteus morganii (Morganella morganii)	4	1	1	0	0	0	0	0	0	6
Proteus rettgeri (Prov. rettgeri Bio 5B)	0	2	0	6	2	0	0	0	0	10
Moraxella osloensis	0	0	1	0	0	0	0	0	0	1
Aeromonas hydrophila	2	2	0	0	1	0	5	0	0	10
Haemophilus influenza	0	0	4	0	0	0	0	0	0	4
Yeast-like organism	14	4	15	7	0	0	2	0	0	42
Candida albicans	22	17	43	65	3	3	12	0	0	162
Candida pseudotropicalis	1	0	1	3	0	0	0	0	0	5
Candida rugosa	56	34	10	83	2	0	24	2	0	211
Candida stellatoidea	0	0	1	2	0	0	0	0	0	3
Candida tropicalis	3	0	13	65	1	0	10	0	0	92
Candida zeylanoides	1	0	0	0	0	0	1	0	0	2
Torulopsis glabrata	0	0	1	10	0	0	0	0	0	11
Trichosporon beigelii	1	0	0	2	0	0	3	0	0	6
Number cultured	163	182	113	153	42	17	78	29	8	
Number of specimens	881	2228	642	1038	65	19	239	53	29	

Total isolates: 3915; total specimens: 5193

Total patients on whom one or more cultures were done: 221

The frequency with which individual patients are colonized or infected is of significance in detecting epidemic episodes in a burn ward. Table 2 shows the principal species and their incidence in terms of patients who were positive from various sites. The numerically important species included S. aureus, P. aeruginosa, E. coli, and K. pneumoniae. Enterobacter spp. were not a major factor, in contrast to recent years when Entero. cloacae was the cause of several epidemics. Yeasts were, in terms of number of patients positive, an increasing part of the epidemic infection problem.

Table 2. Antemortem Bacteriology of Burn Patients: Principal Species 1 October 1978 - 30 September 1979

Organism	Source and Number of Patients Positive in Culture					
	Wound	Blood	Sputum	Urine	IV Cath	Biopsy
S. aureus	88	58	85	36	14	23
Alpha-Strep. not Gp D	24	1	81	7	0	0
Gamma-Strep. not Gp D	9	0	37	6	0	1
Strep. pneumoniae	1	2	17	1	0	0
P. aeruginosa	66	30	56	55	7	30
Escherichia coli	23	8	21	27	5	3
Klebsiella pneumoniae	33	7	36	24	2	3
Klebsiella ozaenae	6	1	11	4	1	0
Enterobacter cloacae	17	0	15	8	2	3
Serratia marcescens	4	2	4	2	0	2
Proteus mirabilis	1	3	10	16	2	1
Candida albicans	18	6	28	25	3	6
Candida rugosa	21	13	7	19	2	10
Candida tropicalis	2	0	6	15	1	2
Total no. patients sampled	163	182	113	153	42	78

The percentage of patients cultured for each site and positive for the major species is shown in Table 3. The relative predominance of S. aureus and P. aeruginosa in sepsis is underscored by the relative degree of involvement of these species in blood and biopsy culture.

BURN WOUND BACTERIOLOGY

The burn wound is frequently incriminated as the source of sepsis in burn patients, and monitoring flora of the burn wound is a major concern in management of burns. The therapeutic regimens used in the

Table 3. Percentage of Patients Positive for Each Species at Principal Sites

Species	Source and & of Cultured Patients Positive			
	Wound	Biopsy	Blood	Sputum
S. aureus	53.7	29.5	32.2	75.0
P. aeruginosa	40.7	38.5	16.7	50.0
E. coli	14.2	3.9	4.4	18.6
K. pneumoniae	20.4	3.9	3.9	31.3
Entero. cloacae	10.5	3.9	4.4	12.5

burn ward are presumed to affect the colonizing flora. In the Institute of Surgical Research, topical treatment with Sulfamylon^{R} and silver sulfadiazine creams and with 5% Sulfamylon soaks ensure that the bacterial flora is extensively exposed to these agents.

Predominant species recovered from wound cultures (mainly swab cultures) are summarized in Table 4. Eight species of bacteria and two of yeasts were sufficiently numerous in incidence to merit assessment, out of a total of 41 species recovered. The species recovered from the largest proportion of patients' wounds was S. aureus. However, the largest number of strains, 281 isolates, were P. aeruginosa, which was recovered from 40.7% of the patients cultured. Other species recovered in a significant incidence included K. pneumoniae, E. coli, Entero. cloacae, and Proteus mirabilis, but none of these species represented a major wound-invading threat. Klebsiella was not changed from its incidence in 1977-78, but the other three species showed a significant decrease in incidence since the previous year.

As has been pointed out in previous years of observation, Group A hemolytic streptococci have been ever the object of diligent search in wound cultures. In 1978-79, no wound culture yielded a strain of this important species. In one other previous year, 1976, the incidence of Group A streptococci in wounds was zero. In other years, a small number of strains were typically found.

RESPIRATORY TRACT FLORA IN BURN PATIENTS

Pulmonary involvement is a major cause of morbidity and mortality in severe burns. Inhalation injury is an obvious predisposing factor in pulmonary infection. Table 5 presents the principal species recovered in sputum aspirates and Luken's tube cultures. Again, S. aureus was numerically the most prominent species, with 75% of cultured patients positive and 329 strains recovered; 113 patients were

Table 4. Burn Wound Surface Flora in 164 Patients
1 October 1978 - 30 September 1979

Species	No. of Strains	No. of Patients Positive	% of Cultured Patients Positive
S. aureus	265	88	53.7
S. epidermidis	27	23	14.2
Alpha-hemol. Strep. not Gp D	25	25	14.8
P. aeruginosa	281	66	40.7
Escherichia coli	46	23	14.2
Klebsiella pneumoniae	45	33	20.4
Enterobacter cloacae	26	17	10.5
Proteus mirabilis	32	15	9.3
Candida albicans	22	18	11.6
Candida rugosa	56	21	13.0

Table 5. Principal Species of Bacteria Recovered from Respiratory Tract of 113 Patients, 1 October 1978 - 30 September 1979

Species	No. of Isolates	No. of Patients Positive	% of Cultured Patients Positive
S. aureus	329	85	75.0
S. epidermidis	44	35	30.9
Alpha-hemol. Strep. not Gp D	217	81	71.7
Non-hemol. Strep. not Gp D	57	37	32.7
Beta-hemol. Strep. not Gp A, B or D	33	22	19.5
Gp D not enterococcus	43	26	23.0
Strep. pneumoniae	26	17	15.0
Neisseria	32	23	20.4
P. aeruginosa	284	56	50.0
Escherichia coli	35	21	18.6
Klebsiella pneumoniae	130	36	31.3
Enterobacter aerogenes	51	18	15.9
Candida albicans	43	28	24.8

cultured for sputum flora. The second most common species in sputum was the group designated as alpha streptococci, not Group D, which was recovered from sputum of 71.7% of patients. It is still evidently a group that seldom produces serious clinical illness. Of pathogenic species, P. aeruginosa was recovered from 50% of patients, and K. pneumoniae from 31.3% of patients. Other species of Enterobacteriaceae were found in fewer than 18% of patients. In the previous year, Entero. cloacae was recovered from sputa of 22% of patients. This was during a period when this species was a significant cause of sepsis, but in 1978-1979, only 13% of patients' sputa harbored this species. E. coli was second to K. pneumoniae in sputum cultures in this group of patients.

SEPTICEMIA IN BURNED PATIENTS

The most significant aspect of bacterial infection in burns is the appearance of sepsis, and the contribution of individual species to this critical problem is here examined in detail. In 1978-79, 181 burned patients had blood cultures drawn. Of these, 111 had at least one positive culture. This presentation does not attempt to distinguish between septicemia as a manifestation of early clinical disease and those terminal positive blood cultures which often appear to be simply a reflection of collapse of all defense mechanisms. Table 6 presents the positive blood cultures in totals of isolates and number of patients positive. Numerically, the important species were S. aureus, P. aeruginosa, and S. epidermidis. The latter species appears not to represent a significant cause of clinical disease; most strains were isolated only once from a patient, and that was often early in the patient's course. Escherichia coli and K. pneumoniae were recovered respectively from eight and seven patients. This incidence does not suggest that these enteric species initiated epidemic outbreaks of sepsis during the observation period.

Yeast bacteremia occurred to a greater extent than had previously been observed. There were 13 patients with Candida rugosa bacteremia, and 34 positive blood cultures. This rate was closely followed by C. albicans, with six patients and 17 blood cultures positive.

Most of the patients with bacteremia harbored more than one species of organism during their course. Thirty-six of the bacteremic patients, however, had only one species of organism recovered during their illness. These species are shown in Table 7. Consistent with their predominance in other sites, S. aureus and P. aeruginosa were the species most frequently seen as single species infections.

Mixed blood stream infections were found in 22 patients. These individuals had two or, in two cases, three species recovered. Table 8 presents these totals. Mixed infections in the blood stream in most instances occurred in severely ill patients, many of whom did not survive their injury. There was no consistent pairing of species;

Table 6. Blood Culture Isolates from 181 Burned Patients
1 October 1978 - 30 September 1979

Species	Total No. of Isolates	No. of Patients Positive	% of Cultured Patients Positive
S. aureus	206	58	32.2
S. epidermidis	21	18	10.0
S. saprophyticus	2	2	1.1
Alpha-hemol. Strep. not Gp D	1	1	0.6
Beta-hemol. Strep. not Gp D	1	1	0.6
Gp D not enterococcus	1	1	0.6
Strep. pneumoniae	2	2	1.1
Strep. faecalis	6	6	3.3
Bacillus spp.	3	3	1.7
P. aeruginosa	114	30	16.7
P. putida	1	1	0.6
Acinetobacter anitratus	2	2	1.1
Acinetobacter lwoffii	2	2	1.1
Escherichia coli	10	8	4.4
Klebsiella pneumoniae	9	7	3.9
Klebsiella ozaenae	1	1	0.6
Enterobacter aerogenes	4	3	1.7
Serratia marcescens	5	2	1.1
Proteus mirabilis	6	3	1.7
Proteus morganii (Morganella morganii)	1	1	0.6
Proteus rettgeri (Prov. rettgeri Bio 5B)	2	1	0.6
Aeromonas hydrophila	2	2	1.1
Yeast-like organism	4	3	1.7
Candida albicans	17	6	3.3
Candida rugosa	34	13	7.2
Corynebacterium spp.	3	3	1.7

Underlined species represent numerically important organisms.

as would be expected, the S. aureus - P. aeruginosa combination was most commonly seen.

BIOPSIES OF BURN WOUNDS

Wound biopsy has become a major tool for assessing the status of burn wounds as to presence of invasive infection in distinction to

Table 7. Bacteremia with Only One Species of Bacteria Recovered 1 October 1978 - 30 September 1979

Species	No. Patients with One Species Recovered	Average No. of Positive Blood Cultures Per Patient	No. Deaths	% Mortality for One Species Bacteremia
S. aureus	12	2.5	6	50.0
S. epidermidis	8	1.0	0	0.0
Strep. pneumoniae	1	1.0	1	100.0
Strep. faecalis	1	1.0	0	0.0
Bacillus spp.	3	1.0	0	0.0
Corynebacterium spp.	1	1.0	0	0.0
P. aeruginosa	5	1.2	3	60.0
P. putida	1	1.0	0	0.0
Escherichia coli	1	1.0	0	0.0
Proteus mirabilis	1	2.0	0	0.0
Aeromonas hydrophila	1	1.0	1	100.0
Candida rugosa	1	1.0	1	100.0

surface colonization. The latter condition is often innocuous; the former, life-threatening.

Table 9 summarizes the results of 239 biopsy specimens collected from 78 patients. The invasive potential of P. aeruginosa is shown in that, where in preceding sources it had been the species second most frequent in incidence, here it was recovered from the largest number of patients. This was in contrast to the preceding year, in which S. aureus had been the most frequent invader. Except for these two species, no bacterial species was present with any significant degree of frequency. Candida rugosa was the predominant yeast in biopsy samples. There were 15 species of bacteria recovered -- a small proportion of the total of species collected altogether. The fatality rate for burn patients with P. aeruginosa and for S. aureus in tissues was virtually the same, 63.3% and 60.9%. Patients with Candida albicans and C. rugosa also exhibited a high mortality rate.

CATHETER TIP CULTURES

Thrombophlebitis as a result of infection involving indwelling catheters is a hazard to be considered during treatment of severely burned patients. Culturing of catheter tips when they are removed from a vein is carried out to monitor the presence of bacteria which have reached this site. Table 10 summarizes these findings on

Table 8. Blood Culture Isolates in Patients with Mixed Infections
1 October 1978 - 30 September 1979

Species	No. of Patients
S. aureus, S. epidermidis	1
S. aureus, Strep. faecalis	1
S. aureus, Gp D not enterococcus	1
S. aureus, Beta-hemol. Strep. not Gp A, B or D	1
S. aureus, P. aeruginosa	8
S. aureus, Acinetobacter lwoffii	1
S. aureus, Candida albicans	2
S. aureus, Candida rugosa	1
S. epidermidis, Corynebacterium spp.	1
P. aeruginosa, Candida rugosa	1
Proteus mirabilis, Enterobacter aerogenes	1
S. aureus, P. aeruginosa, Strep. faecalis	1
S. aureus, P. aeruginosa, Alpha-hemol. Strep. not Gp D	1
No. of patients with two species recovered	20
No. of patients with three species recoverd	2
No. of patients with four species recovered	0
No. of patients with:	
S. aureus	18
P. aeruginosa	11
Strep. faecalis	2
Candida rugosa	2
Candida albicans	2
S. epidermidis	1
Proteus mirabilis	1
Enterobacter aerogenes	1
Alpha-hemol. Strep. not Gp D	1
Gp D Streptococcus not an enterococcous	1
Acinetobacter lwoffii	1
Corynebacterium spp.	1

42 patients. Their diversity resembles that seen in wound cultures. It is to be borne in mind that the mechanical process of removing the catheter presents a contamination hazard that makes it impossible to tell which of these catheters actually harbored bacteria while in situ. The positive findings do, however, make this potential source of seeding in the blood stream a basis for vigilant supervision of technic.

Table 9. Bacterial Flora of Biopsies of Burn Wounds of 78 Patients
1 October 1978 - 30 September 1979

Species	No. of Patients Positive	% of Patients Positive	No. of Patients with Positive Cultures Who Expired	% of Patients Who Expired
S. aureus	23	29.5	14	60.9
S. epidermidis	5	6.4	2	40.0
Non-hemol. Strep. not Gp D	1	1.3	0	0.0
Strep. faecalis	1	1.3	1	100.0
Bacillus spp.	1	1.3	0	0.0
Corynebacterium spp.	1	1.3	1	100.0
P. aeruginosa	30	38.5	19	63.3
Acinetobacter anitratus	1	1.3	1	100.0
Escherichia coli	3	3.9	2	66.6
Klebsiella pneumoniae	3	3.9	3	100.0
Enterobacter cloacae	3	3.9	0	0.0
Serratia marcescens	2	2.6	0	0.0
Proteus vulgaris	1	1.3	1	100.0
Proteus mirabilis	1	1.3	0	0.0
Aeromonas hydrophila	2	2.6	2	100.0
Yeast-like organism	1	1.3	0	0.0
Candida albicans	6	7.7	5	88.3
Candida rugosa	10	12.8	7	70.0
Candida tropicalis	2	2.6	2	100.0
Candida zeylanoides	1	1.3	1	100.0
Trichosporon beigelii	2	2.6	1	50.0

Number of specimens: 239 Number of specimens per patient (average): 3.1

Table 10. Bacterial Flora of IV Catheter Tips
1 October 1978 - 30 September 1979

Organism	No. of Isolates	No. of Patients Positive	% Total Patients Positive
S. aureus	15	14	35.0
S. epidermidis	2	2	5.0
Strep. faecalis	2	2	5.0
P. aeruginosa	7	7	17.5
Citrobacter diversus	1	1	2.5
Klebsiella pneumoniae	2	2	5.0
Klebsiella oxytoca	1	1	2.5
Klebsiella ozaenae	1	1	2.5
Enterobacter cloacae	2	2	5.0
Proteus mirabilis	2	2	5.0
Proteus rettgeri (Providencia rettgeri Bio 5B)	1	1	2.5
Aeromonas hydrophila	1	1	2.5
Candida albicans	3	3	7.5
Candida rugosa	2	2	5.0
Candida tropicalis	1	1	2.5

Number of patients cultured: 42 Number of cultures: 62

URINARY TRACT BACTERIA

Urinary tract infection is a constant hazard in patients in whom in-dwelling catheters are commonplace. The results of cultures on 153 patients are presented in Table 11. As would be expected in the urinary tract, a slightly higher proportion of strains of Enterobacteriaceae was recovered than was the case with wound or sputum cultures. P. aeruginosa, recovered from 55 patients, was by far the most commonly encountered species. S. aureus was the second species in order of frequency, but several enteric species, including E. coli and Kleb. pneumoniae, were recovered more frequently than was the case in other sampling sites. Seven species of yeasts were recognized in urine culture, with Candida albicans and C. rugosa the most commonly encountered. The proportion of patients with these two species was markedly different from that observed in candidemia in blood cultures, which fact suggests that the blood stream invasion by yeasts is more probably associated with wound or respiratory tract colonization than with the urinary tract.

Table 11. Urine Cultures on 153 Patients
1 October 1978 - 30 September 1979

Organism	No. of Isolates	No. of Patients Positive	% Total Patients Positive
S. aureus	73	36	23.2
S. epidermidis	15	14	9.3
S. saprophyticus	1	1	0.7
Alpha-hemol. Strep. not Gp D	7	7	4.6
Non-hemol. Strep. not Gp D	6	6	4.0
Gp D Strep. not an enterococcus	2	2	1.3
Strep. pneumoniae	1	1	0.7
Strep. faecalis	19	13	8.6
P. aeruginosa	145	55	36.4
Escherichia coli	59	27	17.5
Klebsiella pneumoniae	48	24	15.9
Klebsiella oxytoca	1	1	0.7
Klebsiella ozaenae	4	4	2.6
Enterobacter cloacae	9	8	5.3
Enterobacter aerogenes	5	3	2.0
Enterobacter agglomerans	1	1	0.7
Serratia marcescens	4	2	1.3
Proteus mirabilis	32	16	9.9
Proteus rettgeri (Providencia rettgeri Bio 5B)	6	1	0.7
Yeast-like organisms	7	6	4.0
Candida albicans	65	25	16.6
Candida pseudotropicalis	3	2	1.5
Candida rugosa	83	19	12.6
Candida stellatoidea	2	1	0.7
Candida tropicalis	65	15	9.9
Torulopsis glabrata	10	4	2.6
Trichosporon beigelii	2	1	0.7

POSTMORTEM BACTERIOLOGY

The bacteriologic findings at autopsy offer definitive information as to the nature and extent of infection in the burn patient. In the reporting interval, 52 autopsies were conducted with collection of tissue samples for qualitative and quantitative bacteriology.

Table 12. Postmortem Bacteriology of 52 Burn Patients, 1 October 1978 - 30 September 1979

Organism	Recovered at Autopsy	Liver	Spleen	Lung	Wound	Blood	Tip IV Cath
S. aureus	158	10	11	62	55	7	13
S. epidermidis	5	0	1	3	1	0	0
S. saprophyticus	3	1	1	0	1	0	0
Alpha-hemol. Strep. not Gp D	12	1	0	10	0	0	1
Non-hemol. Strep. not Gp D	4	0	0	3	1	0	0
Beta-hemol. Strep. not Gp A, B or D	5	0	0	4	0	1	0
Strep. faecalis	8	1	2	3	1	1	0
Strep. durans	1	0	0	1	0	0	0
Bacillus spp.	2	0	1	1	0	0	0
Corynebacterium spp.	1	0	0	0	0	1	0
P. aeruginosa	172	7	2	61	80	7	15
Flavobacterium spp.	1	0	0	0	1	0	0
Escherichia coli	89	7	9	28	17	13	5
Citrobacter freundii	6	1	1	2	0	1	1
Klebsiella pneumoniae	73	6	6	31	17	10	3
Klebsiella oxytoca	13	2	2	5	1	3	0
Enterobacter cloacae	2	0	0	0	2	0	0
Enterobacter aerogenes	17	0	0	7	8	1	1
Enterobacter agglomerans	1	0	0	0	0	1	0
Serratia marcescens	10	1	0	7	1	0	0
Proteus mirabilis	26	4	2	9	7	2	2
Proteus morganii (Morganella morgannii)	3	0	2	1	0	0	0
Yeast-like organisms	3	0	0	0	3	0	0
Candida albicans	23	1	0	4	18	0	0
Candida rugosa	41	2	2	6	28	0	3
Candida tropicalis	9	0	1	1	6	0	1
Trichosporon beigelii	3	0	0	1	2	0	0

The number of isolates for each site are shown in Table 12. Predominant species were S. aureus and P. aeruginosa. There were 22 bacterial species recovered. Lung samples harbored 17 of these, wound samples 14, liver 11, and spleen 12 species. Enteric species of significance included E. coli and K. pneumoniae. Heart blood samples were also cultured. The recovery of clinically significant species from these sources offers a perspective on the relative significance of viscera and heart blood as sources of postmortem information on the cause of sepsis antemortem.

Table 13 compares results from these sources. S. aureus and P. aeruginosa were, in clinical samples, by far the predominant offenders in sepsis in severe burns. The terminal samples of tissues and blood yielded E. coli and K. pneumoniae in comparable numbers for liver and spleen samples. Heart blood yielded higher levels of these enteric forms. Although the difference was not large, it was consistent with the concept that positive heart blood samples are weighted with the colonic flora, which can easily enter the portal blood pool some hours after death.

Table 13. Liver, Spleen and Heart Blood Postmortem Culture Results on 52 Autopsies

Species	No. of Patients Positive and Source		
	Liver	Spleen	Heart Blood
S. aureus	10	11	7
P. aeruginosa	7	2	7
Klebsiella pneumoniae	6	6	10
Escherichia coli	7	9	13

DISCUSSION

Over a period of years, there have been wide differences in the incidence of bacterial species in burn wounds. In the 1978-79 period, the bacterial flora was extremely diverse as to species incidence, but in each category of collection site, the major species numerically were S. aureus and P. aeruginosa. This pattern, in which enteric species present a minor part of the burn patient flora, has persisted for 18 months. It would be gratifying to be able to state that the problem of nosocomial infections due to enteric species has diminished. But it is far more probable that there will emerge a new or else a previously encountered opportunist that will renew the experience of nosocomial epidemics as they have been seen during the past decade.

PUBLICATIONS

Lindberg RB, Mason AD Jr, and Pruitt BA Jr: Species differences in potential of Enterobacteriaceae to incite nosocomial outbreaks in burn patients. Proceedings of Annual Meeting of American Society of Microbiologists, C96:326, 1979.

PRESENTATIONS

Lindberg RB: Nosocomial infections due to Enterobacteriaceae in burn patients: Species difference in ability to cause prolonged epidemics. American Burn Association Annual Meeting, New Orleans, Louisiana, 10 April 1969.

ANNUAL PROGRESS REPORT

PROJECT NO. 3S161102BS05-00, BASIC RESEARCH

REPORT TITLE: STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE OF TROOPS WITH THERMAL INJURY -- THE ROLE OF FUNGI IN BURN WOUND INFECTION: OBSERVATIONS ON BIOPSY AND AUTOPSY TISSUES FROM SERIOUSLY BURNED SOLDIERS

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

1 October 1978 - 30 September 1979

Investigators:

Robert B. Lindberg, PhD
Jack R. Henderson, PhD
William S. Hardy, SSG
Albert T. McManus, Major, MSC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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Biopsy and autopsy tissue samples were routinely cultured on Sabouraud's agar for th presence of fungi. Fungal infections were sporadic and episodic; no epidemic patterns of transmission were recognized. When Candida spp. were excluded, 11 species of fungi were recovered from biopsy, and 10 from autopsy tissue. The predominant genus was Aspergillus. Pathogenic genera included Coccidioides, Mucor and Absidia.

Fungi
Phycomycosis
Burns
Apergillus
Humans

STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE OF TROOPS WITH THERMAL INJURY -- THE ROLE OF FUNGI IN BURN WOUND INFECTION: OBSERVATIONS ON BIOPSY AND AUTOPSY TISSUES FROM SERIOUSLY BURNED SOLDIERS

Fungal infections in burned patients constitute a poorly understood phenomenon. Infection with saprophytic fungi does occur, but it tends to be episodic, and there is no convincing evidence of cross-infection or of patient-to-patient transmission occurring. Species of fungi which are recovered from burn wounds correspond to those found in the air of patient care facilities. Using numbers of strains recovered from biopsies as an index of frequency of occurrence, the totals range from 87 in 1972 to 6 in 1975, with the arithmetical average for the past seven years being 47. These figures refer to fungi exclusive of yeasts, which are vastly more common. The yeasts have been presented in the section on bacterial infections.

FUNGI IN BIOPSY SPECIMENS

There were 78 patients on whom biopsies were collected. Each specimen was cultured for fungi by planting a tissue fragment on the surface of Sabouraud's agar, prepared in a tissue-culture bottle to facilitate safe study of the culture. The results in terms of patients positive and number of strains recovered are shown in Table 1. The 11 species recovered are not unusual in terms of past experience, except for the unusual circumstance of recovery of a strain of Coccidioides immitis. This organism, the causative agent of coccidioidomycosis, has not previously been recovered from any patient in this Institute, and it was in this instance recovered only from a burn wound, not from sputum. The patient had not lived in the Southwest United States, but this geographical localization is not absolute. It is entirely possible that the wound was seeded after his arrival in the Institute of Surgical Research.

The cumulative listing of species recovered from burn patients over a period of years is a useful source of species incidence and predominance. This comparison of fungal recoveries is shown in Table 2. Consistent appearance has been observed only with Aspergillus, Cephalosporium, Fusarium, Penicillium, and Alternaria. The most rapid and lethal fungal mycoses have occurred with Mucor and Rhizopus spp, but these species are still relatively rare in burn wounds. Aspergillus and Fusarium are the most common species over a long term, with the former becoming numerically predominant in the last two years.

FUNGI RECOVERED AT AUTOPSY

Autopsy tissue samples are routinely cultured to detect the presence of fungi. In some years, the rate of fungal colonization has

Table 1. Fungi Recovered from Biopsies - 1978-1979

Genus	No. Patients Positive	No. of Strains Recovered
Aspergillus spp.	9	28
Yeast-like organisms	7	14
Cephalosporium spp.	2	5
Mycelia sterilia	2	2
Curvularium spp.	1	2
Penicillium spp.	1	1
Helminthosporium spp.	1	1
Coccidioides immitis	1	1
Nigosporium spp.	1	1
Fusarium spp.	1	1
Alternaria spp.	1	1

Total of 78 patients cultured for fungi; 11 genera recovered

Table 2. Fungi Recovered from Burn Wound Biopsies - 1973-1979

Genus	1973	1974	1975	1976-7	1977-8	1978-9
Aspergillus	17	5	2	5	23	28
Cephalosporium	5	5	1	4	5	5
Fusarium	23	17	2	4	4	1
Sepodonium	1	0	0	3	0	0
Penicillium	1	3	0	1	0	1
Alternaria	2	3	1	3	6	1
Trichophyton	0	0	0	1	0	0
Mucor	2	0	0	1	4	0
Rhizopus	2	0	0	0	0	0
Curvularium	2	3	0	0	0	2
Helminthosporium	9	2	0	0	0	1
Geotrichum	0	4	0	0	10	0
Coccidioides	0	0	0	0	0	1
Mycelia sterilia	0	0	0	0	0	2
No. patients cultured	106	135	63	113	61	78
No. genera	10	8	4	8	6	9

been almost the same for burn wound samples and for viscera, including lung, liver and spleen. However, in the 1978-1979 period, the proportion of patients whose tissues yielded fungi on culture was over twice that with recoveries from viscera. Table 3 presents the fungal recoveries from autopsies on 52 burn patients. Four genera, Aspergillus, Cephalosporium, Mucor, and Alternaria were found in both wound and viscera. Six were found only in the wound: Fusarium, Absidia, Diplosporium, Geotrichum, Curvularia, and Mycelia sterilia. Two genera, Hormodendrum and Penicillium, were recovered only from viscera. Phycomycetes, including Mucor and Absidia, were recovered; neither of these potentially invasive genera were found in any biopsy samples. They remain a small part of the total fungal population recovered in burn wounds. Aspergillus has been the predominant genus in burn wounds for the past two years. Prior to 1976, it was far less frequent in recoveries than was Fusarium. The frequency of occurrence of various genera is consistent with the hypothesis that such fungal invasion is truly opportunistic with the septate fungi. No genus thus far observed appeared to embody specific virulence characteristics. Continued observation of fungi in autopsies and biopsies is contemplated. The identity of such invading strains is highly desired, in the ongoing study of opportunistic invaders of burn wounds.

Table 3. Fungi Recovered from Viscera and Burn Wounds at Autopsy, 1978-1979

Genus	Wounds		Viscera	
	Patients Positive	No. of Strains	Patients Positive	No. of Strains
Aspergillus	12	25	4	6
Cephalosporium	2	2	1	1
Fusarium	2	2	0	0
Mucor	2	4	1	1
Absidia	1	1	0	0
Hormodendrum	0	0	1	1
Diplosporium	1	1	0	0
Alternaria	2	2	1	1
Penicillium	0	0	1	1
Curvularia	1	1	0	0
Mycelia sterilia	1	1	0	0
Geotrichum	1	1	0	0

PRESENTATIONS/PUBLICATIONS - None

ANNUAL PROGRESS REPORT

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Investigators:

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In a wide variety of approaches, assessment of the mechanisms of virulence of Pseudomonas aeruginosa has been studied. A study of the role of formation and the amount of endotoxin produced by invasive strains compared to noninvasive strains was carried out. The amoebocyte lysate reaction was applied to assessing such endotoxin production. Thus far, a significant difference in endotoxin formation has not been demonstrable, but the factors involved still offer areas of significance to be explored.

Pseudomonas
Endotoxin
Burns
Virulence
Humans

STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE OF TROOPS WITH THERMAL INJURY -- DETECTION OF ENDOTOXIN IN BURNED SOLDIERS WITH SEPSIS

The amoebocyte lysate gelation reaction, in the decade and a half since its recognition by Levin and Bang (1), has been intensively studied, with the primary objective of establishing its correlation with shock due to entrance of endotoxin into the blood stream, and more specifically, to determine whether such endotoxemia could be detected in vitro to furnish a diagnostic tool for recognition of this dangerous syndrome. Although endotoxin can be demonstrated by lysate gelation after its extraction from blood or plasma by a variety of technics, the diagnostic application has proven to be an unrealized hope. As shown by Lindberg et al. (2) and by McManus, English and Lindberg (3), the incidence of endotoxemia was not correlated with sepsis to a sufficient degree to make the reaction a reliable or usable diagnostic procedure. Sepsis without endotoxemia and endotoxemia without sepsis left too small a remainder of patients with endotoxemia and sepsis, or with no endotoxemia and no sepsis.

Another potential use of the reaction was studied in this laboratory as reported in 1977-1978 (4), in a study of invasive and noninvasive strains of Pseudomonas aeruginosa. An in vitro technic based on growth of the organisms in a substrate devoid of endotoxin was used to compare the rate and quantity of endotoxin formation between strains virulent and those nonvirulent for burned rats. There is no complete understanding of the structural or physiologic attributes that result in striking differences in ability of strains of this species to cause invasive burn wound sepsis. It was in search of correlations that bear on this problem that the endotoxin producing potential of virulent and nonvirulent strains was compared.

Strains were seeded in tissue culture medium L-15 and grown in static and then in rotating cultures for arbitrarily designated

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1. Levin J, Bang FB: The role of endotoxin in the extracellular coagulation of limulus blood. Bull J Hopkins Hosp 115:265-274, 1964.
2. Lindberg RB, English VC, Pruitt BA Jr, Mason AD Jr: Detection of endotoxin in burned soldiers with sepsis. USA Inst Surg Res Annual Report FY 1973, Brooke Army Medical Center, Fort Sam Houston, Texas, Section 6.
3. McManus AT Jr, English VC, Lindberg RB: Detection of endotoxin in burned soldiers with sepsis. USA Inst Surg Res Annual Report, FY 1977, Brooke Army Medical Center, Fort Sam Houston, Texas, pp. 293-296.
4. Lindberg RB, McManus AT Jr, English VC: Detection of endotoxin in burned soldiers with sepsis. USA Inst Surg Res Annual Report, FY 1978, Brooke Army Medical Center, Fort Sam Houston, Texas, pp. 241-249.

intervals. The cultures were arrested by heating to 65° C, and the concentration of endotoxin assessed by the gelation reaction, using dilution titrations against a standard Salmonella enteritidis endotoxin. Various concentrations of inoculum were tested to determine an optimum generation technic for detecting differences in endotoxin production between strains. The incubation time was found to be critical; by an early point on the logarithmic phase of reproduction, endotoxin amounts had increased to a quantity that obscured any differences in rate of production.

Despite testing of a variety of endotoxin production parameters, it has not been possible to demonstrate significant differences in endotoxin amount or rate of production that can be correlated with ability of strains to cause experimental invasive burn wound sepsis. Virulent strains and nonvirulent strains each varied in their rate of endotoxin production, but the differences were not quantifiable in a manner that would correlate such production rates with virulence.

It is entirely possible that the hypothesis on which this work was based is correct, and that differences could be shown with appropriate adjustments of technic. However, the present stage of this study does not show promise of such a development. While endotoxin production undoubtedly plays a role in the pathogenesis of Pseudomonas sepsis, differentiating this pattern by lysate gelation assay has not been possible.

PRESENTATIONS/PUBLICATIONS - None

ANNUAL PROGRESS REPORT

PROJECT NO. 3S161102BS05-00, BASIC RESEARCH

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Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

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Type 84 and 84,85 Staphylococcus aureus epidemics have been present in burn ward populations for at least 9 years. Striking and complete fluctuations from susceptible to resistant strains have occurred, over short time intervals, with reference to methicillin, oxacillin and nafcillin. The mechanism of these shifts is innate, not environmentally induced. Beta-lactamase production is indeed the mechanism of resistance, but its induction can be due to endogenous factors.

Staphylococcus
Bacteremia
Burns
Infections
Antibiotics

STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE OF TROOPS WITH THERMAL INJURY -- EMERGENCE OF METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS IN BURNED MILITARY PERSONNEL

The role of Staphylococcus aureus in hospital infection has been persistent; despite continued input of antibiotics effective in vitro against current strains, it has remained ubiquitous. S. aureus is most often the numerically predominant organism recovered in blood cultures of burn patients. However, the clinical implications of staphylococcal bacteremia are not as grave as are those of gram-negative sepsis.

Epidemiologic tracing of S. aureus infections has relied on bacteriophage (phage) typing. The current pattern of infection in the burn wards of the Institute of Surgical Research has been one of alternating predominance of type 84 and type 84,85 strains. No other type has, in the past 9 years, presented numbers to suggest that it was in prospect of becoming predominant. It is of continued interest that the burn ward type has neither colonized the attending staff nor pervaded adjacent wards of Brooke Army Medical Center.

The antibiotic sensitivity of strains of S. aureus has been determined by minimum inhibitory concentration (MIC) technic, using a macrotube dilution procedure. In reviewing the patterns of sensitivity of these strains, a previously unrecorded phenomenon was recognized: spontaneous return of sensitivity to methicillins -- methicillin, oxacillin and nafcillin. Wide fluctuations in sensitivity occurred over a period of several months or even a year or more. These fluctuations were independent of recognizable external influence, of which the obvious one was fluctuation or variation in the clinical use of antibiotics. Demonstration of such innate variation in sensitivity to a group of antibiotics which had been presumed to exhibit a parallel action toward staphylococci has opened a new dimension in understanding the biology of this important pathogenic species.

Epidemic episodes have been studied in close chronologic detail. Extreme lability in appearance and disappearance of methicillin resistance has been demonstrated.

Table 1 summarizes the changes in sensitivity of S. aureus to the methicillin group of antibiotics over a 2-year period. Prior to 1977, staphylococci had for some time been largely sensitive to methicillin, oxacillin and nafcillin. Nafcillin resistance first appeared in 1977, and from that time forward was persistent. In October 1977, methicillin- and oxacillin-resistant strains first appeared. In November 1977, the strains tested had reverted to oxacillin sensitivity, a pattern which persisted for 2 months. In January 1978, methcillin-sensitive strains reappeared, but oxacillin resistance accompanied

Table 1. Percent of Staphylococcus aureus Strains Resistant over 24 Months, 1977-1979
(MIC > 6.25 mcg/ml)

Antibiotic	Year and Month											
	1977			1978								
	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep
Methicillin	97.5	98.7	82.1	0	0	29.1	95.4	93.3	50.0	31.2	11.1	86.0
Oxacillin	90.6	2.6	5.1	90.0	78.9	4.0	13.6	15.3	8.3	6.2	0	5.5
Nafcillin	94.0	87.4	95.6	100	100	100	100	100	100	87.5	100	100
	1978			1979								
	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep
Methicillin	85.3	85.7	52.9	100	70.0	48.0	62.0	72.7	48.3	50.0	60.0	85.7
Oxacillin	12.5	71.4	16.6	25.0	60.0	60.0	54.5	54.5	3.7	33.0	0	14.2
Nafcillin	95.5	100	100	100	100	100	100	100	100	100	100	100

this change. In April 1978, oxacillin sensitivity reappeared and continued until November 1978. Meanwhile, methicillin resistance reappeared in March and persisted over the next 4 months; in July and August, the staphylococci recovered had abruptly shifted to methicillin sensitivity. This sensitivity lasted only 2 months; then methicillin-resistant strains were present until mid-1979, when half of the isolates were sensitive. Oxacillin sensitivity characterized strains recovered in December 1978 and January 1979. The population then became oxacillin-resistant until June 1979, when a sensitive population reappeared.

These fluctuations were studied closely for evidence of linkage to sensitivity to aminoglycosides, the tetracycline group, cephalothins, or glycopeptide antibiotics. None of these exhibited linkage to the methicillin group fluctuations.

The behavior demonstrated here toward a major group of anti-staphylococcal antibiotics is not one that has been recognized as a part of the naturally occurring susceptibility pattern of microorganisms. Cognizance of this phenomenon may be expected to lead to better understanding of mechanisms of acquisition of antibiotic resistance among gram-positive cocci. The role of S. aureus in the pathogenesis of burn wound infection remains incompletely understood.

PRESENTATIONS

Lindberg RB: Fluctuations in susceptibility of Staphylococcus aureus to methicillin-group antibiotics. Interscience Conference on Antimicrobial Agents and Chemotherapy. To be presented 29 October 1979, Boston, Massachusetts.

PUBLICATIONS

None

ANNUAL PROGRESS REPORT

PROJECT NO. 3S161102BS05-00, BASIC RESEARCH

REPORT TITLE: STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE OF TROOPS WITH THERMAL INJURY -- SENSITIVITY TO SULFAMYLON OF PSEUDOMONAS AERUGINOSA RECOVERED FROM BURNED SOLDIERS

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1 October 1978 - 30 September 1979

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Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

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Sensitivity of Pseudomonas aeruginosa to Sulfamylon^{R} has been assessed on an annual basis. In 1978-1979, 715 strains were tested. These isolates included those from two epidemic episodes of intensive seeding in the burn ward population. The strains were more resistant than had been the case over the five preceding years. In 1972, a similar upsurge of resistant strains appeared. In that instance, P. aeruginosa strains returned to a sensitive level the following year. The current episode of resistance is to be followed closely; if resistance persists, it will be a major factor in assessing the performance of Sulfamylon.

Pseudomonas
Sulfamylon
Burns
Topical therapy
Humans

STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE OF TROOPS WITH THERMAL INJURY -- SENSITIVITY TO SULFAMYLON OF PSEUDOMONAS AERUGINOSA RECOVERED FROM BURNED SOLDIERS

The sensitivity to topical therapeutic agents of potentially invasive bacteria has been of continuing interest in the treatment of severe burns. Sulfamylon[R] burn cream and silver sulfadiazine are two major agents in the armamentarium available for topical treatment. Sulfamylon has been in continuous use in the Institute of Surgical Research since its first experimental trial in 1963, and there has obviously been ample opportunity for the selection of Sulfamylon-resistant strains from the bacterial flora which has colonized the burn patient population passing through the Institute of Surgical Research wards during those years. The typical experience with chemotherapeutic agents and antibiotics used against bacteria has been to encounter drug-resistant strains, often under circumstances where they become predominant and negate further use of the antimicrobial agent in question. Sulfamylon, representing an unusual drug structure in being a methylated sulfonamide, has been shown thus far to be one of the small number of bona fide exceptions to this rule. Just as group A hemolytic streptococci have never developed penicillin resistance, so Pseudomonas aeruginosa has not developed Sulfamylon resistance. Isolates of this important burn pathogen have been tested on a continuing surveillance basis. This study assumed added import during the 1978-1979 period, since it covered an interval in which Pseudomonas infection in the burn wards was essentially epidemic. It was also a period in which Sulfamylon was used in alternating applications of silver sulfadiazine, in a treatment regimen directed toward improving control of wound infection.

SENSITIVITY OF PSEUDOMONAS AERUGINOSA TO SULFAMYLON

The period covered in this report extends from 1 October 1978 through 30 September 1979. Seven hundred and fifteen strains were tested. This large number of isolates reflected the high incidence of Pseudomonas infection and colonization that occurred during this year. The technic for testing is one that was devised in this laboratory. It involves seeding strains on agar with Sulfamylon present in descending quantities from 2.5% down to 0.019%. Sensitive strains are those inhibited by 0.312% or less of the drug. This is 1/32 of the clinical concentration of Sulfamylon used in treatment.

The range of sensitivity to Sulfamylon for 1978-1979 is shown in Table 1. The sensitivity levels dropped for this year, compared to the 1977-1978 period. Such fluctuations have occurred before and do not of necessity connote any long-term shift in sensitivity.

Table 1. Sensitivity to Sulfamylon for Pseudomonas aeruginosa
1 October 1978 - 30 September 1979

No. of Strains	Concentration Required for Inhibition (gm/dl)	% of Total Tested
78	1.250	11.0
307	0.625	43.0
193	0.312	27.0
59	0.156	8.3
47	0.078	6.4
16	0.039	2.2
1	0.019	0.1
14	< 0.019	2.0
Total 715		

Sensitivity to Sulfamylon for the eight preceding years is shown in Table 2. The behavior of P. aeruginosa in 1978-1979 most resembled the pattern seen in 1972. In that year, there was an increase in the proportion of strains which required 0.625% of Sulfamylon for inhibition; in 1978-1979, the major rise was in that group and in the number which were inhibited by 0.312%. There was a drop in the number inhibited by 0.078% or less, i.e., the highly sensitive population.

The changes that have appeared are most clearly visualized when cumulative sensitivity levels are compared on an annual basis. This data is shown in Table 3. Comparison of these annual levels makes it clear that up to 1971, no strains were encountered which required more than 0.625% for inhibition; and prior to 1971, fewer than 5% of isolates tolerated more than 0.312%. The most resistant group, in 1972, was one in which 52% of isolates required more than 0.312% for inhibition. From that year until 1978-1979, the proportion of strains tolerating 0.312% was consistently in the range of 15% to 20%. In 1978-1979, this figure rose to 28.5%. The P. aeruginosa population in this past year has thus been a relatively resistant one, although the difference is still far below a level that would place the organism beyond reach of the drug as used clinically.

The median level of inhibitory activity, at which 50% of strains are inhibited, offers another aspect of the behavior of P. aeruginosa to Sulfamylon. This value, for the years from 1968 to 1979, is shown in Table 4. The two high years of sensitivity were 1972 and 1978-1979; the median inhibitory level for those two years exceeded 0.3%. In 1977-1978, the value was one of the lowest recorded. The fluctuations in sensitivity of populations of P. aeruginosa have not been

Table 2. Inhibiting Concentrations of Sulfamylon for Pseudomonas aeruginosa, 1971-1979

Year	No. of Strains	Concentration of Sulfamylon in gm/dl; No. & % of Strains Inhibited							
		1.25	0.625	0.312	0.156	0.078	0.039	0.019	0.019
1971	286	0	48	41	56	57	65	13	0
			16.7	14.3	19.5	19.3	22.7	4.5	
1972	463	29	212	46	88	31	37	15	5
		6.3	45.8	9.9	19.1	6.7	7.9	3.2	1.1
1973	285	4	14	85	85	52	32	12	1
		1.4	4.9	29.8	19.8	18.3	11.2	4.2	0.4
1974	437	5	59	78	97	97	86	11	4
		1.1	13.5	18.0	22.2	22.2	19.7	2.5	0.9
1975	637	13	133	108	155	68	147	28	4
		2.0	17.7	16.9	24.3	10.7	23.1	4.5	0.6
1976-77	698	4	118	135	295	95	18	23	10
		0.6	16.9	19.4	42.2	13.6	2.5	3.3	1.4
1977-78	141	16	17	16	26	48	12	5	1
		11.3	12.0	11.3	18.4	34.0	8.5	3.5	0.7
1978-79	715	78	307	193	59	47	16	1	14
		11.0	43.0	27.0	8.3	6.6	2.0	0.1	2.0
TOTAL	3662	149	888	702	861	495	413	98	39
		4.2	24.3	19.3	23.5	13.5	11.4	2.7	1.1

Table 3. Cumulative Sensitivity to Sulfamylon of Pseudomonas aeruginosa, 1968-1979

Year	No. of Strains	Concentration of Sulfamylon in gm/dl; % of Strains Inhibited							
		1.25	0.625	0.312	0.156	0.078	0.039	0.019	<0.019
1968	294	100	100	95.1	60.4	45.8	14.1	1.7	0
1969	385	100	100	96.5	50.0	26.9	7.7	0.5	0
1970	296	100	100	100	78.0	49.9	21.9	2.0	0
1971	286	100	100	82.9	68.3	48.3	27.9	4.7	0
1972	463	100	93.7	48.0	38.0	19.0	12.3	4.3	1.1
1973	285	100	98.1	81.3	57.0	33.5	16.1	3.2	0.4
1974	437	100	99.0	85.5	67.5	45.3	23.1	2.4	0.9
1975	637	99.8	97.8	80.1	63.2	38.9	24.2	5.0	0.6
1976-77	698	100	99.4	82.5	63.2	21.0	7.3	4.7	1.4
1977-78	141	100	98.1	83.5	64.3	34.3	17.9	4.5	0.9
1978-79	715	100	95.8	71.5	52.2	28.7	15.2	3.8	1.1

Table 4. Median Value of Pseudomonas aeruginosa Sensitivity to Sulfamylon, 1968-1979

Year	No. of Strains Tested	Median Inhibitory Level (gm/dl)
1968	294	0.136
1969	385	0.176
1970	296	0.068
1971	286	0.125
1972	463	0.316
1973	285	0.111
1974	437	0.086
1975	637	0.125
1976-77	698	0.117
1977-78	141	0.089
1978-79	715	0.324

paralleled by comparable fluctuations in Pseudomonas sepsis. However, the monitoring of important agents used in topical therapy as to their effectiveness against major opportunistic invaders is a study that must be maintained.

PRESENTATIONS AND/OR PUBLICATIONS - None

ANNUAL PROGRESS REPORT

PROJECT NO. 3S161102BS05-00, BASIC RESEARCH

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1 October 1978 - 30 September 1979

Investigators:

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Search for unusual oxidative and fermentative gram-negative bacilli in biopsy and autopsy tissue samples has been continued. This population would include the most probable new invasive species, if such indeed were to appear. Seventeen species of unusual gram-negative bacilli were recovered, primarily in sputum samples. There were no indications of epidemic pattern transmission of these unusual opportunistic invading strains. The surveillance is of value for both negative and positive indications of the emergence of new species causing opportunistic infection.

Burns
Oxidative microorganisms
Acinetobacter
Pseudomonas

STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE OF TROOPS WITH THERMAL INJURY -- NON-FERMENTATIVE GRAM-NEGATIVE BACILLI IN BURNED SOLDIERS: NEW POTENTIAL OPPORTUNISTIC PATHOGENS

Burn wound infections have long been recognized as essentially opportunistic. The numerically predominant species in recent years have been Staphylococcus aureus and Pseudomonas aeruginosa. However, extensive epidemics of other species have occurred in patients in the Institute of Surgical Research burn ward, and short-term outbreaks with relatively rare gram-negative bacilli emphasize the importance of searching for and monitoring this category of microorganism. The search for unusual species has been facilitated by extending the incubation period; some species appear as visible colonies only when primary incubation time is extended to 72 or even 96 hours.

UNUSUAL GRAM-NEGATIVE BACILLI ON BURNED PATIENTS

Most strains of this group of unusual gram-negative bacilli are recovered from sputum and burn wounds. Table 1 summarizes the 16 species from clinical bacteriology specimens recovered in 1978-1979, with a total of 108 strains. Five species of Pseudomonas were recovered, but in contrast to previous years, only seven strains were found. Pseudomonas species had, in previous years, made up a much larger part of the total. Acinetobacter spp. (formerly Mima and Herella spp.) were slightly more frequently encountered. The three patients from whom the organisms were recovered in blood were not seriously ill due to Acinetobacter sepsis. Among the other species recovered, a significant proportion were Klebsiella ozaenae, which appeared in brief increments suggesting an epidemic transmission pattern. Biopsy cultures were almost devoid of unusual gram-negative flora. This situation was a change from findings of 4 to 6 years earlier. A potential opportunistic pathogen which has been rare in burn wounds was Enterobacter agglomerans. This organism came to sudden prominence with its occurrence in a widespread episode of contamination of intravenous fluids. It has been reported in a variety of nosocomial infection situations, and is to be included in the list of potential new opportunists. Similarly, Aeromonas hydrophila has appeared in a variety of hospital and surgical infections, and Aeromonas sepsis was present in one of the patients with bacteremia in this series.

Postmortem cultures yielded only a small number of unusual gram-negative isolates. These are summarized in Table 2. There is no obvious reason for the marked drop in recoveries of unusual gram-negative species from autopsy tissues in the last 2 years. Since such findings are affected by technical manipulation, it is possible that changes in procedure have entered the system undetected. A review of technics for autopsy tissue cultures is under way.

Table 1. Unusual Gram-Negative Species Recovered from Clinical Bacteriology Specimens, 1 October 1978 - 30 September 1979

Species	Source and Number of Strains						
	Blood	Sputum	Wound	Biopsy	Urine	IV Cath Tips	Total
Pseudomonas fluorescens	0	1	0	0	0	0	1
Pseudomonas cepacia	0	2	0	0	0	0	2
Pseudomonas putida	1	0	0	0	0	0	1
Pseudomonas alcaligenes	0	0	1	0	0	0	1
Pseudomonas maltophilia	0	2	0	0	0	0	2
Acinetobacter anitratus	2	5	2	1	0	0	10
Acinetobacter lwoffii	2	2	1	0	0	0	5
Group 5E2	0	1	1	0	0	0	2
Escherichia coli AD	0	0	3	0	0	0	3
Citrobacter freundii	0	1	4	0	0	0	5
Klebsiella oxytoca	0	7	3	0	1	1	12
Klebsiella ozaenae	1	25	6	0	4	1	37
Enterobacter hafnia	0	3	1	0	0	0	4
Enterobacter agglomerans	0	8	2	0	1	0	11
Aeromonas hydrophila	2	0	2	5	0	1	10
Haemophilus influenza	0	4	0	0	0	0	4
Total	8	61	26	6	6	3	110

Table 2. Unusual Gram-Negative Species Recovered from Autopsy Bacteriologic Specimens, 1 October 1978 - 30 September 1979

Species	No. of Patients	Source	No. of Isolates
Flavobacterium spp.	1	Wound	1
Citrobacter freundii	2	Liver, spleen, lung, blood, IV tip, wound	6
Klebsiella oxytoca	3	Liver, spleen, lung, wound, blood	13
Enterobacter agglomerans	1	Blood	1

Seven autopsies yielded unusual gram-negative species. All were fermentative rather than oxidative forms. There was no indication that the postmortem flora reflected emergence of new invasive or potentially epidemic species.

PRESENTATIONS/PUBLICATIONS - None

ANNUAL PROGRESS REPORT

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UNCLASSIFIED

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Further studies of metal-sulfonamide compounds as topical chemotherapeutic agents were directed toward manganese sulfadiazine, which was capable of effecting survivals equal to the most effective metal-sulfonamide compounds. Mixed pairs of metal-sulfadiazines showed encouraging results in survival; this approach is to be studied further.

Pseudomonas
Topical therapy
Burns
Burn wound sepsis

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The investigation of metal-sulfonamide complexes as potential agents for topical chemotherapy in burns has continued. Zinc, copper, cerium and chromium, combined with sulfadiazine, with sulfamethoxazole, and with other sulfonamides to form specific compounds, have been made up in creams and tested for prophylactic and therapeutic capacity in control of experimental Pseudomonas burn wound sepsis.

A new compound, manganese sulfadiazine, was prepared, assessed in varying dilutions for chemotherapeutic activity, and then tested extensively in a 1% w/v concentration for chemotherapeutic activity in comparison with the other compounds listed and with silver sulfadiazine. The test system used was the Institute of Surgical Research standard 20% body surface burn on a 200 gram male rat, seeded with an appropriate virulent challenge strain. Efficacy of therapy was assessed by the survival rate.

RESULTS OF COMPARATIVE TESTS

Manganese sulfadiazine was the principal new metal sulfonamide tested, but further evaluation of the other four sulfadiazine compounds was also made. Table 1 summarizes the results obtained. The tests were carried out in increments of four to five animals per experiment; the total samples ranged from 36 to 48 rats per drug.

Manganese sulfadiazine was found to effect survival at a rate comparable to that achieved with zinc sulfadiazine. Only chromium sulfadiazine was more effective in sparing rats burned and seeded with a lethal challenge. Cerium sulfadiazine was the least effective compound, although with strains not extremely virulent it effected survivals comparable to the other sulfadiazine-metal compounds. Silver sulfadiazine was included as a therapeutic control, and it is effective in the range exhibited by zinc, copper, chromium and manganese sulfadiazine.

Another aspect of topical therapy with metal-sulfadiazine compounds was the possibility of using combinations of two metal-sulfonamide preparations, to reduce further the potential for absorption of metallic ions.

Table 2 presents a preliminary assessment of five different mixtures, with pairings of zinc, cerium, copper, chromium and manganese sulfadiazines. The series is not yet extensive enough to confirm the advantageous results, but initially, these mixtures appear promising. Only 15 or 20 rats are involved in each set, and

Table 1. Survival Rates with Topical Metal-Sulfadiazine Compounds

Compound (1% Concentration)	Challenge Strains and % Surviving 12-4-4	8-28-3	VA-134
Zinc diazine	92.3	90.0	63.3
Copper diazine	90.0	84.6	50.0
Cerium diazine	88.0	33.3	0
Chromium diazine	92.3	93.7	64.8
Manganese diazine	96.7	89.4	46.8
Silver sulfadiazine	86.3	84.7	38.0
Controls, untreated	6.6	4.6	0

Table 2. Combinations of Metal-Sulfonamide Compounds in Control of Pseudomonas Burn Wound Sepsis in Rats

Compound Mixture	Challenge Strains and % Surviving 12-4-4	8-28-3	VA-134
.5% Zn diazine + .5% Ce diazine	100.0	93.3	22.0
.5% Zn diazine + .5% Cu diazine	100.0	100.0	46.0
.5% Zn diazine + .5% Cr diazine	86.6	80.0	41.1
.5% Zn diazine + .5% Mn diazine	100.0	90.0	--
.5% Mn diazine + .5% Ce diazine	100.0	75.0	--

further work will be needed to confirm these encouraging survival rates. Blood levels of metal ions used are to be determined, to assess the validity of this hypothesis. If results of combinations of metal-sulfonamides were to be only as good as those achieved with single metal-sulfonamide combinations, the mixed preparation would reduce by half the exposure to metal ions of each element included. While there has been as yet no indication that any of these ions is

toxic in the experimental model, it would be intuitively desirable that their concentration be held to a minimum. The reported advantages of adding cerium to silver sulfadiazine also prompted scrutiny of combinations in view of the possibility that such pairs might produce an additive effect. While this result was not suggested by any of the pairs thus far tested, this possibility merits further exploration.

PRESENTATIONS

Lindberg RB: New metal-sulfonamide compounds as topical agents in control of experimental Pseudomonas burn wound sepsis. XII International Congress of Chemotherapy, Boston, Massachusetts, 30 September 1979.

PUBLICATIONS

None.

ANNUAL PROGRESS REPORT

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REPORT TITLE: STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE OF TROOPS WITH THERMAL INJURY -- ENZYME PRODUCTION AND VIRULENCE OF PSEUDOMONAS AERUGINOSA RECOVERED FROM SOLDIERS WITH THERMAL INJURY

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

1 October 1978 - 30 September 1979

Investigators:

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Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3S161102BS05-00, BASIC RESEARCH

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A study in depth of enzyme and toxin production by Pseudomonas aeruginosa has been instituted, comparing these parameters in virulent, invasive strains and in non-virulent strains. These entities have been described as associated with virulence, but no specific correlation with behavior in burn wounds has been made. Technics have been established, and studies on 288 strains carried out, for caseinase, lipase, amylase and elastase. Activity ranged from 98% positive for caseinase to zero for amylase. Investigation of other systems, including hyaluronidase, collagenase, and lecithinase, is projected.

Burns
Pseudomonas
Virulence
Enzymes
Humans

STUDIES OF INFECTION AND MICROBIOLOGIC SURVEILLANCE OF TROOPS WITH THERMAL INJURY -- ENZYME PRODUCTION AND VIRULENCE OF PSEUDOMONAS AERUGINOSA RECOVERED FROM SOLDIERS WITH THERMAL INJURY

Since burn wound infection with its threat of invasive sepsis due to Pseudomonas aeruginosa remains a significant hazard despite the use of effective topical chemotherapeutic agents, continued study of possible mechanisms of invasion by this ubiquitous organism has been conducted. The basis for this work is the large collection, now numbering over 400 strains, derived from burn patients since 1961 and assessed for virulence by seeding on a 20% burn on 200 gram rats. This controlled infection differentiates consistently virulent, partially virulent and non-virulent strains. The behavior of organisms in these categories is consistent and reproducible, and they have furnished the basis for developing and evaluating therapeutic agents for control of burn wound infection. It is a plausible hypothesis that differences in enzyme or toxin production can be correlated with virulence, to offer a basis for better understanding of the pathogenesis of Pseudomonas infection and thence improved control of such infections.

Enzymes, particularly proteases, are thought to play a role in the pathogenesis of some P. aeruginosa infections (1-3). Most strains of P. aeruginosa produce three proteases (4). Injection of the protease or elastase of this species intradermally in laboratory animals induces hemorrhagic lesions within 10 minutes, and such lesions become necrotic in 24 hours (5). Pulmonary hemorrhages and focal peritoneal hemorrhage frequently are seen with systemic infection with P. aeruginosa, and it is probable that protease or elastase may account for such lesions (6,7). The perivascular infiltration

1. Kawaharajo K, Homma JY, Aoyama Y, Morihara K: In vivo studies on protease and elastase from Pseudomonas aeruginosa. Jpn J Exp Med 45:89-100, 1975.

2. Kawaharajo K, Homma JY, Aoyama Y, Okada K, Morihara K: Effects of protease and elastase from Pseudomonas aeruginosa on skin. Jpn J Exp Med 45:79-88, 1975.

3. Liu PV: Extracellular toxins of Pseudomonas aeruginosa. J Infect Dis 130(Suppl):S94-S99, 1974.

4. Morihara K: Production of elastase and proteinase by Pseudomonas aeruginosa. J Bacteriol 88:745-777, 1964.

5. Liu PV: The roles of various fractions of Pseudomonas aeruginosa in its pathogenesis. II. Effects of lecithinase and protease. J Infect Dis 116:112-116, 1966.

6. Fetzer AE, Werner AS, Hagstrom JW: Pathologic features of Pseudomonas pneumonia. Am Rev Respir Dis 96:1121-1130, 1967.

7. Meinke K, Barum J, Rosenberg B, Berke RS: In vivo studies with the partially purified protease (elastase) from Pseudomonas aeruginosa. Infect Immun 2:583-589, 1970.

and cuffing of microvasculature that are the hallmark lesions of Pseudomonas sepsis are possibly precipitated by an elastolytic protease (8). Ocular infections with P. aeruginosa frequently destroy the cornea with massive ulceration, and this result appears to be due to severe damage to the collagen component (9).

To study the characteristics of proteases and other enzymes and toxins of P. aeruginosa, a collection of enzyme-producing strains isolated from burn patients was needed. To select appropriate technics and screen Pseudomonas strains, a collection of isolates from burn patients on the Institute of Surgical Research burn wards was assembled and studied. The assessment of virulence of recent isolates was carried out on burned rats using the model described above.

TECHNICS

A study of available procedures for enzyme measurement was made.

a. Caseinase: This enzyme was measured by agar plate assay described by Sokol et al (10). Brain heart infusion broth medium was dialyzed for 18 hours against deionized water, after which agar was added to a 3% concentration; 3% (w/v) skim milk was autoclaved, and the two components were combined in equal volumes and poured in Petri dishes. Test strains were seeded by point inoculum. Caseinase was evinced by a clear zone in the opaque substrate at 24 hours.

b. Lipase: Spirit blue agar (Difco) was used as substrate.

c. Amylase: 1% soluble starch in trypticase soy agar was used to test for amylase production. Point inocula were incubated for 24 hours, following which Gram's iodine flooding of the plate was used to detect hydrolysis as a clear zone in the surrounding deep-blue starch-iodine reaction.

d. Elastase: A technic based on the description of Sbarra (11) has been developed to test for this enzyme. Elastin from bovine neck ligament (Sigma) is incorporated as an opaque suspension, together with several inorganic salts of potassium, magnesium, sodium, iron

8. Mull JD, Callahan W: The role of the elastase of Pseudomonas aeruginosa in experimental infection. Exp Mol Pathol 4: 567-575, 1965.

9. Schoellmann G, Fisher E Jr: A collagenase from Pseudomonas aeruginosa. Biochim Biophys Acta 122:557-559, 1966.

10. Sokol PA, Ohman D, Iglewski B: A more sensitive plate assay for detection of protease production by Pseudomonas aeruginosa. J Clin Microbiol 9:538-540, 1979.

11. Sbarra AJ, Gilfillan RF, Bardawil W: A plate assay for elastase. Nature (London) 188:322-323, 1960.

and manganese. The test plate is seeded by point inocula of Pseudomonas strains. Well-defined clear zones in the medium produced by the dissolution of elastin particles indicate elastase activity.

RESULTS

Tests for caseinase, lipase and amylase have been made on 288 strains of recently isolated P. aeruginosa. Cleared zones were measured with an antibiotic zone reader when appropriate. Zone sizes ranged from 0.3 mm to 9.6 mm. The mean zone size for caseinase was 3.5 mm, which was much larger than that described by Sokol. All but four strains tested produced caseinase. Quantitative differences in enzyme production are to be investigated.

Lipase was produced by 82% of the strains tested. Zone diameter was not measured in this determination. Most non-lipolytic strains produced a deep-brown pigment and were clustered among individual patients.

None of the strains produced amylase.

Elastase activity was tested for in 37 strains, of which 25 were elastase-positive. Zones of clearing in this assay typically exhibited pink to lavender color in the cleared agar.

DISCUSSION

Agar plate assay was used to detect amylase, lipase, caseinase and elastase production by P. aeruginosa. The technic is a simple, rapid and reproducible method for the detection of each of these enzymes.

Projected further study includes assays for collagenase, hyaluronidase and lecithinase. A technic for detection of collagenase production by P. aeruginosa is particularly important since true collagenolytic activity of this bacterial species has not been conclusively demonstrated.

By use of technics described here, all demonstrable enzymes produced by selected strains of P. aeruginosa isolated from burn patients will be assayed. Enzyme activity will be correlated with invasive virulence as demonstrated on the burned seeded rat model. Up to this time, no correlation between toxins of P. aeruginosa and invasive capability has been shown. The approach proposed here offers the possibility of localizing invasive virulence of this ubiquitous opportunistic pathogen, and hence can offer direction for improved methods of controlling Pseudomonas infection in burn wards.

PUBLICATIONS/PRESENTATIONS - None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OG 6969	79 10 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
78 10 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	3S161102BS05	00	087
b. ~~CONTRIBUTING~~	62774A	3S162774A820	00	117
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) The Study of Metabolism and Nutritional Effects 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
76 10	Cont	DA	C. In-House

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

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 79	11	251
FISCAL YEAR CURRENT 80	6	264

19. RESPONSIBLE DOD ORGANIZATION
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ASSOCIATE INVESTIGATORS
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NAME: DA

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Nitrogen balance; (U) Burn injury; (U) Temperature regulation; (U) Environmental control; (U) Protein metabolism; (U) Hormones; (U) Glucose metabolism; (U) Humans

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

23. (U) To identify the etiology and humoral mediators of postinjury hypermetabolism and altered thermoregulation in burned soldiers. To assess the nitrogen sparing of varied alimentation regimens. To define alterations and control of blood flow to the wound and other organs, and to determine the rate of nutrient delivery provided by that flow. To describe the effects of thermal injury on hormone production and on protein, glucose, and fat metabolism.

24. (U) The controlled environmental chamber is used to study thermoregulation and altered heat loss following injury. Respiratory exchange gas is measured continuously by mass spectrophotometer and a canopy hood system. Limb plethysmography is employed to assess blood flow in extremities with and without burns. Arterial and venous concentrations of nutrients are assessed and, with blood flow, turnover rate determined. Hormonal mediators are determined in blood and urine and their relationship to the altered metabolism determined. Nitrogen balance is related to dietary, hormonal, and exercise regimens and the septic complications. The effect of injury on blood flow and metabolism has been studied in injured goats, and protein flux into the wound is studied in burned rats.

25. (U) 7810 - 7909 In the clinically unstable burn patient, concentrations of both total and free T_3 and free T_4 are significantly suppressed. These alterations are not influenced by calorie intake. They do not appear to influence metabolic rate. Chemical hypothyroidism is associated with hepatic extraction of free T_3 in septic patients as compared with efflux of free T_3 from liver to plasma in both septic and non-septic burn patients who are chemically euthyroid. These data suggest that the liver in critically injured man may act as a regulator of plasma levels of free T_3 by varying its rate of extraction and secretion of free T_3.

*Available to contractors upon submitter'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. 3S161102BS05-00, BASIC RESEARCH

REPORT TITLE: THE STUDY OF METABOLISM AND NUTRUTIONAL EFFECTS ON BURN INJURY IN SOLDIERS--INCREASED PERIPHERAL AMINO ACID RELEASE FOLLOWING BURN INJURY

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

1 October 1978 - 30 September 1979

Investigators:

L. Howard Aulick, PhD, Major, MSC
Douglas W. Wilmore, M.D.

Reports Control Symbol MEDDH-288(R1)

Unclassified

ABSTRACT

PROJECT NO. 3S161102BS05-00, BASIC RESEARCH

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

Period covered in this report: 1 October 1978 - 30 September 1979

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Reports Control Symbol MEDDH-288(R1)

Through the use of a specially designed venous occlusion plethysmograph, resting leg blood flow was measured in four normal controls and 18 uninfected burn patients (mean burn size 41% of the total body surface). Following these measurements, blood was drawn simultaneously from the femoral vein (FV) of the leg under study and a peripheral artery (A). A and FV plasma concentrations of alanine, glycine, proline, valine, serine, threonine, leucine, isoleucine, methionine and phenylalanine were determined and peripheral exchange rates calculated for these 10 amino acids. Except for phenylalanine, arterial plasma amino acid concentrations were normal or below in these burn patients. The A-FV concentration difference was only significant for alanine ($-9 \pm 2\ \mu$ mole/dl in the patients vs -5 ± 1 in controls, mean $\pm$ S.E., $p < 0.05$). Leg blood flow was elevated in the patients (6.26 ± 0.57 ml/100 ml leg $\cdot$ min vs 2.62 ± 0.57 in controls). Of the ten amino acids, only alanine was released from the leg at consistently higher rates in the burn patients ($0.27\ \mu$ mole/100ml leg $\cdot$ min vs 0.08 ± 0.02 in controls). The rate of peripheral alanine release was unrelated to leg blood flow ($r^2 = 0.002$) or the size of limb injury ($r^2 = 0.15$), but did increase with the extent of total body surface injury and oxygen consumption of the patient. These results indicate that skeletal muscle proteolysis is a reflection of the generalized catabolic response to injury rather than any response to local inflammatory or metabolic events which may occur in the injured limb.

This project has been completed and a paper under the same title has been published in Surgery 84: 560-565, 1979. No further work in this area is anticipated.

ANNUAL PROGRESS REPORT

PROJECT NO. 3S161102BS05-00, BASIC RESEARCH

PROJECT TITLE: THE STUDY OF METABOLISM AND NUTRITIONAL EFFECTS OF BURN INJURY IN SOLDIERS - STUDIES OF DISTURBANCE OF PROTEIN TURNOVER IN BURNED TROOPS: USE OF AN ANIMAL MODEL

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1 October 1978 - 30 September 1979

Investigators:

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Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3S161102BS05-00, BASIC RESEARCH

PROJECT TITLE: THE STUDY OF METABOLISM AND NUTRITIONAL EFFECTS OF BURN INJURY IN SOLDIERS - STUDIES OF DISTURBANCE OF PROTEIN TURNOVER IN BURNED TROOPS: USE OF AN ANIMAL MODEL

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Period covered in this report: 1 October 1978 - 30 September 1979

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Reports Control Symbol MEDDH-288(R1)

Groups of 180-200 g Sprague-Dawley rats were anesthetized with sodium pentobarbital and subjected to a 20% body surface scald burn or sham burn on the dorsum. Half of each group of rats were given a subcutaneous injection of hyaluronidase (150 N.F. units in 1 ml 0.15 M NaCl) into the wound one hour postburn. The rats were housed in individual cages and permitted free access to food and water until time of sacrifice. The four experimental groups were:

Group SU: Sham untreated

Group SHY: Sham hyaluronidase

Group BU: Burned untreated

Group BHY: Burned hyaluronidase

The tissue within the margins of the burn wound, or within the inked outline of the sham wound, was excised through the paniculus carnosa to fascia. The complete tissue sample, approximately 65 cm^2 surface area, is referred to below as wound whether from burned or sham burned rats.

In one set of experiments, ^{125}I-labeled rat albumin was injected into the tail vein of each rat just before burn or sham burn. The rats were sacrificed at intervals up to six days postburn. The amount of ^{125}I-labeled albumin and total albumin content in the plasma and wound were determined as previously described (1).

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1. Brown WL, Bowler EG, Mason AD Jr, Pruitt BA Jr: Protein metabolism in burned rats. Am J Physiol 231:476-482, 1976.

At one hour postburn, 58.6% of the injected dose of ^{125}I-labeled albumin had disappeared from the plasma compartment of rats in Group BU. Approximately 60% of this amount (34.5% of the dose) was present in the burn wound at that time. Of the 28.6% of dose which had disappeared from the plasma of group SU, 3.3% (0.9% of dose) was present in the sham wound at one hour postburn.

The segment of the plasma disappearance curves from 24-144 hours postburn was analyzed by Analysis of Covariance to determine the statistical significance of differences between the groups.

The slopes (fractional disappearance rates of ^{125}I-labeled albumin) of the plasma curves of Groups SU and SHY were not significantly different. The intercept (^{125}I-labeled albumin content) of the plasma ^{125}I-labeled albumin disappearance curve of Group SU was significantly higher than that of Group SHY ($p < 0.001$). The plasma albumin disappearance curves of Groups BU and BHY had common intercepts and slopes.

Both the slopes ($p < 0.01$) and the intercepts ($p < 0.001$) of the plasma ^{125}I-labeled albumin disappearance curves of Groups (BU + BHY) were significantly lower than those of Groups (SU + SHY).

In normal circumstances this might indicate that the sham burned rats were exchanging albumin from plasma to wound at a much faster rate than were the burned rats. In this case, this interpretation is not appropriate because the albumin in the wound of the burned rats accumulated such a large amount of label immediately postburn; this highly labeled albumin returning to the plasma masks the loss from plasma of the burned rats (shown by the hourly rates below).

The slopes of the disappearance curves from the wounds of Groups SU and SHY were not significantly different, but the intercept of Group SU was higher than that of Group SHY ($p < 0.01$). The slopes of the curves of Groups BU and BHY were not significantly different, but the intercept of Group BU was higher than that of BHY ($p < 0.001$). For the comparison of Groups (SU + SHY) and (BU + BHY), the intercepts of Groups (SU + SHY) were significantly lower ($p < 0.0001$) and the slopes significantly higher ($p < 0.001$) than those of Groups (BU + BHY). Although the fractional disappearance rates of ^{125}I-labeled albumin from wounds of sham burned rats were larger than those of burned rats, the absolute quantity of albumin transferred from the burn wound was much greater.

In another set of experiments, ^{125}I-labeled rat albumin was injected at selected times postburn and the rats were sacrificed one hour after injection.

With the exception of the 0-1 hour measurement of the burned rats, the plasma disappearance rate averaged 25% an hour for all groups at every time of measurement.

The rate of entry of ^{125}I-labeled albumin into the sham wound averaged approximately 0.8% of dose per hour at each time. The entry rates for Group SHY and BHY were slightly higher than those of their untreated counterparts at three hours postburn and slightly lower later on. At this time, the rate of entry of labeled albumin into burn wounds was about ten times that of entry into the sham wounds. The entry rate for burned rats remained two to three times higher from 24-72 hours postburn.

Although the transfer rate of labeled albumin into the wounds of burned rats was accelerated, the total albumin pool of the wound remained approximately 200 mg for Group BU and 150 mg for Group BHY during the 24-72 hour postburn period. There is no evidence in this rat burn model that any substantive amount of albumin is lost through the surface of the skin. The observed maintenance of a stable wound albumin pool size in the presence of an increased transfer rate led to the conclusion that the wound albumin pool is not static but is in dynamic equilibrium with the plasma pool.

PUBLICATIONS/PRESENTATIONS - None

ANNUAL PROGRESS REPORT

PROJECT NO. 3S161102BS05-00, BASIC RESEARCH

REPORT TITLE: THE STUDY OF METABOLISM AND NUTRITIONAL EFFECTS ON BURN INJURY IN SOLDIERS--THE DEVELOPMENT OF A GOAT MODEL FOR THE STUDY OF WOUND BLOOD FLOW AND METABOLISM

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

1 October 1978 - 30 September 1979

Investigators:

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Unclassified

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Neurogenic, hormonal and metabolic influences on blood flow to a large surface wound were evaluated in anesthetized goats (20-40kg) by measuring arterial blood pressure and external iliac artery flow bilaterally with Doppler flow probes 9-12 days after removal of the skin from one hindlimb. Active changes in peripheral vascular resistance (arterial pressure/leg blood flow) were determined during (a) controlled hemorrhage, (b) intravenous infusion of norepinephrine, epinephrine and glucose, (c) direct application of each of these substances and iodoacetate to the wound surface, and (d) induced hypothermia (ice water infusion into the rumen followed by spinal anesthesia. Basal vascular resistance was lower in the injured leg (IL) and increased at a slower rate with hemorrhage and during a stepwise increase in IV norephinephrine infusion. Topical application of norepinephrine vasoconstricted the wound. Intravenous epinephrine over the same dose range produced pronounced β-vasodilator effects only in the uninjured leg (UL) and had no vasomotor effect when applied directly to the wound. Induced hypothermia caused a relatively insignificant increase in IL vascular resistance while flow in the UL was markedly reduced. Spinal anesthesia returned vascular resistance in both hindlimbs to basal levels. These results indicate that wound vessels constrict in response to circulating α-adrenergic mediators but have no apparent β-receptors or functional neurogenic tone. Infusion of exogenous glucose increased glucose turnover in both hindlimbs but had no effect on leg perfusion or vascular resistance. Likewise, topical application of glucose or blockade of glucose metabolism with iodoacetate did not

alter limb flow or vascular resistance. Therefore, while the local chemical environment may contribute to the observed wound vasodilation and loss of reflex vasomotor control, these local influences apparently are unrelated to glucose metabolism.

Wound blood flow
Neurogenic vascular control
Granulation tissue

THE DEVELOPMENT OF A GOAT MODEL FOR THE STUDY OF WOUND BLOOD FLOW AND METABOLISM

The granulating wound which develops 9-12 days post-surgical removal of the skin from one hindlimb of a goat, has many functional similarities to the surface wounds of burned patients (1). Blood flow to the injured limb (goat and patient) is markedly increased while that to the contralateral, uninjured limb remains normal. This increase in peripheral blood flow, presumably directed to the surface wound, is not in response to increased aerobic metabolism, but is associated with an increase in glucose consumption and lactate production. Since these peripheral circulatory and metabolic adjustments to surface injury are identical to those observed in the thermally injured patient, the goat model appears to be an appropriate means by which to study the control of burn wound blood flow. The purpose of this study is to assess further the neurogenic, hormonal and metabolic infuences on wound blood flow.

METHODS

All experiments in this series were performed on the anesthetized goat model, 9-12 days post-excision as previously described (1). Bilateral hindlimb blood flow was measured by chronically implanted Doppler flow probes (Parks Electronic Company, Beaverton, Oregon) placed around the external iliac arteries.

Neurogenic and Hormonal Vasomotor Control: In three anesthetized injured animals bilateral hindlimb blood flow and axillary artery blood pressure were monitored during a 30-45 minute period of hemorrhage, maintained at a rate of 15 ml/min by withdrawing blood from an external jugular vein. In additional studies, the effect of circulating catecholamines on wound blood flow was determined in anesthetized animals during a stepwise increase of the intravenous infusion of either epinephrine (n=3) or norepinephrine (n=4). Each catecholamine (4 mg/ml) was infused at a constant rate into the external jugular vein. By varying pump speed five infusion doses (1.84, 3.68, 9.16, 18.4, and 36.8 µg/min) were utilized for both catecholamines and each dose was administered for a ten-minute period.

In five other animals, the topical or local effects of epinephrine or norephinephrine on the granulating wound were evaluated. In these studies, the injured leg was wrapped in gauze dressings, saturated

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1. Aulick LH, Baze WB, Wilmore DW: The development of a goat model for the study of wound blood flow and metabolism. Annual Research Progress Report, US Army Institute of Surgical Research, Brooke Army Medical Center, Fort Sam Houston, Texas, 1978.

with solutions of either epinephrine (8 mg/L saline, n=2) or norepinephrine (8 mg/L, n=3). The dressings were kept saturated by reapplying 50-75 ml of the test solution every 20 minutes. At the end of the experiment the physiologic properties of the solution were confirmed by injecting 5 ml intravenously and monitoring alterations in blood pressure and flow.

Another technique utilized to assess neurogenic control of wound blood flow was to monitor changes in hindlimb vascular resistance during induced hypothermia. The animals (n=2) were maintained on light inhalation anesthetic and pancuronium (0.06 mg/kg) administered to prevent shivering and other spontaneous muscle activity. Following stabilization of arterial pressure and extremity blood flows on controlled ventilation, fifteen minutes of steady state measurements were obtained. Then 2.5 liters of ice water were infused into the animals' rumen via a previously placed nasogastric tube. Rectal temperature was measured at 5-minute intervals throughout the study. Following 20 minutes of induced hypothermia, the animal was turned on its side and sympathetic denervation of the hindlimbs achieved by administering 10 cc of 1% xylocaine as a spinal anesthetic. The animal was turned back into a supine position and pressure and flow measurements continued.

Glucose and Glucose Metabolism: The relationship between peripheral blood flow and glucose metabolism was evaluated in six anesthetized animals. Serial, arterial and femoral venous blood samples were drawn at 20 minute intervals. Following two basal measurements in two goats, an intravenous infusion of a 10% glucose solution was started in the jugular vein and maintained at a rate of 1.5 ml/min for an hour. In two additional animals basal measurements and blood samples were obtained and then the same 10% glucose solution was applied topically to the granulating wound by saturating gauze dressings, which were wrapped around the injured leg. This dose of topical glucose was maintained for 30 minutes followed by a 30-minute application of 50% glucose. Serial blood samples were drawn at 15 minute intervals during the entire experimental period and blood pressure and hindlimb blood flows monitored continuously. In two additional goats, iodoacetate (50 mg/ml), was applied topically (7 ml initially followed by 2 ml at ten minute intervals) to the injured leg following basal measurements and blood samples. Serial blood samples were obtained at 20-minute intervals following application of this inhibitor of the Embden-Meyerhof pathway and leg blood flows and arterial pressure were continuously monitored.

RESULTS

Neurogenic and Hormonal Vasomotor Control: The peripheral vascular response to controlled hemorrhage of three anesthetized animals was characterized by a progressive increase in limb vascular

resistance (mean arterial pressure/leg blood flow) bilaterally. The initial level of resistance was lower in the injured leg and its rate of change with hemorrhage less than that in the contralateral control limb (Figure 1).

A stepwise increase in the rate of intravenous norepinephrine infusion also increased vascular resistance in both the control and injured legs of three other anesthetized goats similar to that observed with hemorrhage (Figure 2). Intravenous infusion of epinephrine, however, demonstrated pronounced β-vasodilator effects only in the uninjured leg of another three anesthetized animals (Figure 3).

Topical epinephrine also had no effect on vascular resistance in the injured leg but the same dose of norepinephrine caused vasoconstriction when applied directly to the wound (Figure 4). The direct α-effects of norepinephrine were maintained despite systemically induced alterations in peripheral vascular tone evident in the contralateral control limb.

With induced hypothermia (a fall in rectal temperature from 39 to 35°C in 15 minutes), blood flow to the control leg approached zero and vascular resistance increased dramatically, while relatively minimal alterations in flow and vascular resistance were observed in the injured leg (Figure 5). Subarachnoid block immediately reduced vascular resistance in the two hindlimbs to the same level.

Glucose and Glucose Metabolism: With the infusion of exogenous glucose, glucose turnover in both legs increased but this was not associated with major alterations in limb perfusion or vascular resistance. The topical application of 10% and 50% glucose solutions resulted in glucose absorption through the wound with femoral venous glucose concentrations in the injured leg consistently exceeding arterial concentrations. However, in spite of this provision of excess quantities of glucose applied directly to the wound surface, no alterations in blood flow or peripheral resistance were observed. Similarly, topically applied iodoacetate blocked the uptake of glucose in the injured extremity and inhibited lactate production. Despite this profound alteration in local glucose metabolism no change in leg perfusion or vascular resistance was observed.

DISCUSSION

These results suggest that changes in extrinsic control of the neovasculature via the nervous system and/or circulating neurohormones may contribute to the observed vasodilation. Patient studies (2)

2. Aulick LH, Wilmore DW, Mason AD Jr, Pruitt BA Jr: Influence of the burn wound on peripheral circulation in thermally injured patients. Am J Physiol 233(4):H520, 1977.

have shown that vessels in granulating burn wounds had a degree of basal tone which could be modified by changes in the local thermal environment, but reflex heating studies suggested that neurogenic control of the wound was limited (3). This apparent denervation may be structural, the result of actual physical disruption of vasomotor nerves or poorly developed smooth muscle receptors in the wound, or functional, if innervated vessels are competitively inhibited by the local metabolic or inflammatory environment. Numerous potential vasodilators have been identified in injured tissue - i.e., prostaglandins, kinins, lactic acid and potassium ions (1,4-7).

In the goat model, neurogenic control of wound blood flow was evaluated in several ways. First, the peripheral vascular response to hemorrhage was found to be more pronounced in the uninjured hindlimbs, presumably as both skeletal muscle and skin vessels constricted reflexly. The rather limited changes in vascular resistance observed in the injured leg most likely reflect a combination of appropriate vasomotor adjustments in the underlying skeletal muscle without comparable reflex changes in the surface wound. Secondly, spinal anesthesia had minimal effect on vascular resistance in the injured limb while causing an immediate and dramatic loss of vascular tone in the control limb. Both of these studies suggest that goat wound vasculature is not innervated. Whether this is the result of (1) actual disruption of vasomotor nerves at the time of injury and limited nervous regeneration and reinnervation of wound vessels or (2) the absence of α-receptors and/or vascular smooth muscle in granulation tissue was not established.

When the α-adrenergic neurotransmitter, norepinephrine, was applied directly to the wound surface, vascular resistance in the treated limb rose above that of the untreated, control leg. This indicated that the apparent loss of neurogenic control of wound vessels

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3. Aulick LH, Wilmore DW, Mason AD Jr, Pruitt BA Jr: Peripheral blood flow in thermally injured patients. Fed Proc 36:417, 1977.

4. Anggard E, Johnson DE: Efflux of prostaglandin in lymph from scalded tissue. Acta Physiol Scand 81:440, 1971.

5. Arturson G: Prostaglandins in human burn wound secretions. Burns 3:112, 1978.

6. Edery H, Lewis GP: Kinin-forming activity and histamine in lymph after tissue injury. J Physiol, (London), 169:468, 1963.

7. Wilmore DW, Aulick LH, Mason AD Jr, Pruitt BA Jr: Influence of the burn wound on local and systemic responses to injury. Ann Surg 186(4):444, 1977.

was not the result of either faulty receptor activity or end organ (vascular smooth muscle) function. Therefore, these studies taken together suggest that the wound is functionally sympathectomized. Other studies have indicated that this lack of nervous control in a wound has a structural basis.

Sympathetic nerve regeneration and vascular reinnervation of granulation tissue have been shown to involve a considerable period of time (8). Through the use of transparent chambers permitting direct visualization of vessels in the granulating wound, 3-4 weeks elapsed post injury before active arteriolar contractions were observed. In some animals, arterioles were 3.5-7.5 months old before definite active contractions were noted. As a consequence, there is a considerable period of time between the appearance and differentiation of wound vessels and the development of neurogenic vasomotor control. During this time, changes in wound blood flow may occur passively, secondary to variations in perfusion pressure, or actively due to changes in vascular smooth muscle tone in response to either variations in the local environment or the presence of circulating vasoactive substances.

A common result of all forms of major injury is the increased elaboration of catecholamines. To evaluate the effects of these circulating vasoactive substances on wound blood flow, norepinephrine and epinephrine were infused intravenously and changes in leg vascular resistance determined over a wide range of catechol doses. For a given increase in catechol dose, the absolute change in limb vascular resistance was always greater in the uninjured leg. This was true for both the α-vasoconstrictor effects of norepinephrine or the predominately β-vasodilator effects of epinephrine. The goat wound has functioning α-receptors, as previously demonstrated, which may explain a portion of the vasoconstriction in the injured limb with intravenous norepinephrine. In contrast, local application of epinephrine to the surface wound had no demonstrable effect on vascular tone. This suggests that, like normal skin, granulation tissue has no β-receptors and consequently no capacity to dilate in response to β-stimulation.

SUMMARY AND CONCLUSIONS

Despite obvious differences in the animal species, the mechanism of injury and the level of consciousness, changes in blood flow and glucose metabolism in the injured goat hindlimb are comparable to

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8. Clark ER, Clark EL, Williams RG: Microscopic observations in the living rabbit of the new growth of nerves and establishment of nerve-controlled contractions of newly formed arterioles. Am J Anat 55:47, 1934.

those reported earlier in burn patients. Based on these similarities, this animal model provides an excellent approach to the study of the control of blood flow to a large surface wound The results of this investigation indicate that the myriad of new vessels in the granulating wound are dilated because they are without neurogenic vasoconstrictor tone. This vasodilation does not occur because of limitation in oxygen delivery (1) or alterations in glucose metabolism. Moreover, these wound vessles possess vascular smooth muscle and α but not β-receptors, and are therefore capable of responding to appropriate circulatory and/or neurogenic vasoconstrictor drives.

The rapid proliferation of new blood vessels in the granulating wound occurs well in advance of vasomotor nerve regeneration. Without an extrinsic nerve supply, vascular tone in the wound reflects a balance between local effects (metabolic, inflammatory and thermal) and circulating vasoactive agents. Functionally, the loss of neurogenic tone and the presence of local vasodilators dominate. As a consequence, the body's circulatory system must support that newly added vascular bed either through increased cardiac output or compensatory vaso-constriction of other more inactive regions of the body. During this period, wound perfusion appears to vary primarily with changes in systemic pressure. This is a passive response, since the sympathectomized wound is unable to participate actively in neurogenic vasomotor adjustments.

Thus, the wound vasculature becomes a parasite to the circulatory system--with adequate systemic blood pressure, high wound blood flow is insured. Although the interactions between nutrient delivery and wound metabolism are not known, this elevated blood flow appears to be a prerequisite for rapid and normal wound repair.

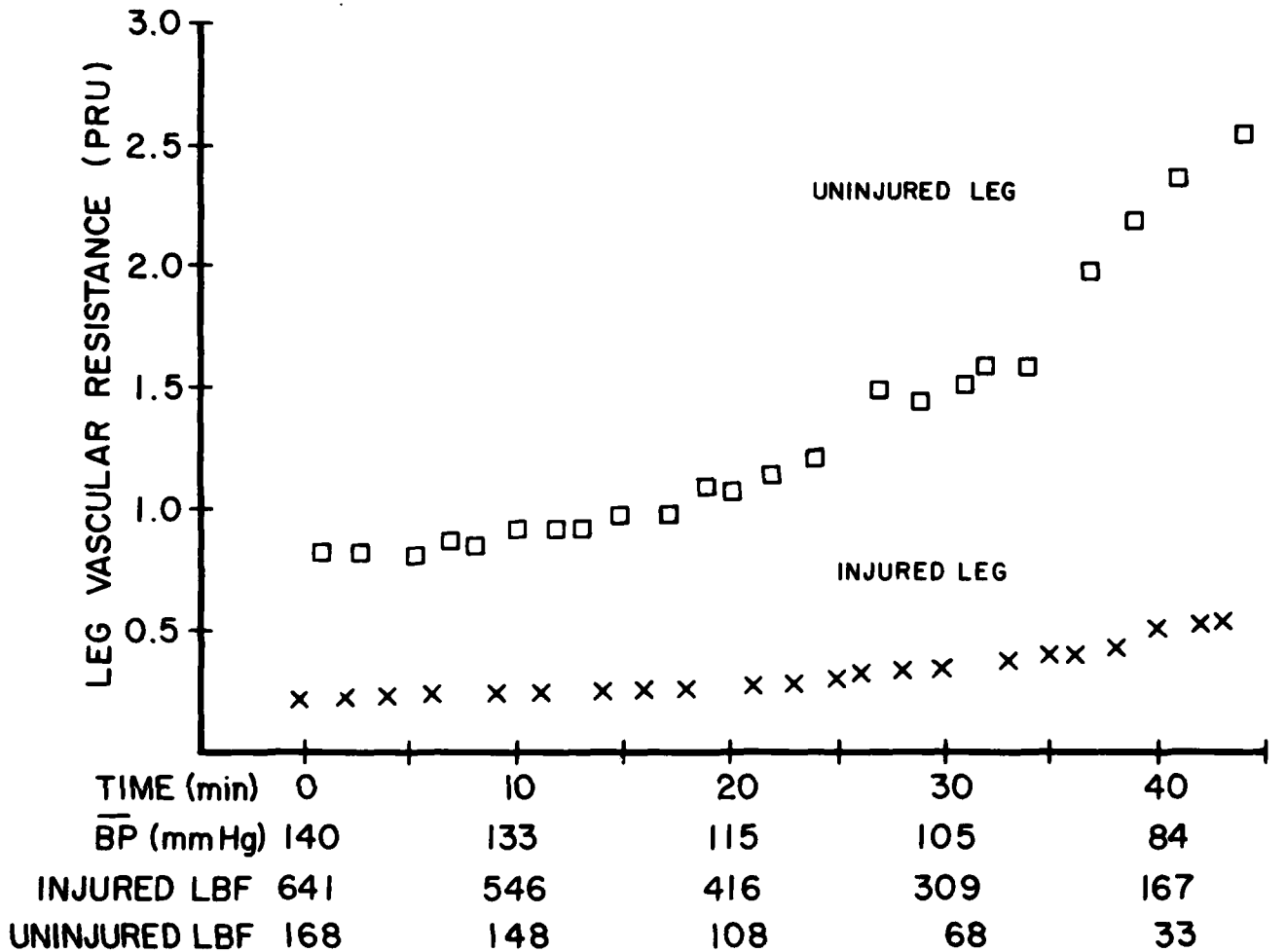

Fig. 1 Vasoconstriction occurs in both injured and uninjured hindlimbs of anesthetized goats (the consistent response as demonstrated in one of three animals) during a controlled hemorrhage (15 ml/min) but the increase in leg vascular resistance was always greater in the uninjured limb. PRU=peripheral resistance units in mmHg/ml·min; $\overline{BP}$=mean blood pressure; LBF=leg blood flow.

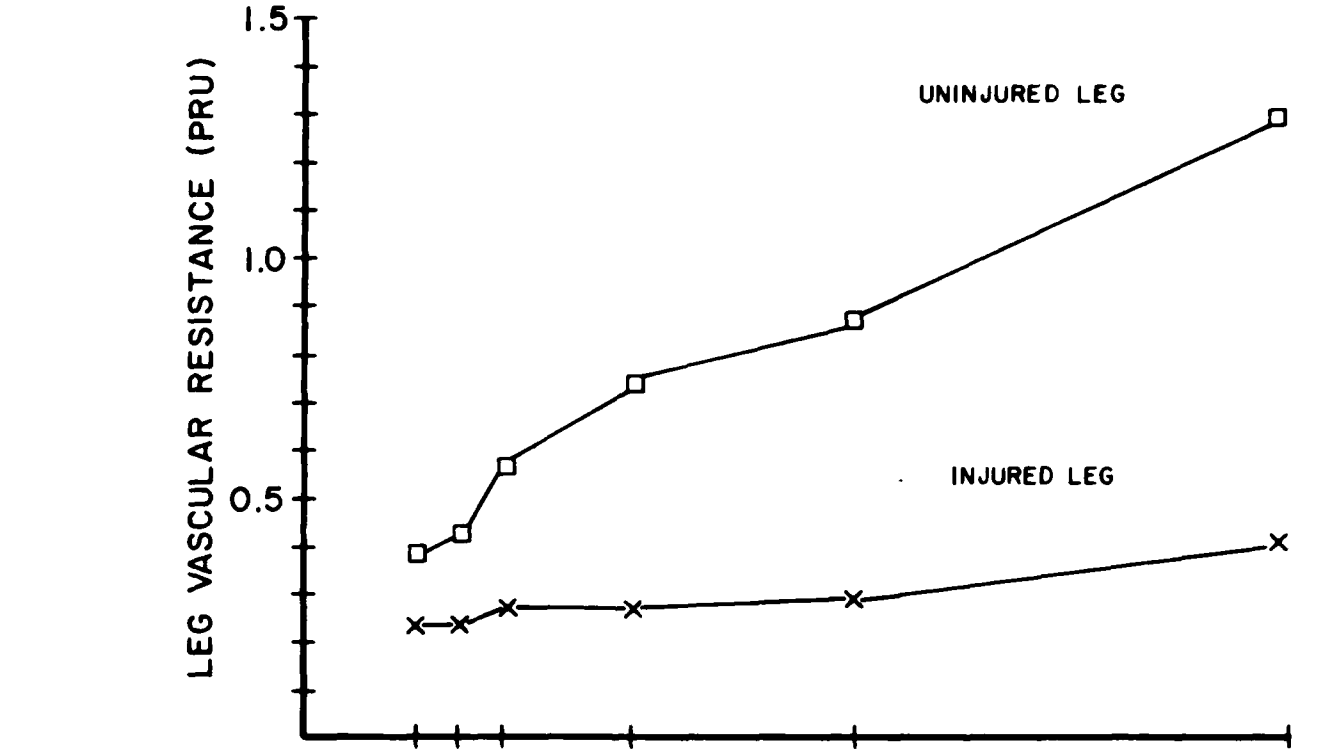

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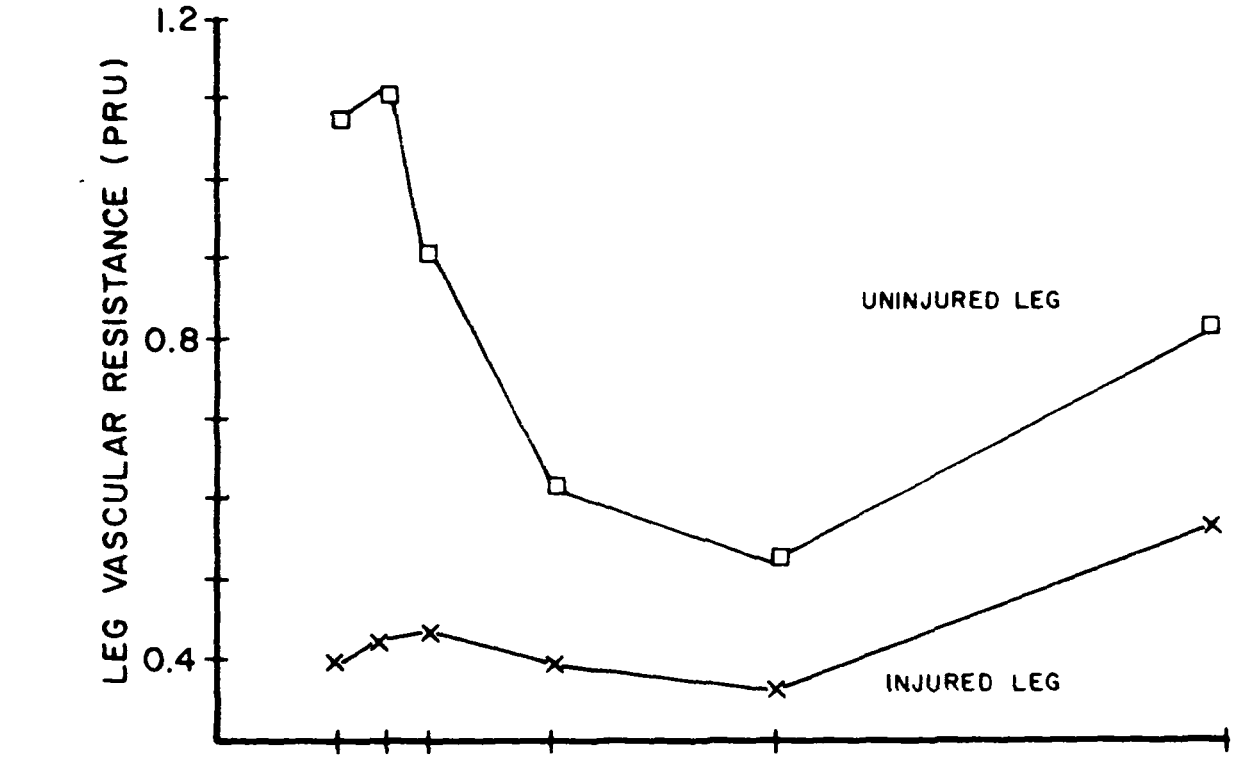
Fig. 2 Intravenous infusion of norepinephrine causes a dose-response increase in bilateral hindlimb vascular resistance of anesthetized goats (as represented by one of the three animals). For any given dose of this α-adrenergic mediator, the absolute change in peripheral resistance is greater in the uninjured leg. Each value represents a steady-state level after 10 minutes of constant infusion. PRU=peripheral resistance units in mmHg/ml·min; $\overline{BP}$=mean blood pressure; LBF=leg blood flow.



EPI INFUSION DOSE (μg/min)	0	1.8	3.7	9.2	18.4	36.8
$\overline{BP}$ (mm Hg)	80		90	100	100	158
INJURED LBF (ml/min)	202		208	251	271	274
UNINJURED LBF (ml/min)	74		99	161	187	190

Fig. 3 The β-vasodilator effect of intravenous epinephrine infusion is most apparent in the uninjured leg of anesthetized goats (representative changes in bilateral leg vascular resistances in one of three animals - PRU = peripheral resistance units in mmHg/ml·min). The lack of vasodilation in the injured leg suggests that wound flow (the major component of limb flow) cannot be increased further by β-stimulation. $\overline{BP}$ = mean blood pressure; LBF = leg blood flow.

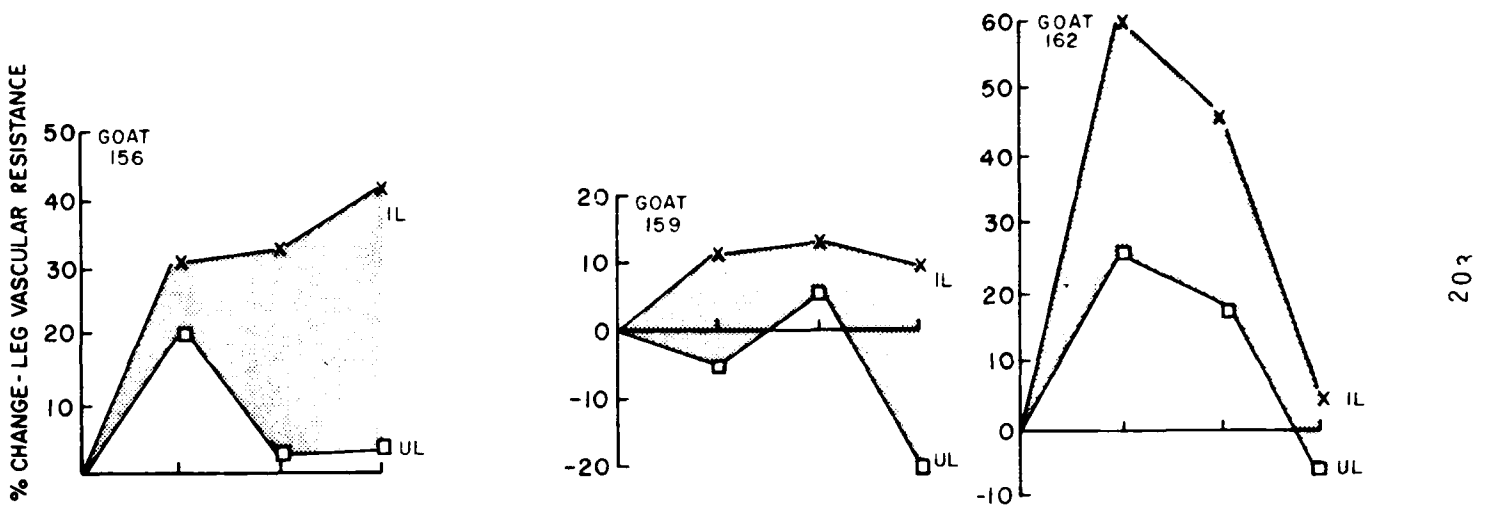


Fig. 4 Topical application of norepinephrine (8 mg/L) directly to the wound caused a proportionally greater increase in leg resistance in the injured leg (IL) than in the untreated, noninjured leg (UL) of three anesthetized animals. Note that in all three studies, the vasoconstrictor effects of locally applied norepinephrine in IL modify the systemic fluctuations in leg vascular resistance as represented by resistance changes of UL. The consistent vasoconstrictor effect of norepinephrine was statistically confirmed by covariant analysis (injured leg resistance before vs after treatment, F ratio = 19.11, $p < 0.01$ as compared to uninjured leg, F ratio = 0.74, N.S.)

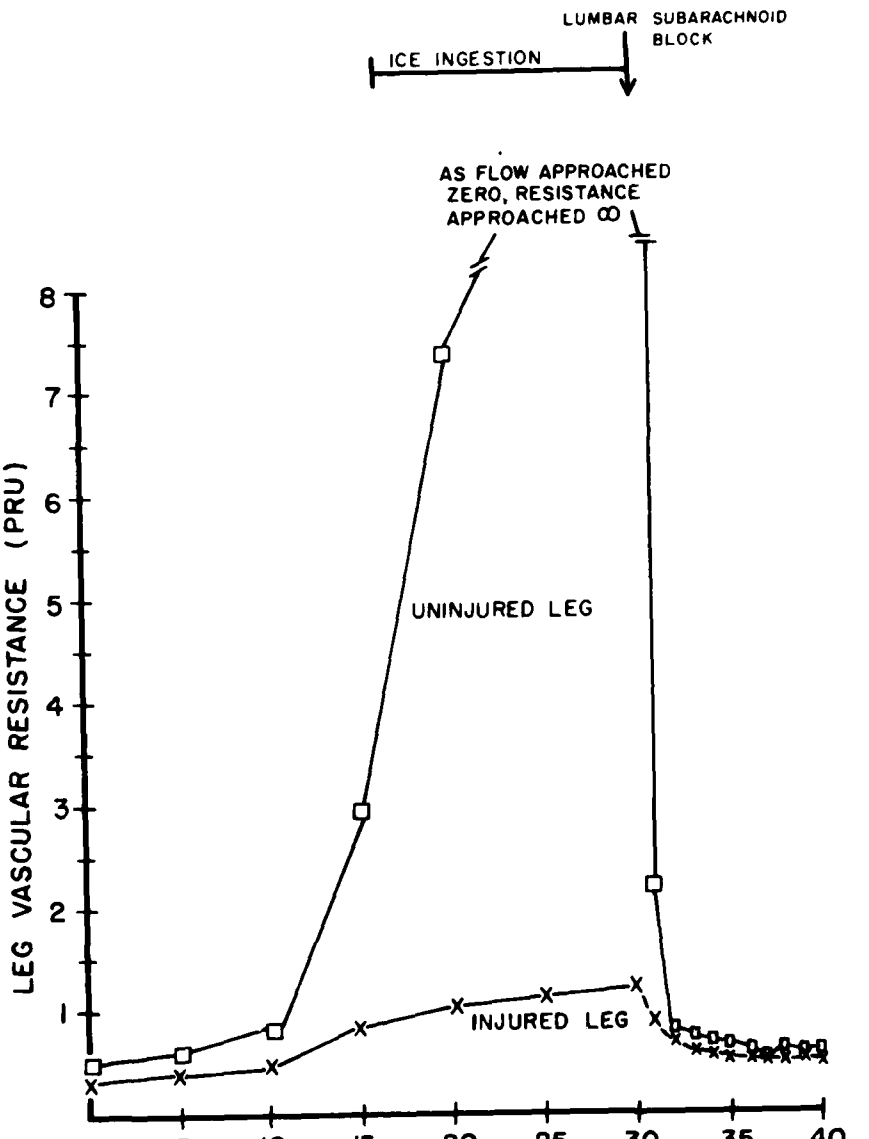


Fig. 5 The induced fall in rectal temperature (T_{re}) apparently initiates changes in mean arterial blood pressure ($\overline{BP}$) and bilateral leg blood flow (LBF) consistent with a major sympathoadrenal outflow. The limited changes in injured leg vascular resistance during development (via hypothermia) and release (via spinal anesthesia) of neurogenic vasoconstrictor drives suggest that the wound is denervated.

ANNUAL PROGRESS REPORT

PROJECT NO. 3S161102BS05-00, BASIC RESEARCH

REPORT TITLE: THE STUDY OF METABOLISM AND NUTRITIONAL EFFECTS ON BURN INJURY IN SOLDIERS--STUDIES OF HEPATIC BLOOD FLOW AND SUBSTRATE TURNOVER FOLLOWING THERMAL INJURY

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

1 October 1978 - 30 September 1979

Investigators:

L. Howard Aulick, Ph.D., Major MSC
Douglas W. Wilmore, M.D.
Cleon W. Goodwin, Jr., M.D., Major, MC
Richard A. Becker, M.D.

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

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To characterize important changes in liver perfusion and glucose metabolism with injury and infection, estimated hepatic blood flow (EHBF) was determined by ICG clearance and simultaneous arterial and hepatic venous blood samples analyzed for oxygen, glucose, lactate and pyruvate in 22 fasted burn patients (mean burn size 57 percent total body surface, range 18 to 83.5) 7 to 29 days post injury. Patients were classified as nonbactermic (n = 10), bacteremic (n = 8) and complicated bacteremic (n = 4). There was no difference among groups in rectal temperature (38.4 $\pm$ 0.2, 38.6 $\pm$ 0.2 and 38.0 $\pm$ 0.5$^{o}C_{\pm}$, group means $\pm$ S.E.), total body oxygen consumption (215 $\pm$ 10, 238 $\pm$ 8 and 244 $\pm$ 12 ml/min $\cdot$ m^2) or cardiac index (7.8 $\pm$ 0.4, 8.8 $\pm$ 0.4 and 7.7 $\pm$ 0.7 l/min $\cdot$ m^2). Estimated hepatic blood flow and hepatic glucose metabolism of these patients were compared with postabsorptive normal subjects from the literature: (mean $\pm$ S.E.)

	Normal	Nonbacteremic (N)	Bacteremic (B)	Complicated Bacteremic (CB)
Blood Flow ($l/min \cdot m^2$)	0.63 - 0.85	1.46 ± 0.10	1.74 ± 0.17	1.19 ± 0.18
Oxygen Uptake ($ml/min \cdot m^2$)	28 - 40	66 ± 4	66 ± 6	73 ± 3
Glucose Output ($mg/min \cdot m^2$)	60 - 73	104 ± 7	150 ± 10	65 ± 11
Lactate Uptake ($mg/min \cdot m^2$)	12 - 15	43 ± 10	39 ± 10	24 ± 10
Pyruvate Uptake ($mg/min \cdot m^2$)	0.5 - 1.0	2.3 ± 0.7	1.6 ± 0.6	1.0 ± 0.4

Thermal injury alone resulted in marked increases in EHBF, hepatic oxygen uptake and glucogenesis. The added insult of bacteremia significantly increased hepatic glucose output; as clinical sepsis progressed, glucose output decreased sharply - $B > N > CB$, $p < 0.05$. These changes in hepatic glucose output in bacteremic patients occurred without significant differences in EHBF, oxygen utilization or lactate and pyruvate uptakes.

THE STUDY OF METABOLISM AND NUTRITIONAL EFFECTS OF BURN INJURY IN SOLDIERS-- STUDIES OF HEPATIC BLOOD FLOW AND SUBSTRATE TURNOVER FOLLOWING THERMAL INJURY

INTRODUCTION

Following successful resuscitation from burn shock, cardiac output rises above normal. The extent of this increase in total body blood flow is generally related to the extent of injury and may reach levels twice normal in the more severely injured patients (1). Peripheral blood flow studies (1-3) have shown that flow to uninjured skin and resting skeletal muscle is essentially normal, while as much as 60 percent of the extra cardiac output is directed to the surface wound. Distribution of the remaining 40 percent (approximately 3 to 4 liters/minute) remains unknown. In this study, hepatic blood flow will be determined in burn patients to ascertain if flow to this visceral organ is increased following thermal injury, varies with the development of bacteremia and significant clinical sepsis and has measurable impact on hepatic glucose production.

Subjects. Twenty-two burn patients were studied (mean burn size 57 percent total body surface, range 18 to 83.5). They were divided into three groups: (1) nonbacteremic (2) bacteremic and (3) complicated bacteremic (Table 1). Nonbacteremic patients never had a positive blood stream culture or demonstrated any clinical evidence of sepsis prior to and including the day of study. The bacteremic group included patients who had more than one positive blood culture and were bacteremic on the day of study. These patients, however, presented no other clinical signs or symptoms which would distinguish them from the nonbacteremic group. Complicated bacteremic patients not only had repeated positive blood cultures but also were obviously septic and presented evidence of multiple organ system dysfunction (i.e. ileus, confusion, obtundation, pulmonary complications requiring ventilatory support). There was no significant difference in age, body size, extent of injury or time post burn between nonbacteremic and bacteremic groups.

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1. Wilmore DW, Aulick LH, Mason AD, Jr., and Pruitt BA, Jr: Influence of the burn wound on local and systemic responses to injury. Ann Surg 186:444-458, 1977.

2. Aulick LH, Wilmore DW, Mason AD, Jr, and Pruitt BA, Jr: Influence of the burn wound on peripheral circulation in thermally injured patients. Am J Physiol 233(4).H520-H526, 1977.

3. Aulick LH, Wilmore DW, Mason AD, Jr, and Pruitt BA, Jr: Muscle blood flow following thermal injury. Ann Surg 188:778-782, 1978.

TABLE 1. PHYSICAL CHARACTERISTICS OF PATIENTS*

	Nonbacteremic	Bacteremic	Complicated Bacteremic
Number of Patients	10	8	4
Number of Studies	11	8	5
Age (yrs)	28 (17-38)	26 (18-39)	31 (25-40)
Body Weight (kg)	75.0 (52-95.9)	67.2 (50.5-80.1)	83.9 (56-100)
Body Surface Area (m^2)	1.90 (1.56-2.19)	1.81 (1.60-1.87)	2.03 (1.90-2.25)
Percent Total Body Surface Burn	49.5 (18-83.5)	62.0 (47-72)	63.0 (56-74)
Post Burn Day Studied	10 (7-14)	13 (6-20)	15 (3-29)

*Mean (range)

Study Design. All patients were studied in the early morning after a six-hour fast. They were taken to the Radiology Department where a 7 French Cournand catheter was advanced under fluoroscopic control through the femoral vein and inferior vena cava to deep within the right hepatic vein. The subject was then moved into an environmental chamber (ambient temperature 30°C., relative humidity 50%) and placed supine in bed. An arterial catheter was inserted into an available peripheral artery and an intravenous cannula inserted if one was not already in place as required for clinical care. After at least 30 to 60 minutes of undisturbed rest in this warm, quiet room, arterial (A) and hepatic venous (HV blood samples were drawn and subsequently analyzed for oxygen, glucose, lactate and pyruvate content as previously described (1). A bolus injection of indocyanine green dye (0.5mg/kg) was then given intravenously and serial timed arterial and hepatic venous blood samples drawn over the next 10 to 12 minutes for the calculation of estimated hepatic

blood flow (4). Cardiac output and total body oxygen consumption were then measured as previously described (1, 5).

The three groups were compared by the Scheffe technique for multiple group comparison. This rigorous statistical technique for inter-group comparison was utilized to identify clear differences among groups. The disadvantage of this approach is that some actual between-group differences may not be detected by this statistical analysis.

RESULTS

In general, all patients were febrile, hypermetabolic and maintained a hyperdynamic circulation (Table 2). In these patients, bacteremia and advancing sepsis had no major impact on core temperature, metabolic rate or total body blood flow.

TABLE 2.

EFFECTS OF THERMAL INJURY AND BACTEREMIA ON BODY TEMPERATURE, OXYGEN CONSUMPTION AND BLOOD FLOW
(GROUP MEAN $\pm$ S.E.)

	Nonbacteremic	Bacteremic	Complicated Bacteremic
Rectal Temperature (^{o}C.)	38.4 $\pm$ 0.2	38.6 $\pm$ 0.2	38.0 $\pm$ 0.5
Total Body Oxygen Consumption ($ml/min \cdot m^2$)	215 $\pm$ 10	238 $\pm$ 8	244 $\pm$ 12
Cardiac Index ($l/min \cdot m^2$)	7.765 $\pm$ 0.393	8.793 $\pm$ 0.407	7.668 $\pm$ 0.717

Estimated hepatic blood flow (EHBF) was above the normal range in all burn patients (Table 3).

4. Rowell LB: Measurement of hepatic-splanchnic blood flow in man by dye techniques. In: Dye Curves: The Theory and Practice of Indicator Dilution. DA Bloomfield (Ed). Baltimore: University Park Press, 1974, pp 209-229.

5. Aulick LH, Hander EH, Wilmore DW, Mason AD, Jr, and Pruitt BA, Jr: The relative significance of thermal and metabolic demands on burn hypermetabolism. J Trauma 19: 559-566, 1979.

TABLE 3.

EFFECTS OF THERMAL INJURY AND BACTEREMIA ON ESTIMATED HEPATIC BLOOD FLOW (MEAN ± S.E.)

	Normal Range*	Nonbacteremic	Bacteremic	Complicated Bacteremic
Estimated Hepatic Blood Flow ($l/min \cdot m^2$)	0.63 - 0.85	1.463 ± 0.096	1.740 ± 0.172	1.194 ± 0.176
Percent of Cardiac Index	20 - 25	19	20	16

*From literature (6, 7)

6. Rowell LB: The splanchnic circulation. In: Physiology and Biophysics: Circulation, Respiration and Fluid Balance. TC Ruch and HD Patton (Eds). Philadelphia: W.B. Saunders Co, 1974. pp 215-233.

7. Bradley, SE: The hepatic circulation. In: Handbook of Physiology, Section 2: Circulation, Volume II. WF Hamilton and P Dow (Eds). Baltimore: Williams and Wilkins Co, 1963. pp 1387-1438.

TABLE 4.

EFFECTS OF THERMAL INJURY AND BACTEREMIA ON HEPATIC AEROBIC METABOLISM (MEAN ± S.E.)

	Normal Range*	Nonbacteremic	Bacteremic	Complicated Bacteremic
Arterial Oxygen Content (ml/dl)	19.5 - 21	13.7 ± 0.8	13.8 ± 0.6	14.0 ± 0.8
A-V Oxygen Difference (ml/dl)	4 - 5	4.6 ± 0.4	4.05 ± 0.5	6.7 ± 1.0
Hepatic Oxygen Consumption ($ml/min \cdot m^2$)	28 - 40	65.7 ± 3.9	65.9 ± 5.3	73.4 ± 3
Percent Total Body Oxygen Consumption	24 - 27	31 ± 2	28 ± 2	30 ± 1

*From literature (8, 9, 22)

8. Ahlborg G, Felig P, Hagenfeldt L, Handler R, and Wahren J: Substrate turnover during prolonged exercise in man. J Clin Invest 53: 1080-1090, 1974.

9. Kibler RF, Taylor WJ, and Myers JD: The effects of glucagon on net splanchnic balances of glucose, amino and nitrogen, urea, ketones, and oxygen in man. J Clin Invest 43: 904-915, 1964.

22. Myers JD, Brannon ES, and Holland, BC: A correlative study of the cardiac output and the hepatic circulation in hyperthyroidism. J Clin Invest 29: 1069-1077, 1950.

Associated with the increase in EHBF was a rise in hepatic oxygen consumption ($\dot{V}_{O_2}$) (Table 4). This rise in hepatic $\dot{V}_{O_2}$ was not significantly different in the three patient groups. Arterial-hepatic venous oxygen concentration differences were within the normal range in the two stable patient groups. In general, hepatic $\dot{V}_{O_2}$ represented a greater portion of the total body oxygen consumption following thermal injury.

Hepatic glucose production rose above normal with injury, became even more elevated with the onset of bacteremia and then fell markedly with clinical deterioration - bacteremic > nonbacteremic > complicated bacteremic, $p < 0.05$, (Table 5). The rise in liver glucose release was clearly evident, as A-HV glucose difference remained within the normal range despite a marked increase in EHBF after injury. The further increase in hepatic glucogenesis with bacteremia occurred without a significant change in liver perfusion. The drop in hepatic glucose production with progressive clinical sepsis occurred as the combined result of nonsignificant decreases in EHBF and A-HV glucose differences.

These changes in liver glucose metabolism following thermal injury were associated with increases in hepatic lactate and pyruvate uptakes (Table 6). The rise in hepatic lactate and pyruvate uptakes were comparable in all three groups of burn patients. Likewise, arterial content and A-HV concentration differences for both lactate and pyruvate were essentially the same in all patient categories.

DISCUSSION

Hepatic Blood Flow. The results of this study clearly demonstrate that estimated hepatic blood flow is approximately twice normal in resting, nonseptic burn patients. Estimated hepatic blood flow represented 19 percent of the total body circulation, and this increase in liver perfusion accounted for 15 to 20 percent of the increase in cardiac output after injury. Since approximately 60 percent of the extra total body circulation is directed to the wound (1, 2), the addition of the increased splanchnic flow will now account for as much as 80 percent of the total increase in the cardiac output of

TABLE 5.

EFFECTS OF THERMAL INJURY AND BACTEREMIA ON HEPATIC GLUCOSE PRODUCTION (MEAN $\pm$ S.E.)

	Normal Range*	Nonbacteremic	Bacteremic	Complicated Bacteremic
Arterial Glucose Content (mg/dl)	80 - 100	99 $\pm$ 6.5	128.0 $\pm$ 22.6	113.4 $\pm$ 10.5
A-HV Glucose Difference (mg/dl)	-8 to -10	-7.2 $\pm$ 0.4	-9.1 $\pm$ 0.9	-6.4 $\pm$ 1.6
Hepatic Glucose Production (mg/min $\cdot$ m^2)	60 - 73	103.9 $\pm$ 7.2	150.5 $\pm$ 9.7	65.2 $\pm$ 10.8

*From literature in postabsorptive man (8, 10-12)

10. Felig P and Wahren J: Influence of endogenous insulin secretion on splanchnic glucose and amino acid metabolism in man. J Clin Invest 50: 1702-1711, 1971.

11. Wahren J, Felig P, Cerasi E, and Luft R: Splanchnic and peripheral glucose and amino acid metabolism in diabetes mellitus. J Clin Invest 51: 1870-1878.

12. Meyers JD: Net Splanchnic glucose production in normal man and in various disease states. J Clin Invest 29: 1421-1429, 1950.

TABLE 6.

HEPATIC LACTATE AND PYRUVATE UPTAKES FOLLOWING THERMAL INJURY AND BACTEREMIA (MEAN ± S.E.)

	Normal Range*	Nonbacteremic	Bacteremic	Complicated Bacteremic
Arterial Lactate Content (mg/dl)	5 - 20	10.4 ± 1.0	13.0 ± 2.3	13.8 ± 3.5
A-HV Lactate Difference (mg/dl)	1.4 - 1.9	3.0 ± 0.9	2.5 ± 0.7	1.9 ± 0.6
Hepatic Lactate Uptake (mg/min · m^2)	12 - 15	42.9 ± 9.9	38.9 ± 9.6	21.4 ± 9.7
Arterial Pyruvate Content (mg/dl)	0.6 - 0.9	0.8 ± 0.05	0.9 ± 0.07	1.0 ± 0.15
A-HV Pyruvate Difference (mg/dl)	0.2 - 0.25	0.2 ± 0.06	0.1 ± 0.03	0.1 ± 0.04
Hepatic Pyruvate Uptake (mg/min · m^2)	0.05 - 1.0	2.3 ± 0.7	1.6 ± 0.6	1.0 ± 0.4

*Postabsorptive man - from literature (8, 10-12)

these injured nonbacteremic patients. Gump et al (13) found a slightly more limited increase in EHBF of three older burn patients; but, like the current study, they reported that the rise in hepatic perfusion was less than the increase in total body circulation.

A similar visceral-to-peripheral shift in total body flow also occurs in normal uninjured subjects exposed to exercise and/or heat stress. But, the combined increases in visceral and peripheral perfusion are unique to the burn patient, since in these other forms of stress (where cardiac output and peripheral flows reach levels comparable to those in the burn patient) liver and renal flows are markedly reduced (14, 15).

Since hepatic and mesenteric vessels have no cholinergic, sympathetic, vasodilator innervation, the increase in splanchnic blood flow following thermal injury may occur only through a reduction in sympathetic vasoconstrictor tone. As burn injury is associated with an increase in sympathoadrenal activity (16), the increase in EHBF is most likely the result of metabolic or other circulating vasodilators which override neural vasoconstrictor drives rather than any decrease in neuronal activity. Such "autoregulatory escape" develops rapidly in animals to restore splanchnic perfusion during neurogenic vasoconstrictor stimulation (6). Man , however can sustain reflex visceral vasoconstriction for longer periods of time (14).

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13. Gump FE, Price JB, Jr, and Kinney JM: Blood flow and oxygen consumption in patients with severe burns. Surg Gynecol Obstet 130:23-28, 1970.

14. Rowell LB: Human cardiovascular adjustments to exercise and thermal stress. Physiol Rev 54:75-159, 1974.

15. Radigan LR and Robinson S: Effects of environmental heat stress and exercise on renal blood flow and filtration rate. J Appl Physiol 2:185-191, 1949.

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

The increase in liver metabolism during the "flow phase" of injury is well recognized (17, 18) and may provide a basis for the observed rise in EHBF. In both groups of stable burn patients, arterial-hepatic venous oxygen difference was normal, suggesting that the increase in local blood flow was appropriate for the associated rise in splanchnic metabolism. A direct cause-and-effect relationship cannot be assumed, however, since numerous studies have demonstrated that variations in hepatic aerobic metabolism have little direct effect on local perfusion (19). This is particularly true under conditions of increased sympathetic outflow where splanchnic perfusion falls despite an increase in hepatic metabolism (20). This disparity between hepatic flow and metabolism has also been described in various disease states. For example, splanchnic oxygen consumption increases in the anemic patient despite a drop in liver perfusion (21). Likewise, hyperthyroidism and leukemia (without anemia) are two conditions which increase hepatic metabolism without affecting flow (21, 22). Therefore, while splanchnic flow and metabolism are both increased in the burn patient, the rise in EHBF cannot be considered solely the result of metabolic autoregulation. The observed increase in hepatic glucose production probably also has no direct impact on local blood flow, since intravenous infusions of glucose, amino acids, insulin and glucagon have no effect on splanchnic perfusion in man (19).

If local metabolic events do not necessarily determine hepatic blood flow, there are two potential circulating vasodilators which are found in these patients and may contribute to the observed increase in EHBF. The first is epinephrine which will significantly increase splanchnic circulation when given intravenously at physiological levels (19). The other is pyrogen which causes liver

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17. Wilmore DW: Carbohydrate metabolism in trauma. In: Clinics in Endocrinology and metabolism. KGMM Alberti (Ed). London: WB Saunders, 1976, pp 731-745.

18. Wilmore DW, Aulick LH, McDougal WS, Heimberger S, Mason AD, Jr, and Pruitt BA, Jr: Glucose flow in injured and septic man. In: Metabolic Response to Stress. S Allison (Ed). London: Academic Press. (In press)

19. Greenway CV and Stark RD: Hepatic vascular bed. Physiol Rev 51:23-65, 1971.

20. Rowell LB, Brengelmann GL, Blackmon JR, Twiss RD, and Kusumi F: Splanchnic blood flow and metabolism in heat-stressed man. J Appl Physiol 24:475-484, 1968.

21. Myers JD: The circulation in the splanchnic area. Transactions of the Fourth Conference on Shock and Circulatory Homeostasis. Josiah Macy, Jr, Foundation, New York, 1955, pp 121-165.

hyperemia at even subfebrile doses (23, 24). Endogenous pyrogens have been identified in nonseptic burn patients (25) which makes their vasodilator potential particularly interesting, since this is one obvious difference between the febrile injured patient and the hyperthermic exercise or heat stressed normal. As such, it may explain the maintenance of high hepatic blood flow in the burn patient when the normal subject reflexly vasoconstricts the viscera to sustain increased peripheral flows.

The physiologic and metabolic impact of bacteremia was difficult to distinguish in these burn patients, since there was no significant change in core temperature, oxygen consumption or cardiac index when burn patients became bacteremic. Likewise, the average EHBF of the bacteremic patients was not significantly different from that of the noninfected group. Since sepsis in other patients has been shown to increase liver perfusion (26), the failure to do so in the burn patient suggests that either (1) injury alone has so dilated the splanchnic bed that the additional vasodilator effects of sepsis are masked, or (2) other systemic vasomotor drives preclude further visceral vasodilatation. The later appears more reasonable, since higher flows have been reported following splanchnic sympathectomy or secretin infusion (27, 28). It is important to realize, however, that while septic and injury vasomotor influences may be different, their combined effects on splanchnic perfusion are not additive in the burn patient.

23. Bradley SE and Cowan NJ: Estimated hepatic blood flow and bromosulphalein extraction in normal man during the pyrogenic reaction. J Clin Invest 26:1175, 1947.

24. Hamrick LW and Myers JD: The effects of subfebrile doses of bacterial pyrogens on splanchnic metabolism and cardiac output. J Lab and Clin Med 45:568-572, 1955.

25. Wilmore DW, Mason AD, Jr, Aulick LH, and Pruitt BA, Jr: Studies on the effect of variations of temperature and humidity on energy demands of the burned soldier in a controlled metabolic room. In: Annual Research Progress Report, US Army Institute of Surgical Research, Brooke Army Medical Center, Fort Sam Houston, Texas, 1 July 1975 - 30 September 1976, pp 252-261.

26. Gump FE, Price JB, Jr, Kinney JM: Whole body and splanchnic blood flow and oxygen consumption measurements in patients with intraperitoneal infection. Ann Surg 171:321-328, 1970.

27. Wilkins RW, Culbertson JW, and Ingelfinger FJ: The effect of splanchnic sympathectomy in hypertensive patients upon estimated hepatic blood flow in the upright as contrasted with the horizontal position. J Clin Invest 30:312-317, 1951.

28. Hultman E: Blood circulation in the liver under physiological and pathological conditions. Scand J Clin Lab Invest 18 (Suppl 92):27-41, 1966.

Hepatic Metabolism. Hepatic oxygen consumption was twice normal in all three groups of burn patients. This is consistent with earlier studies in injured man and is considered to reflect an increase in fat oxidation (29). As previously mentioned, the increase in aerobic metabolism matched the increase in hepatic blood flow in the two stable patient groups; with the general but nonsignificant fall in EHBF in the clinically septic group, the accelerated hepatic oxygen consumption was maintained through an increase in O_2 extraction. Generally, hepatic oxygen consumption represented an above normal portion of total body oxygen uptake, a finding which emphasizes the central role of the liver in the hypermetabolic response to injury and infection. The relative increase in splanchnic heat production associated with the visceral-to-peripheral shift in total body blood flow described earlier will increase visceral heat storage. Such an hepatic heat load contributes to the rapid rise in intravenous temperature as blood passes through this region (30).

As previous indirect studies would predict (17), splanchnic glucose production was increased in these injured, nonbacteremic, burn patients. Stable bacteremic patients, however, maintained even higher rates of hepatic glucose output. This rise in glucose flow with bacteremia was not apparently the result of increased glucogenic substrate delivery, since EHBF and blood levels of lactate and pyruvate were essentially the same in these two groups of burn patients. Likewise, hepatic lactate and pyruvate extraction fractions (arterial-hepatic venous concentration/arterial concentration) and uptake rates were equal in both groups suggesting that intrahepatic processing of these glucogenic precursors was also unaffected by the development of bacteremia. Increased glycogenolysis and/or uptake and conversion of gluconeogenic precursors (i.e. amino acids and glycerol) are the only other possible explanations for the observed rise in hepatic glucose release when burn patients become bacteremic. Long and associates (31) have shown that hepatic conversion of alanine to glucose is markedly increased in infected patients, and previous work at this institute has demonstrated that

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29. Wilmore DW and Aulick LH: Metabolic changes in burned patients. In: Surgical Clinics of North America. JA Boswick, Jr, (Ed). Philadelphia: WB Saunders Co, 1978, pp 1173-1187.

30. Wilmore DW, Aulick LH, and Pruitt BA, Jr: Metabolism during the hypermetabolic phase of thermal injury. In: Advances in Surgery. C Rob (Ed). Chicago: Year Book Medical Publishers, 1978, pp 193-225.

31. Long CL, Spencer JL, Kinney JM, and Geiger JW: Carbohydrate metabolism in men: Effect of elective operations and major injury. J Appl Physiol 31: 110-116, 1971.

alanine flux from skeletal muscle is elevated in noninfected burn patients (32). Whether peripheral release and hepatic uptake of this and other gluconeogenic precursors are elevated further with the advent of bacteremia remains to be evaluated.

Progressive sepsis and clinical deterioration resulted in a marked decline in hepatic glucose production. This drop in hepatic glucose output was not related to substrate deficiency (lactate and pyruvate arterial blood levels remained elevated) as demonstrated earlier in the septic dog model (33) and other bacteremic patients (34). Microorganisms, on the other hand, have a direct "toxic" effect on the hepatocyte, which by altering enzyme function and mitochondrial energy production impair liver glucose production (35-39). Hepatic oxygen consumption remained elevated in these patients which taken together with the decline in glucose production support the concept of a reduction in efficiency of the hepatocyte.

32. Aulick LH and Wilmore DW: Increased peripheral amino acid release following burn injury. Surgery 85: 560-565, 1979.

33. Groves AC, Wolf LI, O'Regan PJ, Beach C, Hasinoff C, and Sutherland WH: Impaired gluconeogenesis in dogs with E. Coli bacteremia. Surgery 76: 533-541, 1974.

34. Wilmore DW, Mason AD, Jr, and Pruitt BA, Jr: Impaired glucose flow in burn patients with gram-negative sepsis. Surg Gynecol Obstet 143: 720-724, 1976.

35. LaNoue KF, Mason AD, Jr, and Daniels JP: The impairment of gluconeogenesis by gram-negative infection. Metabolism 17: 606-611, 1968.

36. McCallum RE and Berry LJ: Effects of endotoxin on gluconeogenesis, glycogen synthesis and liver glycogen synthase in mice. Infect and Immun 7: 642-654, 1973.

37. Mela L, Bacalzo LV, and Miller LD: Defective oxidative metabolism of rat liver mitochondria in hemorrhagic and endotoxic shock. Am J Physiol 220: 571-577, 1971.

38. Shumer W, Das Gupta TK, Moss GS, and Nyhus LM: Effect of endotoxemia on liver cell mitochondria in man. Ann Surg 171: 875-882, 1970.

39. McDougal WS, Heimburger S, Wilmore DW, and Pruitt BA, Jr. The effect of exogenous substrate on hepatic metabolism and membrane transport during endotoxemia. Surgery 84: 55-61, 1978.

Despite this drop in hepatic glucose production, these patients remained hyperglycemic. This suggests that, without a major reduction in the glucose space, peripheral glucose uptake was also limited in this patient group. Such alterations in peripheral glucose metabolism has also been previously documented in bacteremic burn patients (31).

SUMMARY AND CONCLUSIONS

The results of this study have clearly demonstrated that hepatic blood flow is significantly increased following thermal injury and thereby contributes to the well-documented hyperdynamic circulation. The development of positive blood stream cultures has little or no impact on hepatic perfusion but is associated with an increase in hepatic glucose production. With advancing clinical sepsis, these burn patients remained febrile, hypermetabolic and hyperdynamic, but hepatic glucose metabolism was markedly depressed.

ANNUAL PROGRESS REPORT

PROJECT NO. 3S161102BS05-00, BASIC RESEARCH

REPORT TITLE: THE STUDY OF METABOLISM AND NUTRITIONAL EFFECTS ON BURN INJURY IN SOLDIERS--THE RELATIVE SIGNIFICANCE OF THERMAL AND METABOLIC DEMANDS ON BURN HYPERMETABOLISM

US ARMY INSTITUTE OF SURGICAL RESEARCH
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1 October 1978 - 30 September 1979

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Reports Control Symbol MEDDH-288(R1)

Unclassified

ABSTRACT

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Reports Control Symbol MEDDH-288(R)

Swedish investigators have claimed that the hypermetabolic response to burn injury can be eliminated by making the patient warm and comfortable (Danielsson, W. et al. Burns 2:107, 1976). To test this thesis, resting metabolic rates of 34 postabsorptive, noninfected burn patients (mean burn size 46.5% total body surface range 9.5 - 86%) and 10 control subjects were determined by indirect calorimetry utilizing a canopy hood system. In a 30° C. environment (40-50% relative humidity), metabolic rates of 20 sleeping patients increased in a curvilinear fashion with burn size (metabolic rate in $kcal/m^2 \cdot min = 36.8 + 0.8731\%$ burn size - 0.0055% burn size^2, $r^2 = 0.73$). This relationship between metabolic response and extent of injury is comparable to one established earlier from Douglas bag data (Wilmore, D.W. et al. Ann Surg 180:653, 1974). These results indicate that the principle determinant of injury hypermetabolism is burn size rather than technical differences in measurement methods. In seven patients, metabolic rate was continuously monitored as the room was heated. While rectal temperatures rose from 38.6 ± 0.3 to 39.4 ± 0.2° C. (mean ± S.E.M., $p < 0.001$) resting metabolic rate remained essentially unchanged (76.5 ± 3.7 $kcal/m^2 \cdot h$ before and 73.9 ± 3.5 after heating). When five normals and seven patients were allowed to adjust room temperature and select a comfortable thermal environment, patients chose an

ambient temperature of 31.5 $\pm$ 0.7^{o} C. compared to 28.6 $\pm$ 0.7 selected by controls. In this "preferred" comfortable environment, sleeping burned patients remained hypermetabolic (63.9 $\pm$ 5.7 kcal/m^{2} $\cdot$ h). It is, therefore, obvious that the hypermetabolic response to burn injury cannot be eliminated by making patients warm and comfortable. The Q_{10} effect of hyperpyrexia (an increase in BMR of about 13% per degree centigrade rise in rectal temperature above normal) accounts for only 20-30% of the hypermetabolism observed in these patients. Therefore, while burn hypermetabolism is temperature sensitive, it is not the primary result of altered thermoregulatory drives. A more likely explanation is related to the energy cost of wound healing and tissue repair.

This study has been completed, and a paper under the same title has recently been published in the Journal of Trauma, 19: 559-566, 1979.

ANNUAL PROGRESS REPORT

PROJECT NO. 3S161102BS05-00, BASIC RESEARCH

REPORT TITLE: THE STUDY OF METABOLISM AND NUTRITIONAL EFFECTS ON BURN INJURY IN SOLDIERS--A LARGE ANIMAL MODEL OF POST INJURY HYPERMETABOLISM

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Reports Control Symbol MEDDH-288(R1)

Unclassified

ABSTRACT

PROJECT NO.: 3S161102BS05-00, BASIC RESEARCH

REPORT TITLE: THE STUDY OF METABOLISM AND NUTRITIONAL EFFECTS ON BURN INJURY IN SOLDIERS--A LARGE ANIMAL MODEL OF POST INJURY HYPERMETABOLISM

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Reports Control Symbol MEDDH-288(R1)

Many basic components of injury hypermetabolism elude clinical investigators due to the constraints of human research. To evaluate a large animal model, resting oxygen consumption ($\dot{V}_{O_2}$) was measured by closed-circuit spirometry in seven young tracheostomized goats (20-40 kg) before and one week after receiving a twenty percent total body surface injury (excision to fascia of the skin from the back and both sides). The wound was treated closed and food and water were given ad libitum. Animals were studied while standing quietly in a room maintained at 25-28°C. Resting $\dot{V}_{O_2}$ increased from 155 ± 19 to 195 ± 26 ml/min (mean ± S.E.M.) following injury ($p<0.05$, paired t-test). Average body weight declined from 29.7 ± 2.4 to 26.5 ± 2.3 kg ($p< 0.05$). Rectal temperature measured in four animals, rose from 39.5 ± 0.6 to 40.2 ± 0.2°C following injury ($p< 0.05$). Two animals became bacteremic and were not included in data analysis. $\dot{V}_{O_2}$, rectal temperature and body weight remained unchanged in two uninjured control animals studied in the same manner. The rise in aerobic metabolism following injury was significant since simple weight loss alone is associated with a decrease in resting metabolic rate in both man and the ruminant. These results indicate that the injured model goat may be an acceptable approach to further study of injury hypermetabolism.

Hypermetabolism
Animal model

A LARGE ANIMAL MODEL OF POST INJURY HYPERMETABOLISM

The hypermetabolic response to major injury was first described in patients with long bone fractures in 1930 (1). Since that time numerous investigators have confirmed and extended these initial observations and found that the increase in metabolic rate is (a) common to a wide variety of surgical patients; (b) related to the extent of injury, and (c) greatest in patients with severe burns (2,3).

Many basic components of post injury hypermetabolism continue to elude clinical investigators, however, due to the constraints of human research. Attempts to develop small animal models of the hypermetabolic burn patient have been hampered both by the animals' size and the relatively limited metabolic response to injury (4-6). Sheep (7) and dogs (8) have been utilized as large animal models of acute cardiovascular adjustments to burn injury, but no such models are available for metabolic studies. The purpose of this study is to determine if surface injury will result in a reproducible and, therefore, predictable increase in the resting metabolic rate of the goat.

1. Cuthbertson DP: The disturbance of metabolism produced by bony and non-bony injury, with notes of certain abnormal conditions of bone. Biochem J 24:1244, 1930.

2. Moore FD: Metabolic care of the surgical patient. Philadelphia, W.B. Saunders, 1959.

3. Wilmore DW, Aulick LH, Pruitt BA Jr.: Metabolism during the hypermetabolic phase of therapy injury. In Advances in Surgery 12:193, 1978.

4. Caldwell FT Jr, Osterholm JL, Sower ND, et al. Metabolic response to thermal trauma of normal and thyroprivic rats at three environmental temperatures. Ann Surg 150:976, 1959.

5. Herndon DN, Wilmore DW, Mason AD Jr.: Development and analysis of a small animal model simulating the human postburn hypermetabolic response. J Surg Res 25:394, 1978.

6. Moyer CA.: The metabolism of burned mammals and its relationship to vaporizational heat loss and other parameters. In Research in Burns, ed by CP Artz, Philadelphia & Washington, F.A. Davis Co. and American Institute of Biological Sciences, 1952.

7. Traber DL, Bohs CT, Carvajal HF, et al: Early cardiopulmonary and renal function in thermally injured sheep. Surg Gynec Obstet 148:753, 1979.

8. Moncrief JA.: Effect of various fluid regimens and pharmacologic agents on the circulatory hemodynamics of the immediate postburn period. Ann Surg 164:723, 1966.

This animal was selected for study because of our previous experience with injured goats and the wealth of information available on normal ruminant metabolism and temperature regulation (9-11).

MATERIALS AND METHODS

Seven, young, male and female goats of mixed breeds (Spanish goats) weighing 20-40 kg were utilized as experimental animals. They were dewormed and housed individually in outdoor runs for at least two weeks prior to study. All studies took place in the vivarium of the ISR laboratory where room temperature was maintained between 25 and 28°C.

Animals were conditioned to stand quietly on a restraining cart. A meshed sling prevented the goat from stepping off the cart while the horns of the animal were secured to an overhead bar to limit head movement. To simulate test conditions, a spirometer was positioned next to the animal and respiratory tubing from the spirometer attached to the goat's neck. The conditioning program was conducted daily, Monday through Friday, until the animal stood quietly in the experimental setting. Approximately three weeks of such conditioning was required.

Once conditioned, the animal was anesthetized and a tracheostomy performed. On the next day, a cuffed tracheostomy tube was inserted and the animals oxygen consumption measured by closed circuit spirometry utilizing a standard, 9 liter Collins spirometer filled with 100 percent oxygen. The animal inhaled pure oxygen through a two-way Rudolph valve attached to the tracheostomy tube and the exhaled gas passed through a carbon dioxide trap of soda lime before reentering the spirometer. The rate of gas volume decrease within the spirometer was a measure of the animal's aerobic metabolism. Each such determination took approximately 20 minutes and was conducted twice daily for 2-3 days. The lowest recorded rate of oxygen consumption (expressed in milliliters O_2 per minute and corrected to standard conditions) was considered the best estimate of resting aerobic metabolism in an uninjured animal. At the end of each test session rectal temperature was taken with a standard Mercury thermometer and body weight determined.

9. Blaxter KL.: The energy metabolism of ruminants. London, Hutchinson Scientific and Technical, 1967.

10. Jessen C.: Interactions of air temperature and core temperatures in thermoregulation of the goat. J. Physiol 264:585, 1977.

11. Andersson B.: Central nervous and hormonal interation in temperature regulation of the goat. In Physiological and Behavioral Temperature Regulation, ed by JD Hardy, AP Gagge and JAJ Stolwizk, Springfield, CC. Thomas, 1970.

Following these control studies, the animal was placed under deep anesthesia (methoxyfluorane and 100 percent oxygen) and the skin from the back and sides excised to fascia over an area representing approximately 20 percent of the total body surface. The animal was allowed to recover, given an ample supply of food and water but no antibiotic therapy. The wound was covered in gauze dressings which were changed every other day. Nutritional support was provided, daily for all injured animals (Ensure-Ross Laboratories, 40 calories/kg).

For the next 7 days, the injured goat continued the conditioning program but oxygen consumption measurements were not performed. After a week, a second tracheostomy was performed and measurements repeated twice daily for the next two to three days. Once again, the lowest recorded level of oxygen consumption was considered the best estimate of resting aerobic metabolism for the injured animal. Venous blood cultures were performed on the last two days of study and the animal sacrificed. Two control, non-injured goats were studied in the same way. Hair was clipped from the back and sides of controls to simulate a wound. This area was covered in gauze dressings and the animal fed the supplemental diets.

RESULTS

The metabolic response to injury was evaluated in seven of nine animals which remained nonbacteremic after injury. In this group of nonseptic animals there was a highly variable but consistent increase in resting oxygen consumption following injury (Table 1). Resting oxygen consumption for the group increased by 26 percent (range 1-67 percent) following injury when expressed in absolute terms (ml/min); but, when normalized for body weight and expressed in ml/min•weight$^{0.73}$, oxygen consumption increased by 35 percent (range 9-77). Rectal temperatures, of four animals monitored, increased from 39.5 ± 0.6 to 40.2 ± 0.2^{o}C (mean ± S.E., $p < 0.05$ by paired-t test). Body temperature and resting oxygen consumption were essentially unchanged in two control animals which were not injured but otherwise treated in the same manner.

DISCUSSION

Surgical removal of the skin from twenty percent of the surface area of a young goat increased resting oxygen consumption by the end of one week post injury. This increase was highly variable but present in every animal despite an associated loss in body weight. Since a one day fast in sheep with its attendant weight loss results in a 20 percent decrease in resting heat production (9), the observed rise in oxygen consumption of the injured goats probably would have

been even greater had nutritional support been more vigorous and caloric intake matched expenditure.

Table 1. The effects of a twenty percent total body surface injury on body weight and resting oxygen consumption of young goats

GOAT	BODY WEIGHT (kg)		OXYGEN CONSUMPTION (ml/min)		(ml/mn·weight$^{0.73}$)	
	Before*	After	Before	After	Before	After
144	30.0	27.3	123	205	10.27	18.34
146	27.2	24.1	131	171	11.75	16.75
137	22.7	20.2	104	105	10.64	11.71
140	25.9	20.1	123	132	11.43	14.72
173	40.0	36.4	138	286	16.11	20.74
171	36.4	32.6	212	286	15.37	22.48
185	25.4	24.5	156	180	14.72	17.42
MEAN	29.7	26.5	155	195	12.90	17.45
± S.E.	2.4	2.3	19	26	0.91	1.36
**p<	0.05		0.05		0.01	

*Before and 7-10 days after injury
**paired t-test

These animals were generally more active before injury. Such differences in pre- and post injury restlessness would, like the associated caloric deficit, minimize the apparent metabolic effects of injury. Therefore, the observed 26-35% increase in oxygen consumption should be considered an underestimate of the actual change in aerobic metabolism following injury.

The increase in rectal temperature following injury is consistent with the rise in metabolic heat production and suggests that, like the injured patient, the injured animal is febrile. Whether this increase in body heat content is a reflection of an upward reset in the hypothalamic temperature controller or simply an imbalance

between heat production and loss remains to be determined. However, for the model to be considered representative of the burn patient, the febrile response must reflect an alteration in hypothalamic regulation of body temperature (12). Likewise, the model is only valid if the hypermetabolic response is temperature sensitive but not temperature dependent.

SUMMARY AND CONCLUSIONS

The results of this study suggest that the injured goat is an appropriate model for the metabolic adjustments to thermal injury. The observed increase in resting oxygen consumption was considered to be an underestimate of the hypermetabolic response due to the inability to achieve true resting levels of aerobic metabolism before injury as well as the failure to provide sufficient nutritional support after injury. Nevertheless, the animal's metabolic and body temperature responses to this excision type injury are similar to those following a thermal burn. A subsequent study is currently underway to evaluate the metabolic and thermoregulatory responses of the goat to a third degree burn covering the same 20% of the body surface.

12. Aulick LH, Hander EW, Wilmore DW, et al.: The relative significance of thermal and metabolic demands on burn hypermetabolism. J Trauma 19:559, 1979.

ANNUAL PROGRESS REPORT

PROJECT NO. 3S161102BS05-00, BASIC RESEARCH

PROJECT TITLE: THE STUDY OF METABOLISM AND NUTRITIONAL EFFECTS ON BURN INJURY IN SOLDIERS-SPLANCHNIC EXTRACTION OF MELATONIN IN BURN PATIENTS

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Reports Control Symbol MEDDH-288(R1)

Unclassified

ABSTRACT

PROJECT NO. 3S161102BS05-00, BASIC RESEARCH

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Reports Control Symbol MEDDH-288(R1)

Melatonin is being investigated primarily because of its probable role in controlling the hypothalamus. Hypothalamic abnormalities occur in burn patients. Circulating melatonin (MEL) has a very short half-life (3-10 min) in sheep. In humans, we have previously detected rapid rises and falls in MEL concentration, with peaks 8-12 minutes apart. An appreciable amount (30-40%) of human circulating MEL is not bound to plasma proteins. These findings suggest that some organ(s) extracts this hormone from blood, perhaps rapidly enough to allow detection of a concentration gradient. The liver has been implicated in MEL metabolism in the rat. Therefore, we obtained a set of blood samples from the femoral artery (FA), hepatic vein (HV), and renal vein (RV) on 13 occasions at 0730 hours, after an overnight fast, from nine patients with burn injury, mean age 28 years, mean burn size 56%. Just after obtaining sample sets for MEL radioimmunoassay, indocyanine green was injected to determine estimated hepatic plasma flow (EHPF). The data from each sample set were entered into analysis separately, though some patients had more than one sample set. Mean MEL (pg/ml ± SE) was 112 ± 18 in FA, 128 ± 26 in RV (not significant, paired t test), and 70 ± 11 in HV ($p < .001$ vs simultaneous FA values). Both FA-HV concentration gradient (0-94 pg/ml) and FA-HV splanchnic extraction (gradient x EHPF, 0-93 ng/min/M^2) were linearly correlated with FA MEL concentration ($p < .001$). Conclusions: It is possible to detect and quantitate net MEL extraction by the human splanchnic bed. Whether MEL is also secreted into portal or hepatic veins is unknown. If such a contribution is small, the linear relationship between FA-HV gradient and FA concentration is compatible with a hepatic or intestinal binding site capable of extracting about 1/3 of the presented total MEL.

PRESENTATIONS/PUBLICATIONS

Vaughan GM: Splanchnic extraction of melatonin in burn patients. Accepted for presentation at the VI International Congress of Endocrinology, 10-16 February 1980, Melbourne, Australia.

Burn
Melatonin
Splanchnic
Human

ANNUAL PROGRESS REPORT

PROJECT NO. 3S161102BS05-00, BASIC RESEARCH

REPORT TITLE: THE STUDY OF METABOLISM AND NUTRITIONAL EFFECTS OF BURN INJURY IN SOLDIERS -- PLASMA NOREPINEPHRINE, EPINEPHRINE, AND THYROID HORMONE INTERACTIONS IN SEVERELY BURNED PATIENTS

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Reports Control Symbol MEDDH-288(R1)

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ABSTRACT

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Period covered in this report: 1 October 1978 - 30 September 1979

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Reports Control Symbol MEDDH-288(R1)

A prospective study of thyroid catecholamine interactions was performed in 15 thermally injured patients. Two patient groups were studied. Nine patients, receiving 200 µg/day of triiodothyronine (T_3) constituted the T_3-treated group. Twenty-two studies were performed in these patients, who had a mean age of 28 years and a mean burn size of 65%. The mean postburn day of study was day 28. Eight additional patients constituted the untreated group. Seventeen studies were performed in these patients, who had a mean age of 31 years and a mean burn size of 57%. The mean postburn day of study was day 13. Mean serum concentrations of T_3 were significantly lower in the untreated group, 61 ng/dl, than in the T_3 treated group, 139 ng/dl, $p < .01$. Mean serum concentrations of thyroxine(T_4) were significantly higher in the untreated group, 4.5 mcg/dl, than in the T_3 treated group, 2.5 mcg/dl, $p < .01$. The mean plasma norepinephrine concentration in the untreated group, 818 pg/ml, was significantly greater than that of the T_3 treated group, 461 pg/ml, $p < .05$. In the untreated group, plasma norepinephrine was significantly correlated with serum T_3, $r = -.7$, $p < .01$. Similarly, in the untreated group, plasma concentrations of epinephrine were significantly correlated with serum concentrations of T_3, $r = -.56$, $p < .025$. Metabolic rate was followed longitudinally in a single additional patient who received T_3 treatment for chemical hypothyroidism accompanying a severe burn (total body surface burn 81%).

The patient's metabolic rate was not affected by either hypothyroidism or restoration of euthyroid status by T_3 treatment. These data suggest that a reciprocal relationship exists between plasma concentrations of both T_3 and norepinephrine and epinephrine in untreated burn patients and that treatment with the metabolically active hormone, T_3, does not alter the level of hypermetabolism accompanying thermal injury.

Norepinephrine
Epinephrine
Triiodothyronine
Thyroxine

PLASMA NOREPINEPHRINE, EPINEPHRINE, AND THYROID HORMONE INTERACTIONS IN SEVERELY BURNED PATIENTS

The increased metabolism which accompanies thermal injury in man correlates positively with the rate of urinary catecholamine excretion,[1] a manifestation of accentuated sympathetic nervous system activity. This hypermetabolic state contains many of the features associated with hyperthyroidism. Early studies, however, demonstrated normal ^{131}I uptake and normal serum concentrations of protein bound iodine in thermally injured patients.[2] Recent studies suggest that a reciprocal relationship between thyroid hormones and sympathetic nervous system activity may occur in states of hypo- and hyperthyroidism.[3] Recently our laboratory has documented a suppression of serum concentrations of 3,3'5-l-triiodothyronine (T_3, the metabolically active thyroid hormone) and an increase in serum concentrations of 3,3',5'-l-triiodothyronine (rT_3, a metabolically inactive thyroid hormone) in burn patients. Serum concentrations of free T_3 were also suppressed beneath the normal range in patients undergoing clinical deterioration. Serum concentrations of both total and free l-thyroxine (T_4, the precursor of T_3 and rT_3) were within the normal range.[4] Similar alterations in peripheral thyroid hormone concentrations have been observed following a variety of other critical illnesses.[5] The question of whether such alterations represent functional hypothyroidism is unanswered. However, tissue concentrations of T_3 have been measured at autopsy and found to be decreased in patients who died after prolonged illness, compared to victims of sudden accidents.[6] In summary, it is now recognized that a significant

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2. Cope O, Nardi GL, Quijano M, et al: Metabolic rate and thyroid function following acute thermal trauma in man. Ann Surg 137:165, 1953.

3. Landsberg L: Catecholamines and the sympathoadrenal system, in Ingbar SH (ed): The Year in Endocrinology, Plenum Medical Book Company, 1976, pp 177-231.

4. Becker RA, Wilmore DW, Goodwin CW, et al: Free T_4, free T_3, and reverse-T_3 in critically ill, thermally injured patients. J Trauma (in press).

5. Cavalieri RR, Rapaport B: Impaired peripheral conversion of thyroxine to triiodothyronine. Ann Rev Med 28:57, 1977.

6. Sullivan PRC, Bollinger JA, Reichlin S: Selective deficiency of tissue triiodothyronine: a proposed mechanism of elevated free thyroxine in the euthyroid sick. J Clin Invest 52:832, 1973.

proportion of patients with thermal injury, as well as other severe systemic illnesses, have low circulating levels of total and free T_3 and normal or high free-T_4 levels, and that in most, if not all cases, this reflects impaired peripheral conversion of T_4 to T_3. These biochemical alterations may reflect functional hypothyroidism.

Because of both the suggested reciprocal relationship between catecholamines and thyroid hormone and the reported heightened sensitivity to catecholamines observed in states of hyperthyroidism,[3] the present study was designed to assess thyroid hormone catecholamine relationships in a state of inherently high catecholamine secretion, the severely burned patient. Such information would be of special clinical interest because of the severe catabolic state which the burn patient suffers and which may be mediated by catecholamines, and because of the known effects of thyroid hormone on wound healing[7,8,9,10] and leukocyte metabolism.[11,12,13]

MATERIALS AND METHODS

Patients-T_3 Treated

Twenty-two studies were performed in nine patients with a mean age of 28 years, a mean burn size 65%, and mean postburn day of study, day 28. The patient group designated as T_3-treated, received 200 mcg of T_3 daily by mouth, in four divided doses, adjusted to maintain serum T_3 concentrations within the normal range. All patients in this group received T_3 for at least five days prior to the first day of study.

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7. Lauber HF: Münch Med Wschr 434, 1930.
8. Kosdoba AS: Arch Klin Chir 179:551, 1934.
9. Barclay THC, Cuthbertson DP, Isaacs A: The influence of metabolic stimulants on wound healing; the influence of thyroid and 2-4-A-dinitrophenol. Q J Exp Physiol 32:309, 1944.
10. Glicksman AS, Rawson RW, Nickson JJ: Modification of late radiation injury with L-triiodothyronine. Radiology 73:178, 1959.
11. Kurland GS, Krotkov MV, Freedberg AS: Oxygen consumption and thyroxine deiodination by human leukocytes. J Clin Endocrinol Metab 20:35, 1960.
12. Alexander WD, Bisset SK: The correlation of thyroid function with the rate of oxygen uptake of human leukocytes. Q J Exp Physiol 46(1):46, 1961.
13. Alexander WD, Bisset SK: The effect of intravenous injections of triiodothyroacetic acid and L-triiodothyronine on the oxygen consumption of circulating human leukocytes. Q J Exp Physiol 46(1): 50, 1961.

Patients-Untreated

Seventeen studies were performed in eight patients, mean age 31 years, mean burn size 57%, and mean postburn day of study, day 13.

Study Design

Blood specimens were obtained at approximately 0600 hours after the patient was in the supine position overnight. Specimens for plasma catecholamine assay were obtained within seconds after the patient was awakened and informed that blood was to be obtained. Specimens for catecholamine analysis were collected into chilled tubes containing EGTA and glutathione and were maintained at 4°C until plasma was separated by refrigerated centrifugation and stored at -60°C until assay. Blood for thyroid hormone assay was obtained at the same time.

Assays

Serum T_4 and T_3 were measured by radioimmunoassay using commercial kits obtained from Ortho Diagnostics. Free hormone concentrations were determined by equilibrium dialysis at the Nichols Institute, San Pedro, California. Plasma norepinephrine, epinephrine, and dopamine were measured by radioenzymatic assay using the catechol-o-methyltransferase technique described by Passon and Peuler.[14] Data obtained between groups were compared by "t" test analysis.

RESULTS

Figure 1

Plasma concentration of T_3 in the T_3-treated group (139 ± 10 ng/dl, mean ± S.E.) was higher ($p < .01$) than in the untreated group (61 ± 5 ng/dl). The lower value ($p < .01$) for T_4 in the T_3-treated group (2.6 ± 0.4 μg/dl) compared with that in the untreated group (4.6 ± 0.3 μg/dl) provides additional evidence for the suppressive effect of T_3 treatment on thyroid hormone homeostasis in these patients.

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14. Passon PG, Peuler JD: A simplified radiometric assay for plasma norepinephrine and epinephrine. Anal Biochem 51:618, 1973.

Figure 2

Plasma concentration of norepinephrine in the untreated group was 818 ± 151 pg/ml (mean ± S.E.), as compared with 460 ± 56 pg/ml in the T_3 treated group ($p < .01$). Plasma concentration of epinephrine in the untreated group was 184 ± 44 pg/ml (mean ± S.E.) as compared with 154 ± 33 pg/dl in the T_3 treated group. This difference was not significant. Plasma concentration of dopamine in the untreated group was (mean ± S.E.) 121 ± 38 pg/ml, as compared with 128 ± 40 pg/ml, in the T_3-treated group. This difference was not significant.

Figure 3

These data describe the linear regression analysis between plasma norepinephrine, pg/ml, and plasma T_3, ng/dl, for the untreated patient group, $NE = -21.5\ T_3 + 2143$, $r = -.7$, $p < .01$. When multiple regression analysis was performed on data from the untreated group with plasma norepinephrine (pg/ml) as the dependent variable, and T_3 (ng/dl), percent total body surface burn, and age in years as independent variables, the following equation was derived: $NE = -.203\ T_3 + .249$% total body surface burn + .23 age (years) -.105, $r^2 = .789$, $p < .001$ (graph not shown). These data are consistent with previous observations of both increasing plasma norepinephrine concentrations with increasing age[15] and a positive correlation between increased urinary catecholamine excretion and percent total body surface burn.[1]

Figure 4

These data describe the linear regression analysis for plasma epinephrine, pg/ml, and T_3, ng/dl, $EPI = -4.91\ T_3 + 489$, $r = -.562$, $p < .025$.

Figure 5

These data describe the response of metabolic rate and thyroid hormone concentration in a single additional patient, 18 years old, with a burn size of 81%, studied longitudinally over a 108 day hospital course. Between the fifth and the tenth postburn days, the patient experienced two episodes of sepsis and on the tenth postburn day, appeared to be undergoing a rapid clinical deterioration. On the tenth postburn day, free-T_4 was 1.3 ng/dl, normal range 1.3-3.8 ng/dl, and free-T_3 was 184 pg/dl, normal range 230-669 pg/dl. TSH was at the upper limit of the normal range, 5.2 μU/ml. Metabolic rate, determined by indirect calorimetry, was 75 kcal/hr · m^2, or about twice the

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15. Ziegler MG, Lake CR, Kopin IJ: Plasma noradrenalin increases with age. Nature 261:333, 1976.

predicted value. On the tenth postburn day, T_3 replacement therapy was instituted at 200 µg/day, and on the fourteenth postburn day, increased to 300 µg/day and continued at that level until the fiftieth postburn day when T_3 therapy was discontinued. In response to T_3 treatment, serum free-T_3 rose to 238 pg/dl on the fourteenth day, 510 pg/dl on the twenty-third day, 848 pg/dl on the thirty-seventh postburn day, and 1,000 pg/dl on the fiftieth postburn day. With cessation of T_3 replacement, serum free-T_3 decreased to 202 pg/dl by the fifty-seventh postburn day, and returned to the normal range where it remained until the patient was discharged. Free T_4 levels and TSH levels were appropriately suppressed by T_3 therapy. A TSH rebound effect was noted on the sixty-seventh postburn day, 6.0 µU/ml. Free T_4 levels rose in response to the TSH increase, 507 ng/dl on the ninety-first postburn day, and returned to the normal range prior to discharge. The patient's metabolic rate was unchanged during the period of thyroid manipulation, remaining approximately twice the predicted value until the eighty-second postburn day, when it decreased to 54 kcal/hr · m^2 and decreased further to 48 kcal/hr · m^2 by the ninety-first postburn day. Metabolic rate appears independent of the patient's status of hypo- or hyperthyroidism. As has been previously reported by our Institute, metabolic rate returned to the normal range only when the patient's wounds were closed and the healing process well advanced. Catecholamine measurements are not available in this patient.

DISCUSSION

Hypermetabolism accompanies thermal injury and has been ascribed to increased sympathetic activity.[1] Previous observations of catecholamines in patients with primary thyroidal diseases have suggested, indirectly, a reciprocal relationship between catecholamines and thyroid hormone.[3] We have previously demonstrated suppression of thyroid hormones following thermal injury in man. Significant suppression of serum concentrations of T_3 and elevation of serum concentrations of reverse-T_3 were observed in the clinically stable burn patient. However, with clinical deterioration, a significant decrease in the serum concentrations of free T_4 and free T_3 were observed.[4] Metabolic rate was followed longitudinally in a single patient who received T_3 treatment for chemical hypothyroidism accompanying a severe burn (total body surface burn 81%). The patient's metabolic rate was not affected by either hypothyroidism or restoration of euthyroid status by T_3 treatment. In summary, the hypermetabolic burn patient represents physiology altered both by an increase in catecholamine secretion and by altered peripheral thyroid metabolism, including a block in T_4 to T_3 conversion. We now report plasma concentrations of norepinephrine, epinephrine, and thyroid hormone in untreated burn patients and in burn patients receiving T_3 supplement to maintain T_3 concentrations within the normal range.

In untreated burn patients, both plasma norepinephrine and epinephrine are significantly correlated with serum concentrations of T_3. These data describe the first direct evidence of a significant correlation between plasma catecholamine and thyroid hormone concentrations in man. These findings are consistent with previous reports of a suggested reciprocal relationship between catecholamines and thyroid hormone in other states of thyroid dysfunction.

When multiple regression analysis was performed on data from the untreated group, with plasma norepinephrine as the dependent variable and T_3, percent total body surface burn, and age as independent variables, a greater statistical significance was found. This multiple regression analysis establishes that in addition to the reciprocal relationship between plasma norepinephrine and serum T_3, plasma norepinephrine is positively correlated with patient age and burn size. This relationship is consistent with the previously reported increase in plasma norepinephrine with age.[15] Further, our laboratory has previously reported a positive correlation between increased urinary catecholamine excretion and percent total body surface burn.[1]

In burn patients treated with T_3, the mean plasma concentration of norepinephrine was significantly lower than in the untreated patient groups. Although age and burn size were well matched for these patient groups, the mean postburn days of study were not, days 28 and 13 respectively. While both patient groups were hypermetabolic at the time of study, it is possible that catecholamines may decrease with time after injury. Further, plasma norepinephrine and epinephrine did not correlate with serum T_3 concentration in the T_3-treated patients. Given the reciprocal relationship between catecholamines and T_3 observed in untreated patients, the absence of a similar relationship in T_3-treated patients suggests that T_3 may not be the single controlling factor in catecholamine-T_3 interactions. Further study is indicated to determine what other factors may influence this relationship in T_3-treated burn patients.

PRESENTATIONS:

Becker RA: Suppression of norepinephrine by triiodothyronine (T_3) in thermally injured man. Presented at the Western Surgical Association Meeting, Colorado Springs, Colorado, November 13, 1979.

PUBLICATIONS:

Becker RA, Vaughan GM, Goodwin CW, Ziegler MG, Mason AD, Jr, Pruitt BA: Plasma norepinephrine, epinephrine, and thyroid hormone interactions in severely burned patients. Arch Surg (in press).

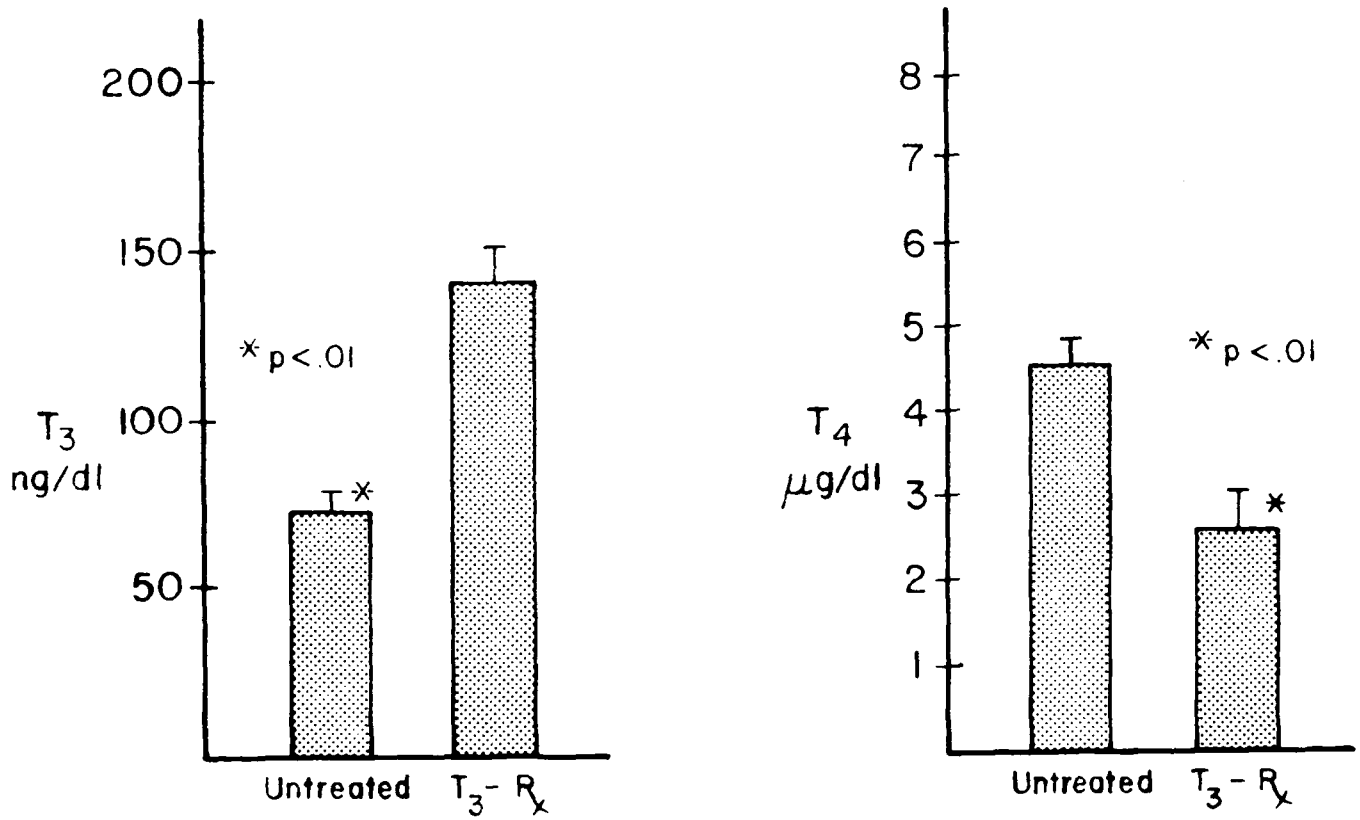

Figure 1 - The bar graphs represent the mean + S.E. for T_3 and T_4 in Untreated and T_3-treated patients. *p <.01.

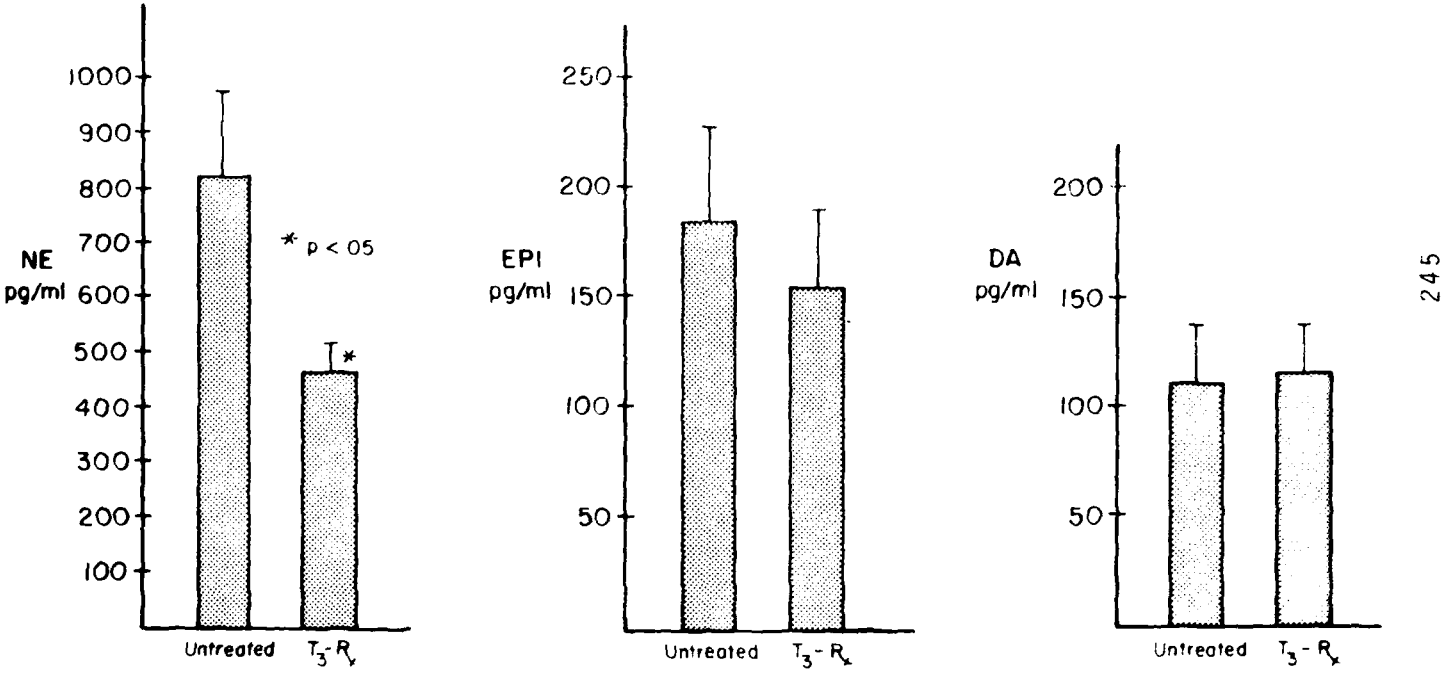

Figure 2 - The bar graphs represent the mean + S.E. for NE (norepinephrine), EPI (epinephrine), DA (dopamine), in pg/ml in Untreated and T_3-treated patients. *p <.05.

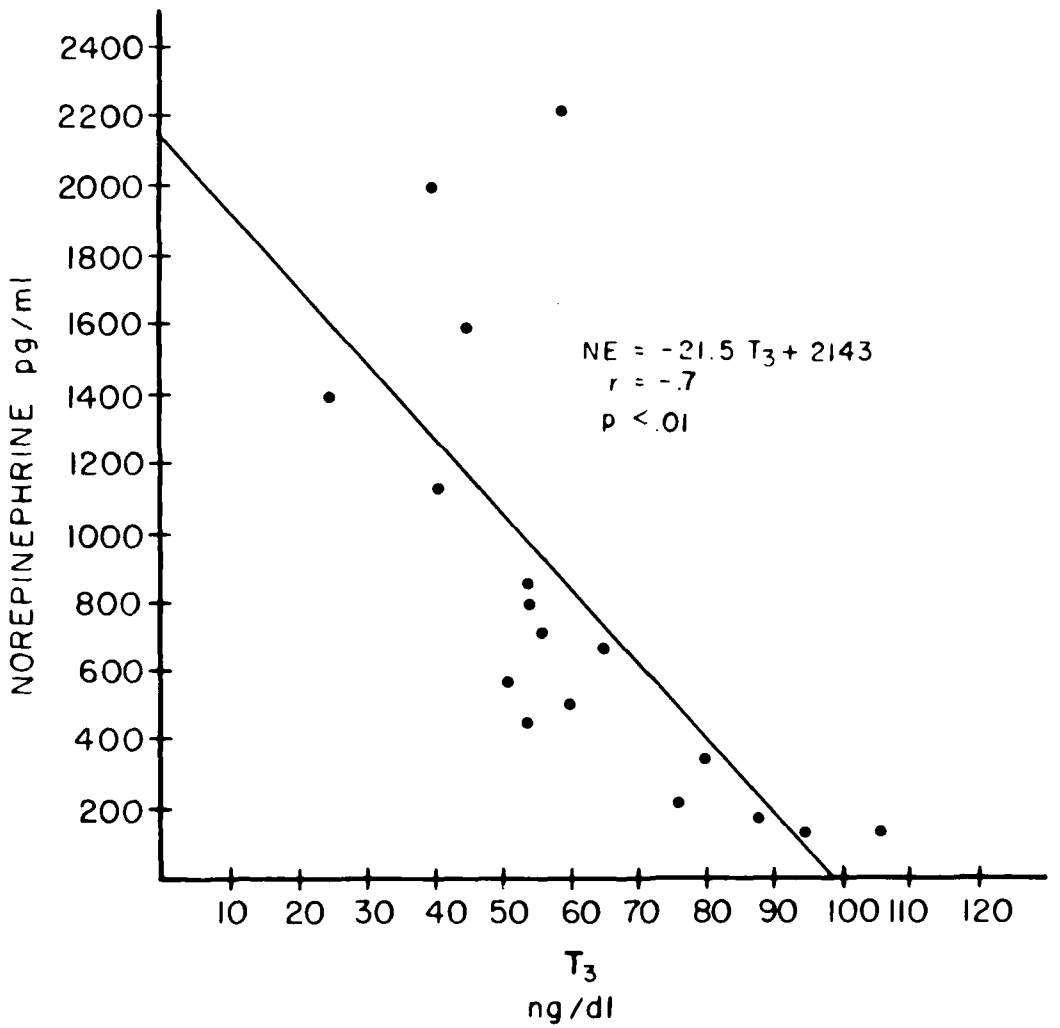

Figure 3 - Linear regression analysis of plasma norepinephrine (pg/ml) and serum T_3 (ng/dl) in Untreated patients, r = -.7, p <.01.

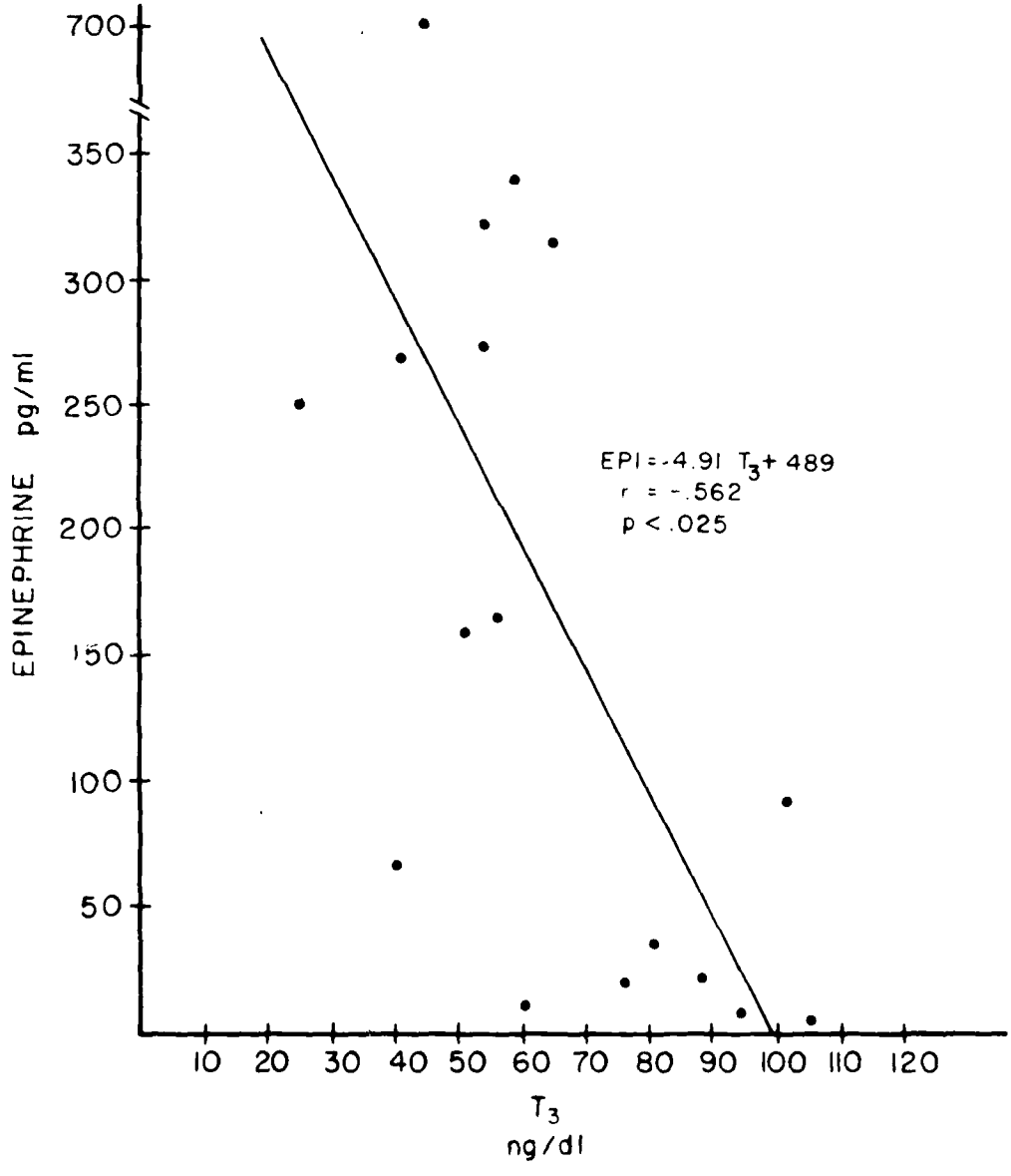

Figure 4 - Linear regression analysis of plasma epinephrine (pg/ml) and serum T_3 (ng/dl) in Untreated patients, r = -.562, p <.025.

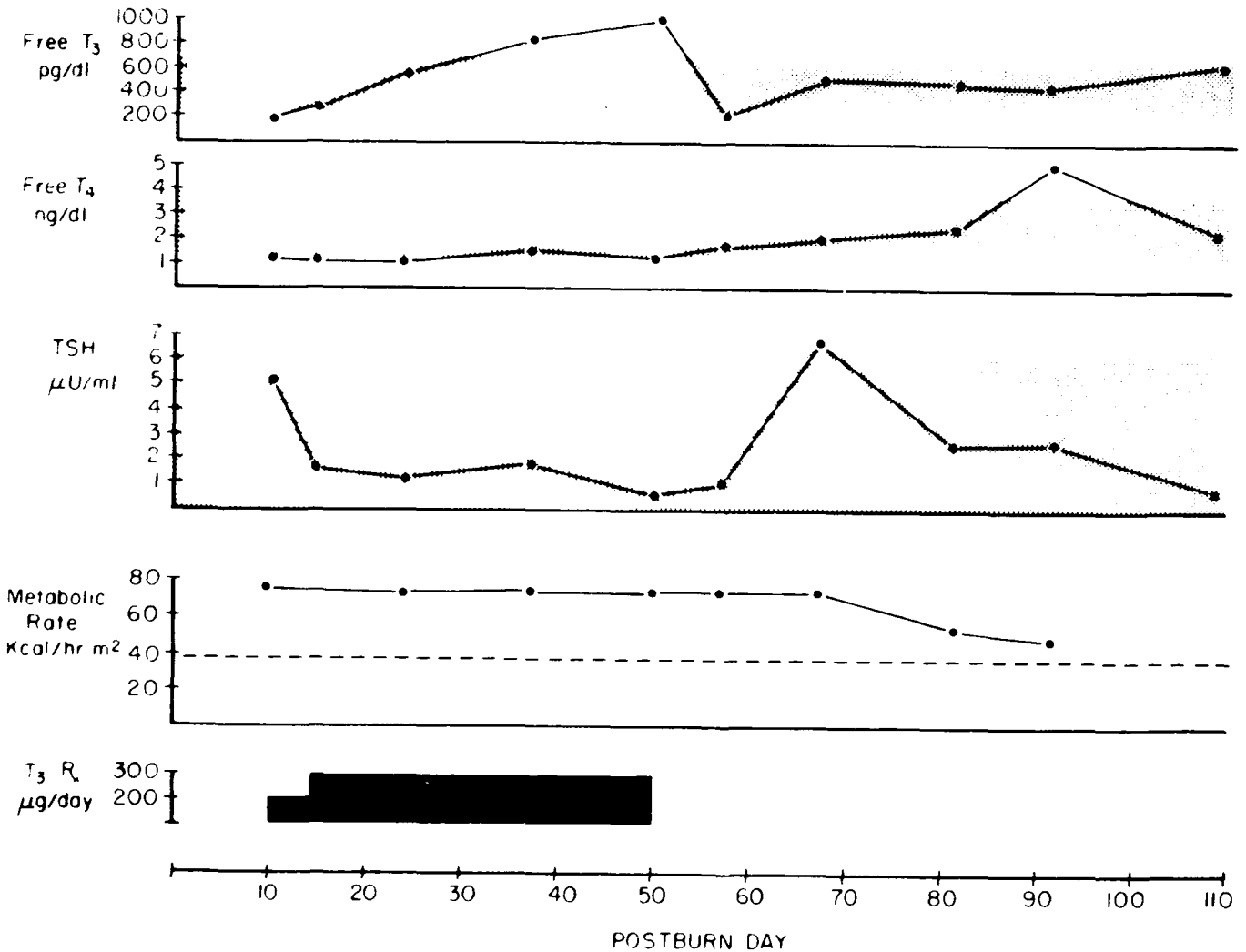

Figure 5 - Free T_3 (pg/dl), free T_4 (ng/dl), TSH (μU/ml, and metabolic rate (kcal/hr · m^2) in a single patient over a 108 day hospital course. Hatched areas represent the normal range for each thyroid hormone. The dashed line represents the predicted eumetabolic rate. The T_3 treatment dose is indicated by the solid bar between days 10 and 50.

ANNUAL PROGRESS REPORT

PROJECT NO. 3S161102BS05-00, BASIC RESEARCH

REPORT TITLE: THE STUDY OF METABOLISM AND NUTRITIONAL EFFECTS ON BURN INJURY IN SOLDIERS--COMPUTER-ASSISTED NUTRITIONAL ASSESSMENT OF THE THERMALLY INJURED PATIENT

US ARMY INSTITUTE OF SURGICAL RESEARCH
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1 October 1978 - 30 September 1979

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Douglas E. Mills, First Lieutenant, MSC
Richard A. Becker, M.D.
Cleon W. Goodwin, Jr., M.D., Major, MC

Reports Control Symbol MEDDH-288(R1)

Unclassified

ABSTRACT

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Nutritional balance studies were carried out on 50 thermally injured patients over a 12-month period. All food items were prepared and individually weighed in the metabolic kitchen. Beverages and parenteral solutions were reconstituted according to desired nitrogen and calorie content. Twenty-four hour dietary intake was classified by its total calories and by the proportional content of carbohydrate, fat, and protein, and these data were stored in each patient's dietary profile. Daily nitrogen loss was estimated by 24-hour urea production corrected for stool and wound losses. Metabolic expenditure was usually determined by computer-stored nomograms which interrelate degrees of prior weight loss, changes in metabolic expenditure, preferred nitrogen to calorie ratio, as well as body weight and burn size. With difficult-to-manage patients whose caloric requirements change with time or with the onset of septic complications, energy expenditure was measured by indirect calorimetry in an environmental chamber. To achieve positive energy balance, administered calories were closely predicted by the sum of 1,000 plus the resting metabolic expenditure. Nitrogen was provided in a ratio to calories of 1 to 150. Daily computer-generated profiles tabulated predicted caloric requirements, actual intake, nutritional surpluses or deficits, and weight change. All data were summarized on a continuous graphics plot so that trends in nitrogen balance, caloric intake, energy balance, and daily weight could be analyzed. Daily weights were compared to the patient's preburn weight and to a 10% reduction limit to that weight.

Use of such assessment techniques was successful in maintaining the majority of patients above the 10% weight loss limit. Departure below this limit prompted reappraisal of nutritional status and often led to initiation of the use of more advanced techniques of enteral and parenteral support.

PRESENTATIONS/PUBLICATIONS

Zitzka CA: Computer-assisted nutritional assessment of the thermally injured patient. Accepted for presentation at the Twelfth Annual Meeting of the American Burn Association, San Antonio, Texas, March 27-29, 1980.

Nitrogen balance
Energy balance

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OG 1842	79 10 02	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
79 10 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	3S161102BS05	00	092
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) Assessment of L-Triiodothyronine Therapy in Thermally Injured Patients (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 Clinical Medicine 002300 Biochemistry

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
79 08	Cont	DA	C. In-House

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

18. RESOURCES ESTIMATE		a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR	PRECEDING 79	0	0
	CURRENT 80	2	155

19. RESPONSIBLE DOD ORGANIZATION
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RESPONSIBLE INDIVIDUAL
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20. PERFORMING ORGANIZATION
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PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
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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) L-Triiodothyronine; (U) Therapy; (U) Burn patients; (U) Hypothyroidism

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

23. (U) To assess the efficacy of L-triiodothyronine (T_3) treatment in thermally injured patients.

24. (U) A prospective, single-blinded, randomized study will be performed. Serum concentrations of T_3 will be maintained within the normal range. Specific endocrine, microbiologic, and pathologic parameters will be monitored.

25. (U) 7908 - 7909 Twenty-eight patients are currently in the protocol and undergoing active study at this time. Our preliminary observations suggest that T_3 levels can be maintained within the normal range in critically injured burn patients without major effect on metabolic rate. Assessment of the marginating leukocyte pool suggests that the marginating pool is present but composed of leukocytes which are immature and of limited metabolic reserve. Collection of urine and plasma specimens for multiple hormonal analysis is underway. The efficacy of T_3 treatment in these patients cannot be assessed until the study is completed and the randomized treatme t code is broken. The results of this study are anticipated during the next year.

* 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. 3S161102BS05-00, BASIC RESEARCH

REPORT TITLE: ASSESSMENT OF L-TRIIODOTHYRONINE THERAPY IN THERMALLY INJURED PATIENTS--FREE T_4, FREE T_3, AND REVERSE T_3, IN CRITICALLY ILL THERMALLY INJURED PATIENTS

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Basil A. Pruitt, Jr., MD, Colonel, MC

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A prospective study of thyroid function was performed in 25 thermally injured patients. These patients are divided into two groups. The first group contains 5 patients with greater than 50% burn size studied longitudinally during the first 15 days following thermal injury. Significant suppression of serum concentrations of 3,5,3'-triiodothyronine (T_3) and elevation of serum concentrations of 3,3',5'-triiodothyronine (rT_3) were seen. The free thyroxine index and serum TSH concentrations remained within the normal range. To assess the clinical significance of these alterations in peripheral thyroid hormone concentrations, a second group of 20 patients was studied. We measured the free serum levels of T_4 (FT_4) and T_3 (FT_3) in 10 patients, mean age 34 years, mean burn size 56%, studied during a period of clinical deterioration, and in 10 patients of comparable age and burn size who were clinically stable. Both FT_4 and FT_3 values were significantly lower in the unstable patients, $p < .01$. All FT_3 values for the unstable patients (M $\pm$ SE), 193 $\pm$ 14 pg/dl, were beneath the normal range for FT_3 of 230-669 pg/dl. This correlation of biochemical hypothyroidism with clinical deterioration may have functional significance for the critically ill trauma patient.

Free thyroxine
Free triiodothyronine
Reverse triiodothyronine
Energy balance

ASSESSMENT OF L-TRIIODOTHYRONINE THERAPY IN THERMALLY INJURED PATIENTS--FREE T_4, FREE T_3, AND REVERSE T_3, IN CRITICALLY ILL THERMALLY INJURED PATIENTS

The recovery course of the severely burned patient is characterized by hypermetabolism. Although severely burned patients demonstrate many of the clinical features associated with severe hyperthyroidism, such as elevated basal metabolic rate, tachycardia, hyperventilation, hyperpyrexia, hyperkinesia, and severe weight loss, studies of thyroid function by Cope and Stanbury and their colleagues in 1953, disclosed normal ^{131}I uptake and normal concentrations of serum protein bound iodine in thermally injured man (1). In 1970, Caldwell showed no increase in thyroid activity during periods of increased oxygen consumption associated with full-thickness burn in an animal model (2). In 1974, Wilmore and his colleagues in our laboratory related the hypermetabolism following thermal injury to increased sympathetic nervous system activity (3). Metabolic rate correlated closely with the urinary catecholamine excretion rate, and post-traumatic hypermetabolism could be attenuated by administration of beta but not alpha adrenergic blocking agents.

Alterations in peripheral thyroid economy occur following a wide variety of stress and disease states. The changes which generally occur are characterized by a decrease in serum concentrations of 3,5,3'-triiodothyronine (T_3) and a rise in 3,3',5'-triiodothyronine (rT_3). It has been proposed that these reciprocal alterations in serum concentration of T_3 and rT_3 reflect a shift in the metabolism of thyroxine (T_4) from pathways which lead to generation of T_3 to pathways which lead to the generation of rT_3, a compound with diminished physiologic effect. It has also been proposed that a decrease in metabolic clearance rate of rT_3 without an increase in production rate accounts for the increase in serum concentrations of rT_3 in situations characterized by this

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1. Cope O, Nardi GL, Quijano M, et al: Metabolic rate and thyroid function following acute thermal trauma in man. Ann Surg 137:165-174, 1953.

2. Caldwell F: The role of the thyroid gland in the reproduction of the hypermetabolic state occurring in rats with full thickness burns. Endocrinology 67:363-367, 1970.

3. Wilmore DW, Long JM, Mason AD, et al: Catecholamines: mediator of the hypermetabolic response to thermal injury. Ann Surg 180:653-669, 1974.

alteration (4,5). Decreased serum concentrations of T_3 and increased levels of rT_3 have been observed during starvation (6), anorexia nervosa (5,7), severe trauma and hemorrhagic shock (8), hepatic dysfunction (9,10), surgery (11) and severe infection (9,12-14).

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4. Einsenstein A, Hagg S, Vagenakis A, et al: Observations on the peripheral metabolism of 3,3',5'-triiodothyronine (reverse T_3, rT_3) in fed and fasted patients. Clin Research 25:294A, 1977.

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6. Vagenakis AG, Burger A, Portnay GI, et al: Diversion of peripheral thyroxine metabolism from activating to inactivating pathways during complete fasting. J Clin Endocrinol Metab 41:191, 1975.

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8. Wahl R, Grussendorf M, Magnus J, et al: Changes of thyroid hormone concentration after severe trauma and in hemorrhagic shock. Eur Surg Res 9:(suppl 1), 1977.

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12. Burger A, Suter P, Nicod P, et al: Reduced active thyroid hormone levels in acute illness. Lancet 1:653-655, 1976.

13. McLarty DG, Ratcliffe WA, McColl K, et al: Thyroid hormone levels and prognosis in patients with serious non-thyroidal illness. Lancet 2:275, 1975.

14. Wartofsky L, Burman KD, Dimond RC, et al: Studies on the nature of thyroidal suppression during acute falciparum malaria: Integrity of pituitary response to TRH and alterations in serum T_3 and reserve T_3. J Clin Endocrinol Metab 44:85-90, 1977.

Because of both the known alterations in hypothalamic function and the hypermetabolism which accompany thermal injury, this study was designed to assess thyroid function following thermal injury. In addition, we present data correlating clinical deterioration of burn patients with significant depression of serum concentrations of free thyroxine (FT_4) and free triiodothyronine (FT_3).

MATERIALS AND METHODS

Patients - Group I

Longitudinal measurements of T_3, rT_3, and TSH were made in 5 males, mean age 29 years, mean burn size 66.5% (range 53-78%) who were injured simultaneously in a gasoline explosion. T_3 uptake (T_3U) and thyroxine (T_4) were also measured and expressed as the free thyroxine index (FTI). Venous blood specimens were obtained at 7 AM, at two to three day intervals beginning on the third post-burn day and continuing for the first fifteen days following thermal injury. Serum was immediately separated by centrifugation and the samples frozen until analysis. All but one of these patients developed bacteremia within the first week of admission, but all cleared their sepsis with appropriate antibiotic treatment and were free of systemic infection in the second week of observation.

Patients - Group II

Serum concentrations of T_4, T_3, FT_4, FT_3, and TSH were measured in 10 patients, mean age 24 years, mean burn size 59%, who were studied at a time of clinical deterioration, as indicated by either positive blood stream cultures, ileus, hypothermia, or altered mental status. In addition, similar hormonal determinations were made in 10 patients of comparable age and burn size, mean age 34 years, mean burn size 56%, but who were clinically stable and with sterile blood stream cultures. None of the patients received either dopamine or exogenous steroids, nor any other medications known to influence thyroid metabolism.

ASSAYS

All samples from each group were measured in a single assay. Serum T_4 was measured by competitive protein binding (15) and T_3U by silica gel uptake. Previously described radioimmunoassays were

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employed for the measurement of TSH, T_3, and rT_3 (16). Free hormone concentrations were determined by equilibrium dialysis at the Nichols Institute, San Pedro, California. Measurements obtained during the different study periods were compared by "t" test analysis.

RESULTS

Group I (Figure I)

T_3U - Values for T_3U ranged between 42±2% and 35±2% (mean ± S.E.) during the 15 day recovery period. Most values were at or above the upper limit of normal, probably reflecting the known fall in thyroid binding globulin capacity associated with severe trauma or illness.

T_4 - Mean (± S.E.) serum T_4 ranged from 2.5±0.5 to 5.0±0.8 µg/dl. Seven of eight values were below the normal range.

FTI - The FTI is the product of the T_3U and T_4 and correlates well with measured free thyroxine levels. Values for FTI ranged from 3.0±0.7 to 6.3±1.1 (mean ± S.E.). With the exception of the 3rd postburn day, all were within the normal range.

TSH - Serum TSH values ranged between 2.2±0.6 and 4.7±0.8 µIU/ml (mean ± S.E.). One patient had an elevated TSH value of 11 µIU/ml on the 3rd postburn day.

(Figure 2)

T_3 - Serum T_3 ranged from 16±4 to 52±12 ng/dl (mean ± S.E.). All values were beneath the normal range.

rT_3 - Serum rT_3 levels ranged from 104±10 to 56±9 ng/dl (mean ± S.E.). With the exception of day 13, all values were above the normal range.

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16. Burman KD, Dimond RC, Wright FD, et al: A radioimmunoassay for 3,3',5' l-triiodothyronine (Reverse T_3): Assessment of thyroid gland content and serum measurements in conditions of normal and altered thyroidal economy and following administration of thyrotropin releasing hormone (TRH) and thyrotropin (TSH). J Clin Endocrinol Metab 44:660-671, 1977.

Group II

Clinical and hormonal data from 10 control patients are presented in Table I. These patients were stable during the hypermetabolic phase of convalescence from burn injury, with sterile blood cultures and without clinical evidence of sepsis. All patients were febrile, with a mean body temperature 101.4°F, consistent with previous measurements in the uncomplicated burn patient (3). Serum concentrations of T_4 and T_3 were within the normal range, or slightly beneath it, consistent with the decreased plasma concentration of thyroid binding globulin which accompanies severe injury. Accordingly the FT_4 and FT_3 values were within the normal range, except in patient #5, whose TSH value was elevated as well. TSH elevations were noted in patients 5 and 8. The mean TSH value for all 10 patients (6.5 μU/ml) was within the normal range.

Table II presents clinical and hormonal data from 10 patients studied at a time of clinical deterioration as judged independently by their attending physicians. Most patients had positive blood or urine cultures. All ten patients were receiving appropriate antibiotic therapy at the time of study. Although the mean body temperature of all patients was 100.4°F, patients 12,14,16,19 and 20 exhibited relative hypothermia, a clinical sign of sepsis in the burn patient. All patients demonstrated a change in their clinical course, consistent with clinical sepsis, either ileus or altered mental status. Blood cultures, drawn once each day at 0600, showed positive growth in five patients. Urine cultures were positive for gram negative organisms in four patients. Serum T_4 concentrations were slightly beneath the normal range. As in the control patients, serum FT_4 values were, with the exception of patient #15, within the normal range. However, all of the serum concentrations of T_3 and FT_3 were beneath the normal range. In addition, patients 12, 15 and 20 exhibited elevated TSH values.

Table III presents a comparison of clinical and hormonal data between control and septic patients. Clinical indices of age, postburn day studied, percent total body surface burn, and temperature were not different between groups. Significant differences between control and septic patient groups were found for FT_4, $p < .01$, T_3, $p < .05$, and FT_3, $p < .001$. T_4 and TSH were not significantly different between groups.

Tables IV and V present the daily caloric assessment and net energy balance available in most patients. All food items were prepared and individually weighed in the metabolic kitchen. Beverages and parenteral solutions were reconstituted according to desired nitrogen and calorie content. Each item was classified by

its total calories and by the proportional content of carbohydrate, fat, and protein, and these data were stored in each patient's dietary profile. Daily nitrogen loss was estimated by 24-hour urea production corrected for stool and wound losses. Metabolic expenditure was usually determined by computer-stored nomograms which interrelate degrees of prior weight loss, changes in metabolic expenditure, preferred nitrogen to calorie ratio, as well as body weight and burn size. With difficult-to-manage patients whose caloric requirements change with time or with the onset of septic complications, energy expenditure was measured by indirect calorimetry in an environmental chamber (3). To achieve positive energy balance, the estimated metabolic requirement was predicted by the sum of 1,000 plus the resting metabolic expenditure. Daily computer-generated profiles tabulated predicted caloric requirements, actual intake, and nutritional surpluses or deficits. The estimated energy balance was taken as the difference between total caloric intake and estimated metabolic requirement. These values were not significantly different between the clinically stable and clinically unstable patient groups.

DISCUSSION

During the hypermetabolic recovery phase of thermal injury (3), longitudinal measurements (Group I patients) of serum concentrations of T_3 and T_4 disclose low to low-normal values. FTI and basal serum TSH levels generally fall within the normal range, and serum levels of rT_3 are markedly elevated.

The alterations in peripheral thyroid hormonal concentrations are similar to previous observations in patients with other catabolic disorders (6-9,11,12,14,17). Because these changes may occur with fasting or reduced food intake (6,7), the effect of diminished energy intake on these hormonal changes should be separated from the effect of increased sympathetic nervous system activity. During the first 48 hours following burn injury, fluid resuscitation of burn shock is a major priority. With restoration of blood volume, nutritional intake increases, and high protein-high calorie feedings are instituted. In the Group I patients studied longitudinally for the first two weeks postinjury, weight loss below preinjured body weight was minimal, although decreased dietary intake did occur in the early resuscitative phase. In the Group II patients, vigorous nutritional support was achieved, and alterations in thyroid hormone concentrations were observed with the onset of posttraumatic complications. This would suggest that the dietary influences in these patients are minimal in initiating the hormonal alterations observed.

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The changes in peripheral thyroid hormone concentrations associated with the stress of illness, may be a reflection of altered peripheral hormone turnover, changes in pool size, or altered production or release of these hormones. It has been suggested that the degradative mechanism for T_4 is reduced in infectious disease (14,18). Others have reported that increased turnover of T_3 occurs with certain bacterial infections (19,20), although serum concentrations of this hormone may not be altered. However, recent studies by Einsenstein et al and Suda et al suggest that elevations in serum rT_3 associated with catabolic states are due to a decreased metabolic clearance rate of rT_3 (4,5), perhaps reflecting the concomitant fall in both serum T_3 and metabolic rate. It is unlikely that this mechanism can be applied to the severely hypermetabolic burn patient. Thyroid hormone kinetic studies are needed to address this question in the burn patient. Finally, it has been proposed by Dratman that the T_3 distribution space may be related to the function of the sympathetic nervous system or the functional mass of tissue innervated by the autonomic nervous system (21). T_3, like its precursors, phenylalanine and tyrosine, which also exhibit increased turnover following severe thermal injury (22), may be taken up by the adrenergic nerve for further metabolism and storage or may, itself, serve as a neurotransmitter. Interactions of this type would explain in part the apparent reciprocal relationship between thyroid hormone and catecholamine activity in various states of thyroid dysfunction (23), as well as thermal injury.

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18. Wartofsky L, Burman KD, Dimond RC, et al: Studies on the nature of thyroidal suppression during acute falciparum malaria: Integrity of pituitary response to TRH and alterations in serum T_3 and reserve T_3. J Clin Endocrinol Metab 44:85-90, 1977.

19. Gregerman RI, Solomon N: Acceleration of thyroxine and triiodothyronine turnover during bacterial pulmonary infections and fever: Implications for the functional state of the thyroid during stress and senescence. J Clin Endocrinol Metab 27:93, 1967.

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22. Herndon DN, Wilmore DW, Mason AD, Jr, et al: Significance of abnormalities of phenylalanine and tyrosine kinetics in septic and nonseptic burn patients. Ann Surg (In press).

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An intact hypothalamo-pituitary axis is required for normal thyroid function, and T_3 and T_4 are thought to affect TSH elaboration through feedback mechanisms. Recent studies suggest that glucocorticoids or T_4, alone, may effectively suppress TSH (24,25). Thus, a normal serum TSH may not exclude hypothyroidism. Rather, it may reflect a normal serum free T_4 in the presence of significantly suppressed serum T_3 and FT_3 values or suppression of the pituitary thyrotroph by the high levels of cortisol observed in burn patients, Although five of our patients had elevations of TSH, thyrotropin releasing hormone (TRH) tests were within normal limits (data not shown) in similar burn patients whose basal levels of TSH were within the normal range. Accordingly, intact pituitary responses have been reported in other patients with infective febrile illnesses (14,26).

The clinical significance of the alterations of peripheral thyroid metabolism which accompany severe injury or illness is unknown. In ten patients studied during a period of clinical deterioration, we observed significant depression of serum concentrations of FT_4 and FT_3 when compared with a matched control group. All of these unstable patients exhibited FT_3 levels beneath the normal range, but only one patient had a FT_4 level beneath the normal range. Accordingly, three of these patients had only slight elevations of TSH, suggesting that T_4, and especially FT_4, may have a significant role in feedback inhibition of the hypothalamo-pituitary axis. Further, the disparity between FT_4 and FT_3 levels in these patients suggests either a significant block in hepatic conversion of T_4 and T_3 or altered clearance, again pointing to the need for kinetic studies in the burn patient.

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24. Larsen PR, Frumess RD: Comparison of the biological effect of thyroxine and triiodothyronine in the rat. Endocrinology 100: 950, 1977.

25. Sowers JR, Carlson HE, Brautbar N, et al: Effect of dexmethasone on prolactin and TSH responses to TRH and metoclopramide in man. J Clin Endocrinol Metab 44:237-241, 1977.

26. Talway KK, Sawhney RC, Rastogi GI: Serum levels of thyrotropin, thyroid hormones and their response to thyrotropin releasing hormone in infective febrile illness. J. Clin Endocrinol Metab 44: 398. 1977.

In summary, in the uncomplicated burn patient, serum concentrations of free thyroid hormones are generally within normal limits. This finding is consistent with the observation of euthyroidism in burn patients made by Cope and Stanbury in 1953. However, in the clinically deteriorating burn patient, as determined by clinical evidence of sepsis and positive blood and urine cultures, serum concentrations of both total and free T_3 and free T_4 are significantly suppressed. This association of clinical deterioration with chemical hypothyroidism may have functional significance for the critically ill trauma patient.

PRESENTATIONS

Becker RA: Are critically ill trauma patients hypothyroid? Presented at American Association for the Surgery of Trauma, Chicago, Illinois, 13-15 Sep 79.

PUBLICATIONS

Becker RA, Wilmore DW, Goodwin CW Jr, Zitzka CA, Wartofsky L, Burman KD, Mason AD Jr, Pruitt BA Jr: Free T_4, free T_3, and reverse-T_3 in critically ill, thermally injured patients. Arch Surg (In press).

TABLE I

Characteristics of Group II Burn Patients - Clinically Stable

Pt.	Age (Years)	Postburn Day Studied	% Total Body Surface Burn	Temp. (°F)	Clinical Evidence of Sepsis	Culture Results	T_4 µg/dl (nl,5-11.5)	FT_4 ng/dl (nl,1.3-3.8)	T_3 ng/dl (nl,100-200)	FT_3 pg/dl (nl,230-669)	TSH µU/ml (nl,<5.0)
1	16	22	60	101^2	None	No Growth	5.1	2.5	94	770	5.3
2	23	19	56	100^0	None	No Growth	5.3	2.65	58	280	6.2
3	33	27	49	101^8	None	No Growth	4.2	1.3	68	298	4.4
4	31	15	60	102^0	None	No Growth	4.6	2.25	77	434	4.9
5	31	10	46	102^6	None	No Growth	4.2	1.76	54	257	11.2
6	30	6	65	101^4	None	No Growth	6.4	2.69	178	707	5.3
7	56	9	55	100^6	None	No Growth	4.8	2.35	85	404	3.1
8	62	9	53	100^6	None	No Growth	5.3	2.76	54	270	10.2
9	23	40	56	101^0	None	No Growth	8.7	2.96	132	466	6.7
10	31	36	60	102^8	None	No Growth	5.9	2.48	90	474	3.3

TABLE II

Characteristics of Group II Burn Patients - Clinically Unstable

Pt #	Age (Years)	Postburn Day Studied	% Total Body Surface Burn	Temp. (°F)	Clinical Evidence of Sepsis	Culture Results	T_4 µg/dl (nl,5-11.5)	FT_4 ng/dl (nl,1.3-3.8)	T_3 ng/dl (nl,100-200)	FT_3 pg/dl (nl,230-669)	TSH µU/ml (nl,<8.5)
11	32	40	57	101.4	Obtundation	S. aureus*	5.3	1.48	78	225	5.3
12	26	19	68	98.6	Ileus	Ps. aeruginosa**	3.8	1.86	35	205	11.4
13	17	19	80	102.0	Obtundation	S. aureus*	5.1	1.33	49	184	5.2
14	30	48	57	99.2	Ileus	E. coli**	5.0	2.05	55	205	5.5
15	19	42	61	102.0	Obtundation	S. aureus*	4.0	1.00	53	225	9.7
16	19	20	57	99.2	Ileus	No growth	4.9	1.86	60	224	8.4
17	42	21	51	101.8	Obtundation	Ps. aeruginosa*	5.9	1.71	72	223	4.2
18	24	10	51	101.6	Obtundation	Ps. aeruginosa*	7.0	1.89	40	118	4.3
19	18	20	53	99.8	Ileus	Ps. aeruginosa**	5.0	2.60	47	213	6.3
20	19	8	60	99.0	Ileus	Ps. aeruginosa**	4.1	1.93	24	111	12.7

* Blood culture
** Urine culture

TABLE III

Comparison of Data (Mean ± SE) Between Clinically Stable and Unstable Patients

	Age (Years)	Postburn Day Studied	% Total Body Surface Burn	Temp. (°F)	T_4 µg/dl (nl,5-11.5)	FT_4 ng/dl (nl,1.3-3.8)	T_3 ng/dl (nl,100-200)	FT_3 pg/dl (nl,230-669)	TSH µU/ml (nl,<8.5)
Stable Patients	33 ± 5	19 ± 4	56 ± 2	101.4 ± .3	5.5 ± .4	2.37 ± .15	94 ± 14	430 ± 59	6.5 ± .8
Unstable Patients	25 ± 3	24 ± 5	60 ± 3	100.4 ± .5	5.0 ± .3	1.77 ± .13	51 ± 5	193 ± 14	7.3 ± 1
p	N.S.	N.S.	N.S.	N.S.	N.S.	<.01	<.01	<.001	N.S.

TABLE IV

Caloric Assessment* and Energy Balance* in Group II Burn Patients - Clinically Stable

Pt #	Carbohydrate Gm/Day	Protein Gm/Day	Enteral KCal/Day	Parenteral KCal/Day	Total Caloric Intake KCal/Day	Estimated Metabolic Requirement KCal/Day	Estimated Energy Balance KCal/Day
1	959	197	0	4014	4014	4000	-14
2**							
3	655	228	3341	1200	4542	4735	-193
4	220	191	288	1577	1865	4615	-2750
5	909	323	13	4301	4314	4570	-256
6**							
7	613	340	1630	2460	4090	3850	+240
8	433	327	1463	1811	3274	4050	-776
9	534	68	3200	0	3200	4750	-1550
10	428	491	0	4133	4133	4750	-617

*All values represent the mean of the three days preceding the day of study.
**Caloric information not available. Patients eating regular diet and maintaining body weight.

TABLE V

Caloric Assessment* and Energy Balance* in Group II Burn Patients - Clinically Unstable

Pt #	Carbohydrate Gm/Day	Protein Gm/Day	Enteral KCal/Day	Parenteral KCal/Day	Total Caloric Intake KCal/Day	Requirement KCal/Day	Energy Balance KCal/Day
11**							
12	616	161	4377	0	4377	4155	+222
13	447	250	3883	0	3883	3990	-115
14	506	232	3225	874	4097	3500	+597
15	645	86	3530	0	3530	4700	-1170
16**							
17	309	97	237	1247	1483	2850	-1367
18	334	169	3414	0	3414	3400	+14
19**							
20**							

*All values represent the mean of the three days preceding the day of study.
**Caloric information not available. Patients eating regular diet and maintaining body weight.

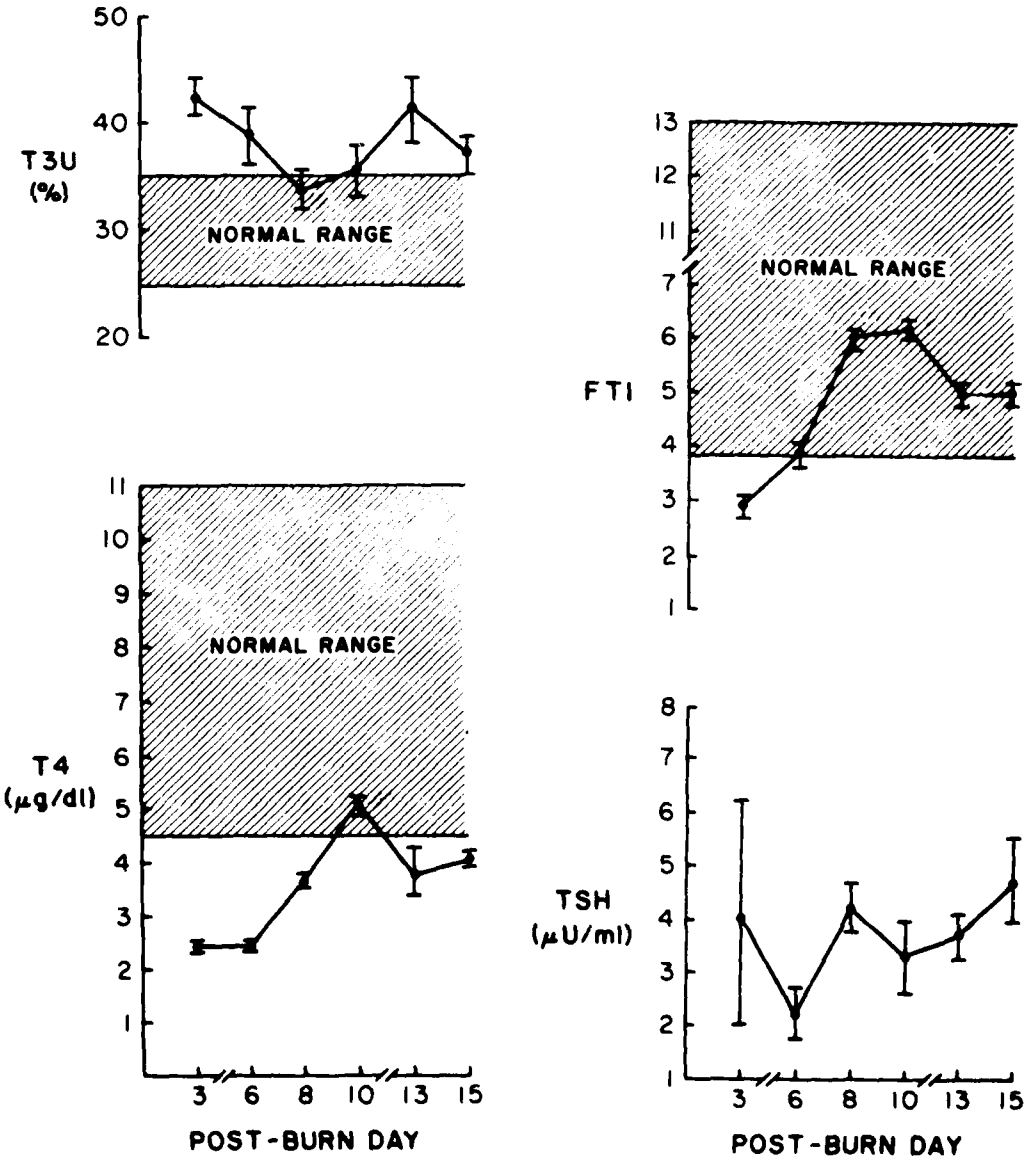

Figure 1 - Serum values (mean ± S.E.) for T_3U %, T_4 μg/dl, FTI, and TSH μU/ml in Group I patients during the first fifteen days following thermal injury. Shaded areas indicate the normal range. Normal values for TSH are less than 10 μU/ml.

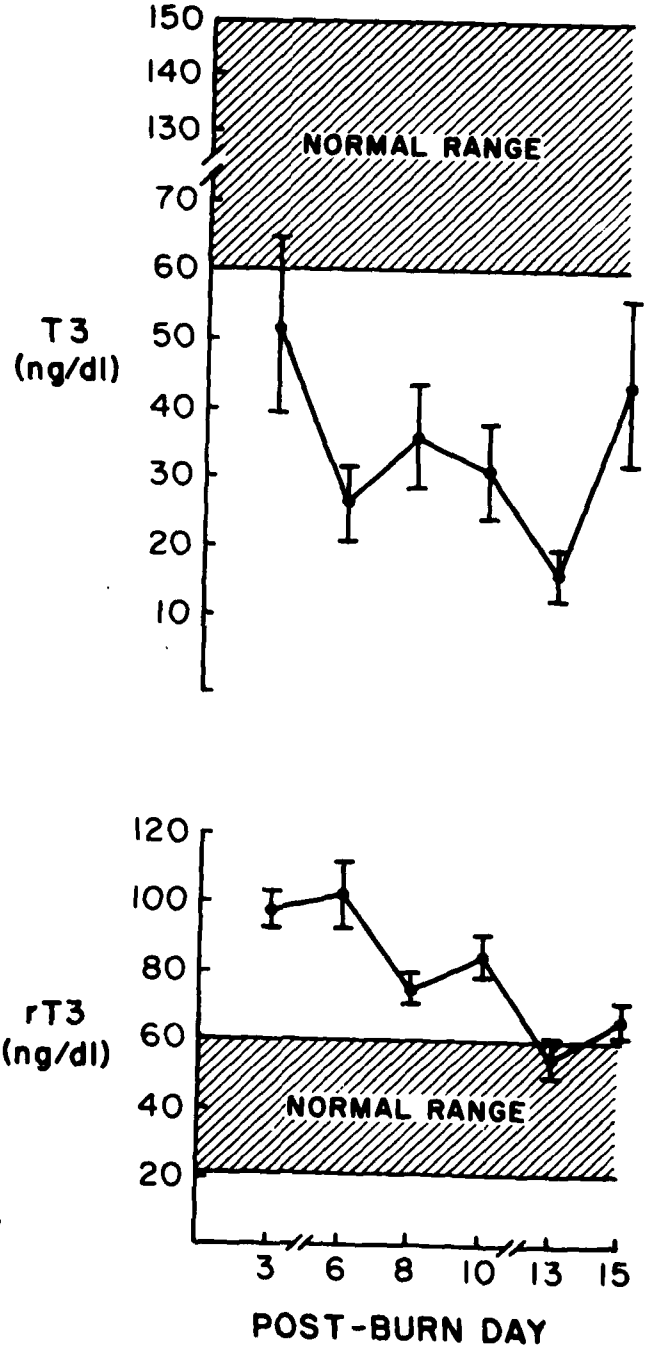

Figure 2 - Serum values (mean ± S.E.) for T_3 ng/dl and rT_3 ng/dl during the first fifteen days following thermal injury. Shaded areas indicate the normal range.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OG 6950	79 10 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
78 10 01	K. COMP.	U	U	NA	NL	[X] YES [ ] NO	A. WORK UNIT

10. NO. CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	077
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)

(U) Hepatic and Muscle Membrane Kinetics in The Endotoxemic Dog: A Preliminary Study For Assessment of Membrane Function in The Septic 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
76 07	79 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 79	.5	20
FISCAL YEAR: CURRENT		

19. RESPONSIBLE DOD ORGANIZATION

NAME: US Army Institute of Surgical Research
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RESPONSIBLE INDIVIDUAL
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20. PERFORMING ORGANIZATION

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

PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
NAME: Louis H. Aulick, PhD, MAJ, MSC
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) Liver; (U) Membrane transport; (U) Sepsis; (U) Indocyanine Green; (U) Dogs

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

23. (U) To define the alterations in liver function which occur following sepsis by determining hepatic transport characteristics of indocyanine green dye, hepatocyte membrane electrical potential and whole organ metabolic function. Once these abnormalities have been determined, to evaluate the effect of various therapy of these derangements.

24. (U) Plasma clearance and biliary secretion of indocyanine green dye was determined in 34 dogs before and after endotoxin administration. Hepatocyte membrane potential difference, liver blood flow and hepatic substrate arteriovenous difference were measured in selective experiments. The effect of infusion of amino acids and glucose-insulin was examined as potential treatments for the hepatic derangements.

25. (U) 7810 - 7909 Endotoxin injection causes a decrease in hepatic cell membrane active transport in concert with a decrease in liver cell electrical potential. Infusions of glucose and amino acid solution or of glucose plus insulin exert a protective effect on the hepatocyte as indexed by extraction of indocyanine green. Infusions of equal caloric amounts of glucose or amino acid solutions have similar and additive effects on nitrogen balances. Nutritional support of injured man should include both carbohydrates and amino acids.

*Available to contractors upon originator's approval.

ANNUAL PROGRESS REPORT

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: HEPATIC AND MUSCLE MEMBRANE KINETICS OF THE ENDOTOXEMIC DOG: A PRELIMINARY STUDY FOR ASSESSMENT OF MEMBRANE FUNCTION IN THE SEPTIC THERMALLY INJURED SOLDIER

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

1 October 1978 - 30 September 1979

Investigators:

W. Scott McDougal, M.D.
Steven Heimburger, M.D.
Douglas W. Wilmore, M.D.
Basil A. Pruitt, Jr., M.D., Colonel, MC

Reports Control Symbol MEDDH-288(R1)

Unclassified

ABSTRACT

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US Army Institute of Surgical Research, Brooke Army Medical Center, Fort Sam Houston, Texas 72834

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Alterations in hepatic function are central to the body's adjustments to severe infection. To characterize these adjustments, the following measurements were performed in 34 postabsorptive anesthetized dogs before and after receiving a constant infusion of E. coli endotoxin: (1) hepatic artery and portal vein blood flow via Doppler ultrasonic flow probes; (2) arterial and hepatic venous Indocyanine green (ICG) disappearance curves; (3) ICG bile excretion rate; and (4) net hepatic exchange of oxygen, carbon dioxide, lactic acid, glucose and insulin. Nine animals received 50% dextrose in water and insulin with the endotoxin infusion while five others received a 8.5% crystalline amino acid solution. Liver cork bore biopsies were obtained in selected animals and hepatocyte membrane potential measured in Ringer's lactate using glass microelectrodes. Endotoxemia in anesthetized dogs 1) had no measurable effect on hepatic artery and portal vein blood flows, 2) reduced average arterial (A) and hepatic venous (HV) ICG disappearance rate constants (A from 0.1026 to 0.0886 min^{-1} and HV from 0.1064 to 0.0720, respectively), 3) lowered mean ICG excretion rate from 0.0255 to 0.0100 mg/min, 4) increased hepatic glucose and lactate release and decreased oxygen consumption, and 5) reduced hepatocyte membrane potential from -46.4 $\pm$ 1.2 mV to -33.7 $\pm$ 1.6. Glucose-insulin infusion restored hepatic ICG uptake and excretion, oxygen consumption and hepatocyte membrane potential and converted the liver from an organ of glucose release to one of glucose uptake.

Isocaloric quantities of intravenous amino acids, however, had no apparent effect. These results taken together suggest that endotoxin reduces hepatic energy turnover and shifts metabolism toward anaerobic glycolysis. The resultant limitations in cellular energy utilization are manifested by the impaired active transport of ICG and fall in membrane potential of the hepatocyte. Glucose and/or insulin appear to correct this energy deficit.

This project has been completed, and a paper entitled "The Effect of Exogenous Substrate on Hepatic Metabolism and Membrane Transport During Endotoxemia" was published in Surgery 84: 55-61, 1978. No further work is planned.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OG 6976	79 10 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
78 10 01	D. CHANGE	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO./CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	089
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) Use of a Laminar Flow Isolator to Control Infection 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
77 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 79	.4	15
	CURRENT 80	.5	12

19. RESPONSIBLE DOD ORGANIZATION

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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) Burn injury; (U) Infection; (U) Laminar flow; (U) Humans

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

23. (U) It has been well known in recent years that the development of infection has been the most common cause of death in burned soldiers. As the vast majority of these cases result from invasive infection of the burn wound, methods of reducing burn wound contamination would be expected to result in improved survival. In addition, studies have shown that cross-contamination colonization causes more invasive burn wound infections than auto-contamination colonization. These facts generated interest in the use of laminar air flow isolator units as part of burn care.

24. (U) The Sci-Med Company of Minneapolis, Minnesota, was contracted to develop a laminar air flow unit to meet certain specifications. Following initial temporary installation, initial testing, and initial patient trials, numerous modifications and new developments were thought to be necessary and were undertaken. These have been completed, and the unit is now installed in its permanent form. Comparison of burn wound microbiology between laminar flow and conventionally treated patients will be made and related to septic complications and mortality in both groups.

25. (U) 7810 - 7909 The airborne microbial flora of the isolator unit was compared with the conventional ICU environment with the unit air flow operating and not operating. The isolator, when not operating, acquired a high count of saprophytic fungi, particularly on the plastic enclosure curtains. The genera recovered which were predominately Aspergillus, Penicillium and Alternaria, could be reduced by thorough cleaning of the curtain before use. In operating trials the isolator unit reduced the airborne bacterial count to 6.1% of that in the conventional environment (average bacterial colony count per cubic foot of air in 4 trials 5.25 vs 82.51). The mechanical system of the unit has been found inadequate for long term patient support and an improved airhandling system is being obtained.

*Available to contractors upon originator's approval

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

ANNUAL PROGRESS REPORT

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDPEENDENT RESEARCH

REPORT TITLE: USE OF A LAMINAR FLOW ISOLATOR TO CONTROL INFECTION IN BURNED TROOPS

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

1 October 1978 - 30 September 1979

Investigators:

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Robert B. Lindberg, Ph.D
Arthur D. Mason, Jr., M.D.

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: USE OF A LAMINAR FLOW ISOLATOR TO CONTROL INFECTION IN BURNED ~~TROOPS~~

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

Period covered in this report: 1 October 1978 - 30 September 1979

Investigators: William F. McManus, M.D., Lieutenant Colonel, MC
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Reports Control Symbol MEDDH-288(R1)

The effectiveness of a laminar flow isolator unit to protect the burn patient from bacterial colonization is currently under evaluation. Comparison of patients treated for ten days within the laminar flow isolator unit with those patients managed with routine aseptic precaution in beds adjacent to the laminar flow unit will determine whether there is any significant advantage to the burn patient from such a protected environment.

This study as of the date of this report has been delayed pending installation of a new laminar flow bed in the ICCU.

Burn injury
Infection
Laminar flow
Humans

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OG 6977	79 10 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
78 10 01	D. CHANGE	U	U	NA	NL	[X] YES [ ] NO	A. WORK UNIT

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a. PRIMARY	61101A	3A161101A91C	00	090
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)

(U) Measurement of Pulmonary Tissue Volume 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
77 04	Cont	DA	C. In-House

17. CONTRACT/GRANT: Not Applicable

a. DATES/EFFECTIVE: EXPIRATION:
b. NUMBER:
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e. KIND OF AWARD: f. CUM. AMT.

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 79	.6	19
FISCAL YEAR CURRENT 80	.4	20

19. RESPONSIBLE DOD ORGANIZATION

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NAME: DA

21. GENERAL USE

FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Burn injury; (U) Pulmonary function tests; (U)Plasma oncotic pressure; (U) Pulmonary extravascular water; (U) Resuscitation; (U) Humans

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

23. (U) To evaluate the significance of pulmonary extravascular lung water changes in burned soldiers and assess the effectiveness of conventional therapy.

24. (U) With the disappearance of an indicator soluble gas during a rebreathing maneuver, pulmonary extravascular water will be determined and correlated with changes in arterial blood gas tension and body weight.

25. (U) 7810 - 7909 Serial measurements of lung tissue volume, cardiac output and colloid osmotic pressure have been carried out during and after resuscitation in a group of 19 patients having an average lung size of 42% who received an average volume of resuscitative fluid of 3.56 ml/kg/%. No evidence of myocardial depression was found in this group. Colloid osmotic pressure was not predictive of pulmonary extravascular water volume. Pulmonary capillary blood flow rose progressively during the first 72 hours post burn. A mean increase in lung water occurred with time post burn. The volume of resuscitation fluids appears to affect lung tissue volume more significantly than their composition.

*Available to contractors upon originator's approval

ANNUAL PROGRESS REPORT

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: MEASUREMENT OF PULMONARY TISSUE VOLUME IN THERMALLY INJURED SOLDIERS

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

1 October 1978 - 30 September 1979

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Cleon W. Goodwin, M.D., Major, MC
Dianne L. Martin, SP5

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: MEASUREMENT OF PULMONARY TISSUE VOLUME IN THERMALLY INJURED SOLDIERS

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

Period covered in this report: 1 October 1978 - 30 September 1979

Investigators: Victor Lam, M.D., Major, MC
Cleon W. Goodwin, M.D., Major, MC
Dianne L. Martin, SP5

Reports Control Symbol MEDDH-288(R1)

Colloid oncotic pressures are correlated with the rebreathing method for determination of pulmonary tissue volume in the early resuscitation phase of thermal injury. Data from 19 patients show no relation between colloid oncotic pressure and pulmonary tissue volume.

We conclude that venous colloid oncotic pressures are not valuable in regulating albumin therapy to reduce transcapillary fluid balance of the lung.

Pulmonary extravascular water
Adult respiratory distress syndrome
Pulmonary capillary blood flow
Pulmonary capillary permeability
Plasma oncotic pressure
Resuscitation

MEASUREMENT OF PULMONARY TISSUE VOLUME IN THERMALLY INJURED SOLDIERS

The efficacy of albumin in the resuscitation of the thermally injured patient remains unproven. Physiologic considerations include the effect of colloid on cardiac output, organ perfusion and transcapillary fluid balance. Cost is another important criterion for the use of albumin in resuscitation protocols (1,2).

In thermally injured patients, large fluid volumes are frequently required in the first 24 hours postburn to maintain adequate tissue perfusion and organ function. Adult respiratory distress syndrome may occur in this patient population during the first week of hospitalization. Proposed etiologies for the development of pulmonary edema include myocardial depression, reduced intravascular oncotic pressure, sodium loading, and increased pulmonary capillary permeability.

Colloid oncotic pressure measurements have recently been reported to be an effective guide to albumin therapy during resuscitation (3). Using a noninvasive rebreathing method to study pulmonary tissue volume (4), we investigated the effect of colloid oncotic pressure on pulmonary extravascular water. The initial study period encompassed the first three days postburn, when fluid requirements are maximal and complicating bacteremia and renal failure are rare. Correlation of plasma colloid oncotic pressure and pulmonary tissue volume during resuscitation allows analysis of the role of colloid oncotic pressure as a determinant of net capillary fluid balance of the lung.

METHODS

Colloid Oncotic Pressure

Colloid oncotic pressures are measured in venous plasma using an Instrumentation Laboratory oncotic pressure measurement chamber with Amicon PM 30 membranes. Two hundred microliters of plasma are

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1. Skillman JJ: The role of albumin and oncotically active fluids in shock. Crit Care Med 4:55-61, 1976.
2. Virgilio RW, Rice CL, Smith DE, James DR, Zarins CK, Hobelmann CF, Peters RM: Crystalloid vs. colloid resuscitation: Is one better? A randomized clinical study. Surgery 85:129-139, 1979.
3. Rackow EC, Fein IA, Leppo J: Colloid osmotic pressure as a prognostic indicator of pulmonary edema and mortality in the critically ill. Chest 72:709-713, 1977.
4. Petrini MF, Peterson BT, Hyde RW: Lung tissue volume and blood flow by rebreathing: Theory. J Appl Physiol: Respirat Environ Exercise Physiol 44:795-802, 1978.

placed in the sample chamber, and pressure transducer output is recorded. Oncotic pressures are accepted after a 30-second steady value. Five percent IL albumin solutions are used as standards (5).

Lung Tissue Volume

All studies are performed in the Institute of Surgical Research Pulmonary Function Laboratory with a modified Perkin-Elmer medical mass spectrometer. Modifications to the mass spectrometer include a heated stainless steel capillary inlet, dimethyl ether (DME) plate at mass 15, and Swagelok Inlet connector.

A bag-in-box with a 16" pillow rebreathing bag (Calibrated Instruments) is connected with large bore tubing to an Ohio 843 data acquisition dry spirometer for a volume signal output. A four-way Hans-Rudolph pulmonary breathing valve with a mouthpiece selects room air or the rebreathing bag. A Honeywell 1858 fiberoptic recorder with a frequency response of 5,000 Hertz is used to record the helium, DME, and bag volume signals.

The initial bag volume is adjusted with a test gas mixture of 1.5% DME, 7% helium, 30% oxygen, and balance nitrogen to approximate the forced expiratory volume in one second. The bag concentration of test gas is measured prior to the rebreathing maneuver, and a volume calibration is obtained.

The subject with nose clip in place is asked to breathe quietly through the mouthpiece and then instructed to empty lungs to residual volume. The valve then selects the rebreathing bag, and a maximal rebreathing maneuver begins for five breaths. Finally, the mixed expired bag gas concentrations are determined.

The signal tracing is digitized and stored in a Hewlett-Packard 9830A minicomputer. Entry of calibration data for volume, time, and initial gas concentration is stored by the BASIC program. Correction of time for passage of gas through the anatomic dead space is performed manually. The first end-expiratory point is corrected in value for anatomic and apparatus dead space.

Calculated values include lung tissue volume (Vt, Equation 2), residual lung volume (RV), alveolar lung volume (V_A, Equation 4), and inspired lung volume corrected to body temperature pressure saturated (BTPS). Lung tissue volume ratio is lung tissue volume Vt normalized by alveolar volume to reduce individual variation.

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5. Morissette MP: Colloid osmotic pressure: Its measurement and clinical value. Can Med Assoc J 116:897-900, 1977.

EQUATIONS

Initial volume of test gas = volume in alveolar space + volume dissolved in tissue.

(1) $V_A\ (100\%) = V_A\ (\text{intercept } \%) + Vt\ \alpha_t\ (\text{intercept } \%)$

(2) $$Vt = \frac{V_A}{\alpha_t} \left[\frac{100}{\text{intercept}} - 1 \right] \frac{760}{P_B - 47}$$

Vt = pulmonary tissue volume

V_A = alveolar volume

P_B = barometric pressure

α_t = solubility coefficient in lung tissue

Slope of Disappearance Curve

(3) $$\dot{Q}_C = \frac{V_A \frac{760}{P_B - 47} + \alpha_t\ Vt}{\alpha_b\ (t_1 - t_2)} \ln \frac{F_{A(t_2)}}{F_{A(t_1)}}$$

F_A = concentration of soluble gas in alveoli

$\dot{Q}_C$ = pulmonary capillary blood flow dilution of inert gas

α_b = solubility coefficient in blood

(4) $$V_A = V_I \frac{(F_I)}{(F_E)}$$

V_I = volume inspired

F_I = inspired concentration of insoluble gas (initial)

F_E = expired concentration of insoluble gas (mixed)

Pulmonary capillary blood flow ($\dot{Q}_C$, Equation 3) is determined from the slope of a logarithmic normal regression plot. A correlation coefficient r is determined from the goodness of fit of end tidal points. Rebreathing dead space is obtained from the helium equilibration trace (4).

The validity of individual lung water measurements is analyzed by several criteria. Poor fit of data to a linear regression ($r^2 < 0.98$) is rejected, as are incomplete emptying of rebreathing bag, large residual volumes, poor equilibration of helium trace and evidence of recirculation prior to four breaths.

THEORY

The composition of the lung normally includes 80% water by weight. Measurement of pulmonary tissue volume is therefore a good indication of pulmonary extravascular water space (6).

Tissue volume measurements by rebreathing methods have been shown to accurately reflect lung water in patients with pulmonary edema (7). The intravascular double indicator dilution method requires access both to the pulmonary artery (i.e., Swan-Ganz) and to an arterial sampling site and involves a moderate loss of blood during repeat measurements. Thus, it is an invasive and potentially hazardous procedure and may not be indicated in the mildly ill patient.

The theoretical basis and original breath-holding technique have been reported by Cander and Forster (6). When a soluble gas is inspired into the airways, there will be an equilibrium between the alveolar gas and lung parenchyma (Vt) in about 10 milliseconds (see Fig. 1).

The initial drop in soluble gas concentration is a direct function of both the solubility of the gas in lung tissue and the volume of lung tissue. Then in the following 15 seconds, the concentration of soluble gas in the alveoli will decrease expotentially as it equilibrates with and is transported away by pulmonary capillary

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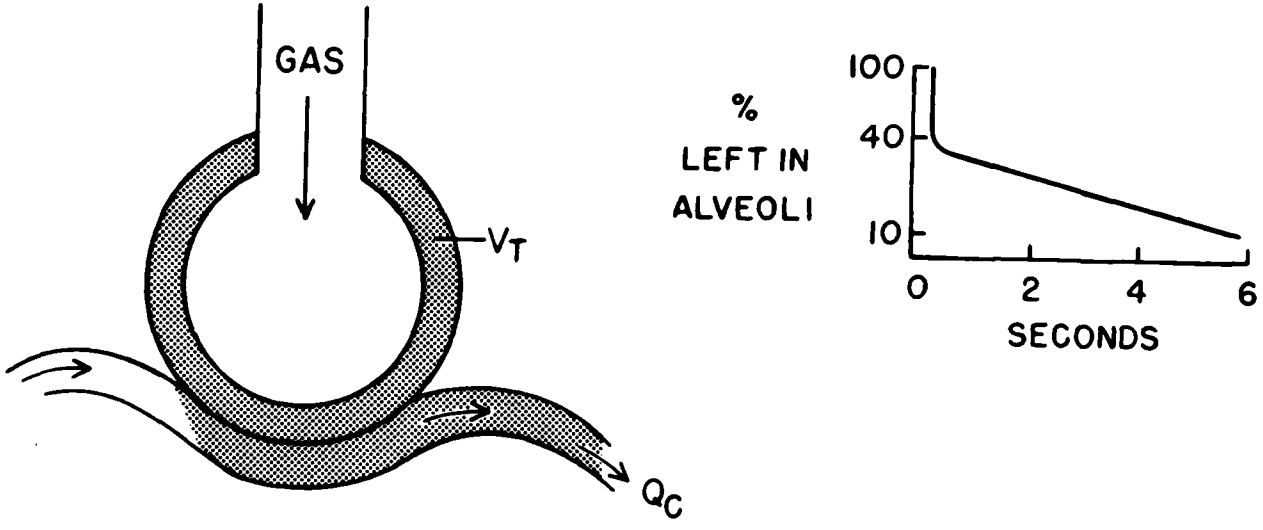
4. Petrini MF, Peterson BT, Hyde RW: Lung tissue volume and blood flow by rebreathing: Theory. J Appl Physiol: Respirat Environ Exercise Physiol 44:795-802, 1978.

6. Cander L, Forster RE: Determination of pulmonary parenchymal tissue volume and pulmonary capillary blood flow in man. J Appl Physiol 14:541-551, 1959.

7. Peterson BT, Petrini MF, Hyde RW, Schreiner BF: Pulmonary tissue volume in dogs during pulmonary edema. J Appl Physiol: Respirat Environ Exercise Physiol 44:782-794, 1978.

Fig. 1

LUNG MODEL-SOLUBLE GAS



circulation. Recirculation occurs after 15 seconds.

By obtaining serial alveolar gas samples, a disappearance curve can be produced. If plotted as a semilogarithmic graph, the intercept and slope can be determined. The choice of a suitable soluble test gas is dependent on its solubility characteristics. If the gas has too high a solubility, the gas will dissolve into the airway before arriving at the alveoli.

A low solubility gas such as acetylene will produce a small depression in the intercept, and the calculated Vt will be prone to more scatter because of measurement error. Dimethyl ether has been shown to give more precise measurements of Vt with its solubility characteristics α_t = 9.0. However, acetylene has been better as an indicator for pulmonary capillary blood flow.

PROTOCOL

Patients between 18 and 60 years old sustaining a thermal injury requiring intravenous resuscitation therapy were included in the study, after giving informed consent. Control by the investigators of fluid resuscitation within the first 6 hours postburn was required for inclusion into the study. Intravenous therapy was begun with either Ringer's lactate solution or Ringer's lactate solution with 2.5% albumin to sustain an hourly urine output of 30 to 50 ml. Measurements obtained at least every 12 hours included lung tissue volume and pulmonary capillary blood flow, plasma colloid oncotic pressure, arterial blood gas tensions, body weight, intake and output records and spirometry.

Xenon ventilation perfusion lung scan and fiberoptic bronchoscopy were performed to exclude patients with inhalation injury.

RESULTS

The relationship between colloid oncotic pressure and pulmonary tissue volume during early resuscitation have been studied in 19 thermally injured patients. Patient characteristics included a mean age of 29 years, 41% total body surface area burn and a mean of 3.56 ml/kg/% burn for fluid resuscitation. In the first 72 hours postburn, statistically significant changes occurred in colloid oncotic pressure, lung tissue volume ratio and cardiac output. No significant change occurred in alveolar-arterial oxygen gradient or body weight. Patient characteristics are listed in Table 1.

Figure 2 shows the time course of lung tissue volume for each of the individuals tested during the first 72 hours postburn. The mean lung tissue volume ratio increased 15% from .134 to .154.

Fig. 2

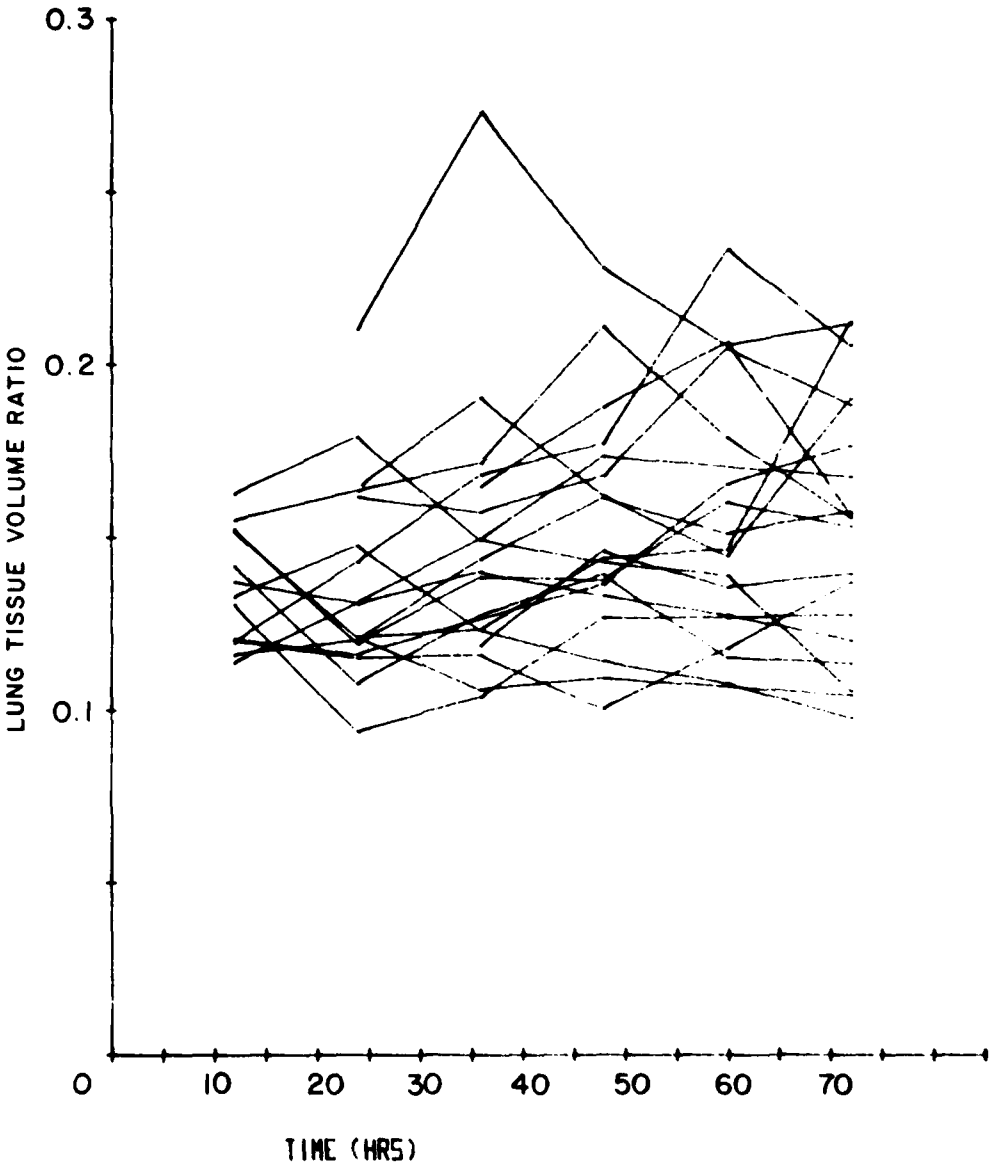


Table 1. Patient Characteristics

Number	19
Age (years)	29.5 ± 9.2*
% Burn	41.4 ± 15.9*
% Male	95
Resuscitation (ml/kg/% burn)	3.56 ± 2.00*

* Mean ± S.D.

Figure 3 depicts the individual time course of all patients for colloid oncotic pressure. The mean colloid oncotic pressure rose 17% from 19.2 to 22.5 torr during the first 3 days following burn injury.

Figure 4 shows cardiac output versus time for all 19 patients. There was a 36% mean elevation in cardiac output.

Data for colloid oncotic pressure and pulmonary tissue volume ratio for all individuals are plotted in Fig. 5. Linear regression analysis yielded a correlation coefficient of .03.

Linear regressions were performed for colloid oncotic pressure and lung tissue volume ratio for each individual, and the distribution of individual correlation coefficients is displayed in a histogram (Fig. 6). The mean of the individual correlation coefficients is 0.43.

Table 2 indicates the 72-Hour time course of mean colloid oncotic pressure, mean lung tissue volume ratio, cardiac output, body weight, and alveolar-arterial oxygen (A-a O_2) gradient.

DISCUSSION

Pulmonary capillary blood flow measured by the soluble gas rebreathing technique shows a progressive increase after the first postburn day. The normal alveolar-arterial oxygen gradient reflects the lack of inhalation injury and allows the equating of pulmonary capillary blood flow with cardiac output, since little right-to-left shunting occurs.

These data confirm those of Dorethy, who found no evidence of myocardial depression. In that study, velocity of circumferential fiber shortening and ejection fraction were normal or above normal

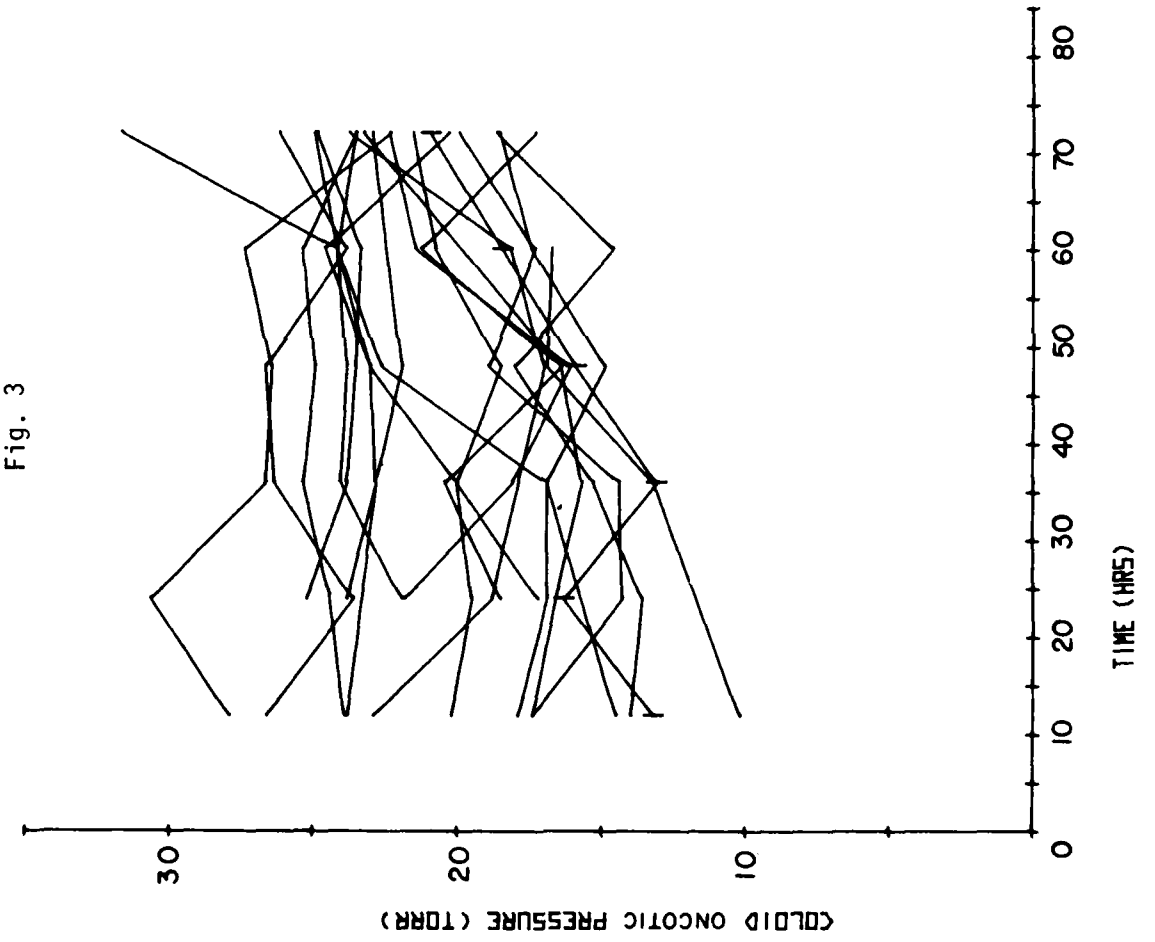

COLOID ONCOTIC PRESSURE (TORR)
30
20
10
0
10
20
30
40
50
60
70
80
TIME (HRS)

Fig. 3

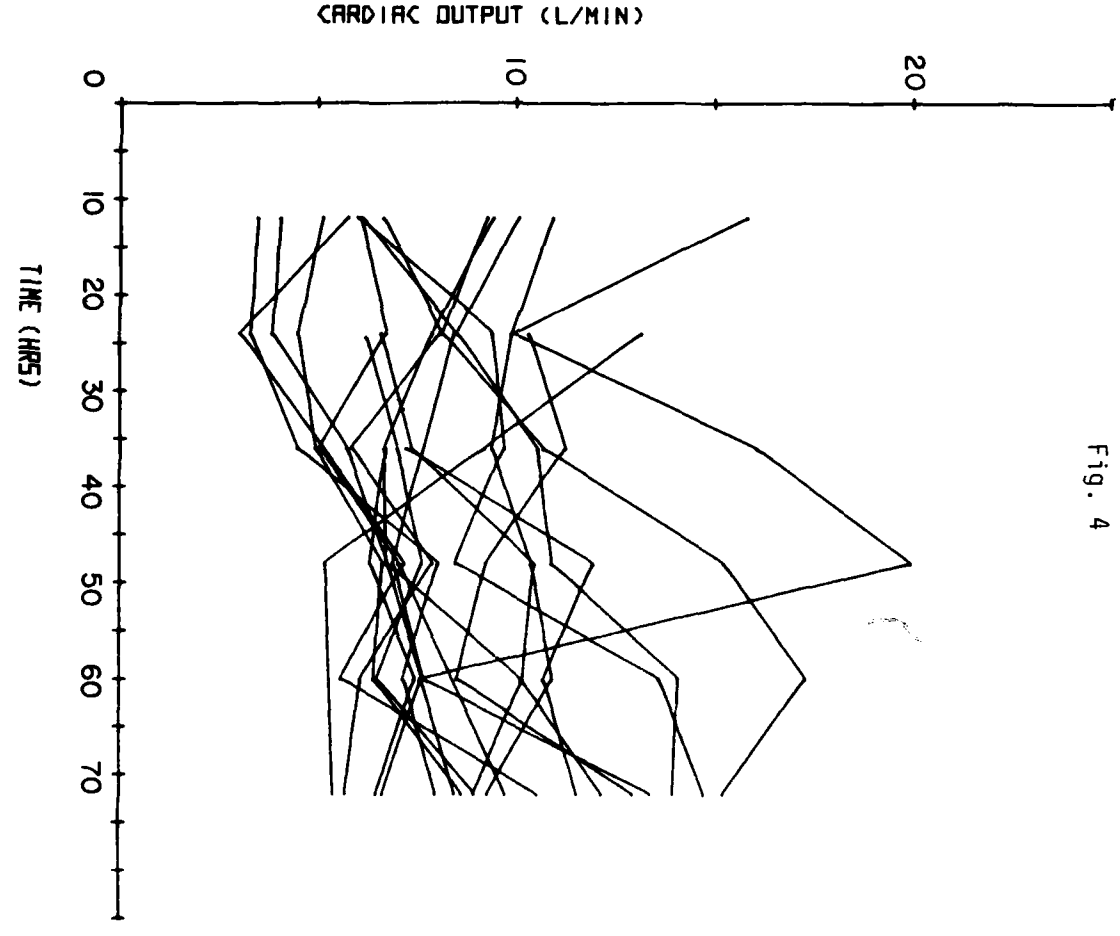


Fig. 4

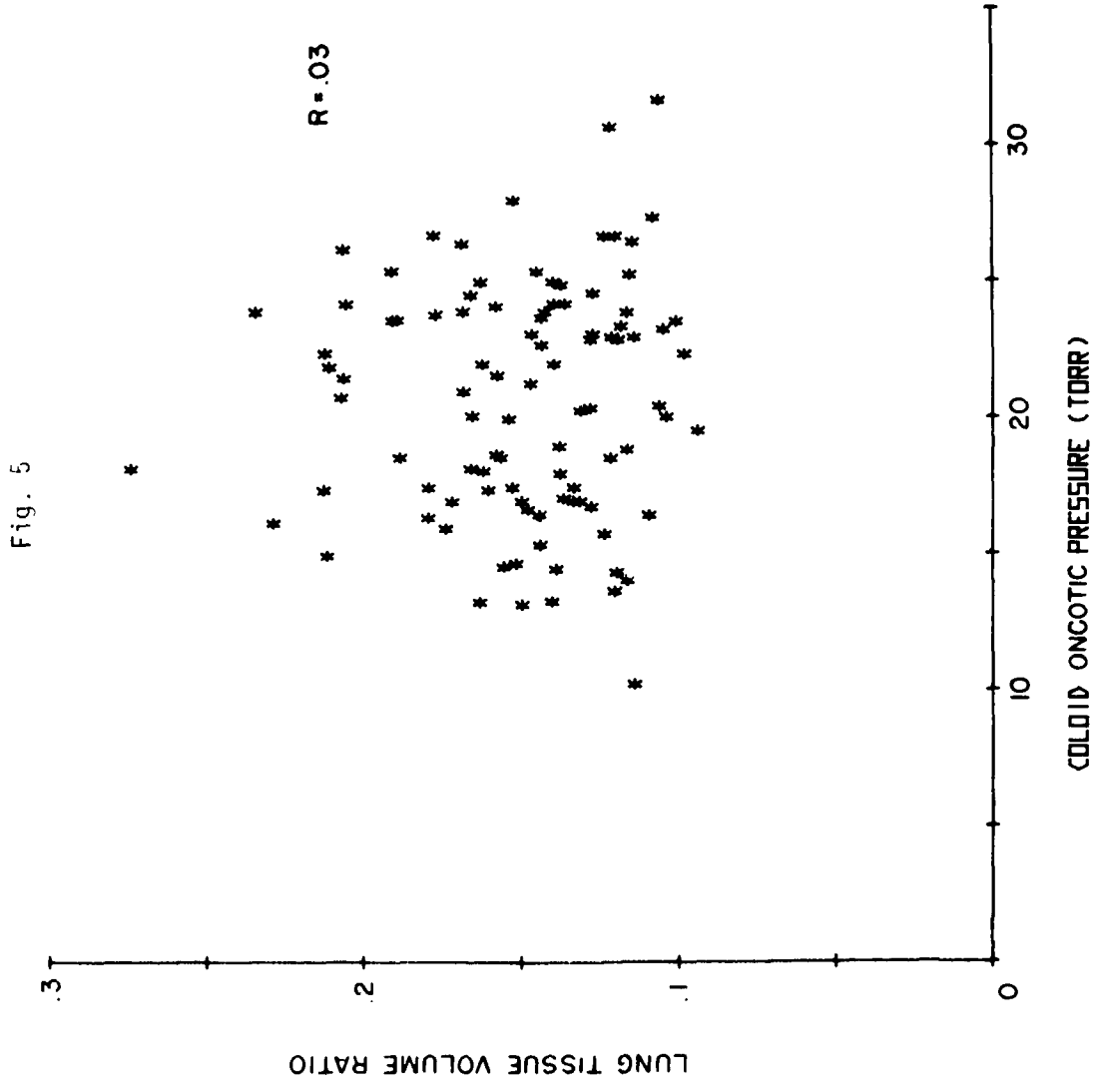

R=.03
LUNG TISSUE VOLUME RATIO
.3
2
1
0
10
20
30
COLOID ONCOTIC PRESSURE (TORR)

Fig. 5

Fig. 6

INDIVIDUAL CORRELATION COEFFICIENTS BETWEEN LUNG TISSUE VOLUME RATIO AND COLLOID ONCOTIC PRESSURE

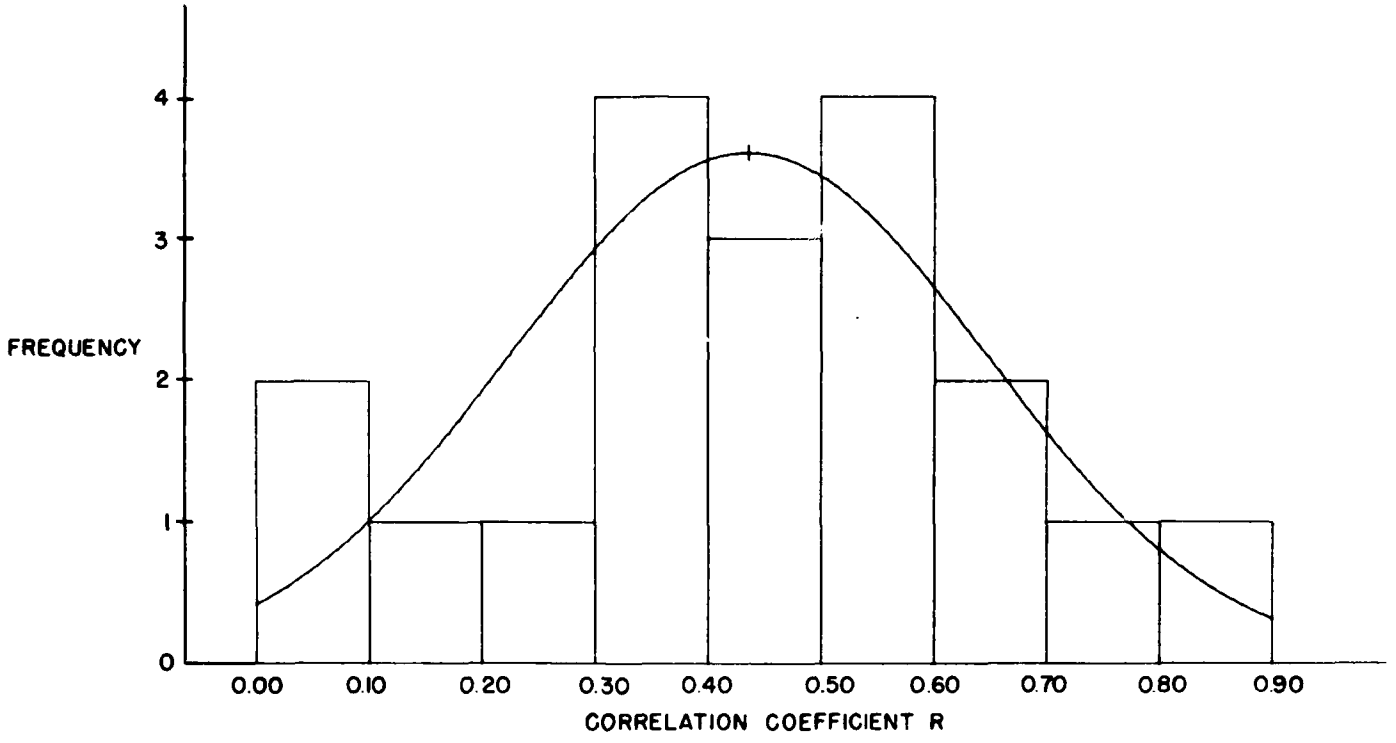


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Table 2. Results

Hours Postburn	Colloid Oncotic Pressure (torr)	Lung Tissue Volume Ratio	Cardiac Output (L/min)	Body Weight (kg)	A-a O_2 Gradient (torr)
12	19.2 ± 1.5	.134 ± .004	7.28 ± .93	82.1 ± 4.1	20.0 ± 3.8
24	20.2 ± 1.1	.137 ± .007	7.25 ± .70	83.3 ± 4.3	18.0 ± 3.0
36	19.8 ± 1.1	.155 ± .008	7.93 ± .70	82.1 ± 3.5	19.8 ± 5.9
48	20.2 ± 0.9	.155 ± .008	8.99 ± .78	85.9 ± 4.2	15.5 ± 2.2
60	21.3 ± 0.9	.159 ± .009	9.14 ± .75	82.4 ± 3.9	15.3 ± 3.3
72	22.5 ± 0.8	.154 ± .008	9.91 ± .67	84.3 ± 4.5	16.8 ± 3.4

Mean ± S.E.

and confirmed increased myocardial contractility (8). We have chosen not to normalize the mean cardiac output to body surface area (9).

Pulmonary extravascular water, as estimated by lung tissue volume ratio, increased during the 3-day study period. Some of the increase may reflect changes in pulmonary capillary blood volume; however, the individual changes in lung tissue volume are greater than the 100 ml pulmonary capillary blood volume found by diffusion methods.

The individual changes in lung tissue volume ratio or absolute lung tissue volume are consistent with data presented by Morgan et al (10). They determined lung thermal volumes, which required the additional risk of infection from the Swan-Ganz catheterization.

The mechanism for adult respiratory distress syndrome appears to be due to a change in vascular permeability rather than microvascular pressure gradients. Pulmonary artery wedge pressures are low to normal in this patient population, indicating that hydrostatic pressure is not the most important factor in changes of lung fluid balance (J.F. Dorethy, personal communication). If colloid oncotic pressure is the major factor in lung fluid balance in the thermally injured patient, then plasma oncotic pressure changes would be opposite to changes in lung water. Although both the mean lung water and mean colloid oncotic pressure increase in burn patients, changes in lung water and colloid oncotic pressure show no consistent relation for any given individual.

With a range of 10 to 30 torr for colloid oncotic pressure and lung tissue volume ratios of .09 to .27, the correlation coefficient of .03 indicates a random relationship. The histogram of individual correlation coefficients shows that colloid oncotic pressure is not a predictor of pulmonary extravascular water. It is not altogether surprising that there is no simple relation between colloid oncotic pressure and lung tissue volume, since the Starling balance of forces across the capillary involves three additional factors. Recently, Demling et al, using sheep with chronic lymphatic fistulae, showed

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that colloid oncotic pressure-pulmonary capillary wedge pressure gradient does not reflect net transcapillary fluid flux accurately after experimental thermal injury (11).

Changes in pulmonary vessel permeability could explain increased pulmonary extravascular water in the face of low pulmonary capillary wedge pressures and lack of correlation between oncotic pressures and lung water. The mean increase in lung water is consistent with a delayed mobilization of fluid into the pulmonary interstitium. The majority of the resuscitation fluid is administered in the first 24 hours. Vascular permeability changes may be decisive, since pulmonary pressures and colloid oncotic pressures cannot explain the delayed accumulation of fluid in the lung. We cannot rule out changes in interstitial tissue pressures affecting fluid balance of the lung; however, lung volumes are not grossly changed in this patient population.

The rebreathing method for lung tissue volume allows the early detection of pulmonary edema despite the lack of clinical signs such as rales, tachypnea and hypoxia. Since these clinical signs are evidence of late stage *pulmonary edema* with alveolar flooding, increased lung tissue volume identifies an earlier stage of edema formation and allows more timely therapeutic intervention.

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PRESENTATIONS

Lam V: Does pulmonary extravascular water vary with colloid oncotic pressure after burn injury? Annual Meeting of American Thoracic Society, Las Vegas, Nevada, 16 May 1979.

PUBLICATIONS

Lam V, Goodwin CW Jr, Treat RC, Martin DL, Mason AD Jr, Pruitt BA Jr: Does pulmonary extravascular water vary with colloid oncotic pressure after burn injury? Am Rev Respir Dis 118:139, 1979.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1 AGENCY ACCESSION	2 DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OG 6952	79 10 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
78 10 01	D. CHANGE	U	U	NA	NL	[X] YES [ ] NO	A. WORK UNIT

10 NO / CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	082
b. CONTRIBUTING				
c. CONTRIBUTING				

11 TITLE (Precede with Security Classification Code): (U) Laboratory Investigation of The Mechanisms of Acquired Leukocyte Dysfunction 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
75 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 79	1.0	32
FISCAL YEAR CURRENT 80	1.0	33

19 RESPONSIBLE DOD ORGANIZATION
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ADDRESS: Fort Sam Houston, Texas 78234

RESPONSIBLE INDIVIDUAL
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NAME: Albert T. McManus, MAJ, MSC
TELEPHONE: 512-221-3411
SOCIAL SECURITY ACCOUNT NUMBER

21 GENERAL USE: FOREIGN INTELLIGENCE NOT CONSIDERED

ASSOCIATE INVESTIGATORS
NAME: Arthur D. Mason, Jr., MD
NAME: DA

22 KEYWORDS (Precede EACH with Security Classification Code): (U) Rat model; (U) Burns; (U) Leukocytes; (U) Glucose oxidation; (U) Latex phagocytosis; (U) Stress hormones; (U) Cyclic neucleotides

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

23. (U) Efforts will be made to establish one or more metabolic basis for acquired leukocyte dysfunction following thermal injury. Establishment of specific nutritional or environmental effects may allow for corrective management.

24. (U) Initial efforts will be to measure glucose metabolism in normal and burned patients' leukocytes. Purified and washed granulocyte populations will be examined for oxidization of Carbon 14 labeled glucose. Hexose monophosphate shunt and glycolysis activity will be estimated by release of $^{14}CO_2$ from respectively 1-^{14}C glucose. Measurements will be made on resting and latex particle (=0.8 u) stimulated cells. Leukocyte function will be examined in the burned rat. The effect of burn rat serum on leukocyte glucose metabolism and cyclic neucleotide levels will be examined. Establishment of an animal model of burn associated leukocyte dysfunction will allow examination of corrective procedures in vivo.

25. (U) 7810 - 7909 Significant elevations in urine and plasma catecholamine in the burned rat model have been established. The hypercatecholamine state temporally matched the previously noted depression of in vivo chemotaxis. Additionally, in vivo examination of granulocyte adherence, a requirement for chemotaxis, has been found to be greatly depressed. Examination of granulocyte kinetic changes following intravenous injection of Epinephrine failed to demonstrate a significant marginating pool of granulocytes. This fact fits well with the depression of in vitro adherence. The present data suggest that although the total circulating granulocyte counts are elevated, burned rats are functionally neutropenic. That is, despite apparently adequate numbers of circulating cells, burned rats have a depressed inflammatory response.

* Available to contractors upon originator's approval

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

ANNUAL PROGRESS REPORT

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: LABORATORY INVESTIGATION OF THE MECHANISMS OF ACQUIRED LEUKOCYTE DYSFUNCTION FOLLOWING THERMAL INJURY IN BURNED SOLDIERS

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

1 October 1978 - 30 September 1979

Investigators:

Albert T. McManus, Major, MSC
Arthur D. Mason, Jr., M.D.

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: LABORATORY INVESTIGATION OF THE MECHANISMS OF ACQUIRED LEUKOCYTE DYSFUNCTION FOLLOWING THERMAL INJURY IN BURNED SOLDIERS

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

Period covered in this report: 1 October 1978 - 30 September 1979

Investigators: Albert T. McManus, Major, MSC
Arthur D. Mason, Jr., M.D.

Reports Control Symbol MEDDH-288(R1)

The high incidence of serious opportunistic infection following human burn injury has been well documented. Investigations of the mechanisms of this acquired susceptibility have demonstrated several defects in phagocytic defenses. An established rat burn infection model was modified to study neutrophil function in animals with a 60% burn injury. These rats (350 g) received 35 ml saline resuscitation. When not further stressed, survival to healing occurred in more than 80%. Burned animals were found to have decreased inflammatory responses to ip injections of heat-killed Pseudomonas aeruginosa, Staphylococcus aureus, and sterile sodium caseinate. These reductions could not be explained by neutropenia. Prior immunization with heat-killed Pseudomonas did not improve the inflammatory response to ip injection of homologous organisms. Levamisole treatment did improve the inflammatory response to ip Pseudomonas. Epinephrine injection (iv) showed burned animals to have a markedly reduced proportion of marginated neutrophils but an increase in total peripheral neutrophil counts. The stress hormones, corticosterone and catecholamines were elevated during times of decreased inflammatory responsiveness; additionally, burned animals had decreased nylon fiber adherence. The possible role of burn-related stress adaptation and human immunosuppression has been studied.

Burns
Leukocytes
Glucose oxidation
Latex phagocytosis
Stress hormones
Cyclic neucleotides
Rat model

LABORATORY INVESTIGATION OF THE MECHANISMS OF ACQUIRED LEUKOCYTE DYSFUNCTION FOLLOWING THERMAL INJURY IN BURNED SOLDIERS

Susceptibility to Pseudomonas aeruginosa appears to be primarily a host-dependent variable; that is, human Pseudomonas infection is most frequently associated with or perhaps a symptom of underlying immunosuppression. The association of Pseudomonas infection with burn injury is a frequently quoted example. Although Pseudomonas infection of burn wounds has become less frequent, it remains a good example of opportunistic infection.

The immunologic basis for burn trauma associated susceptibility to opportunistic infection has been the subject of numerous investigations. Patients with large burns frequently display immunologic defects (1). The polymorphonuclear leukocyte has been the most intensively studied host defense system. Following severe injury, in vitro neutrophil defects have been found to include altered chemotaxis (2-5), decreased microbicidal activity (6-8), decreased lysosomal enzyme content (5,9), decreased oxygen consumption (10), decreased NBT tests

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(11), decreased chemiluminescence (12), altered NADH-NADPH oxidase activity (13) and decreased glucose oxidation (14). Additionally, decreased inflammatory responsiveness as measured by skin windows has been described (15).

An established rat burn model of Pseudomonas susceptibility has been examined for possible underlying abnormalities. The Walker-Mason rat burn model was used (16,17); this model has been used extensively at this Institute and elsewhere to investigate antimicrobial chemotherapies (18), vaccines (16) and trauma-associated metabolic changes (19). These investigations indicate that Pseudomonas infected animals succumb and noninfected burned rats show maximal stress-related altered metabolism early in the second week following injury.

METHODS AND MATERIALS

Rat burn model. Adult male, 340-360 g Sprague-Dawley rats were anesthetized with sodium pentobarbital (1 mg/25 g); the dorsal and ventral surfaces were clipped with a number 40 Oster blade. A 30% full-thickness scald injury (17) was first inflicted on the dorsum by immersion for 10 seconds in boiling water. The animals were then removed from the burn mold and injected intraperitoneally with 35 ml of

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normal saline; they were next returned in the prone position to the mold and a 30% ventral burn was inflicted by immersion in boiling water for 3 seconds. Following burning, the animals were individually caged and fed and watered ad libitum in a controlled temperature (20-25° C) environment with controlled lighting.

Examination of peripheral leukocyte patterns. Total and differential white cell counts were performed in a group of six animals prior to burning and daily for the first 10 days postburn. Blood was obtained from unanesthetized animals by tail venipuncture. Total white counts were determined with a Coulter counter (Model F), and differential cell counts were made on Wright-Giemsa stained smears.

Examination of intraperitoneal inflammatory responses. Burned animals were examined for their ability to muster a neutrophilic exudate response following ip injection of leukoattractants. Sterile casein (20), heat-killed Pseudomonas aeruginosa or heat-killed Staphylococcus aureus were used. The peritoneal challenge inocula were 10 ml of sterile 10% sodium caseinate, 10 ml of saline-suspended heat-killed Pseudomonas (10^8/ml), or 10 ml of saline-suspended heat-killed Staphylococcus (10^7/ml). The response to Pseudomonas was examined in animals injected with heat-killed organisms 30 days and 10 days prior to burning. These animals were also examined for agglutinating antibodies at the time of ip injection. Burned control animals were used to measure total hemolytic complement (21) activity at the times of intraperitoneal challenge. Unburned injected animals were used as controls.

Exudates were examined 18 to 24 hours after ip injection by injecting 60 ml of heparinized saline (10 units/ml) into the peritoneum of Penthrane® (Abbot Laboratories, North Chicago, Illinois) anesthetized rats, opening the peritoneum by midline laparotomy and collecting the exudate into graduated cylinders. The peritoneal cavities were liberally washed with heparinized saline and the total recovered volume measured. Total cell recoveries and differential counts were determined.

Circulating neutrophil response to iv epinephrine. Total peripheral neutrophil counts (circulating plus marginating) (22) were measured in sodium pentobarbital anesthetized rats. Blood samples were drawn

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via a cannula placed in the abdominal aorta prior to iv injection of epinephrine into the penile vein. Epinephrine (0.1 ml) was used at a 1:10,000 dilution (22). Burned animals were examined on the ninth day postburn. Unburned animals were used as controls.

Circulating neutrophil adherence to nylon fiber. Neutrophil adherence to nylon fiber was measured by a modification of the method of MacGregor (23). Heparinized (10 units/ml) blood was taken from the abdominal aorta immediately following Penthrane anesthesia. This blood was passed over nylon fiber columns packed in Pasteur pipettes as originally described. The flow rate was limited by using an iv flow valve placed on a small length of tubing attached to the bottom of the pipettes. Neutrophil counts before and after passing the column were used to determine percent adherence. One ml of heparinized blood was used per column, and all specimens were examined in triplicate. Experiments were conducted at 37° C with a flow time of 5 to 7 minutes.

Corticosterone and catecholamine determinations. Serum corticosterone levels were measured fluorometrically (24). Total plasma catecholamines were determined by the Cat-A-Kit^{R} radioenzymatic assay (Upjohn, Kalamazoo, Michigan). Blood samples were taken from the abdominal aorta immediately following Penthrane anesthesia. Total 24-hour urinary output of catecholamines was determined in animals housed in metabolic cages, using the Oxford^{R} fluoremetric catecholamine assay (Oxford Laboratories, Foster City, California). These animals were conditioned for 1 week prior to burning.

Levamisole treatment. The effect of levamisole (25) on the peritoneal response to heat-killed Pseudomonas was examined. The drug was infused continuously, using the Alzet^{R} 1702 implantable minipump (Alza Corp., Palo Alto, California), at doses of 1 mg/kg or 10 mg/kg per day (26). Saline-loaded pumps were used to infuse sham controls. Nonburned and burned untreated animals were used as additional controls. The pumps were implanted subcutaneously over the skull immediately following burning. Pseudomonas challenge was as described above.

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Statistical analyses. Data were compared by analysis of variance or Student's t test. Control and experimental animals in each experiment were from the same shipment lot.

RESULTS

When not further stressed, the 60% burned 350 g rat had greater than 80% survival (30 days). The majority of deaths occurred within 48 hours postburn. Animals with spontaneous culture proven sepsis were excluded from these studies.

Burned animals showed a peripheral leukocyte temporal pattern (Fig. 1) similar to that reported for burned man (27). An initial leukocytosis was followed by a moderate depression which was succeeded by a second elevation that persisted for the period studied. Differential counting showed that neutrophils were principally responsible for the observed elevations (Fig. 2). Lymphocyte counts showed a significant lymphopenia on day 1 through 3 (Fig. 3). Lymphocyte counts were within the preburn values by day 4 and thereafter remained at that level. Immature cells (metamyelocytes) constituted less than 10% of the granulocytes counted during the observation period. At no time did the absolute neutrophil count drop below the preburn values.

Examination of peritoneal inflammatory responses following ip casein or staphylococcus showed significant reductions of neutrophil accumulation on day 3 and day 9 postburn (Figs. 4 and 5). Comparison of circulating neutrophil counts following casein showed burned animals to have equal or greater counts than injected unburned controls (Fig. 4).

Despite preburn exposure to homologous heat-killed Pseudomonas, burned animals had a significantly depressed peritoneal response (Fig. 6). Unburned and burned rats had equal reciprocal geometric mean antibody responses, 71.83 ± 1.8 and 80.63 ± 2.33 respectively.

Measurement of total hemolytic complement (C'50) in three groups of five rats showed no significant differences among unburned, 3 day burned or 9 day burned rats. Values were 39.8 ± 2.03, 38.6 ± 2.93 and 45.4 ± 3.80 respectively. The human control C'50 was 32.

The circulating neutrophil response of an anesthetized normal rat to epinephrine injection is shown in Figure 7. There was a rapid rise in the absolute neutrophil count which returned to normal by about 10 minutes postinjection. Red blood cell counts taken from the same specimen showed no significant elevation; the neutrophil increase does not represent hemoconcentration. The ratio of the postinjection

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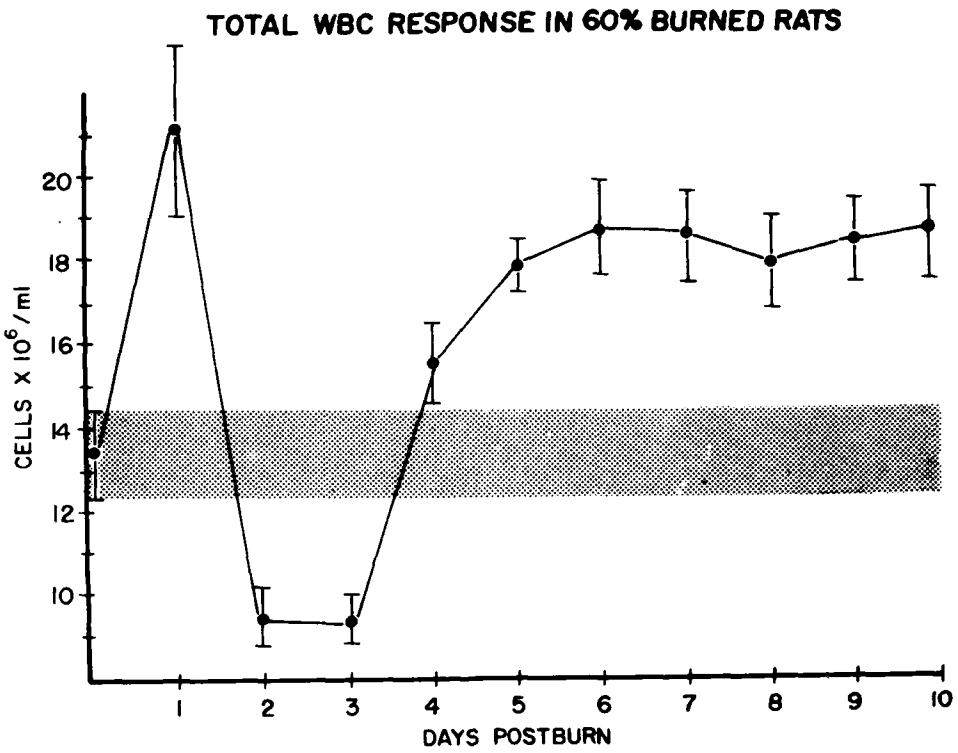

Fig. 1. Temporal effect of 60% burn injury of rats on the total white blood count. Values represent serial means of six animals. Shaded area represents 2 standard errors of the preburn mean.

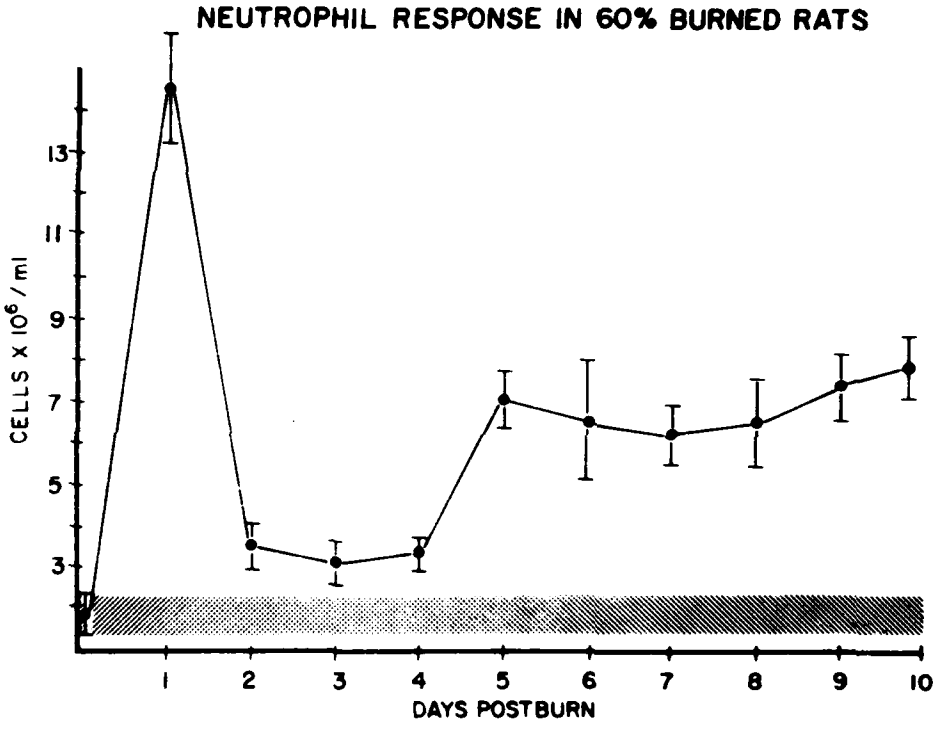

Fig. 2. Temporal effect of 60% burn injury of rats on the absolute neutrophil count. Values represent serial means of six animals. Shaded area represents 2 standard errors of the preburn mean.

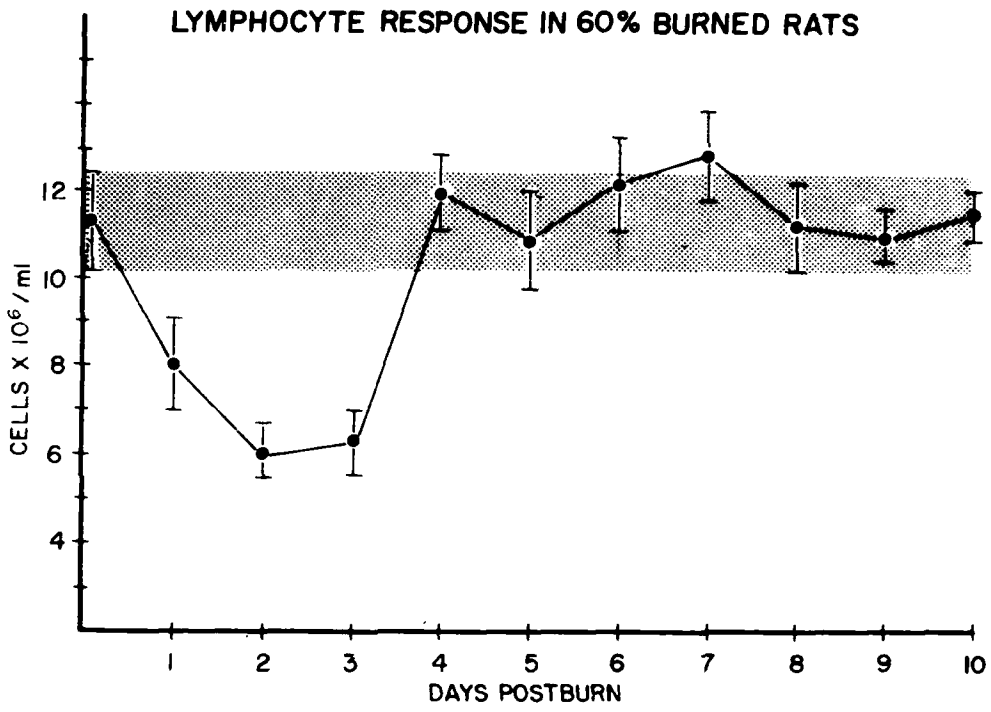

Fig. 3. Temporal effect of 60% burn injury of rats on the absolute lymphocyte count. Values represent serial means of six animals. Shaded area represents 2 standard errors of the preburn mean.

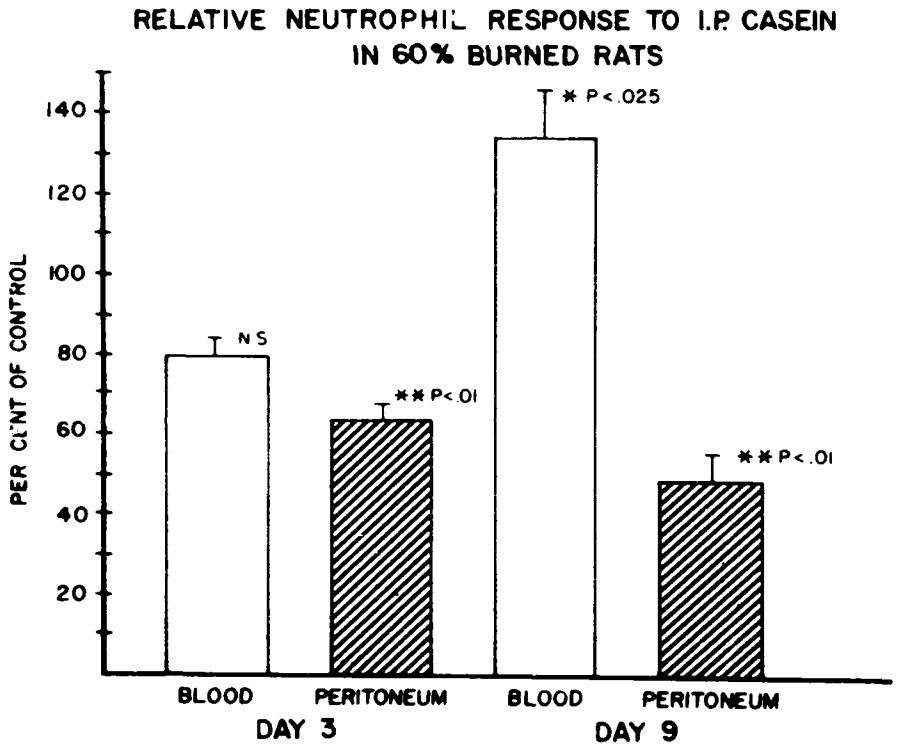

Fig. 4. Comparison of absolute neutrophil count and peritoneal neutrophil recovery following ip injection of casein in the 60% burned rat to the response of injected unburned rats. Burned animals were examined on the third and ninth days postburn.

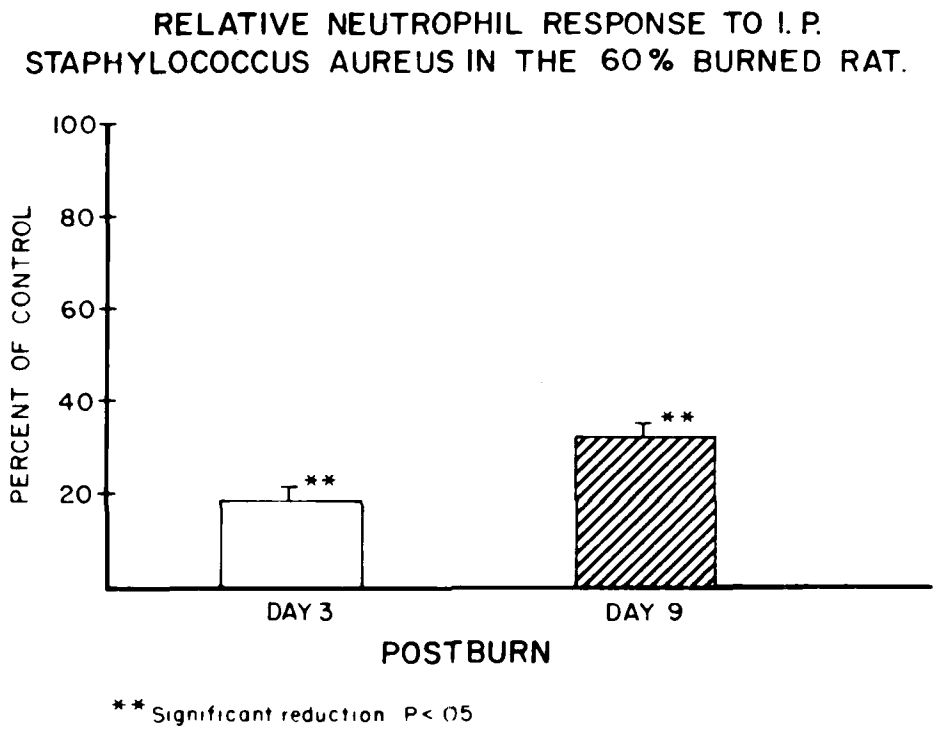

Fig. 5. Comparison of the peritoneal neutrophil recovery following ip Staphylococcus aureus between unburned and 60% burned rats.

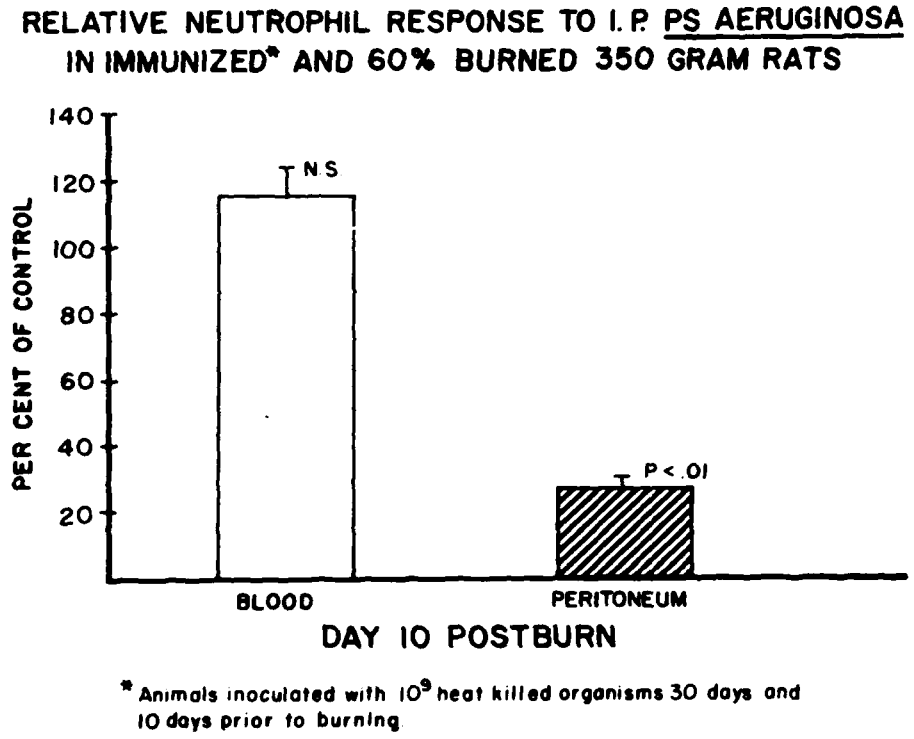

Fig. 6. Comparison of the peritoneal neutrophil recovery following ip Pseudomonas aeruginosa between immunized 60% burned rats and immunized unburned rats. Animals were examined 10 days following burn injury.

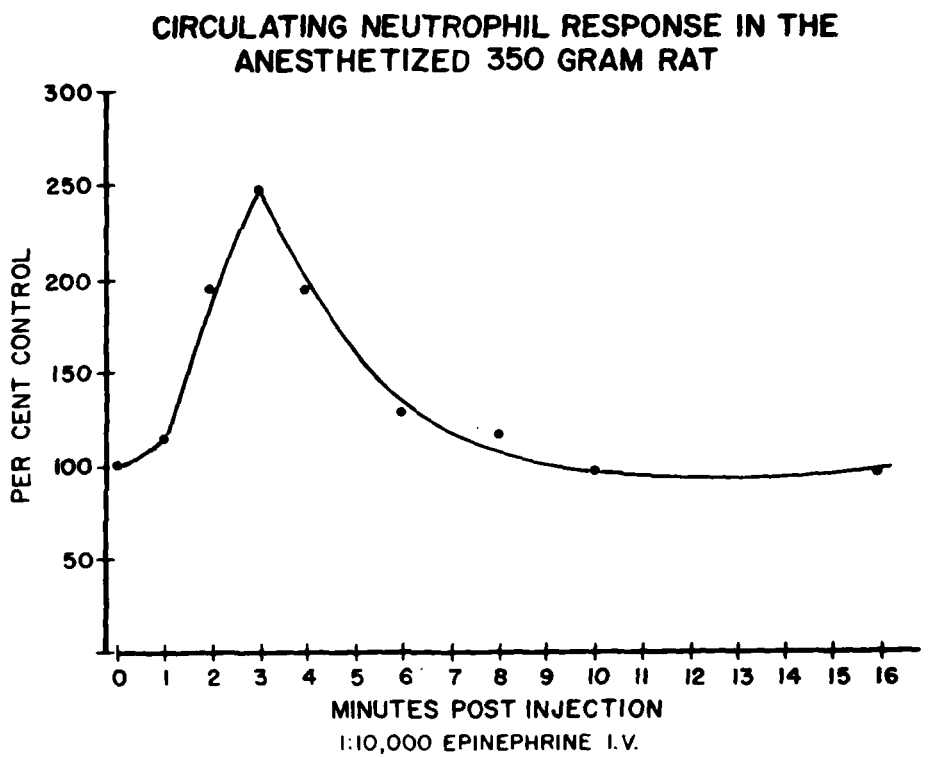

Fig. 7. Effect of 0.1 ml of 1/10,000 diluted iv injection on absolute neutrophil count of an anesthetized 350 gram rat.

neutrophil count to the preinjection value was taken as the proportion of the total peripheral neutrophil count that was marginated. The peak neutrophil response of normal (n = 5) and 9 day burned (n = 5) rats is shown in Table 1.

Table 1. Neutrophil Response to IV Epinephrine in 350 Gram Anesthetized Rats

	Preinjection neutrophil count/mm^3	Postinjection neutrophil count/mm^3	Percent increase
		Normal	
	2014	4988	148
	1040	3025	190
	1630	3915	140
	1206	3350	177
	1936	4290	122
Mean	1565	3914	155
		9-Day burned	
	3131	4052	29
	4781	5474	14
	3003	4298	43
	5161	6613	28
	3920	5368	37
Mean	3999	5261	30

Burned animals showed an elevated preinjection neutrophil count ($P < 0.01$). Both burned and normal rats showed neutrophil elevations postinjection ($P < 0.01$). Burned animals showed a larger than control total peripheral neutrophil count ($P < 0.05$). However, burned rats had a markedly reduced proportion of marginating cells as reflected by the difference in the percent increase between the two populations ($P < 0.001$).

The dose response relationship of normal rat neutrophil adherence to varying weights of nylon fiber is presented in Figure 8. Pooled blood from three normal rats was used in the experiment. A weight of 80 mg was selected for comparing burned and normal rat adherence. Burned rat and normal rat neutrophil adherence was compared on day 9 postburn. Each group contained six rats. As shown in Figure 9, burned rats had significantly reduced adherence to nylon fiber.

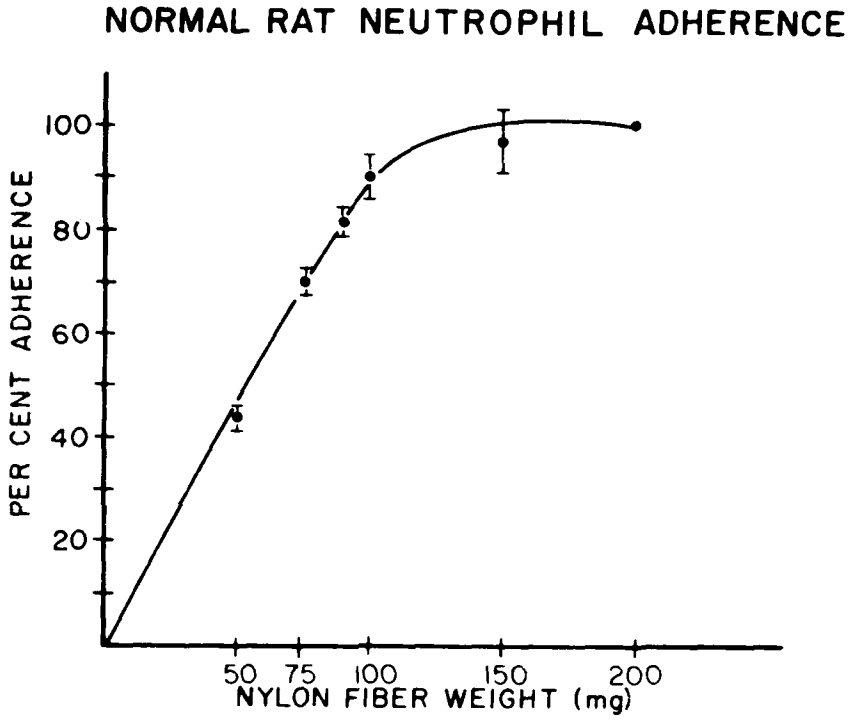

Fig. 8. Dose response relationship of normal rat neutrophil adherence to increasing weights of nylon fiber.

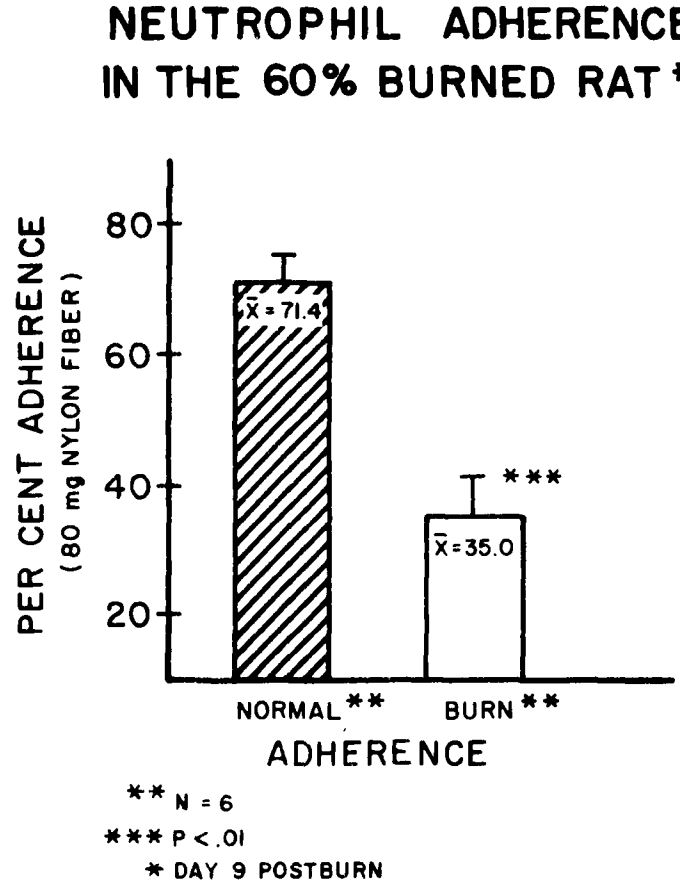

Fig. 9. Relative neutrophil adherence to 80 mg of nylon fiber between normal and 60% burned rats. Animals were compared on the ninth day postburn.

Serum corticosterone was measured in groups of five rats on the third and the ninth day postburn. Control rats had a mean of 27.31 ± 2.07 µg/100 ml. Burned rats showed 51.85 ± 2.30 µg/100 ml on day 3 postburn and 33.31 ± 1.18 µg/100 ml on day 9. Analysis of variance (Scheffe) showed the elevation on day 3 postburn to be significant ($P < 0.001$). Day 9 values, though suggesting elevation, were not significantly different from control.

In separate experiments, day 9 burned rats had markedly elevated plasma and urinary catecholamine levels ($P < 0.01$). Urine values ($n = 6$) were 0.363 ± 0.104 µg/day for controls and 2.27 ± 0.218 µg/day for burned rats. Plasma levels ($n = 5$) were 8,235 ± 259 pg/ml for controls and 16,634 ± 2,578 pg/ml for experimental animals.

Continual infusion of levamisole significantly increased the inflammatory response to ip Pseudomonas in burned rats (Fig. 10). At both doses, treated animals had significantly elevated cell counts when compared to burned or burned and sham-operated animals ($P < 0.01$). Levamisole treatment did not, however, return the responses to those of unburned animals.

DISCUSSION

Human and rat burn wound infections with Pseudomonas aeruginosa follow a very similar bacteriological and histopathological course (28). Basically, the infection is a progression of bacterial growth from colonization of nonviable surface tissue to massive accumulation of bacteria in the deeper eschar, with subsequent invasion of viable tissue and bacteremia. At autopsy, disseminated infections with metastatic lesions of the lungs, kidneys, spleen, liver and other organs are frequently observed. The similarity between human and experimental rat infection makes the rat burn model an excellent tool to investigate virulence mechanisms of Pseudomonas aeruginosa. Unlike mouse burn injection models used to demonstrate proposed virulence factors (29,30), the rat burn surface model requires expression of microbial aggressive properties needed to invade the host. Virulence factors may be missed or misinterpreted by injecting characterized strains beneath the burn

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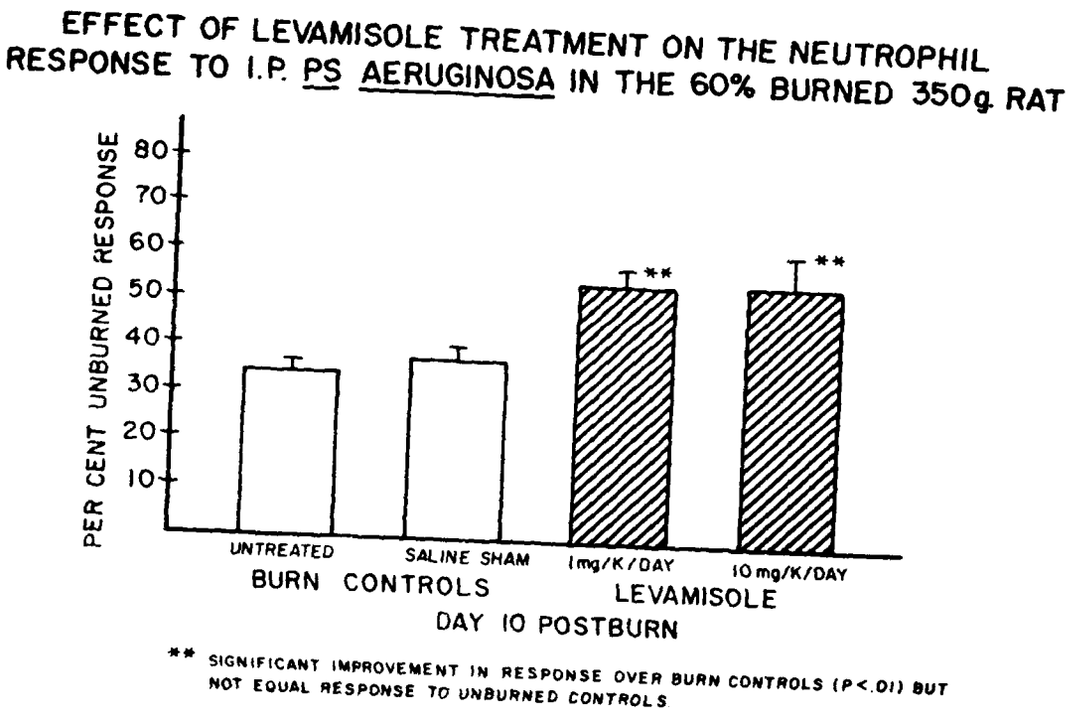

Fig. 10. Effect of levamisole on ip response of 60% burned rats to ip Pseudomonas aeruginosa injection. Animals were compared on the tenth day postburn.

surface (31).

The intent of this investigation was to use the rat model to examine the other side of the burn infection coin. The noninfected burned rat has been found to have decreased peritoneal inflammatory capacity. This finding could not be explained by decreased numbers of circulating cells or decreased complement activity. The presence of antibody did not normalize the response to ip injected killed Pseudomonas. Burned rats had a decreased proportion of marginated neutrophils and decreased neutrophil adherence to nylon fiber. This relationship is similar to that seen when demarginated human blood is examined for neutrophil adherence to nylon fiber (32). The elevation in the examined hormones of the burned rat may be responsible for the observed altered adherence. The partially restorative effect of levamisole supports this concept in that levamisole is known to reverse selected neutrophil dysfunctions that follow exposure to trauma-related hormones (33,34). If one assumes inflammation to be an important component in host defense against Pseudomonas, predictably the burned rat would be a susceptible host.

The metabolic alterations that follow burn injury are the most extreme seen in any hospitalized population (35). Burn patients demonstrate hypermetabolic, hypercatabolic, and hyperhemodynamic adaptations to burn stress (35-37). These responses are burn size related. Wilmore and others have explained the metabolic adaptation to be mediated by burn size related elevations in catacholamines

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and corticosteroids (38,39). It is interesting to consider in concert the dose response curves of burn size related hypermetabolism (38) and decreased probability of survival (40) and relate such to the concept that there are limits to survivable stress as proposed by Selye (41). Perhaps infection is a symptom of burn disease rather than the most easily explainable cause of death. It is hoped that the rat burn infection model may be used further to test this possibility and evaluate immunomodulatory therapies which may improve resistance in the chronically stressed host.

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PRESENTATIONS

McManus AT: Examination of altered host resistance in an animal model of burn trauma. 15th National Meeting of the Reticuloendothelial Society, 6-9 December 1978, Charleston, South Carolina.

McManus AT: Examination of neutrophil function in a rat model of decreased host resistance following burn trauma. Accepted for presentation, Symposium on Pseudomonas aeruginosa Infections, Walter Reed Army Medical Center, Washington, DC, 6 December 1979.

PUBLICATIONS

None.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OG 6978	79 10 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
78 10 01	D. CHANGE	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO. CODES:	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	078
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Procede with Security Classification Code): (U) Monitoring and Modification of The Metabolic and Physiologic Alterations Associated With 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
78 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 79	.8	15
FISCAL YEAR — CURRENT 80	.6	10

19. RESPONSIBLE DOD ORGANIZATION
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SOCIAL SECURITY ACCOUNT NUMBER

21. GENERAL USE: FOREIGN INTELLIGENCE NOT CONSIDERED

ASSOCIATE INVESTIGATORS
NAME:
NAME: DA

22. KEYWORDS (Procede EACH with Security Classification Code): (U) Pathogenesis; (U) Evaluation of therapy; (U) Patient profile; (U) Plasma; (U) Urine; (U) Enzymes; (U) Proteins; (U) Metabolites; (U) Rats

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

23. (U) To develop a profile of plasma/urine constitutents which accurately reflect the severity of the thermal trauma, the presence of infection, and the healing process so as to allow objective assessment of the efficacy of therapeutic measures and to further elucidate the pathophysiology of thermal injury with the ultimate goal of lessening morbidity and mortality due to severe thermal trauma as well as hastening convalescence.
24. (U) Animal and clinical studies will be run concomitantly when feasible. Animal studies, which can be rigorously controlled, will be used to test hypotheses and to expand upon the findings from patient studies. The rat burn model developed by Walker and Mason, suitably modified, will be the primary animal model employed. Initial patient studies will focus on patients who according to age and burn size, are deemed to have a 40-60% chance of survival.
25. (U) 7810 - 7909 (1) Zinc has been found to accumulate in the livers of burned and burned-infected rats greatly in excess of that lost from plasma and in proportion to the severity of stress, i.e., burned-infected > burned. This accumulation may be related to nitrogen redistribution from muscle and may be a means by which the body stores zinc for later use in synthetic, healing processes. (2) There appear to be at least two substances, one fluorescent, in perchloric acid filtrates of whole blood which reflect the presence of infection in burned rats. (3) Increases in plasma acute-phase proteins have been observed in a burned quadriplegic with additional increases evident during infectious episodes indicating that adrenergic stimuli are not responsible for all the metabolic sequelae of thermal injury and infection. (4) Thermal injury depresses the fasting whole blood ketone concentration in rats; infection superimposed on burn injury causes a further dimunition in ketonemia.

*Available to contractors upon originator's approval

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

ANNUAL PROGRESS REPORT

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: MONITORING AND MODIFICATION OF THE METABOLIC AND PHYSIOLOGIC ALTERATIONS ASSOCIATED WITH THERMAL INJURY

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

1 October 1978 - 30 September 1979

Investigators:

Michael C. Powanda, PhD, Major, MSC
John Dubois, B.S.
Calvin R. Kennedy, B.S.
Haywood E. Murray
Ysidro Villarreal, B.S.
Harrel L. Walker, M.S.

Reports Control Symbol MEDDH-288(R1)

Unclassified

ABSTRACT

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REPORT TITLE: MONITORING AND MODIFICATION OF THE METABOLIC AND PHYSIOLOGIC ALTERATIONS ASSOCIATED WITH THERMAL INJURY

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Hypozincemia and hepatic sequestration of zinc occur in rats receiving a non-lethal scald injury as well as in rats with a lethal infection superimposed on the burn injury, apparently in proportion to the severity of the injury. The amount of zinc accumulated by the liver is far in excess of that lost from serum and may be related to altered nitrogen distribution and reflect the need to store zinc for later use in wound healing.

Burn injury ± infection in rats causes minor alterations in the plasma concentration of glycerol, cholestrol and triglycerides, but a marked reduction in the whole blood fasting concentration of β-hydroxybutyrate which may indicate increased utilization of Ketone bodies or, less likely, reduced synthesis of ketone bodies.

There appear to be two substances in perchloric acid filtrates of blood, one fluorescent in the plasma and one associated with the cellular components of blood, which reflect the presence of infection in burned rats. The cell associated substance does not change with the extent of injury but only increases during infection. The fluorescent plasma substance is greater in burned-infected, than in burned rats. If applicable to patients these substances will aid in the diagnosis of infection in burn patients and the decision to use antibiotics.

In a burned patient with preexisting paralysis, post injury increase in specific serum proteins indicative of inflammatory stress

have been noted with a further increase occurring during infection. These findings indicate that the neurohormonal stimuli are not the sole mediators of post injury metabolic change.

Thermal injury	Plasma and tissue zinc
Infection	Plasma proteins
Patients	Indices of infection
Rats	Lipid metabolism

MONITORING AND MODIFICATION OF THE METABOLIC AND PHYSIOLOGIC ALTERATIONS ASSOCIATED WITH THERMAL INJURY

TISSUE ZINC ALTERATIONS

Burns covering 30% or more of a total body surface place a severe metabolic demand on the host, a demand that often exceeds even that imposed by some infections (1). Though burns elicit alterations in amino acid, trace metal and protein metabolism akin to those observed during infection, injury and inflammation (1-3), there is a recent report by Oh, et al which indicates that burns might differ from other inflammatory stresses in that zinc did not accumulate in the liver despite a significant decrease in serum zinc (4). The absence of zinc sequestration by the liver in burned animals, if true, might be the result of leakage from the wound or a differential deposition within the wound itself (5), perhaps carried along on the albumin which accumulates in the wound area (3). The latter possibility is intriguing in that during myocardial infarction, zinc appears to accumulate preferentially at the site of the damage in organelles associated with biosynthesis (6,7). Finally, there is evidence, in that in zinc deficient animals zinc aids in wound healing (8).

Another reason to reinvestigate whether hepatic zinc accumulation occurred in burned animals is that hepatic zinc accumulation has

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always preceded or accompanied acute-phase globulin synthesis during inflammation and infection, even in zinc deficient animals (9-11). Since burned rats are known to exhibit increased synthesis of acute-phase globulins (2), the absence of enhanced zinc uptake by the liver would indicate that this apparent association between zinc influx to the liver and increased acute phase globulin synthesis was indeed merely coincidental and neither essential nor causal.

Infection is often a complication of burn injury and infection per se is accompanied by both hypozincemia and enhanced hepatic uptake of zinc (10,11). We therefore also studied burned-infected rats to ascertain whether they would exhibit the pattern of zinc redistribution typical of infection or that said to be associated with burns, i.e. hypozincemia without hepatic sequestration of zinc. Reduced food intake is common in infections, but not always a component of burn injury, thus we examined the effect of fasting as well as ad libitum food intake on zinc distribution in burned and burned-infected rats.

MATERIALS AND METHODS

Male albino rats (180-200g) were used in all studies (Holtzman Co., Madison, Wisconsin). In the first study there were three groups of rats: control, burned, and burned-infected; food was available ad libitum to all groups. Eight rats per group were killed on days 1,2,3,4, and 7 postburn. In the second study there were four groups of rats: fed control, fasted control, fasted burned, and fasted, burned-infected; food was removed from the last three groups just prior to scalding and/or infection.

A 30% total body surface, full thickness burn of the dorsum was achieved by immersing anesthetized, shaved rats, which had been placed in a mold to define the extent of injury, in boiling water for 10 seconds (12). No resuscitation was carried out. Infection was accomplished by placing 1 ml of a 16-hour broth culture of Pseudomonas aeruginosa on the burn followed by swabbing to distribute

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the organisms over the entire burned surface. A clinical isolate of P. aeruginosa was used, strain 12-4-4, and the culture was adjusted to yield 10^8 organisms and inoculation of the animals took place within 2 hours of burning.

At scheduled intervals the rats were anesthetized by the intraperitoneal injection of 0.5-1 mg sodium pentobarbital/25 g body weight, the body cavity opened and bled from the hepatic vein. The livers were then perfused with physiologic saline and samples of liver and kidneys were taken for zinc determinations. The tissues were solubilized by the addition of an equal volume of 25% tetramethylammonium hydroxide (13) followed by incubation in 37^{o} in a shaking water bath until the particulate matter had entirely dissolved. Serum and tissue zinc was determined by atomic absorption spectrophotometry. Serum albumin was measured with bromcresol green reagent (14). Analysis of variance was used to assess statistical significance.

RESULTS

In the first study where food was provided ad libitum, burned rats exhibited a significant decrease in serum zinc concentration three days postburn and this hypozincemia persisted through day 7. Burned-infected rats displayed significant hypozincemia on day 2 with an additional diminution on day 3 and an even further decline on day 7. From days 3 through 7, the burned-infected rats had a significantly lower serum zinc concentration than burned rats (Fig. 1). Despite the fact that the serum zinc content of burned rats remained within the normal range for the first two days postburn, there was a 50% increase in liver zinc concentration on day 1 in these animals and the liver zinc content remained significantly elevated through day 7. Burned-infected rats displayed a similar degree of zinc sequestration by the liver as burned rats on day 1, but continued to accumulate zinc within the liver up to 120% above control values by day 7 (Fig. 1).

Though all groups were initially closely matched for weight, burned rats did not gain as much weight as controls, even with food available, and burned-infected rats lost weight during the week of the study. There was no significant difference in total liver weight among these groups (Table 1). Using these data one can estimate the amount of zinc ost from the plasma and gained by the liver. Using the day 7 values to estimate total serum or liver zinc, in the first

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case one simply multiplies the total body weight times 0.035, the factor representing the proportion of body weight which is serum, times the zinc content per ml of serum, e.g., 264 x 0.035 x 1.4 = 13 µg; in the case of liver, one merely multiplies total liver weight times the value per gram of liver, e.g., 12.6 x 12.2 = 154 µg. It is clear that the liver has accumulated zinc in great excess of that lost by the serum (Table 1).

Table 1. Body and Liver Weight, Plasma and Liver Zinc Concentration One Week Postburn

	Weight (g) a			Zinc (µg)		Liver Gain-Plasma Loss
	Body		Liver	Plasma	Liver	
	Initial	Final	Total	Total (Δ)	Total (Δ)	(µg)
Control	188 ± 2	264 ± 3	12.63 ±0.37	13	154	
Burned	187 ± 2	213 ± 7	11.22 ±0.43	8 (-5)	184 (+30)	+ 25
Burned-Infected	190 ± 2	155 ± 4	10.95 ±0.59	2 (-11)	330 (+176)	+165

a = Mean ± SEM

To obviate differences in food intake caused by burning and/or infection, rats were fasted following burning and/or seeding with microorganisms. Fasting of itself results in a decrease in serum zinc on day 2, with an additional decrement on day 5. Burned rats incurred a 33% greater fall in plasma zinc concentration as compared with fasted rats on day 1 with little change thereafter. Burned-infected rats not only had a one-third decline in serum zinc concentration on day 1, but the serum zinc concentration continued to plummet in a linear fashion to a low of 18 µg/dl by day 5 (Fig. 2).

Thermal injury with or without infection superimposed, induced only slight, generally, nonsignificant increases in kidney zinc

concentration as compared to fasting. Fasting itself resulted in a 15-30% increase in kidney zinc concentration which was statistically significant from the second day on (Fig 2).

Since about 50-70% of zinc in serum is bound to albumin, serum albumin was measured by using bromcresol green dye. Serum albumin was not depressed by fasting; burned rats had a somewhat lower serum albumin concentration than fasted rats on days 2,3, and 4. In contrast, burned-infected rats had significantly depressed serum albumin concentration on day 1 which further decreased until by day 5 their albumin concentration was little more than 2 g/dl (Fig 2).

DISCUSSION

The present data clearly indicate that nonfatal thermal injury in rats not only induces hypozincemia, but also results in an accumulation of zinc within the liver. The difference between the present data and that of Oh, et al (4) cannot be resolved on the basis of diet, since both fasted and fed rats displayed hepatic zinc sequestration. Nor is the dissimilitude merely a matter of zinc accumulating in the liver in some form other than metallothionein, since Oh, et al (4) measured both metallothionein and whole tissue zinc concentration and neither increased as a result of the burn. Resolution of the disparity in findings may have to do with the size of the lesion, in Oh's study the burned area was 2 cm in diameter (about 1% total body surface), while in the present study, the whole back was scalded (30% total body surface). Conceivably, one could have comparable depressions in serum zinc concentration, yet markedly dissimilar degrees of sequestration of zinc by the liver, for example, fasted rats have the same serum zinc concentration as burned, fasted rats on days 5 and 6, yet significantly different hepatic zinc contents. The present data also indicate that the converse is at times possible, i.e., dissimilar serum zinc concentrations and yet comparable accumulations of zinc within the liver, as was observed on day 2 when the rats were allowed food ad libitum (Fig 1) and in the fed versus fasted rats (Fig 2).

It is clear that the amount of zinc accumulated by the liver is far in excess of that lost by the serum. It appears that hepatic sequestration reflects the degree of injury and/or severity of infection. That fasted rats sequester more zinc than fed animals is puzzling, yet may indicate that zinc is of such import either in protecting against disease and/or injury, or in repairing the damage thereof (15), that the body goes to great lengths to store it.

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Normally some 40-50% of the serum concentration of zinc is an integral part of the $alpha_2$-macroglobulin (16,17) a protease inhibitor, and so unless this protein decreases in concentration, the lowest serum zinc concentration one would expect to find if all the labile zinc, i.e., that bound to albumin and amino acids, were removed from circulation is 60-75 µg/dl. Burned-infected rats, whether fed or fasted, have serum zinc concentrations in the agonal stage which are so low as to suggest that the $alpha_2$-macroglobulin concentration has decreased in these animals. $Alpha_2$-macroglobulin turnover has, in fact, been shown to be increased even in patients with small burns (10-15%)(18). Moreover a decrease in $alpha_2$-macroglobulin would not be unlikely considering that the infection was with a microorganism whose virulence may in part depend on elaboration of proteases (19) and $alpha_2$-macroglobulin and $alpha_1$-antitrypsin interact or eliminate released proteolytic enzymes (16) with the result that $alpha_2$-macroglobulin is consumed.

Since liver accumulates more zinc than serum loses, the source of the zinc, especially in fasted animals, is in question. Muscle is a most likely source since marked muscle wasting occurs in both injury and infection (1). If the 40% weight loss that burned-infected rats experience within 5 days were primarily a loss of muscle tissue and if one accepts an average muscle zinc content of 6-8 µg/g (20), then sufficient zinc would be made available (480-640 µg) to account for liver sequestration as well as the excess urinary excretion which is observed following thermal trauma (21,22). It is interesting that

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Davies and Fell found a highly significant correlation between the amounts of zinc and creatinine excreted (22) in light of the accumulation of zinc by the liver. If Davies and Fell's finds are not merely fortuitous, but truly represent a constant stoichiometric release of zinc and nitrogen from peripheral tissue, then the accumulation of zinc by the liver may yield an estimate of nitrogen released from muscle which is available for recycling or reutilization, perhaps in the form of plasma proteins (15,23). Assuming 7.25 µg zinc and 33 mg nitrogen per gram of muscle tissue and using the data in Table 1, burned rats would have 136 mg of nitrogen available for reutilization or enough to replace the total plasma content twice over while burned-infected rats would have 801 mg of nitrogen available for recycling or enough for almost a 12-fold replacement of circulating plasma proteins. If one assumes a value of 30 mg zinc and 33 mg nitrogen per gram of muscle (22) then the available nitrogen would be decreased by a factor of 4. Interestingly enough, these estimates are compatible with data on incorporation of radiolabel into total serum protein (23) and increases in acute phase globulin synthesis/turnover in burns and infection (2,3,23-26). Finally, these data provide additional circumstantial evidence that zinc and nitrogen redistribution during injury and infection may indeed be coupled. In patients with gastro-intestinal disease who are receiving totalparenteral nutrition there does appear to be a link between the metabolism of protein and that of zinc (27).

PART II:

Tissue fat is the major energy reservoir in man comprising about 85% of the total available calories (1). Despite the fact that RQ values and calculations indicate that endogenous fat is metabolized during thermal injury and injury in general (1,28), our understanding

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25. Dobryszycka W, Zeineh R, Ebroon E, Kurkel JC: Metabolism of plasma proteins in injury states. II. Incorporation of [^{14}C] of glucosamine and [^{35}S] methionine into human and canine haptoglobin. Clin Sci 36:231-240, 1969.

26. Kurkel JC, Zeineh R, Dobryszycka, Pollitt J, Stone N: Metabolism of plasma proteins in injury states. I. Turnover rates of fibrinogen in burned patients labelled with [^{35}S] methionine. Clin Sci 36:221-230, 1969.

27. Wolman SL, Anderson GH, Marliss EB, Jeejeebhoy KN: Zinc in total parenteral nutrition: requirements and metabolic effects. Gastroenterology 76:458-467, 1979.

28. Kinney JM, Duke JH Jr, Long CL, Gump FE: Tissue fuel and weight loss after injury. J Clin Path 23 Suppl 65-72.

of lipid metabolism during injury is limited. Though exogenous fat by itself appears not to spare nitrogen (29), in conjunction with amino acids it reduces body wasting and favors the synthesis of plasma proteins (30) and this may be a valuable source of calories in the nutrition of the seriously ill.

Our preliminary studies of lipid metabolism in burned (30% total body surface) and burned-infected (P. aeruginosa) rats indicated the following pertubations in lipid metabolism.

(a) In regard to beta-hydroxybutyrate, the major ketone body produced in rats, fasting elicits a marked increase in the whole blood concentration of this substance; burn injury ± infection causes a reduction in the ketonemia on days 2 and 4 post burn. (Fig 3)(top left).

(b) In regard to plasma triglycerides the major storage and transport form of fat, fasting for 2 days decreases their concentration equal to that of injury ± infection. By 4 days the triglyceride level returns to control values in burned rats but is still depressed in burned-infected rats. Fasting appears to act synergistically in the latter group (fig 3)(bottom left).

(c) In regard to glycerol, a product of triglyceride degradation and a potential gluconeogenic precursor, there is little relationship of whole blood glycerol levels to injury ± infection except in the fasted, burned infected rats on day 4 (Fig 3)(top right).

(d) Initially cholesterol levels are elevated by injury and in the case of fed rats, further increased by superimposed infection. However, by four days there is only a slight elevation in plasma cholesterol induced by burn injury while fasted, burned-infected rats display a decrease (Fig 3)(bottom right).

PART III:

Massive injury such as that produced by burns compromises a soldier's resistance to infection. However, due to the loss of the skin barrier, it becomes more difficult to determine whether a person is truly infected or merely has colonized wounds. This distinction is not trivial

29. Long JM III, Wilmore DW, Mason AD Jr, Pruitt BA Jr: Effect of carbohhydrate and fat intake on nitrogen excretion during total intravenous feeding. Ann of Surg 185:417-422, 1977.

30. Wnnemacher RW Jr, Kaminski MV, Neufeld HA, Dinterman RE, Bostian KA, Hadick CL: Protein-sparing therapy during Pneumocosal Infection in Resus monkeys. J Parenteral and Enteral Nutr 2:507-518, 1978.

in that many antibiotics have toxic side effects and should not be used unnecessarily. A rapid, reliable indicator of actual infection would aid in the decision to use antibiotics.

In the course of analyzing perchloric acid (PCA) extracts of whole blood from burned and burned infected rats for various metabolites, Mr. John Dubois noted that the filtrates from burned-infected rats possessed significantly greater background fluorescence than those from either burned or control rats. A like observation has previously been made in regard to samples from seriously ill patients (31). The extracts from burned-infected rats also had a pinkish cast. Initial studies indicated that 398 nm might be an appropriate wavelength to monitor absorption and 420 nm to detect emission. Fig. 4 depicts the increase in light absorption that occurs with time in PCA whole blood filtrates from burned (30%) infected (10^8 P. aeruginosa) rats. Thermal injury of itself only elicited a modest, transient, albeit significant increase on day 3. Fig. 5 shows the change in background or native fluorescence observed in PCA whole blood extracts. Both burned and burned-infected rats exhibit a decrease on day 2, but by day 3 only the samples from the burned infected rats showed a significant increase. Increased burned size (60% vs 30%) does not significantly increase light absorption at 398 nm and only slightly enhances fluorescence at 420 (Table 2). The increase in both absorption and fluorescence occurs

Table 2. The Effect of Burned Size on Light Absorption Emission of Perchloric Acid Whole Blood Filtrates

	ABSORPTION 398 nm		EMISSION 420 nm	
	Day 4	Day 7	Day 4	Day 7
CONTROL	0.055 ±0.005	0.072 ±0.003	10.0 ±1.0	6.9 ±0.4
BURNED 30%	0.048 ±0.005	0.078 ±0.012	9.3 ±0.4	7.0 ±0.3
BURNED 60%	0.046 ±0.008	0.066 ±0.013	11.8 ±1.4	8.8 ±0.7
BURNED 30%, INFECTED	0.236 ±0.010	0.349 ±0.036	17.9 ±1.0	13.0 ±1.1

Mean ± SEM, n = 6

31. Lloyd B, Burrin J, Smythe P, Alberti KGMM: Enzymic fluorometric continuous-flow assays for blood glucose, lactate, pyruvate, alanine, glycerol and 3-hydroxybutyrate. Clin Chem 24:1724-1729, 1978.

when the rats are infected with Proteus mirabilis as well as with P. aeruginosa (Table 3) suggesting that the effect is independent of etiologic agent.

Table 3. Absorption and Fluorescence of Perchloric Acid Extracts of Whole Blood

		OPTICAL DENSITY at 398 nm	FLUORESCENCE UNITS at 420 nm
(10)	CONTROL	0.045 ± 0.002	14.6 ± 0.5
(10)	BURNED, 30%, 72 hrs	0.049 ± 0.009	22.3 ± 2.3^{a}
(14)	BURNED, 30% 96 hrs INFECTED, P. AERUGINOSA	$0.210 \pm 0.010^{a,b}$	$65.1 \pm 6.6^{a,b}$
(8)	BURNED, 30%, 48 hrs	$0.129 \pm 0.011^{a,b,c}$	$59.1 \pm 6.9^{a,b}$

Mean ± a = $p < 0.01$ vs control
b = $p < 0.01$ vs burned
c = $p < 0.01$ vs burned, P. aeruginosa

The fluorescent factor appears to be localized in the plasma (Table 4) while the substance or substances which absorb light at

Table 4. Light Emission at 420 nm of Perchloric Acid Filtrates

	WHOLE BLOOD	PLASMA	CELLS
CONTROL	10.0 ±1.0	10.3 ±1.5	4.8 ±0.5
BURNED	9.3 ±0.4	11.4 ±0.9	4.4 ±0.3
BURNED, INFECTED	17.9 ±1.0	24.3 ±1.8	4.4 ±0.5

Mean SEM, N = 6

1 ml of whole blood or 1 ml of plasma or ½ ml of packed red cells plus ½ saline were precipitated with 4 ml of 0.8 m perchloric acid. The samples were read against a 0.5 m perchloric acid blank, excitation 355 nm.

418 nm are not but seem associated with the cellular components of blood. Additional evidence that there are at least 2 distinct substances is the fact that the pattern of response differs (compare figure 4 and 5) and that when a linear regression was attempted using the individual data from the burned infected rats whose mean values are shown in Table 3 an R^2 of 0.017 was found.

Work is in progress to better characterize these potential biochemical indices of infection and assess their applicability to patient care.

PART IV:

Alterations in plasma proteins have been documented in burned patients and animals (2,3,24-26). Additional studies on the regulation and metabolism of plasma proteins are warranted (1) to complement the amino acid metabolism studies carried by Wilmore and Aulick (32) so as to assess how much of the nitrogen released from muscle tissue is recycled as plasma proteins and (2) because there are indications that plasma proteins may be of value to the host in defense against disease and the repair of impaired tissue (23).

At present quantification of plasma proteins in samples from a trans-hepatic catherization study is underway in an attempt to assess turnover rates of specific proteins and quantify the amount of nitrogen incorporated into these proteins. Not all these data have been accumulated as yet.

A quadriplegic with a 22% total body surface burn (19% 3rd degree) had an uneventful course of recovery for 1½ months during which time acute phase proteins such as α_1-antitrypsin, haptoglobin and α_1-acid glycoprotein were increased in concentration. Moreover, during a transient episode of infection on or about day 52 post burn and in response to an exploratory laparotomy and appendectomy on day 62 post burn, α_1-antitrypsin and α_1-acid glycoprotein displayed transient increments in concentration and C-reactive protein markedly increased (Fig. 6). This patient did not respond to infection or surgery with fever but did display leucocytosis and hyperglycemia during each episode. These data suggest that though the sympathetic nervous system appears to be involved in inducing the hypermetabolism associated with major injury, not all of the metabolic and physiologic sequelae of injury/infection are adrenergically regulated.

32. Wilmore DW, Aulick LH. Metabolic changes in burned patients. Surg Clin N.A. 58:1173-1187, 1978.

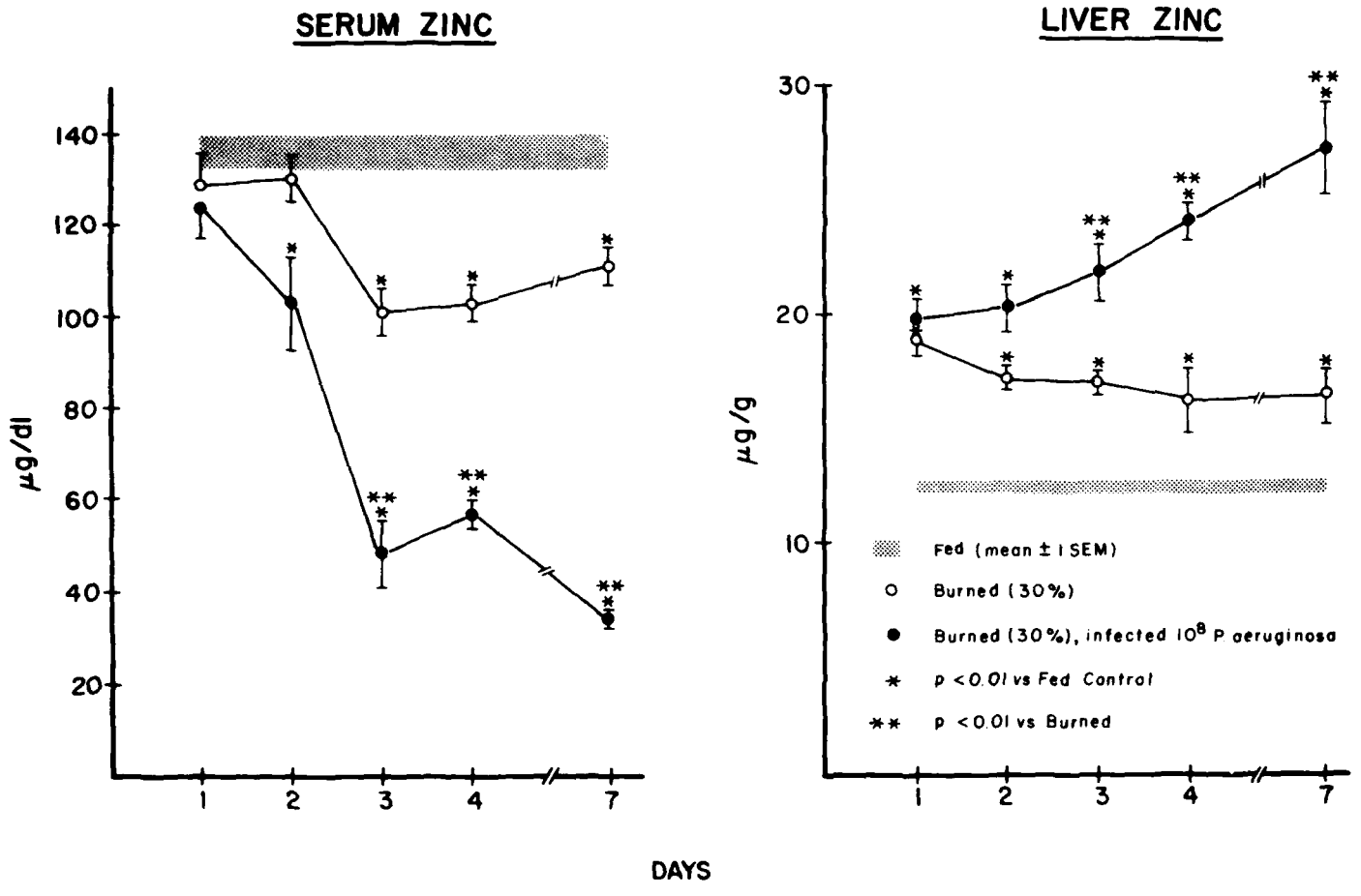


Fig. 1. Serum and zinc concentrations in burned and burned-infected rats given food ad libitum.

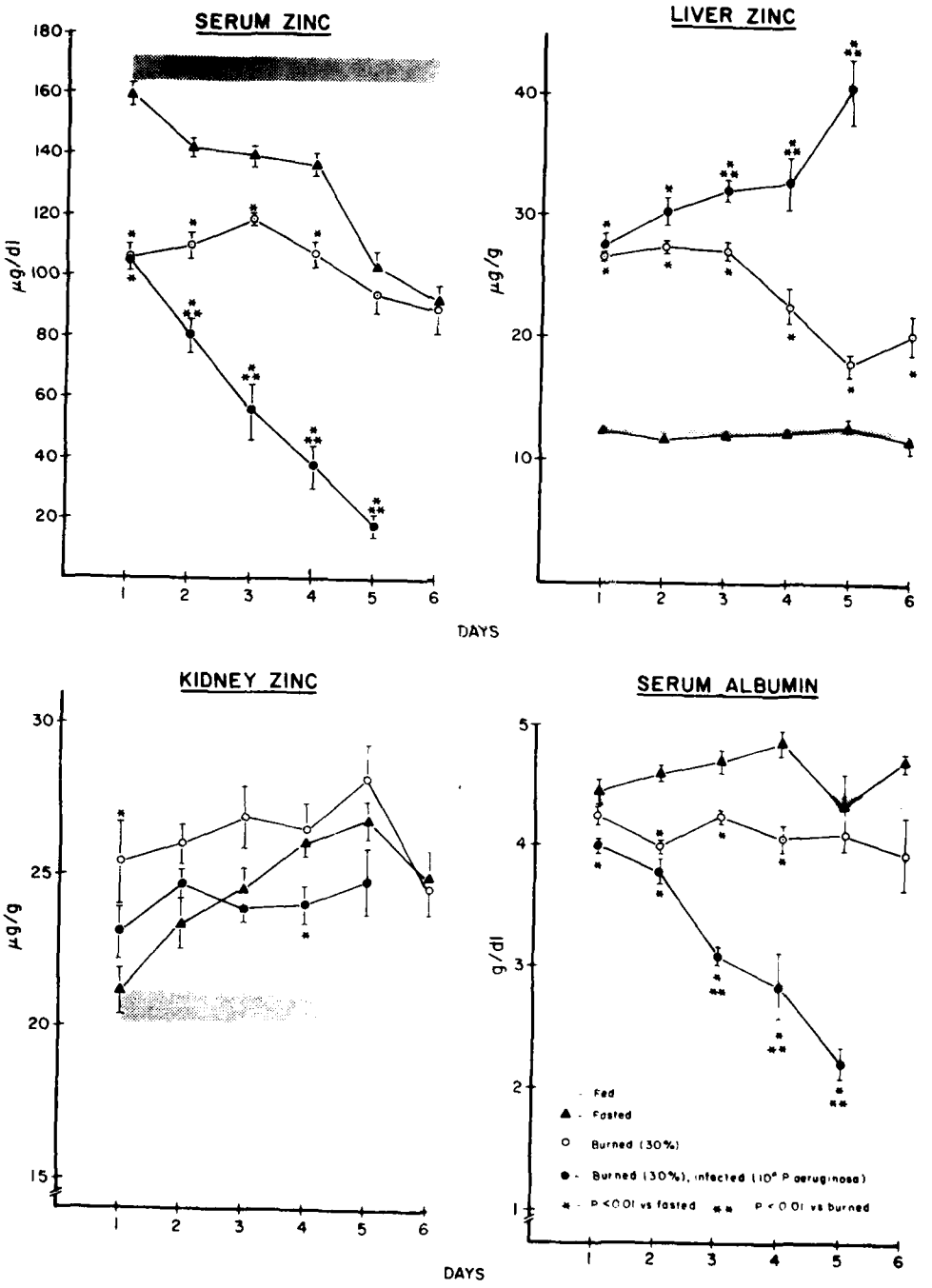


Fig. 2. The effect of fasting on serum, liver and kidney zinc concentrations and serum albumin in burned and burned-infected rats.

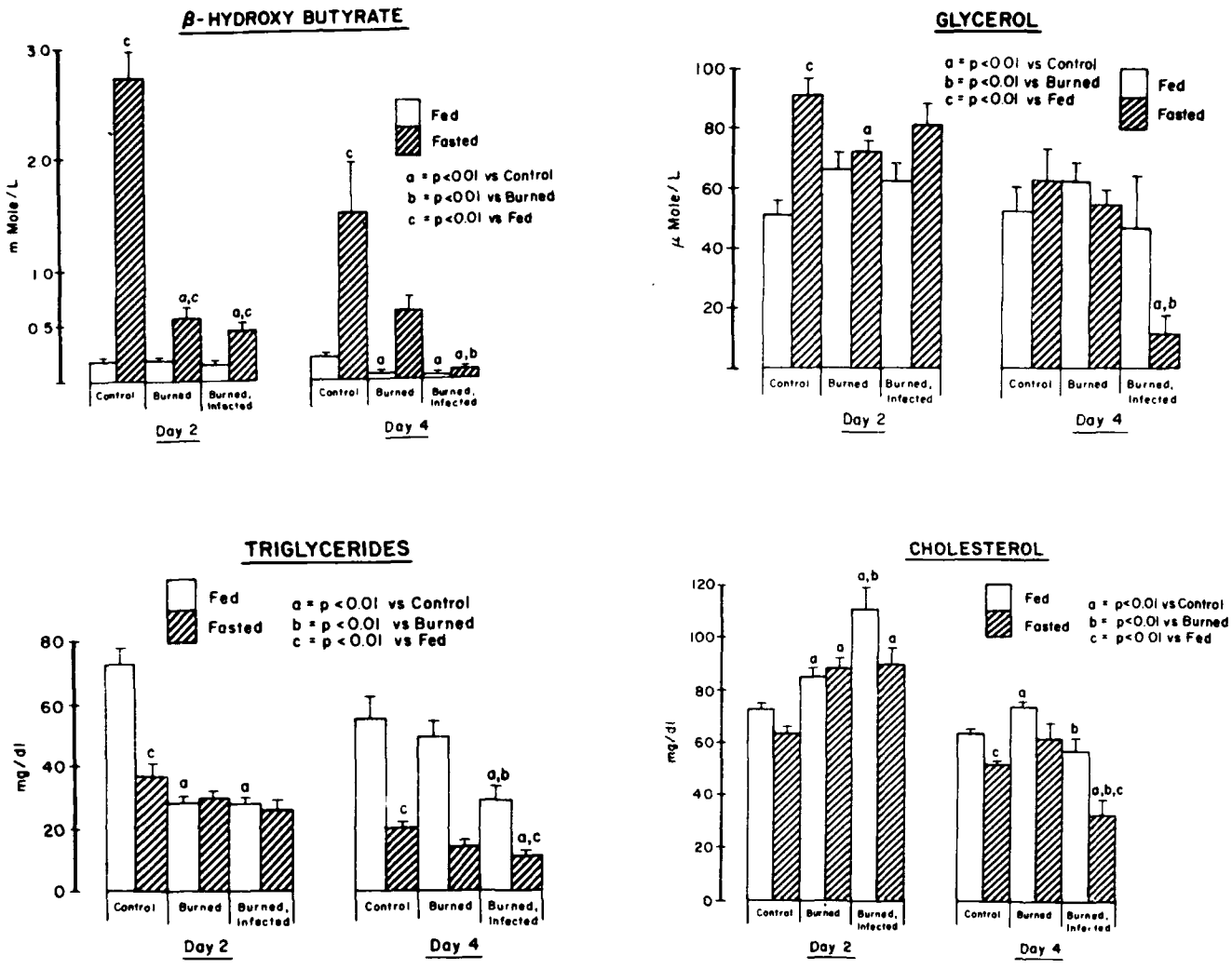
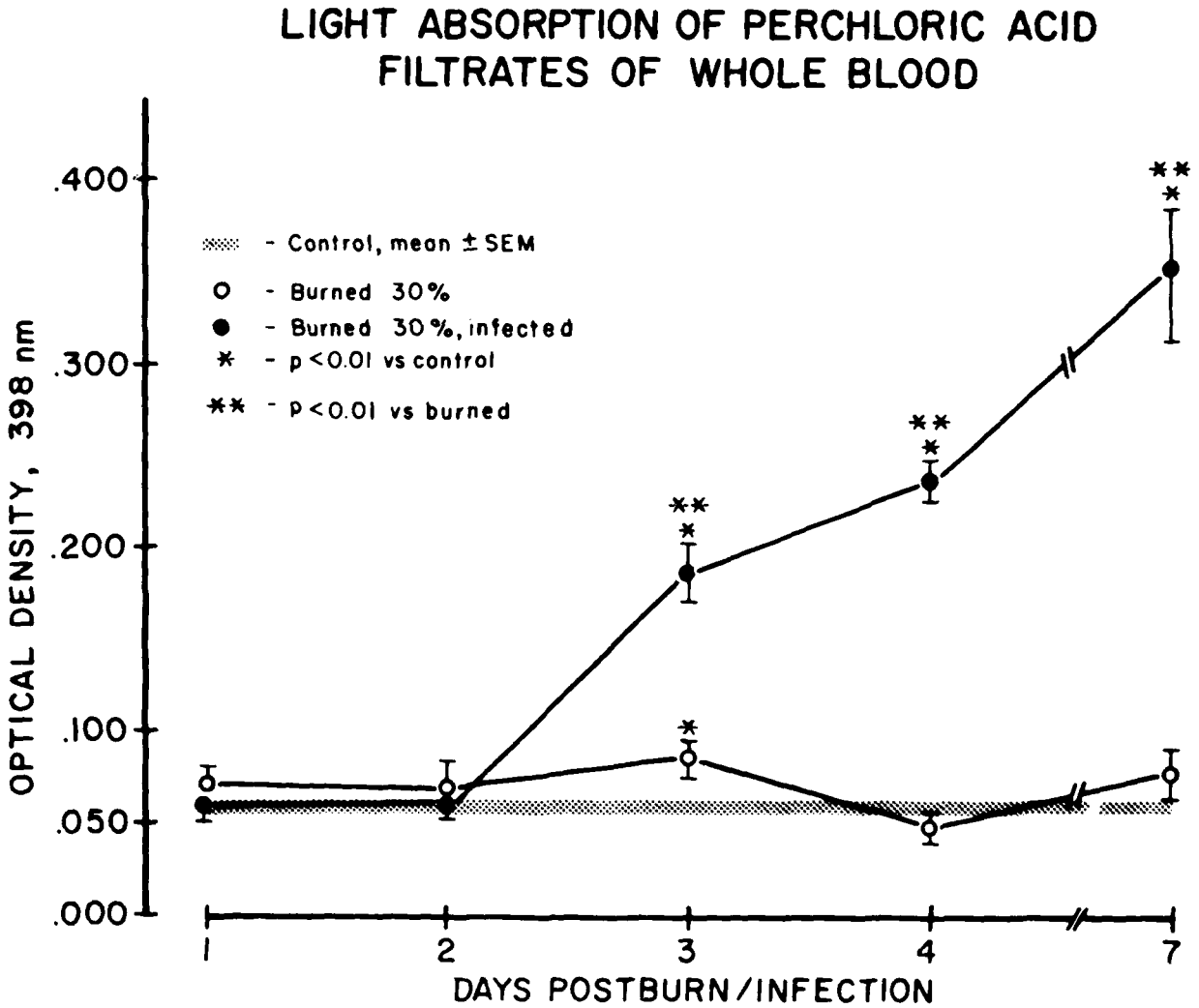


Fig. 3. Alterations in whole blood β-hydroxybutyrate and glycerol and in plasma triglycerides and cholesterol as a function of time after injury ± infection and availability of food.



337

Fig. 4. Variation in O.D. @ 398 of perchloric acid filtrates of whole blood from burned and burned infected rats.

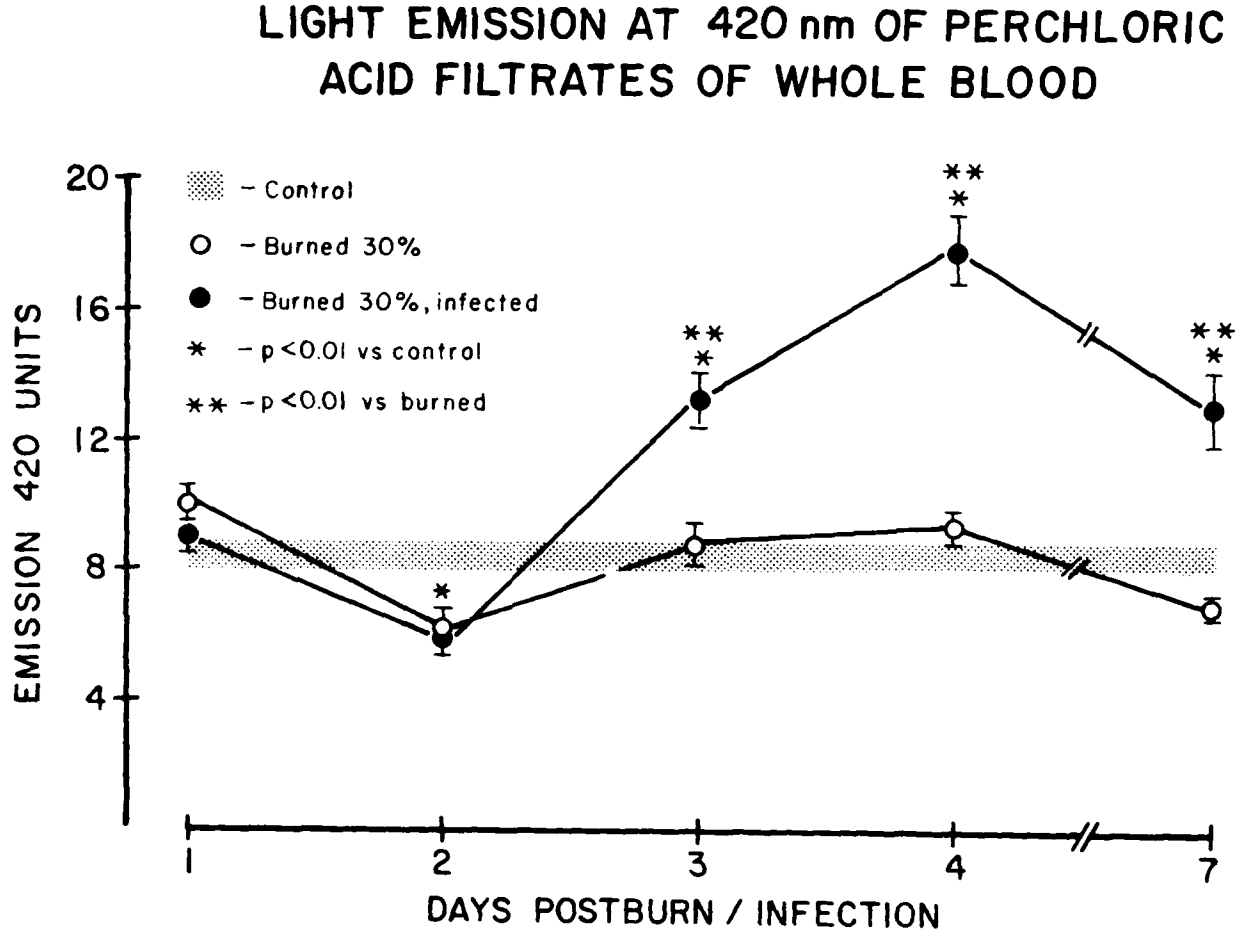


Fig. 5. Fluorescence of perchloric and filtrates of whole blood from burned and burned infected rats.

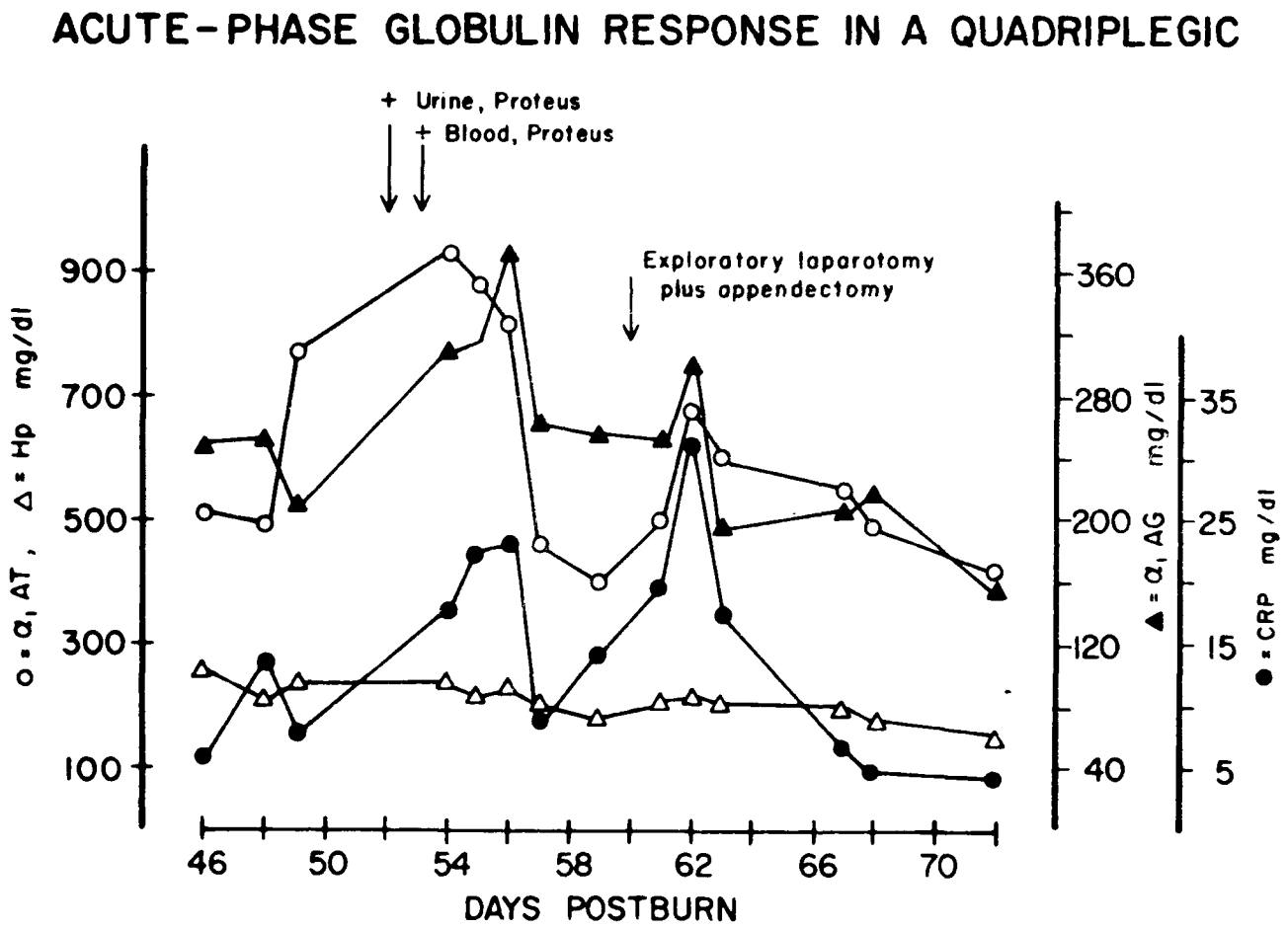


Fig. 6. Acute-phase globulin response in a quadriplegic.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OG 1845	79 10 02	DD DR&E AR(A)6

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
79 10 01	D. CHANGE	U	U	NA	NL	[X] YES [ ] NO	A. WORK UNIT

10. NO. CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	075
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code)
(U) Micromethod For Assessment of Serum Opsonic Capacity in The Burned Patient (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS
003500 Clinical Medicine 002300 Biochemistry

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
79 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 79	.4	11
FISCAL YEAR CURRENT 80	.5	17

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

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) PMN Leukocytes; (U) Chemiluminescence; (U) Opsonization; (U) Immunoglobulins; (U) Complement; (U) Burn injury

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

25. (U) The nonspecific opsonic capacity of sera from patients following burn injury will be compared to normal control sera. Qualification of opsonic capacity will be based upon the rate and magnitude of oxidative microbicidal activation as measured by amplified chemiluminescence using a set number of functional polymorphonuclear leukocytes (PMN) challenged with a set concentration of either zymosan or bacteria (Staphylococcus aureus or Pseudomonas aeruginosa). By holding zymosan and PMN leukocyte number constant, chemiluminescent activity will reflect the opsonic activity of sera.

24. (U) These functional measurements will be correlated with immunologic data, such as serum complement and immunoglobulin, and quantified by immunoelectrophoretic and immunodiffusion techniques.

25. (U) 7906 - 7909 The chemiluminescence assay has been modified to enhance sensitivity and to permit detection of low levels of opsonic activity. The assay has been established for quantifying both classical and alternate pathways of complement activation and specific antibody antigen reactions. Collection of serum from burn patients has begun.

*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

PROGRESS REPORT

PROJECT NO. 3A161101A91C, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: MICROMETHOD FOR ASSESSMENT OF SERUM OPSONIC CAPACITY IN THE BURNED PATIENT

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

1 June 1979 - 30 September 1979

Investigators:

Robert C. Allen, M.D., PhD, CPT, MC
Basil A. Pruitt, Jr., M.D., COL, MC

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3A161101A91C, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: MICROMETHOD FOR ASSESSMENT OF SERUM OPSONIC CAPACITY IN THE BURNED PATIENT

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

Period covered in this report: 1 June 1979 - 30 September 1979

Investigators: Robert C. Allen, M.D., Ph.D., Cpt., MC
Basil A. Pruitt, Jr., M.D., Col., MC

Reports Control Symbol MEDDH-288(R1)

The opsonic capacity of sera from patients with burn injuries is being studied throughout the patients' course of hospitalization, and the activities of these sera are compared with simultaneously drawn control sera. Quantification of opsonic capacity is based upon the rate and magnitude of opsonin-mediated activationof polymorphonuclear leukocyte metabolism. This measurement is based on chemical amplification of the luminescence (chemiluminescence) response associated with certain oxidation reactions that follow polymorphonuclear leukocyte activation. Non-specific opsonic capacity, mediated by alternative pathway complement, is titrated using zymosan as the stimulant. Specific opsonic capacity, mediated by antigen-specific IgG, IgM and classical pathway complement, will be titrated against bacteria commonly associated with post-burn infection (eg. S. aureus). These functional measurements of opsonins will be compared to results obtained from immunodiffusion and immunoelectrophoretic techniques. Sera are presently being collected, and initial studies employing chemiluminescent technique and radial immunodiffusion are underway. The techniques for immunoelectrophoresis are in the development stage at present.

Polymorphonuclear Leukocytes
Chemiluminescence
Opsonization
Immunoglobulins
Complement
Burn injury

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OG 1840	79 10 02	DD DR&E AR(Q)6

3. DATE PREV SUMRY	4. KIND OF SUMMARY	5. SUMMARY SCTY	6. WORK SECURITY	7. REGRADING	8A. DISP'N INSTR'N	8B. SPEC. DATA CONTRACTOR ACCESS	8. LEVEL OF SUM
79 10 01	D. CHANGE	U	U	NA	NL	[X] YES [ ] NO	A. WORK UNIT

10. NO. CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	076
b. CONTRIBUTING				
CONTRIBUTING				

11. TITLE (Precede with Security Classification Code) (U) Distribution and Control of Peripheral Blood Flow Following Extensive Leg Surface Injury in Burned Soldiers (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS
003500 Clinical Medicine 012900 Psychology

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
79 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 79	.3	1
FISCAL YEAR CURRENT 80	.6	19

19. RESPONSIBLE DOD ORGANIZATION
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NAME

21. GENERAL USE
FOREIGN INTELLIGENCE NOT CONSIDERED

22. KEYWORDS (Precede EACH with Security Classification Code) (U) Vasomotor control; (U) Granulation tissue; (U) Arteriovenous shunts; (U) Nutrient blood flow

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

23. (U) To establish vascular dimensions in large surface granulating wound and determine extent of functional innervation of the neovasculature.

24. (U) A large animal model has recently been established in order to study, in greater detail, the anatomical and physiological basis for wound directed peripheral blood flow. The wound is created in one hindlimb of a 20-40 kg goat by surgically removing the skin from that leg from groin to hock. Over the next week the wound develops into a rich bed of granulation tissue and blood flow to that limb increased to twice that of the contralateral, uninjured extremity. Peripheral glucose uptake and lactate production increased in the injured leg, comparable to changes previously observed in burn patients, suggesting similar local chemical environments are present in both granulating wounds. The current study is designed to partition these total leg blood flow measurements into wound, skin, and muscle compartments through the entrapment of radiolabelled microspheres. Through the use of different size spheres, one can also describe the vascular dimensions in the wound (capillaries vs A-V shunts). Once peripheral flow distribution and vascular dimensions are known, various features of vasomotor control will be evaluated. Of primary concern is degree of neurogenic control but this model is also ideally constructed for additional studies in local chemical influences on wound perfusion.

25. (U) 7810 - 7909 Initial studies with radiolabelled microspheres (Ce-141 and Sr-85, 15 μ in diameter) suggest that the granulating wound contains large numbers of arteriovenous shunts. Technical refinements and additional studies are required, however, before more specific conclusions can be drawn.

*Available to contractors upon originator's approval

DD FORM 1 MAR 66 1498 PREVIOUS EDITIONS OF THIS FORM ARE OBSOLETE. DD FORMS 1498A 1 NOV 68 AND 498 1 MAR 68 FOR ARMY USE ARE OBSOLETE

ANNUAL PROGRESS REPORT

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: DISTRIBUTION AND CONTROL OF PERIPHERAL BLOOD FLOW FOLLOWING EXTENSIVE LEG SURFACE INJURY IN BURNED SOLDIERS

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

1 October 1978 - 30 September 1979

Investigators:

Louis H. Aulick, PhD, Major, MSC
Douglas W. Wilmore, M.D.

Reports Control Symbol MEDDH-288(R1)

Unclassified

ABSTRACT

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: DISTRIBUTION AND CONTROL OF PERIPHERAL BLOOD FLOW FOLLOWING EXTENSIVE LEG SURFACE INJURY IN BURNED SOLDIERS

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

Period covered in this report: 1 October 1978 - 30 September 1979

Investigators: Louis H. Aulick, PhD, Major, MSC
Douglas W. Wilmore, M.D.

Reports Control Symbol MEDDH-288(R1)

In a continuing effort to study reflex vasomotor control of the burn wound, two water filled, venous occlusion plethysmographs were designed and constructed to fit the forearms of burn patients. Forearm blood flow (FBF) was then measured bilaterally in injured and uninjured extremities of one control and one patient before and after induced hypothermia. FBF in the uninjured control decreased by 60% bilaterally with reflex cooling. In the patient, however, a comparable cool stress results in a 48% drop in FBF in the uninjured limb as compared to only a 21% decreased in the injured limb. Further studies are necessary before definitive conclusions can be established.

Reflex vasomotor control
Wound blood flow
Plethysmography

DISTRIBUTION AND CONTROL OF PERIPHERAL BLOOD FLOW FOLLOWING EXTENSIVE LEG SURFACE INJURY IN BURNED SOLDIERS

A series of direct and indirect heating studies have demonstrated that the wound vasculature is functionally denervated in that it will respond appropriately to direct changes in surface temperature but is unresponsive to reflex vasodilator signals.

While such studies demonstrated a loss of reflex vasomotor control in the burn wound, they have been criticized in two areas. First, simultaneous studies on injured and uninjured limbs of the same patient have not been performed. Secondly, while a decrease in neurogenic vasodilation was well established, the lack of reflex vasoconstriction had never been demonstrated. In an effort to answer both criticisms, two water-filled, forearm venous occlusion plethysmographs were constructed and validated. To study reflex control of forearm blood flow (FBF), the subject rested supine in a 30°C room and plethysmographs were positioned bilaterally. Water temperature in both plethysmographs was maintained at 35°C throughout the study. After a one hour equilibration period, blood flow to the hand was occluded for five minutes and a series of 3-5 measurements performed. Immediately thereafter similar measurements are performed on the other arm. To stimulate cutaneous vasoconstriction, the room was then cooled slowly and the subject given ice to eat over the next 30-45 minutes.

To date, the effect of reflex cooling on bilateral FBF has been demonstrated in one uninjured control subject and in one fully healed burn patient. In the control subject, FBF dropped upon cooling from 8.02 to 3.11 ml/min·100 ml while contralateral FBF decreased from 7.10 to 2.49 ml/min·100 ml. This study both demonstrated the uniformity in response of the two limbs (a 61% decrease in FBF bilaterally) as well as provided some index of the relative magnitude of surface vasoconstriction to be anticipated in this kind of experiment.

In the patient, FBF in the control arm dropped from 4.75 to 2.46 ml/min·100 ml with reflex cooling; flow to the healed injured forearm, however, fell from 10.18 to 8.03 ml/min·100 ml. This suggests that reflex vasoconstriction is reduced in the wound, since flow to uninjured skin decreased by almost 50% as compared to only a 21% reduction in the contralateral limb. Additional studies are planned as soon as injured patients become available.

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OG 6979	79 10 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
78 10 01	D. CHANGE	U	U	NA	NL	☒ YES ☐ NO	A. WORK UNIT

10. NO. CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	085
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code) (U) Mitochondrial Oxidative Function in The Burn Wound and The Effect of Resuscitation 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
78 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 79	.7	20
FISCAL YEAR CURRENT 80	.7	23

19. RESPONSIBLE DOD ORGANIZATION
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ASSOCIATE INVESTIGATORS
NAME
NAME

DA

22. KEYWORDS (Precede EACH with Security Classification Code)
(U) Metabolism; (U) ATP; (U) Oxygen; (U) Cytochromes; (U) Goats

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

23. (U) To define pathologic alterations in cellular function produced by thermal injury and to assess efficacy of treatment in reversing the process in burned soldiers.

24. (U) Initial studies will utilize the burned goat as a model for human burns. Following specific interval of time, sample of granulation tissue from burned areas will be sampled for separation of its subcellular components. Mitochondrial oxidative phosphorylation will be assayed by appropriate techniques, oxygen uptake by polarographic electrode, cytochrome content and activity by the double beam dual wave length spectrophotometry, and calcium transport by its reaction with murexide. After these baseline data are obtained, the effects of various resuscitation formulae in improving tissue perfusion will be assessed by changes in cellular functions described above.

25. (U) 7810 - 7909 Coupling is a standard measure of mitochondrial integrity and is best expressed as the respiratory control ration (RCR). Uncoupling results in a higher and inefficient rate of utilization of substrates and oxygen. Liver mitochondria from burned, but not from control, animals are uncoupled, with decreased RCR's (7.96 control vs 6.50 burn, $p \leq 0.05$). Furthermore, uncoupling increases (and RCR decreases) with time post burn ($RCR = -0.75 + 154.3/PBD$, $r^2 = 0.79$, PBD = post burn day), and may be the cellular basis for post burn hypermetabolism.

* Available to contractors upon originator's approval

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

ANNUAL PROGRESS REPORT

PROJECT NO. 3A161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: MITOCHONDRIAL OXIDATIVE FUNCTION IN THE BURN WOUND AND THE EFFECT OF RESUSCITATION IN BURNED SOLDIERS

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

1 October 1978 - 30 September 1979

Investigators:

Cleon W. Goodwin, M.D., Major, MC
Douglas W. Wilmore, M.D.
Arthur D. Mason, Jr., M.D.

Reports Control Symbol MEDDH-288(R1)

UNCLASSIFIED

ABSTRACT

PROJECT NO. 3S161101A91C-00, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: MITOCHONDRIAL OXIDATIVE FUNCTION IN THE BURN WOUND AND THE EFFECT OF RESUSCITATION IN BURNED SOLDIERS

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

Period covered in this report: 1 October 1978 - 30 September 1979

Investigators: Cleon W. Goodwin, M.D., Major, MC
Douglas W. Wilmore, M.D.
Arthur D. Mason, Jr., M.D.

Reports Control Symbol MEDDH-288(R1)

With the completion of resuscitation, postburn hypermetabolism increases over the succeeding weeks and is accompanied by increases in blood flow to the wound and viscera. This response is paralleled by increases in total body and organ oxygen utilization. The majority of tissue oxygen utilization occurs in the mitochondria, cellular organelles whose function is to convert substrate and oxygen to ATP. Abnormalities of mitochondrial function have been demonstrated in ischemia, hypoxia and endotoxemia. Since many of the physiologic abnormalities of the above conditions are present in hypermetabolic burn patients, mitochondrial function is being investigated, utilizing the hypermetabolic burn rat model previously established at this Institute. Rat liver, the most metabolically active organ, is homogenized and its mitochondria isolated by differential centrifugation. Mitochondrial oxygen uptake is measured polarographically and cytochrome concentrations by dual wavelength spectrophotometry. All values are normalized to mitochondrial protein concentration. Liver mitochondria from burned animals are uncoupled, with decreased respiratory control ratios (7.96 control versus 6.50 burn, $p \leq 0.05$). Uncoupling is a standard measure of the integrity of mitochondrial function and results in a higher and inefficient use of substrates and oxygen. Furthermore, uncoupling increases with time postburn as total body $\dot{V}O_2$ increases (RCR = $-0.75 + 153.4/PBD$, $r^2 = 0.79$), and may be the cellular basis for postburn hypermetabolism.

Mitochondria
Oxidative phosphorylation
Uncoupling
Respiratory control ratio

MITOCHONDRIAL OXIDATIVE FUNCTION IN THE BURN WOUND AND THE EFFECT OF RESUSCITATION IN BURNED SOLDIERS

Following fluid resuscitation of the acutely burned patient, marked hypermetabolism develops and reaches a plateau 7 to 10 days following injury. This hypermetabolism is characterized by increases in cardiac output, oxygen consumption, and metabolic rate and has been related to enhanced catecholamine production (1,2). Partition of blood flow in burned patients has indicated that a substantial portion of this increased flow is directed to the viscera, particularly the liver (3). Although oxygen delivery to the liver is elevated as a consequence of the increased hepatic flow, arteriovenous oxygen content difference remains normal as a result of markedly increased hepatic oxygen utilization. Since more than 90% of tissue oxygen utilization occurs in the mitochondria (4), analysis of the oxidative functions of this intracellular organelle may explain the increased oxygen consumption and hypermetabolism of thermally injured patients.

METHODS

Animal Preparation. Male Holzman rats (475 to 500 grams body weight) were placed in single unit cages and allowed to acclimate for 1 week. Using a modification of a standard burn injury model, a 60% total body surface thermal injury was placed on one animal from each of seven pairs (5). Each injured animal was resuscitated by intraperitoneal injection of 15 ml of balanced electrolyte solution and allowed to recover. Beginning on postburn day 10, a pair of rats was sacrificed every 2 days, and liver tissue was removed for mitochondrial preparations.

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

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Isolation of Mitochondria. Following removal, the tissue (approximately 5 grams) was immediately placed into 0° C medium and chopped into small pieces to facilitate rapid cooling. Mitochondria were isolated in a medium consisting of 0.225 M mannitol, 0.075 M sucrose, 100 μM EGTA, and a final pH of 7.4. The mitochondria were gently homogenized by a motor-driven Teflon pestle in a glass homogenizer. The resulting suspension was centrifuged at 600 X g to remove residue, and the mitochondria were washed four times and recovered at 8000 X g. The medium for the last two washes contained no EGTA. Washed mitochondria were suspended in an EGTA-free medium at 20-30 mg protein per ml.

Mitochondrial Assays. All measurements were carried out in a medium containing 0.225 M mannitol, 0.075 M sucrose, 15 mM TRIS, 10 mM KH_2PO_4, and a final pH of 7.4. Oxygen uptake was measured polarographically with a Clark O_2 electrode in mitochondria respiring in State 4 (excess substrate) and State 3 (excess substrate and ADP), in the presence of 1 mM $MgCl_2$ (6). Respiratory control ratios (RCR) were calculated as the ratio of State 3 to State 4 rates. ADP/O ratios were calculated from the measured O_2 consumption (O_2 capacity of medium 240 nanomoles/ml) with 500 μM ADP as the phosphate acceptor. Protein concentrations of the mitochondrial samples were determined by a modification of the biuret reaction (7). Electron micrographs of selected samples were processed in the Institute of Surgical Research facility.

Statistics. The data were analyzed by paired *t*-tests and analysis of variance. A probability of less than 0.05 was used to judge significant differences between treatment groups.

RESULTS

With glutamate plus malate as substrates (in the presence of magnesium), the RCR of mitochondria isolated from burned rats was significantly lower than that determined in mitochondria from uninjured animals (Table 1). This evidence of uncoupling of oxidative phosphorylation was not observed for other combinations of substrates used in this investigation. Further, when compared with mitochondria from their paired unburned controls, mitochondria from burned rats were not significantly different in any of the other measured oxidative functions (State 4 oxygen utilization rate, State 3 oxygen utilization rate, and ADP/O). In general, the addition of the divalent cation magnesium increased the efficiency of oxygen utilization,

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6. Chance B, Williams GR: The respiratory chain and oxidative phosphorylation. Adv Enzymol 17:65-134, 1956.
7. Gornall AG, Bardawill CJ, David MM: Determination of serum proteins by means of the biuret reaction. J Biol Chem 177:751-766, 1949.

Table 1. The Effect of Burn Injury on Mitochondrial Oxidative Function

	Control	Burn	P_1
I. State 4 (nmoles/min/mg protein)			
G + M - Mg^{++}	2.13	2.53	NS
G + M + Mg^{++}	1.90	2.07	NS
P_2	NS	NS	
Succ + rot - Mg^{++}	4.98	5.96	NS
Succ + rot + Mg^{++}	4.16	4.17	NS
P_2	< 0.003	< 0.015	
II. State 3 (nmoles/min/mg protein)			
G + M - Mg^{++}	13.99	13.77	NS
G + M + Mg^{++}	13.89	12.97	NS
P_2	NS	< 0.03	
Succ + rot - Mg^{++}	21.19	20.47	NS
Succ + rot + Mg^{++}	18.25	17.50	NS
P_2	< 0.003	< 0.001	
III. RCR			
G + M - Mg^{++}	7.52	6.33	NS
G + M + Mg^{++}	7.96	6.51	< 0.05
P_2	NS	NS	
Succ + rot - Mg^{++}	4.45	3.92	NS
Succ + rot + Mg^{++}	4.57	4.50	NS
P_2	NS	< 0.001	
IV. ADP/O			
G + M - Mg^{++}	2.60	2.66	NS
G + M + Mg^{++}	2.36	2.48	NS
P_2	< 0.005	< 0.03	
Succ + rot - Mg^{++}	1.59	1.55	NS
Succ + rot + Mg^{++}	1.49	1.45	NS
P_2	< 0.01	< 0.01	

P_1 = probability of significant differences between means of control and burned groups; P_2 = probability of significant differences between means of $-Mg^{++}$ and $+Mg^{++}$ groups; G = glutamate; m = malate; Mg^{++} = magnesium; succ = succinate; rot = rotenone.

particularly in mitochondria from burned animals with succinate as the metabolic substrate.

The uncoupling of mitochondrial respiration observed in studies of burned rats increased with time (as evidenced by progressively decreasing RCRs): RCR = -0.75 + 153.4/PBD, $r^2 = 0.79$; PBD = postburn day. This weakly hyperbolic relationship is nearly linear (RCR = 12.7 - 0.27 PBD, $r^2 = 0.71$, and it may be more physiologically relevant to consider it as such (see Fig. 1). RCR from unburned control animals remained constant with time. Burned animals progressively lost weight, which stabilized after the first postburn week (425 grams versus 492 grams for preburn weight, $P < 0.01$). The control animals maintained stable weights over the same intervals (495 grams versus 507 grams).

DISCUSSION

In anaerobic glycolysis, only 2 moles of adenosine triphosphate (ATP) are formed from each mole of glucose. This inefficient yield is only 5.6% of the net production of 36 moles of ATP synthesized during oxidative glucose metabolism. Mitochondria utilize 90% of the available cellular oxygen supply under normal conditions. Deficiency of oxygen availability or mitochondrial malfunction would be expected to affect cellular energy supplies. Decreased oxygen availability has been shown to alter gastric secretion (8), fracture union (9), and wound healing (10). The important energy-linked electron transfer carriers and enzymes are located on the inner mitochondrial membrane. Substrate carriers are necessary for transport of Kreb's cycle intermediates into the mitochondrion. The most important of these are malate-phosphate, ketoglutarate-malate, and citrate-malate carriers. Inhibition of any of these shuttle systems blocks the electron transfer reaction to the final electron acceptor, oxygen. Adenine nucleotide translocase, ATPase, and the phosphate carriers are likewise essential for ATP synthesis. And, finally, oxygen transport and utilization by mitochondria is essential for maximal efficiency of the energy producing process.

In the presence of adequate substrates and oxygen, mitochondria assume various states of respiratory activity (11). In State 3,

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$$RCR = -0.75 + \frac{153.4}{PBD} \quad r^2 = 0.79$$

$$RCR = 12.7 - 0.27\ PBD \quad r^2 = 0.71$$

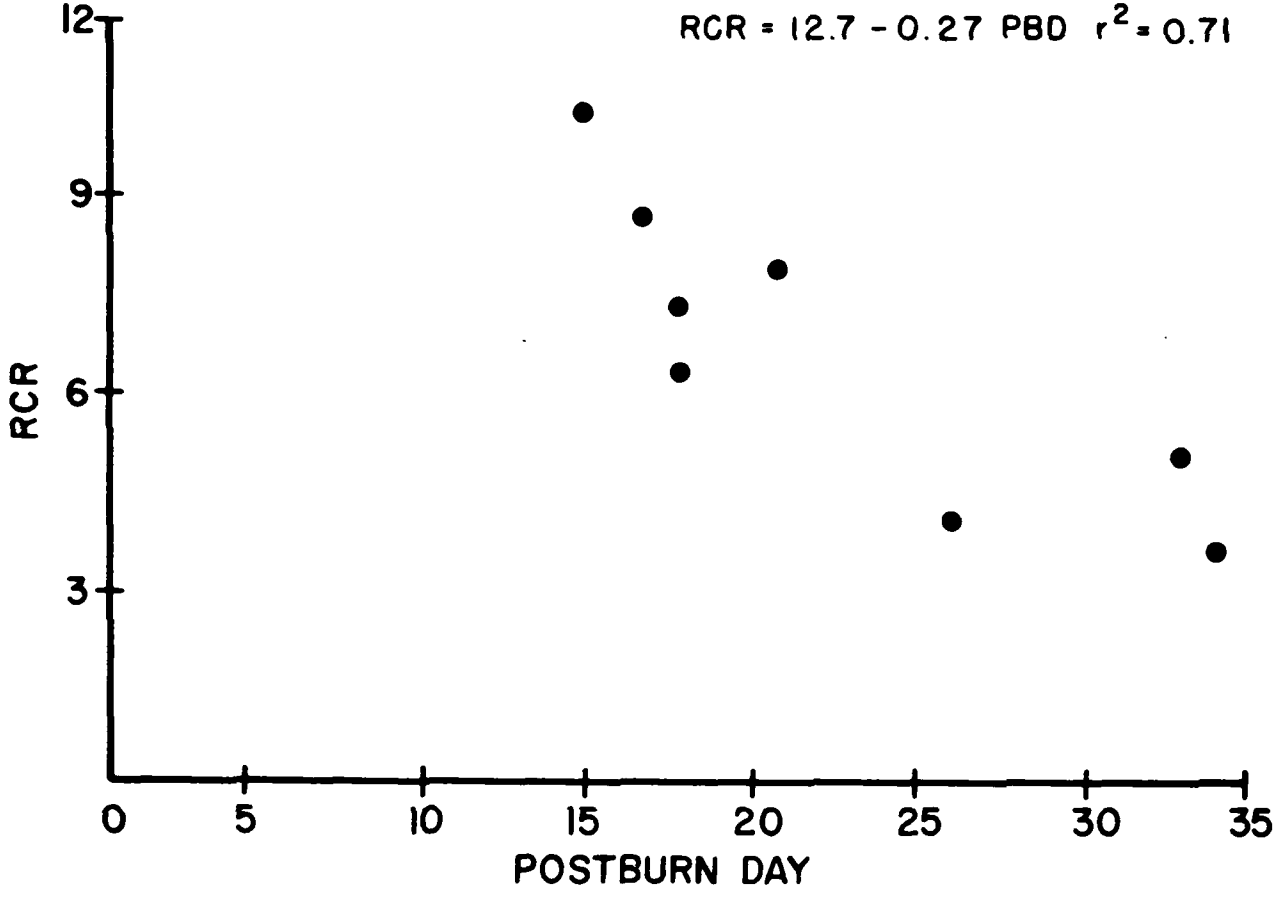

Figure 1

the active state, in the presence of adequate substrate and ADP, maximal oxygen uptake occurs and ATP is produced. When the phosphate acceptor, ADP, is exhausted, the mitochondria return to State 4, the resting state. Oxygen consumption falls and no further ATP is synthesized. Other important mitochondrial functions include calcium accumulation, which is at least partially responsible for regulation of cellular-free calcium. Essential to the design of these experimental conditions is a standardized assay in which substrate, phosphate, and oxygen are present in excess, such that any deviation of activities from control represents an irreversible alteration of mitochondrial function.

Recent innovations in isolation and assay of subcellular organelles have enabled investigators to compare mitochondria from healthy and diseased subjects. Reproducible alterations have been consistently documented in ischemia and shock of varying durations. However, as ischemia progresses, oxygen uptake decreases and, even later, so do the activities of electron transfer enzymes. Similar effects occur with endotoxin shock (12,13). However, this inhibition occurs only in those organs with decreased blood flow (liver, kidney, brain, skeletal muscle) (14).

As long as oxygen supply to the cell is in excess of a critical level necessary for the electron transfer chain to operate, pure tissue hypoxia will not damage or inhibit mitochondrial activity. On the contrary, adaptive responses take place which enhance the ability of the mitochondria to utilize oxygen. At lower levels of oxygenation, mitochondria in heart, liver, kidney, and brain demonstrate an increased respiratory activity. In both acute and chronic hypoxia, calcium accumulation increases, cytochrome concentrations decrease, and oxygen utilization increases (15,16). If the

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13. Mela L, Miller LD, Bacalzo LV, et al: Role of intracellular variations of lysosomal enzyme activity and oxygen tension in mitochondrial impairment in endotoxemia and hemorrhage in the rat. Ann Surg 178:727-735, 1973.

14. Mela L, Hinshaw LB, Coalson JJ: Correlation of cardiac performance, ultrastructural morphology, and mitochondrial function in endotoxemia in the dog. Circ Shock 1:265-272, 1974.

15. Goodwin CW, Mela L, Park CD, Campbell J: Adaption to oxygen by cardiac mitochondria in newborn and hypoxic adult dugs. Surg Forum 25:185-187, 1974.

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oxygen utilization is related to the concentration of the cytochromes, or turnover number, the ability of the respiratory enzymes to react with oxygen is dramatically increased when oxygen availability is decreased. This directly states that in adapting to lower tissue oxygen tensions, the mitochondria utilize oxygen more efficiently, not less so, and function at supramaximal rates when compared to normal. An opposite activity change occurs in the newborn animal's adaptation to normoxia. Calcium transport decreases, cytochrome concentrations increase, and oxygen uptake decreases (17-20). Because more oxygen is available, the respiratory chain operates less efficiently.

The relationship of increased tissue oxygen consumption to abnormalities of mitochondrial function remains unexplored. Oxygen utilization by mitochondria does not directly result in the production of energy in the form of ATP but rather is coupled to energy production through a series of proposed intermediate steps. Further, this process of oxidative phosphorylation is approximately 45 to 50% efficient, with heat production accounting for the remainder of the potentially available energy from this process. Under certain conditions, the passage of substrate-reducing equivalents down the mitochondrial electron transport chain to oxygen, which is reduced to water, can be partially or totally uncoupled from the production of ATP. This uncoupling of oxidative phosphorylation allows oxygen utilization to increase markedly with little energy output and with increased heat production. Uncoupling of mitochondrial oxidative phosphorylation can explain postburn metabolism, which is characterized by elevated oxygen consumption and heat production (21).

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Using glutamate plus malate (with magnesium) as substrate, mitochondria from burned rats displayed significantly less coupling of oxidative phosphorylation when compared with this subcellular fraction isolated under identical conditions from unburned controls. Since this burn injury model reliably exhibits a 50 to 60% increase in resting metabolic rate (22), it is tempting to relate the partial uncoupling to the elevated total body oxygen consumption. However, several factors prevent any definitive connection between these two metabolic variables. The difference in the RCRs between the injured and the controlled groups is of borderline significance, $P < 0.05$, when the data are examined by standard paired t-tests. Further, when the groups are analyzed by analysis of variance, significant intragroup, as well as intergroup, interactions are evident and do not allow a conclusive statement about a direct relationship between the degree of uncoupling and oxygen consumption. Respiratory control ratios in burned rat mitochondria decreased with time postburn in a nearly linear fashion, and this decline with time prevents lumping together burned animals into one treatment group. In addition, significant weight loss occurred in the injured group of animals and may have influenced the observed changes in subcellular function observed in this study. The unburned control animals maintained constant body weights.

Based on the above information, further studies have been designed to eliminate or explain the potential effects of postburn day of study and weight loss. All injured animals will be studied on the fourteenth postburn day, which is well within the stable period of hypermetabolism. Since we have not been able to prevent postburn weight loss by force-feeding the injured animals, a separate group of unburned animals will be fed a restricted diet which produces the same degree of weight loss sustained by the burned animals. Both of these groups will be compared to uninjured rats maintained on a stable ad libitum diet. Thus, the potential effects of weight loss can be separated from those produced by the massive burn.

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22. Herndon DN, Wilmore DW, Mason AD Jr: Development and analysis of a small animal model simulating the human postburn hypermetabolic response. J Surg Res 25:394-403, 1978.

PUBLICATIONS/PRESENTATIONS - None

RESEARCH AND TECHNOLOGY WORK UNIT SUMMARY

1. AGENCY ACCESSION	2. DATE OF SUMMARY	REPORT CONTROL SYMBOL
DA OG 1841	79 10 02	DD-DR&E(AR)636

3. DATE PREV SMRY	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
79 10 01	D. CHANGE	U	U	NA	NL	[X] YES [ ] NO	A. WORK UNIT

10. NO. CODES	PROGRAM ELEMENT	PROJECT NUMBER	TASK AREA NUMBER	WORK UNIT NUMBER
a. PRIMARY	61101A	3A161101A91C	00	080
b. CONTRIBUTING				
c. CONTRIBUTING				

11. TITLE (Precede with Security Classification Code): (U) Assessment of Thyroid Hormone Kinetics in Thermally Injured Patients (44)

12. SCIENTIFIC AND TECHNOLOGICAL AREAS: 003500 Clinical Medicine 002300 Biochemistry

13. START DATE	14. ESTIMATED COMPLETION DATE	15. FUNDING AGENCY	16. PERFORMANCE METHOD
79 08	Cont	DA	C. In-House

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

18. RESOURCES ESTIMATE	a. PROFESSIONAL MAN YRS	b. FUNDS (In thousands)
FISCAL YEAR PRECEDING 79	.2	4
FISCAL YEAR CURRENT 80	.8	30

19. RESPONSIBLE DOD ORGANIZATION
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PRINCIPAL INVESTIGATOR (Furnish SSAN if U.S. Academic Institution)
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TELEPHONE: 512-221-2720
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) Thyroxine; (U) 1-triiodothyronine; (U) 1-reverse-T_3; (U) Kinetics; (U) Burn patients

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

23. (U) To assess metabolic clearance rate and production rate of Thyroxine, 1-T_3, and 1-rT_3 in burn patients.

24. (U) 1-T_3 labeled with ^{125}I, 1-T_4 labeled with ^{131}I, and 1-rT_3 labeled with ^{125}I will be injected intravenously into burn patients and their disappearance from plasma monitored. Both single injection and continuous infusion techniques will be evaluated. A single compartmental model will be used for analyzing data.

25. (U) 7908 - 7909 Approval to begin the studies has just been received. Studies have begun in four burn patients and the samples collected from those patients are being assayed for thyroid hormone values which will be used to calculate kinetic indices.

*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

PROGRESS REPORT

PROJECT NO. 3A161101A91C, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: ASSESSMENT OF THYROID HORMONE KINETICS IN THERMALLY INJURED PATIENTS

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

1 August 1979 - 30 September 1979

Richard A. Becker, M.D.
George M. Vaughan, M.D., MAJ, MC

Reports Control Symbol MEDDH-288(R1)

Unclassified

ABSTRACT

PROJECT NO. 3A161101A91C, IN-HOUSE LABORATORY INDEPENDENT RESEARCH

REPORT TITLE: ASSESSMENT OF THYROID HORMONE KINETICS 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 August 1979 - 30 September 1979

Investigators: Richard A. Becker, M.D.
George M. Vaughan, M.D., Maj., MC

Reports Control Symbol MEDDH-288(R1)

The metabolic clearance rate and production rate of thyroid hormone is being studied in burn patients. Triiodothyronine (T_3) and reverse-triiodothyronine (rT_3), labeled with ^{125}I, and thyroxine, labeled with ^{131}I, are administered intravenously into burn patients, using a single injection technique, and their disappearance from plasma are monitored. A single compartmental model is used for analyzing data. Studies have been performed in two burn patients. In contrast to control subjects, a highly significant increase in both the metabolic clearance rates and production rates of T_3, rT_3, and T_4 was observed in the burn patients.

Thyroxine
Triiodothyronine
Reverse-triiodothyronine
Kinetics
Burn patients

PUBLICATIONS

1 October 1978 - 30 September 1979

Artz CP, Moncrief JA, Pruitt BA Jr: Burns: A Team Approach, WB Saunders Co., Philadelphia, 1979.

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